Antimicrobial effect of Manuka honey and Kanuka honey alone and in combination with the bioactives against the growth of *Propionibacterium acnes* ATCC 6919

A Thesis

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Abstract

Background: Acne vulgaris is a chronic inflammatory disease of the pilosebaceous follicle. The *Propionibacterium acnes (P. acnes)* play a key role in inflammation and the formation of comedones. *P. acnes* has been reported to develop antibiotic resistance, which has generated some interest in developing natural antimicrobial compounds which forms the subject matter of this study. Recently, Manuka honey (*leptospermum scoparium*) has demonstrated strong antibacterial activities against a wide range of pathogens with distinct non-peroxide activity. Kanuka honey has also shown to be effective against many bacterial species. Many natural bioactives were reported to possess strong antibacterial activities, a few of which were studied against *P. acnes*. Therefore, the aims of this study were to investigate the antibacterial activity of Manuka honeys and Kanuka honey with and without catalase against the growth of *P. acnes* alone and to screen the antibacterial activities of five selected nature bioactives alone and in combination with honey against *P. acnes in vitro*.

Methods: The growth of *P. acnes* was evaluated under aerobic and anaerobic conditions. *P. acnes* was cultivated in nutrient broth and fastidious anaerobic agar containing horse blood. Manuka honeys 20+, 15+ and 10+ UMF and Kanuka honeys were tested against the growth of *P. acnes,* ranging from 0.5 % to 12.5 % (w/v) with and without catalase under both aerobic and anaerobic conditions. The artificial honey was used as the control. Manuka tree essential oil (MTO), lavender essential oil (LO), green tea extract (GTE), olive leaves extract (OLE), propolis were screened using disc diffusion method, spectrophotometric assay, viable cell counts to determine the survival of *P. acnes* in the bioactives testing solutions. The combination creams of Manuka honey 10+ UMF (10 %, w/v) with bioactives were studied using viable cell count method to determine the viable cells of *P. acnes.*

Results: *P. acnes* is capable of growing under both aerobic and anaerobic conditions. Manuka and Kanuka honeys exhibited antibacterial activity against the growth of *P. acnes*. Kanuka honey had similar antibacterial activity as Manuka honey 15+ UMF and Manuka honey 20+ UMF without catalase. MIC_{100} of Manuka honey 20+ UMF was 148.90 mg/mL; MIC_{100} of Manuka honey 15+ UMF was 125.81 mg/mL; MIC_{100} of Manuka honey 10+ UMF was 144.43 mg/mL; MIC_{100} of Kanuka honey was 123.28 mg/mL. Manuka honeys possessed non-peroxide activity, but the antibacterial activity of Kanuka honey decreased significantly after the removal of hydrogen peroxide with MIC_{100} of 549.21 mg/mL. Artificial honey did not markedly inhibit the growth of *P. acnes*. Among the five bioactives, only GTE and MTO had bactericidal ability. Honey creams with bioactives showed that cream containing 10 % honey and 1 % GTE caused about five log reductions in the bacterial cell numbers; in contrast, cream of honey (10 %) and MTO (0.125 %) resulted in about two log reductions. No bacterial cells (< 100 CFU/mL) were found in the creams containing honey (10 %), MTO (0.125 %) and GTE (1 %).

Conclusion: Manuka honey exhibited antibacterial activity against the growth of the *P. acnes*. The antibacterial potency of the honey was significantly enhanced by the presence of bioactives in the emulsion cream.

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ABBREVIATIONS

Abs_{595nm}	=	Absorbance at wavelength of 595 nm
ANOVA	=	Analysis of variance
BHI	=	Brain heart infusion
CFU	=	Colony forming unit
CO_2	=	Carbon dioxide
DHA	=	Dihydroxyacetone
DMSO	=	Dimethyl Sulfoxide
EGCG	=	Epigallocatechingallate
FA agar	=	Fastidious anaerobe agar
GTE	=	Green tea extract
HMF	=	Hydroxymethylfurfural
LO	=	Lavender essential oil
MBC	=	Minimum bactericidal concentration
MGO	=	Methylglyoxal
MIC	=	Minimum inhibition concentration
MRSA	=	Methicillin-resistant *Staphylococcus aureus*
MTO	=	Manuka tree oil
OLE	=	Olive leaf extracts
P. acnes	=	*Propionibacterium acnes*
UMF	=	Unique Manuka Factor
SD	=	Standard deviation
SEM	=	Standard error of mean
ϴ	=	Diameter

1.0 Introduction

Acne vulgaris (commonly known as acne) is defined as a chronic inflammatory disease of the pilosebaceous follicle which is notable for open and/or closed comedones (blackheads and whiteheads) and inflammatory lesions including papules, pustules, or nodules on the skin surface of the face, neck, chest, or back (Strauss *et al*., 2007; Burrow, 2009). Acne is known as one of the most prevalent skin conditions affecting approximately 80 % of girls and 90 % of boys in their adolescence years (Porter, 1998). Acne often results in secondary damage of the skin in the form of scarring. Despite its apparently superficial appearance, the disease can penetrate further than the surface of the skin and may place a heavy emotional and psychological burden on the patients (Ayer and Burrow, 2006).

Acne is caused and characterized by many factors, which include *Propionibacterium acnes* (*P. acnes*) activity, increased sebum production, androgenic stimulation, follicular hypercornification, lymphocyte, macrophage, and neutrophil inflammatory response (Porter, 1998). Among all the factors, the *P. acnes* has been suggested to play a significant role in the development, formation and inflammation of acne (Dessinioti *et al*., 2009).

P. acnes is a common component of the bacterial flora of the human skin, being predominantly located within the deeper recesses of pilosebaceous follicles at the sebum-rich sites of human skin preferring moist, oil areas of the skin (Hammer, 2003). Excess sebum in the microcomedo causes proliferation of *P. acnes* (Muizzuddin, 2008). *P. acnes* can break down the triglycerides in the sebum to form free fatty acids which are both comedogenic and proinflammatory (Berson *et al*., 1995). *P. acnes* can secret proinflammatory cytokines including interleukin (IL)-1β, IL-8, and tumour necrosis factor (TNF)-α and chemotactic factors (C5a) that attract neutrophilis (Dessinioti *et al*., 2009). Lysosomal enzymes released from the neutrophils rupture the follicle wall and release proinflammatory mediator, including keratin and lipids into the surrounding dermis. In addition, *P. acnes* can also release lipases, proteases and hyaluronidases that can lead to tissue injury (Dessinioti *et al*., 2009).

Based on the severity of acne, topical therapy is the first safe attempt for a patient with noninflammatory comedones or mild to moderate inflammatory acne without notable side effects (Ayer and Burrows, 2009). A common topical treatment includes benzoyl peroxide, retinoids (tretinoin, tazarotene, adapalene and retinylaldehyde), salicylic acid, antibiotics and anti-inflammatory drugs (Ayer and Burrows, 2009). For moderate to severe inflammatory acne, oral antibiotics are normally added to the treatment (Berson, *et al*., 1997). The oral antibiotic used for acne includes tetracycline, erythromycin, minocycline, doxycycline, and trimetho–prim–sulfamethoxazole. Nevertheless, many noticeable side effects were found for individual patients with the current treatments (Berson *et al*., 1995). Many patients may experience dry skin, headaches, dry eyes, photosensitivity, hair loss, nasal irritation and diminished night vision (Berson, *et al*., 1997). Most importantly, *P. acnes* was reported to develop antibiotic resistance for the first time in 1979. Hence, long exposure to topical antibiotics can induce an enormous selective pressure on the bacterial skin flora of acne patients. This can contribute to treatment failure with the selection and overgrowth of the antibiotic-resistant *P. acnes* (Coate *et al*., 2002). It is therefore of interest to look for alternatives which have good antibacterial activities, especially potential bioactives sourced from natural origin.

The antibacterial activities of honey have been described by many researches (Molan, 1992; Postmes *et al*., 1993; Steinberg *et al*., 1996; Weston *et al*., 2000; Cooper *et al*., 2002; Snow and Manley-Harris, 2004; Molan, 2006). Honey has been shown to be effective in the inhibition of a wide range of bacterial species. However, there have been no reports of any honey being tested against *P. acnes*. This study was therefore undertaken to investigate the susceptibility of *P. acnes* to the antibacterial activity of honey and other bioactives.

The major antibacterial factor in most honeys is hydrogen peroxide, produced in the honey by the action of glucose oxidase, which is inherent in the honey, but some antibacterial activity is due to other substances that are derived from the flowers (Snow and Manley-Harris, 2004). Other antibacterial factors of honey are related to the high sugar content, low water activity and acidity.

However, honey derived from *Leptospermum* species in Australasia contains unique phytochemical components such as methylglyoxal (MGO) that impart additional antibacterial properties and this phenomenon is commonly described as the non-peroxide activity (Molan, 1992; Elvira *et al*., 2008). *Leptospermum scoparium* (Manuka) honey has shown to inhibit the growth of a range of medically significant bacterial species (Cooper, 2007). The honey is sold as a therapeutic agent worldwide. The presence of MGO in the Manuka honey contributes to its uniqueness, which has been termed as the unique Manuka factor (Mavric *et al.,* 2008; Sherlock *et al*., 2010).

Unique Manuka Factors (UMF) are commonly used to represent the potency of the antibacterial activity of the Manuka honey in New Zealand. The UMF numbers are derived from a standard laboratory test with “active” Manuka honey, which have been compared with a standard antiseptic (phenol) to prove its potency (Badet and Quero, 2011). For example, the Manuka honey 20+ UMF could be equivalent to the antiseptic potency of 20 % solution of phenol.

Many other natural bioactives have been used for medicinal purposes due to their strong antibacterial activities against a wide range of pathogens. However, there is lack of information about the antibacterial activity of bioactives against the growth of *P. acnes*. Historically, essential oil distilled from Manuka tree leaves (MTO) has been traditionally used in New Zealand Maori remedies as an external antiseptic (Douglas *et al.*, 2004). Several studies have reported that MTO can inhibit the growth of Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli (E. coli), Pseudomonas aeruginosa* (*P. aeruginosa*) and *Candida albicans* (*C. albicans*) at concentration ranges of 0.039-1.25 % (v/v) (Perry *et al.*, 1999; Porter and Wilkins, 1999; Lis-Balchin *et al*. 2000; van Klink *et al*., 2005).

The olive leaf extracts (OLE) have been used for medicinal purposes for combating fevers and disease such as malaria (Bevavente-garcia *et al.*, 2000). Some reports have shown that OLE has antibacterial activity against a wide range of microorganisms and the bioactive has proved more active against fastidious bacteria including *Campylobacter jejuni* (*C. jejuni)*, *Helicobacter pylori* (*H. pylori)* and MRSA, with MICs as low as 0.31–0.78 % (v/v) (Myers *et al.*, 2006; Sudjana *et al.*, 2009)

Propolis has been commonly used in folk medicine for many years, and there is substantial evidence indicating that the bioactive has many biological activities, such as antiseptic, antifungal, antibacterial, antiviral, anti-inflammatory and antioxidant properties (Pietta, 2002). In studies by Miorin *et al.* (2003), propolis showed higher antibacterial activity against *S. aureus* in comparison to honey; the minimum inhibition concentration (MIC) of propolis ranged from 0.36 to 3.65 mg/mL. Furthermore, 30 % ethanol extract of propolis (90 µL) was highly active against *P. acnes* (Boyanova *et al*., 2006). The antibacterial activity of propolis is attributed to its ability to destroy the cytoplasm membrane and inhibition of bacterial motility and enzyme activity.

Few other natural bioactives have shown potential antibacterial activity against the growth of *P. acne*s (Kudo *et al*., 1992; Soriya, 2007; Elsaie *et al.,* 2009). It has been long known that placing a warm tea bag on acne helps to draw the toxins out of the lesion, promoting quicker healing times (Soriya, 2007). In the most recent clinical study, Elsaie *et al.,* (2009) reported 2 % topical green tea lotion to be a cost-effective treatment for mid-to-moderate acne vulgaris. In addition to this, flavour volatiles of green tea exhibited bacteriostatic ability with MICs between 3.13 and 400 µg/mL *in vitro* (Kudo *et al*., 1992).

Lavender essential oil (LO) has been reported to contain antimicrobial properties against *P. acnes*. According to the study of Chao *et al.* (2006), lavender essential oils exhibited strong bactericidal activities. Lavender essential oils (0.25 %, v/v) were able to kill *P. acnes* completely after 5 minutes treatment.

Although there has been more research in honey and bioactives in the last 20 years, there are still many unanswered questions. The previous studies have used different methods (Molan, 1992; Postmes *et al*., 1993; Steinberg *et al*., 1996; Weston *et al*., 2000; Cooper *et al*., 2002; Snow and Manley-Harris, 2004; Molan, 2006); hence, it is different to compare the results. The present study aims to fill gaps in our knowledge of the antibacterial activity of honey and bioactives against the growth of *P. acnes*. Therefore, the general objectives of the study may be summarised as follows:

1) To understand the growth of *P. acnes* using different growth media, and to monitor the growth kinetics of *P. acnes* in nutrient broth using the microtiter plate under aerobic and anaerobic conditions;

2) To evaluate the growth of *P. acnes* in Manuka honey 10+ UMF, Manuka honey 15+ UMF, Manuka honey 20+ UMF, Kanuka honey and artificial honey ranging from 0.5 % to 12.5 % with and without catalase; to determine the relationship between the percentage of inhibition for the bacterial growth with the honey concentration using dose-dependent curves; to determine the MICs of each honey under aerobic and anaerobic growth conditions;

3) To investigate the antibacterial activities of five selected bioactives in terms of their MICs and MBCs as well study their killing effects against *P. acnes*;

4) To determine the combined effects of Manuka honey and bioactives using killing rates and survival rates of *P. acnes*;

5) To formulate honey combinations with various bioactives contained in the emulsion cream and evaluate their antibacterial activity against *P. acnes*.

2.0 Literature review

2.1 Acne vulgaris

Acne vulgaris (commonly known as acne) is defined as a chronic inflammatory disease of the pilosebaceous follicle. This disease is notable for the open and/or closed comedones (blackheads and whiteheads) and inflammatory lesions including papules, pustules, or nodules on the skin surface of the face, neck, chest, or back (Figure 2.1) (Strauss *et al*., 200; Burrow, 2009). Acne vulgaris is the most prevalent skin disorder and is estimated to affect 85 to 100 % of the world's population at some time in their life (Tayor and Shalita, 2004). Approximately 80 % of girls and 90 % of boys develop acne vulgaris in their adolescent years and some people may continue to have acne in their 40s and 50s (Ayer and Burrows, 2009). For girls, the peak incidence is from 14 to 17 years and for boys it ranges from 16 to 19 years (Rivera, 2008). About 95 % of both sexes with acne could be also affected by some degree of scarring (Rivera, 2008).

Despite the superficial appearance, the disease can penetrate further than the surface of the skin and may place a heavy emotional and psychological burden on the patients that may be far worse than the physical impact (Clayton *et al*., 2009). A survey conducted by Tan (2004) indicated that individuals affected with acne, regardless of disease severity, had social psychological and emotional problems as severe as those recorded for patients with asthma, epilepsy, diabetes, chronic back pain and arthritis. A review carried out by Ayer and Burrows (2006) stated that changing the skin's appearance associated with acne may result in reducing self-esteem, social withdrawal, feeling of insecurity and inferiority, limiting employment opportunities, functional and interpersonal difficulties at work and suicidal tendencies. Therefore, "there is no single disease which causes more psychic trauma, more maladjustment between parent and children, more general insecurity and feeling of inferiority and greater sums of psychic suffering than acne vulgaris" (Rivera, 2008).

a. Comedonal (non-inflammatory)
White head (closed): a dilated hair follicle filled with keratin, sebum and bacteria, with an obstructed opening to the skin. Black head (open): a dilated hair follicle filled with keratin, sebum and bacteria, with a wide opening to the skin capped with a blackened mass of skin debris

b. papulo-puetular (inflammatory)
Papule: small bump less than 5mm in diameter. Pustule: smaller bump with a visible central core of purulent material.

c. Nodular (inflammatory)
Nodule: bump greater than 5mm in diameter

Figure 2.1 Types of Acne vulgaris (Rivera, 2008)

2.1.1 Grading of acne

The severity of acne can be graded using different grading system. The grading of acne helps to determine the appropriate treatment. Numerical pictorial grading system reported by Ayer and Burrows (2009) seems to be the most accurate, reproducible, and rapid method (Figure 2.2) and acne can also be assessed by the psychological impact using tools such as the APSEA questionnaire (Appendix D).

Figure 2.2 Photographs illustrating different grades of acnes (A) Mild acne (grade 2): Comedones: a few small papules and a few pustules; (B) Moderate acne (grade 7): Numerous comedones and small inflammatory papules, numerous pustules; (C) Server acne (grade 12): numerous Comedones, deeper papules and pustules, deep and large lesions, presence of cysts and abscesses (Ayer and Burrows, 2009)

2.1.2 Pathogenesis of acne vulgaris

The development and severity of acne vary greatly in pathogenesis and clinical manifestations due to the individual genetic variation, stress level, hormonal imbalance, amount of androgens, and excess sweating. It also relates to their life-style and eating habits. For instance, clinical study by Schafer *et al*. (2001) suggested the severity of acne is worsening in the active smoker and dosage-dependent of smoking. Common myth related to acne such as lack of exercise, lack of hygiene, greasy hair hanging over the face and masturbation has not been proven to have any influence on acne (Ayer and Burrows, 2006). The pathogenesis of acne is a complex process in the sebaceous follicle units of the skin with strong evidence supporting the involvement of sebaceous hyperplasia, follicular hyper-keratinisation, bacterial hyper-colonization, as well as immune reactions and inflammation (Muizzuddin, 2008). The development of inflammatory acne is shown in Figure 2.4. The four main stages of acne formation are summarized as (1) sebum production by the sebaceous gland; (2) *P. acnes* follicular colonization; (3) alteration in keratinisation process; and (4) release of inflammatory mediators into the skin (Thiboutot *et al*., 2009; Clayton *et al*., 2009). This multi-factorial process results in acne lesions that range from comedones (open and closed) to papules and pustules to nodules and cysts.

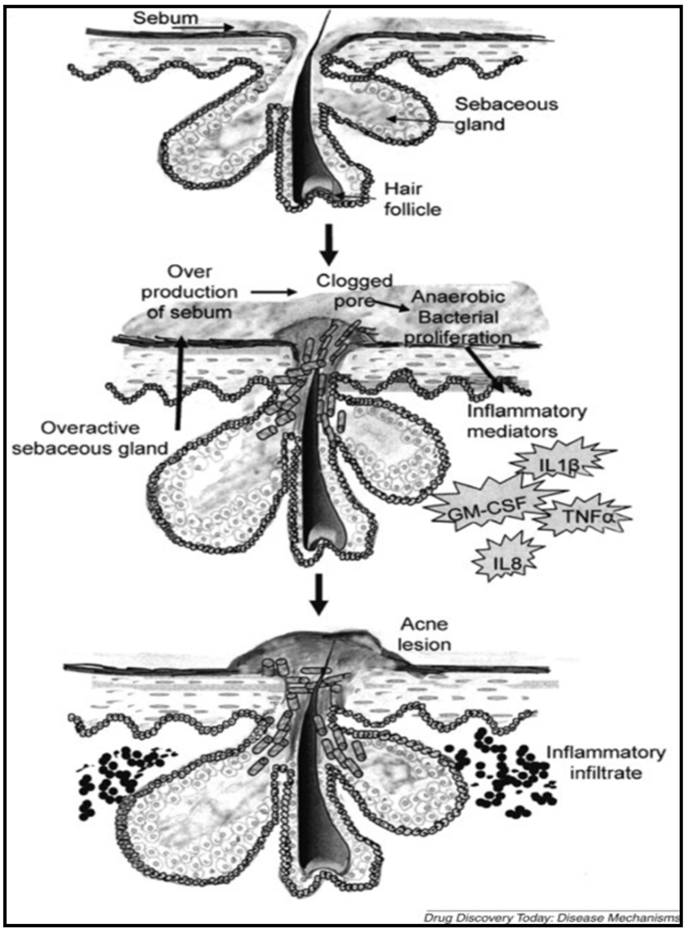


Figure 2.3 Three-stage formation of an acne inflammatory lesion. (1) The pilosebaceous unit normally produce sebum and differentiating keratinocytes, in the presence of the cutaneous flora. (2) Hyper-keratinisation and excess sebum provoke the clogging of the pore. (3) Anaerobic bacteria (*P. acnes*) proliferate, recruit inflammatory cells, which release inflammatory signals (IL-1β, TNF-α, etc.) (Muizzuddin, 2008)

During puberty, elevated androgen levels stimulate the sebaceous glands to enlarge and a large amount of sebum is produced in the sebaceous follicle (Ayer and Burrows, 2006). As a consequence, keratinisation pattern is altered by hyperkeratosis of the follicular epithelium, which leads to clogging of duct by a horny plug (Lavker *et al*., 1981). As a result, microcomedo is formed as increasing accumulation of dense material composed of sebum and keratinous at the clocked duct site of the skin. The formation of microcomedo is a precursor of all acne lesions. A further development of this process leads to open (blackhead) and closed comedones (whitehead). However, Ayer and Burrows (2006) reported that acne is not only related to the amount of sebum secretion but also to the quantity of free fatty acids which are converted by the triglycerides in the follicular site. The increased activity of sebaceous glands can be elicited by androgen.

Excess sebum in the microcomedo causes proliferation of *P. acnes* (Muizzuddin, 2008). *P. acnes* can break down the triglycerides in the sebum to form free fatty acids which are both comedogenic and proinflammatory (Berson *et al*., 1995). *P. acnes* can secret proinflammatory cytokines including interleukin (IL)-1β, IL-8, and tumour necrosis factor (TNF)-α and chemotactic factors (C5a) that attract neutrophilis (Dessinioti *et al*., 2009). Lysosomal enzymes released from the neutrophils rupture the follicle wall and release proinflammatory mediator, including keratin and lipids into the surrounding dermis. In addition, *P. acnes* can also release lipases, proteases and hyaluronidases that could lead to tissue injury (Dessinioti *et al*., 2009). Consequently, the formation of inflammation papules and pustules is followed. Once the host response has occurred; the macrophages and foreign-body react, this leads to cysts and nodules (Berson *et al*., 1995). Among all factors described above, the presence of *P. acnes* on the skin surface is suggested to play a significant role in the development, formation and inflammation of acne.

2.2 ***Propionibacterium acnes* (*P. acnes*)**

Propionibacterium acnes (*P. acnes*) is a common component of the bacterial flora of the human skin. It is a predominant bacterium located on the surface and within the deeper recesses of pilosebaceous follicles at the sebum-rich sites of human skin (Gribbon *et al*., 1993; Gribbon *et al*., 1994). *P. acnes* prefers moist, oily areas of the skin. The concentration of *P. acnes* is similar between patients with acne and controls (Dessinioti *et al*., 2009).

2.2.1 Taxonomic classification

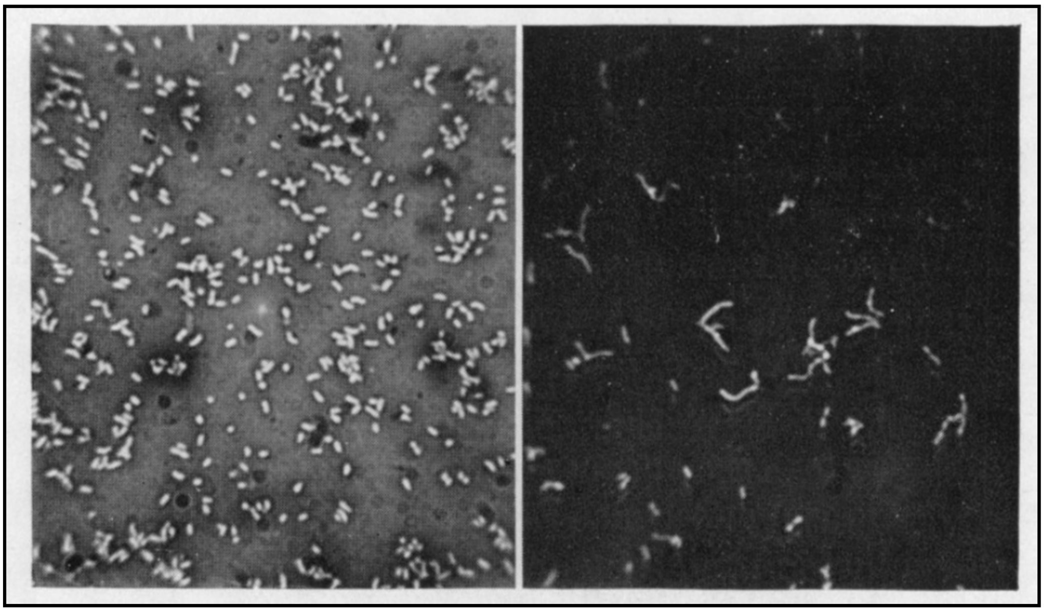
Higher order taxa of the *P. acnes* is *Actinobacteria*, *Actinobacteridae*; *Actinomycetales*, *Propionibacteriaceae* and *Propionibacterium*. It is known by an alternative nomenclature such as *Corynebacterium acnes*, *Bacillus acnes* (Henyl and Forlifer, 1994)

2.2.2 Genomic structure

The genome of *P. acnes* has been sequenced in its entirety. This bacterium consists of a single circular chromosome of 2.56026 base pairs (Bruggemann *et al*., 2004). Its DNA contains 2351 putative genes coding for 2297 known protein products and constituting a 60 % G-C (guanine-cytosine) content.

2.2.3 Cellar and colonial morphology

The observation of colonial and bacterial cell morphology of *P. acnes* were reported similarly (Poppert *et al.*, 2009; Perry and Lambert, 2006; Esteban *et al.*, 1996). Colonial morphology of *P. acnes* is described as opaque, circular, convex colonies of 1 mm or less. Upon longer incubation, the colonies could change from initial small white colonies to larger and darker yellow or pink colour colonies (Anderson *et al.*, 1999). The surface of the colonies varied from 1.5 to 4.0 mm in diameter, and the colour of colonies was pink after 4 - 5 days of anaerobic incubation (Douglase and Gunter, 1946).



(a) (b)

Figure 2.4 Negative stains of four days old slant cultures incubated (a) anaerobically and (b) aerobically (Douglas and Gunter, 1946)

P. acnes is non-spore-forming, non-motile, anaerobic, gram-positive and pleomorphic rod. The bacterium has homogenous cell morphology. The organism ranges from small plump rods to ellipsoids which tend to occur in pairs (Figure 2.4 a).

The size of the individual cells in Gram stains is approximately 0.4 - 0.5 by 0.8 - 0.9 microns (Douglas and Gunter, 1946). *P. acnes* grows better under anaerobic condition (Collins *et al*., 2004) and it can also grow poorly under aerobic condition (Charles *et al*., 1979). Cells from aerobic broth culture appear to be longer and swollen, and may appear to have rudimentary branching (Figure 2.5 b). Aerobic cultured cells of *P. acnes* are more pleomorphic than those under anaerobic condition (Douglas and Gunter, 1946).

2.2.4 Biofilm formation

P. acnes cells residing within the skin surface follicles are capable growing as biofilm (Figure 2.5). Biofilm formation of *P. acnes* is probably responsible for strengthening the resistance to common antimicrobial agents (Coenye *et al*., 2007)

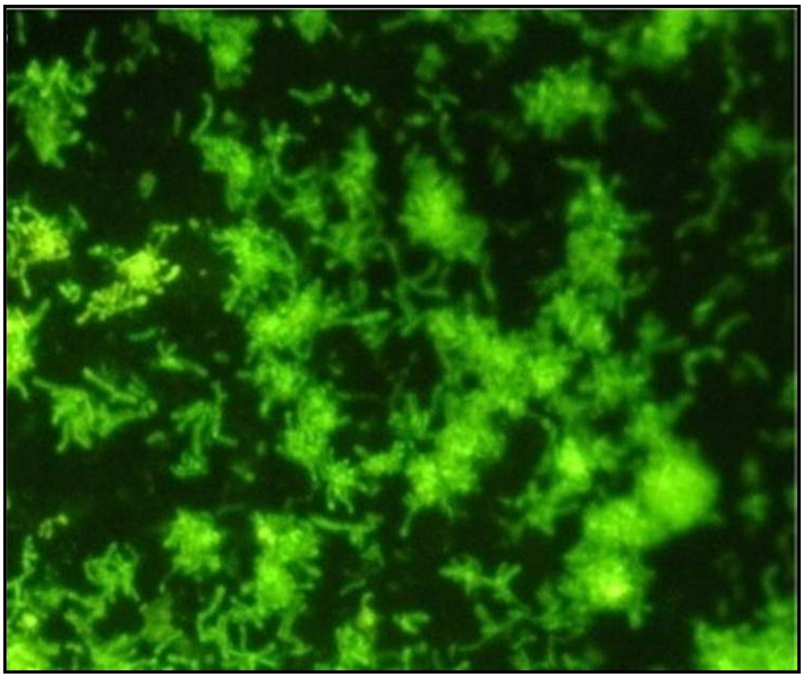


Figure 2.5 Visualization of early stages (24 h) of biofilm formation of *P. acnes* LMG 16711, using SYTO9 and epifluorescence microscopy (magnification: 500×) (Coenye *et al*., 2007)

2.2.4 Biochemical characteristic of *P. acnes*

P. acnes normally produce acids. The spot test for indole production for *P. acnes* was positive (Collins *et al.,* 2004). In the study of Charles *et al*. (1979), catalase tests, Gram's stain, gelatine hydrolysis and indole formation were carried out routinely for checking the purity of the culture.

2.2.5 Metabolism of *P. acnes*

P. acnes is usually regarded as a sticky anaerobe; it can tolerate oxygen up to 100 % saturation but grows at reduced rate (Perry and Lambert, 2006). Hence, the effects of oxygen on the *in vitro* propagation of *P. acnes* were investigated under defined micro aerophilic conditions (Gribbon *et al*., 1994). The metabolic pathways are different under both aerobic and anaerobic conditions. There is more than one mechanism involved in stimulating the aerobic growth of *P. acnes* shown in Figure 2.7 (Evans *et al*., 1979; Bruggemann *et al*., 2004).

When *P. acnes* grows under anaerobic condition, propionate, acetate and carbon dioxide are produced at a ratio of 2:1:1 from lactate or glucose through the Krebs Cycle, Embden-Meyerh of Pathway and the pentose phosphate pathway (Ye *et al*., 1999), deriving energy with the help of enzymes such as nitrate reductase, dimethyl sulfoxide reductase and fumarate reductase (Bruggemann *et al*., 2004).

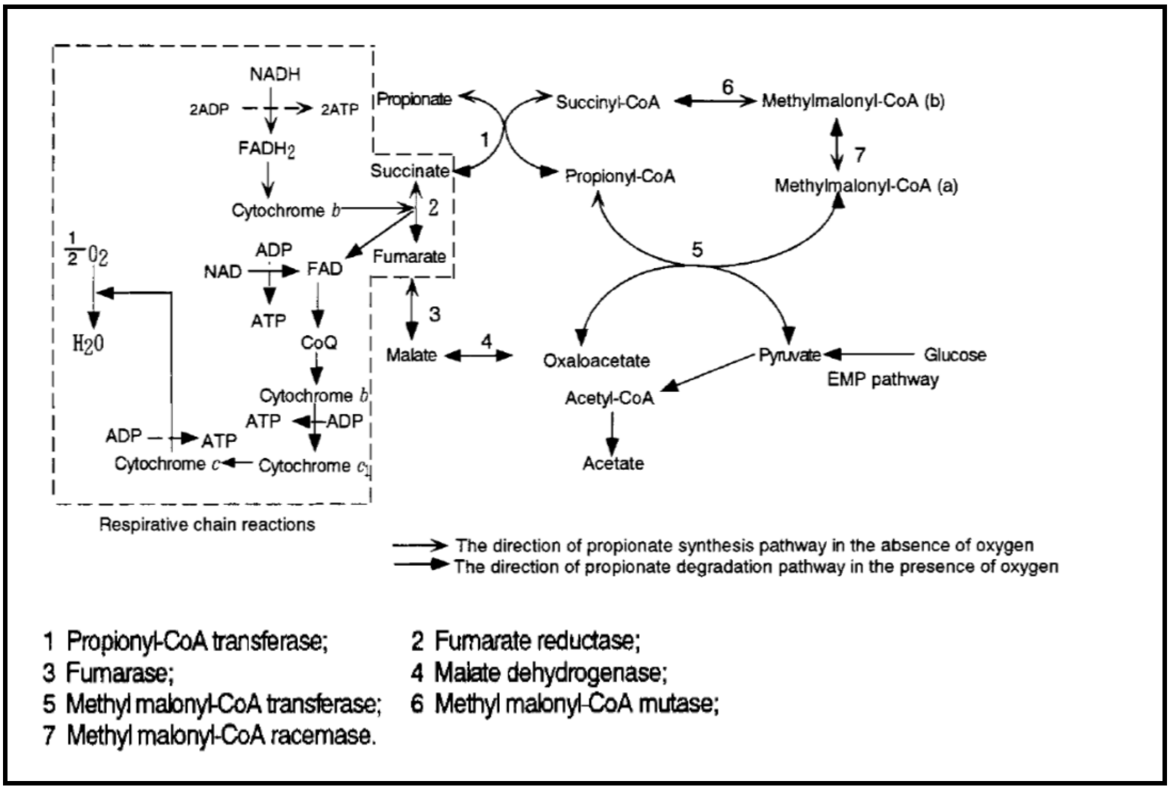


Figure 2.6 Metabolic pathways of *Propionibacterium acnes* growing in oxygen (Ye *et al*., 1999)

The *P. acnes* is capable of growing in 100 % air at reduced rate; the biomass of the bacterium is decreased between 0 to 20 % air-saturation and no further changes at higher air saturations for *P. acnes* (Cove *et al*., 1983). The growth of *P. acnes* seems to be promoted by the utilization of molecular oxygen at low concentration, but at higher concentration, the bacterium induces production of toxic forms of oxygen at concentrations exceeding the capabilities of the protective mechanism by the cells (Gibbon *et al*., 1994). It appears that *P. acnes* carries out an aerobic respiration through formation of succinate by transmitting electrons from cytochromes *b* and *c* and vitamin K_2 to fumarate when oxygen serves as a final electron acceptor (Gibbon *et al*. 1994). It seems that oxygen could activate the reversed randomised pathway, which causes the change of organic acid formation and oxidation of propionate. These pathways are shown in Figure 2.6 (Ye *et al*., 1999).

2.2.7 Incubation temperature of *P. acnes*

The optimum growth temperature for *P. acnes* is 33 °C at pH 5.6 in anaerobic condition using continuous culture technique (Gribbon *et al.*, 1993). However, the optimum growth of the bacterium can be also obtained from 35-37 °C (Schlecht *et al.*, 1997).

2.2.8 Media for cultivation the *P. acnes*

Under anaerobic conditions, *P. acnes* can grow well on media containing glucose, glycerol, ribose, fructose, mannose and N-acetylglucosamine. Different solid media for the cultivation of anaerobes were compared in the study of Heginbothem *et al*. (1990). In this study, *P. acnes* had detectable (moderate to good) growth after 48 h on BHI agar (Brain Heart Infusion agar), FA agar (Fastidious Anaerobe) Agar and GAA (Gibco Anaerobe Agar). In addition, BHI broth is the commonly used liquid medium for growing *P. acnes* culture (Miskin *et al.*, 1997; Chomnawang *et al.*, 2005).

2.3 Treatment of acne

Acne treatment should be based on the disease severity and patient characteristics in order to aid in the selection of appropriate therapeutic agents. More importantly, an effective treatment need to focus on a combination of the four acne formation factors (Berson *et al*., 1995).

In general, topical therapy is the first safe attempt for patient with non-inflammatory comedones or mild to moderate inflammatory acne without notable side effects (Berson *et al*., 1995). Topical therapy prevents the recurrence of the formation of new acne lesions. Topical therapy is only useful on the frequency of application and the place of application and where they are applied. It should therefore be applied daily to all areas of the skin prone to acne (Ayer and Burrows, 2009). A common topical treatment includes benzoyl peroxide, retinoids (tretinoin, tazarotene, adapalene and retinylaldehyde), salicylic acid, antibiotics and anti-inflammatory drugs (Ayer and Burrows, 2009).

For moderate to severe inflammatory acne, and when the patient does not respond to topical treatments, oral antibiotics (systemic drugs) are normally added to the treatment (Berson *et al*., 1997). The oral antibiotic used for acne includes tetracycline, erythromycin, minocycline, doxycycline, and trimetho-prim-sulfamethoxazole. They inhibit the growth of *P. acnes* and decrease the amount of free fatty acids in surface lipid (Berson *et al*., 1997). The common oral antibacterials have potential side effects. For instance, tetracycline is effective to reduce papule and pustule counts in inflammatory acne and decrease the number of *P. acnes* and inhibits lipase, chemotactic factors and neutrophils. However, tetracycline oral treatment may modify the gastrointestinal and mucosal flora leading to vaginal candidiasis and gastrointestinal upset, phototoxicity and possible decrease efficacy of oral contraceptive (Berson *et al*., 1997). Furthermore, the use of oral erythromycin is also restricted because of its gastrointestinal negative effects. Oral antibiotics are usually taken daily for a long period (4 to 6 months). The side effects can be minimized by altering the dose of the drug (Berson *et al*., 1997).

In terms of treating the most severe inflammatory acne (patients with nodulocystic acne); isotretinoin is the most effective treatment (Rivera, 2008). It is the only drug which can affect all four of the pathogenic factors for the acne vulgaris. The drug can suppress sebum production, directly influencing abnormal follicular keratinisation and inhibits sebaceous gland function and diminishes the proinflammatory mediator produced by *P. acnes* (Berson *et al*., 1997). However, the use of the drug needs to be carefully controlled due to the numbers of potential side effects. Patients may experience dry skin, headaches;

dry eyes, photosensitivity, hair loss and nasal irritation, diminished night vision and hormonal approaches are only limited to systemic treatment of female patients. They are estrogens, glucocorticoids and systemic anti-androgens (Berson *et al*., 1997).

Antibiotics reduce the bacterial colonization of *P. acnes* in the deeper parts of the follicles and only lipophilic antibiotics can penetrate the microcomedo (Jappe, 2003). Over the last 30 years, erythromycin, clindamycin, tetracycline and microcyclin have been widely used to treat acne (Coate *et al.*, 2002). In addition of the antibacterial properties, the antibiotics have anti-inflammatory properties by suppressing chemotaxis and decreasing the percentage of proinflammatory free fatty acids in the surface of the skin (Berson and Shalita, 1995). The antibiotics do not have comedolytic effects; hence, they cannot treat the pre-existing comedones. For instance, topical erythromycin is not only a well-accepted antibiotic, it also has anti-inflammatory, suppresses the chemotaxis of inflammatory cells and decreases pro-inflammatory free fatty acids in sebum indirectly by down-regulating either the *P. acnes* metabolism and/or extracellular lipase production (Jappe, 2003). Topical erythromycin has been successful in treating acne by reducing 50 to 60 % of the lesions (Mills *et al*., 1975).

A growing problem of antibiotic use is widespread. The antibacterial-resistance of *P. acnes* was first reported by Crawford (1979). Long exposure to topical antibiotics could induce an enormous selective pressure on the bacterial skin flora of acne patients. This can contribute to treatment failure with the selection and overgrowth of the antibiotic-resistant *P. acnes* (Coate *et al*., 2002). Hence, it is of interest to look for alternatives which have good antibacterial activities.

Benzoyl peroxide is a powerful broad-spectrum bactericidal for *P. acnes* and improves both inflammatory and non-inflammatory (Ayer and Burrows, 2009). It is the most widely used topical agent for acne-treatment today; it is available in both over-the-counter and prescription-only formulations in concentration between 2.5 % and 10 %. Benzoyl peroxide is an oxidizing agent which can kill *P. acnes.* It works by introducing oxygen into follicles. Benzoyl peroxide is a lipophilic molecule and it can penetrate the clogged pore on the stratum corneum and enter the pilosebaceous follicle, and then, it is rapidly degraded to form benzoic acid (Taylor and Shalita, 2004). In the pilosebaceous follicle, the benzoyl peroxide can generate free radicals that oxidize proteins in bacterial cell membranes, and therefore, it can rapidly decrease the population counts of *P. acnes* in the sebaceous follicle. Because of this mechanism of action, *P. acnes* does not develop resistance to benzoyl peroxide.

2.4 Honey and healing

2.4.1 Historical overview of the use of honey in medicine

At present, honey is mainly used for food because of its sweetening ability, highly viscous texture, and glossy colour formation during food preparation (Figure 2.7). However, in the past, the honey had much wider use besides food. Across many ancient communities, honey was used as an important medical treatment for various health problems (Molan, 1992).

The honey used for medicine purpose would date back to at least 4000 years ago (Jones, 2001). The first written record of using honey in medicine was in 2000 B.C. on the Sumerian clay tables. Honey and other honey products were found very essential to the ancient Egyptians. In Egyptian mythology, "when the god re wept, his tears fell to the ground and were turned into bees, the bees began to build and were active on all flowers of any kind belonging to the plant kingdom. Thus wax came into being, thus was created honey from the tears of the God Re" (Bogdanov, 2010). The importance of honey was also recorded on the ancient Egyptian tablets from Tomb of Pabasa, Figures 2.8 (a) shows the importance of the relationship between both man and honey bees. On the other ancient Egyptian tablet, "Honeys poured into a vessel" (Figure 2.7, b) was signifying the value of honey in the eyes of Egyptians. Besides that, the ancient Egyptians offered their god honey combs over flowing with honey as a valuable gift to show devotion and worships and stored honey in the jars buried with the dead as sustenance for the afterlife (Hajar, 2002).

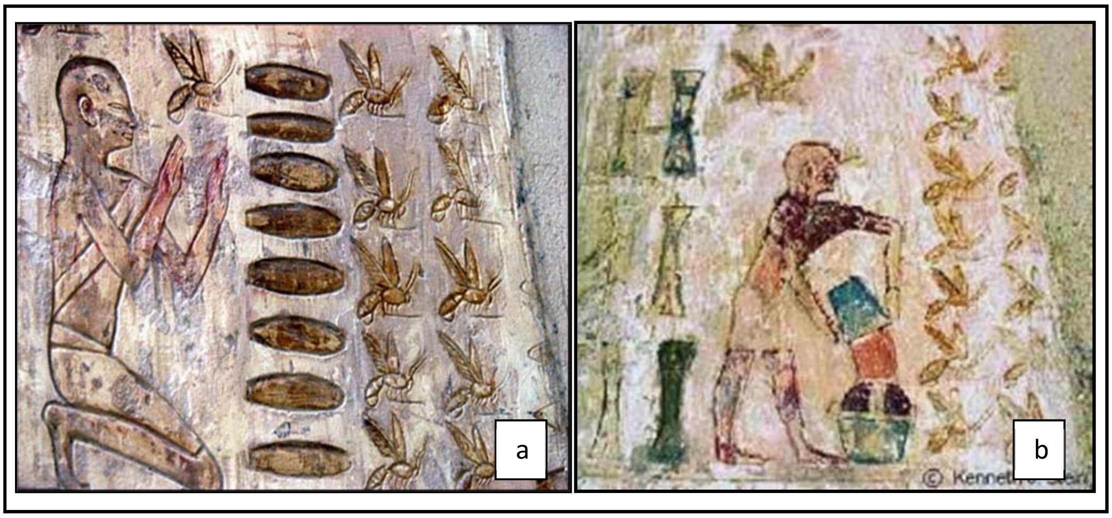


Figure 2.7 Beekeeping in Ancient Egypt (Tomb of Pabasa). Relief shows (a) cylindrical hives made of clay (http://nefertiti.iwebland.com/timelines/ topics/ beekeeping. htm); (b) Honey poured into a vessel (http://pcela.rs/ Egyptian _ Beekeeping_1.htm). Retrieved on 6 June 2010.

Dating back to 1800 B.C, the healing properties of honey was also addressed in treatment procedures for several ailments (Molan, 1992). The Ebers Papyrus is an Egyptian medical papyrus dating to about 1550 BC, which is one of the oldest and most important medical papyri of ancient Egypt. It stated that honey could be a key ingredient used in all the drug recipes for both external and internal use (Hajar, 2002), which includes the use of honey as ointment for wounds and burns, skin irritation, and eye diseases.

However, the ancient Egyptians were not the only people who used honey as medicine. The Ancient Greeks, Romans, Chinese, Indian, and Arabs (Figure 2.8) all used honey alone or in combination with other herbs to treat wounds and various diseases (Hajar, 2002).

Harvesting bee's product dates back to at least 3400 B.C. in the ancient Greeks, several well-known physicians and philosophers (Democrit of Abderos, Zenos and Pythagoras) claimed consuming and drinking honeys were keys to their long life. Aristotle (384-322 BC), the famous Greek philosopher has the same beliefs that eating honey could prolong life. Hippocrates (460-377 BC) was also an ancient Greek physician of the Age of Pericles (Classical Athens), and is considered one of the most outstanding figures in the history of medicine, who has been known as the father of Western medicine. He once said that "I eat honey and use it in the treatment of many diseases because honey offers good food and good health" (Henriques, 2006). He uses honey to treat numbers of different conditions, including stomach and ophthalmologic problems.

Figure 2.8 Dioscorides, De Materia Meica, 15th c. Iviron Monastery, Mount Athos, (B) Illustration from De Materia Medica of Dioscorides, Baghdad, AD 1224. (http://www.hmc.org.qa/hmc/heartviews/h-v-v3%20n4/9.htm). Retrieved on 7 June 2010.

In Romans, beekeeping was continued as the same as in Ancient Egypt and Greece. Records from Virgil and Plinius the Elder recommended honey in conjunction with fish oil as a topical treatment for wound and ulcers. Paulus Aegineta (607-690) mentioned the used of cooked honey as an astringent and raw honey was used to clean wounds (Forrest, 1982)

In the first written records of ancient Chinese Medicine “Shennong” (many years BC), honey is firstly mentioned in written form at around 200 AD. There were many prescriptions and medical indications which contains honey (Dharamananda, 2004). In ancient India, Ayuruvedic medicine used honey for many purposes. According to the Ayruveda classic Astanga Hridaya (about 500 AD), honey can be used against many diseases, including healing and cleaning wounds against different internal and external infections.

Until the beginning of the 20th century, honey was still commonly used for medical purposes. Even in World War I, honey was part of the combat gear (Bergman *et al.*, 1983). However, with the discovery of penicillin from *Penicillium notatum* by Sir Alexander Fleming in 1928, honey was gradually relegated to Folk Medicine, and was no longer seen as suitable for medical use in modern medicine (Molan, 2001). Nevertheless, it was not until the 1980’s that interest in the healing properties of honey was rekindled and clinical research started (Henriques, 2006)

2.4.2 The characteristics of honey

Honey is a sweet substance produced by bees, using nectar from flowers and occasionally from the sap of plants. Nectar (Figure 2.9 a) is an aqueous solution containing sugars, amino acids, proteins, lipids, minerals, and other components (Ball, 2007). This complex sugar solution of nectar is produced by the glands of flower to attract the insect or bird to visit the flower for the purpose of proceeding cross-pollination. The composition of the nectar has a definite impact on the flavour and quality of the finished honey.

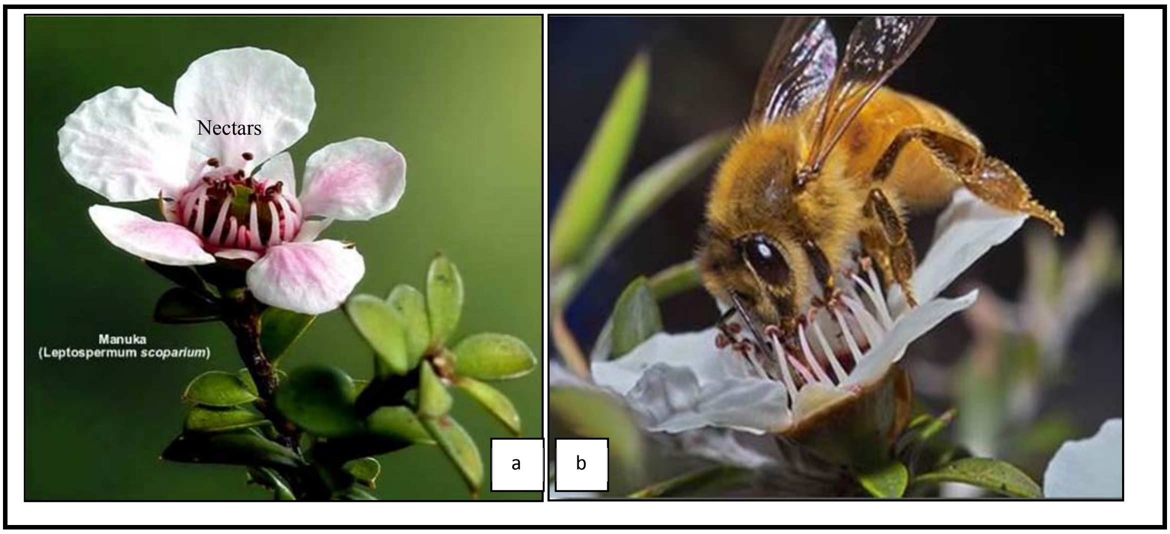


Figure 2.9 (a) Nectars of Manuka flowers (b) honey bees collect nectar from Manuka flowers (http://www.airborne.co.nz/Manuka .shtmL). Retrieved on 6 June 2010.

When work bee drinks the nectar (Figures 2.9 b) from a flower, about 25 mg of nectar is stored at the honey stomach (or nectar sac) (Ball, 2007). During this process, the nectar is mixed with secretions from both salivary and the hypopharyngeal. Different enzymes in the secretions are subsequently mixed with nectar, leading to the chemical alteration of the nectar. These enzymes include diastase and invertase, both of which are capable of breaking down large saccharides (especially sucrose) into monosaccharides (Henrique, 2006). In the bee hives, house bees drink the nectar carried by worker bee. Many more enzymes are added into the nectar at this stage, and moisture content is reduced through a series of regurgitations and ingestions over a period of 15 to 20 minutes. Eventually, the nectar droplet is placed into the honey comb for further ripening. Honey is super-saturated with sugar; therefore the first process of ripening is to evaporate about 80 % of the total nectar. The second is continually converting sucrose into glucose and fructose, which may take about 1 - 3 days before enclosing each individual honey comb cells with a cap (bees wax) (Ball, 2007).

2.4.3 Sensory and physical characteristics of honey

Sensory properties of honey are important determinants of the quality of the product. Colour, aroma and taste are the main attributes of the sensory properties of honey. Those sensory parameters largely vary due to differences in botanical origins, regions, season and processing conditions and storage period of honey. For example, the main sensory attributes of chestnut honeys were described as candy, toasty caramel, fruity, ripe fruit, licorice, woody, herbaceous, spicy, and floral notes (Castro-Vázquez *et al.*, 2010). Indian honeys were described as flowery, fruity, waxy, jaggery-like, chemical and caramelised (Anupama *et al*., 2002).

In terms of physical characteristics of honey, it is a supersaturated sugar solution with high osmolarity. The gross physical attributes of honey are largely determined by the types and concentrations of the carbohydrates (Tchoumboue *et al*., 2003). The high content of sugar also leads to high osmotic pressure for the survival of bacteria. Hence, honey has a broad spectrum of antimicrobial activity.

Honey is a viscous waxy solution, which is highly related to water content, temperature and floral source. Viscosity of honey ranges from 3.01 poise for Alfalfa honey and 4.11 poise for Sumac honey. The viscosity of honey is temperature-dependent. High viscosity of honeys is due to their high sugar concentrations and protein profiles.

Other physical characteristics that can help in the identification of the different honey types are colour (varying from water white, through amber tones, to almost black) (Bogdanov *et al*., 2004). The thermal conductivity (helps to distinguish between floral and honeydew honeys) and hygroscopicity (the capacity of each honey to absorb humidity) are also key physical characteristics of honey (Henrique, 2006).

2.4.4 Chemical characterises of honey

Honey is a complex substance (Figure 2.10), made up of hundreds of substances (Johns, 2001). Sugars are the main components of honey, comprising about 95 % of honey by dry weight (Bogdanov *et al*., 2004). Using various testing chromatographic methods, such as HPLC with refractometric detection, ion exchange chromatography with pulsed amperometric detection and gas chromatography with FID detection can help to define about 15 different sugars in honey (Henriques, 2006).

The main sugars present in honey are fructose (38 %), glucose (31 %), maltose (7.2 %), and sucrose (1.5 %) and higher sugar (1.5 %) (White, 1979). This high sugar concentration leads to a high osmolarity, which restricts microbial growth (White *et al.,* 2005).

Honey contains many organic acid with a pH value ranging from 3.5-5.5 (Bogdanov *et al*., 2004). Even though honey is acidic, the high sugar content tends to mask the acidity in taste. There are at least 30 different organic acids in the honeys (Anklam, 1998). The common ones are glyconic acid, acetic acid, citric acid, lactic acid, succinic acid and formic acid (Henriques, 2006). The presence of glyconic acid is the results of action of bee glucose-oxidase on nectar glucose (Mato, 2003). The origins of the minor organic acids in the honey are not fully understood. However, the minor organic acids might be the intermediates from the Krebs cycle. The organic acids contribute to honey flavour and colour as well as contributing to physical and chemical characteristics such as pH, acidity and electrical conductivity and even stability against microbial spoilage.

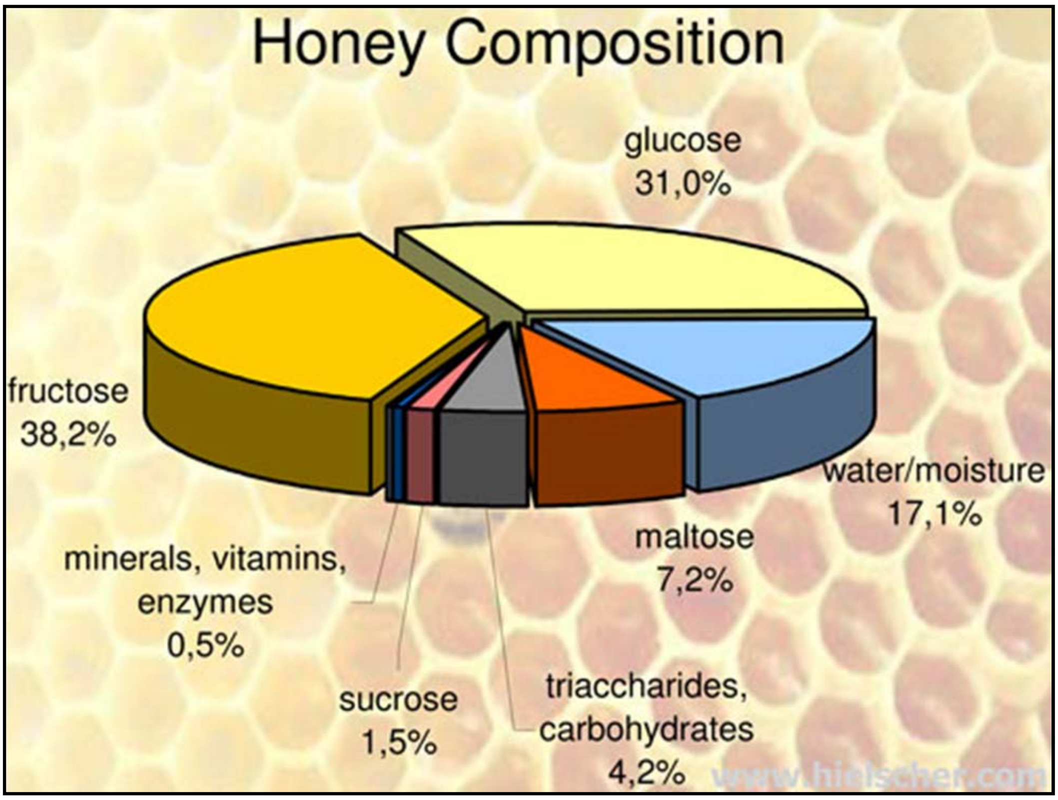


Figure 2.10 Average chemical composition of honey (http://www.hielscher.com/image/honeycompositionp0500.jpg); Retrieved on 8 June 2010.

Honey contains very little amount of nitrogen (0.041 % w/v), which is the key building block of different proteins, enzymes and free amino acid. The presence of protein is minor, which may be partially responsible for the viscous characteristic of honeys. The presence of enzymes in the honey is crucial. Most of the enzymes are introduced by honeybees into the honey. The various honey types showed considerable differences in enzyme activities (Bodganov *et al.*, 2004). The major enzymes in honey include diastase, invertase, glucose-oxidase and other minor enzymes (catalase and an acid phosphatase), most of which aid the transformation of nectar into honey (Henriques, 2006). The enzyme activity in the honey decreases after storage and heating of honey.

Free amino acids are also found in honey. They are also derived from honeybees, not the nectar or the pollen. Among all, proline is the main amino acid of honey, which is important for honey ripeness and helps to verify the characteristic properties in different unifloral honeys (Bodganov *et al.*, 2004).

Honey contains very small amounts of minerals (0.1 %) in the form of ash. Ash is more abundant in the dark colour honey and monofloral honey has lower content of ash. Potassium makes up around half of the total ash, other minerals found in honey are calcium, copper, sodium, magnesium, manganese, and chlorine salts (Henriques, 2006).

Honey contains trace amounts of vitamins, flavonoids and antioxidant components and phytochemical components. Hence, honey can not be considered as good sources of vitamins (Anklam, 1998). Small amounts of other beehive products are also present in honey such as propolis, royal jelly and wax (Ball, 2007).

Many factors may affect the chemical composition of honey. Typical factors include the floral origin of the nectar, the year and time of year the honey is collected. There are many types of honeybees; each of them produces different varieties of honey with distinct chemical profiles. The chemical characteristics of honey may be altered during production by the addition of sugars, protein, moisture, hydroxymethylfurfural (HMF). The authentic honey could be detected using certain chemical markers (Mota *et al.*, 2003). In addition, HMF is a product of sugar breakdown in honey as a result of either heating or storage.

2.4.5 Antibacterial activity of honey

Honey has been reported to have antibacterial activity against a wide spectrum of general bacteria, food spoilage related and other clinical-associated pathogenic bacteria (Molan, 1992). The antibacterial activity is dependent upon a variety of actions and relies on honey composition.

Generally, honey is supersaturated with sugar (fructose, glucose, maltose and sucrose); thus, the high sugar concentration leads to high osmolarity. As known, water molecules has very high affinity to sugar, therefore, only a small amount of free water is available to support the growth of micro-organisms (White *et al.,* 2005). Moreover, a complex mixture of acids in honey contributes to low acidity. The low pH environment does not promote the growth of microorganisms.

Antibacterial effect is not only due to high sugar content in the honey, but also relies on other factors. In a study by Sackett (1919), the antibacterial activity of honey was increased when honey was diluted with water. In 1937, a term “inhibine” was used to explain the involvement of an antimicrobial substance in the honey. By 1962, the identification of the “inhibine” was first recognized as hydrogen peroxide that is a well-known antimicrobial agent in clinical practice (Molan, 2001). This “inhibine” was observed to be light and heat sensitive.

Hydrogen peroxide is generated by the action of glucose oxidase. The enzyme originates from the hypopharyngeal glands of bees, and during nectar collection, it is introduced into honey processing in the hive (White *et al.*, 2005). The enzyme glucose oxidase is activated by dilution and coverts glucose into gluconic acid and hydrogen peroxide in the presence of oxygen (Equation 1).

$$C_6H_{12}O_6 + H_2O + O_2 \rightarrow C_6H_{12}O_7 + H_2O_2 \qquad (1)$$

The rate of hydrogen peroxide production is depended on the honey variety and dilution. Slow release of hydrogen peroxide inhibits microorganisms without damaging human cells and tissues (White, 1963). The amount of hydrogen peroxide in honey is typically around one mmol/Land it is about 1000 times less than the concentration of hydrogen peroxide used as clinical aseptic solution (3 %) (Molan, 2001).

It is known that the antibacterial activity of honey is multi-factorial, which depends on the action of different components present in the honey, and it varies from honey to honey (Molan, 1992). High sugar concentration, low water content and low pH prevents the growth of many bacteria. Despite that hydrogen peroxide has been recognized as an important parameter responsible for the antibacterial activity of honey, other chemical components (aromatic acids, natural flavonoids, phenolic acids) may be also part of the antibacterial action against different microorganisms (White *et al.,* 2005). The study of Weston *et al*. (1998) reported that the aromatic acids isolated from some type of honey also exhibit antibacterial activity towards a range of bacteria.

2.4.6 Manuka honey, a unique honey

Antibacterial activity of honey shows remarkable difference among floral sources. Manuka honey showed high antibacterial activity (Table 2.1), which makes it one of the most popular honeys in medicinal honeys (Molan, 1992; Weston, 2000; Snow and Manley-Harris, 2003). Recently, the unique antibacterial property of Manuka honey due to the nature of non-hydrogen peroxide activity has been reported (Mavric *et al*., 2008). The honey is now being formulated into several medical products for the external skin treatments, and the products are being exported from New Zealand to many countries. The pronounced antibacterial activity of Manuka honey is an important commercial property, which is referred to in marketing as "Unique Manuka Factor" (UMF). The Factor is based on the premium products based on the microbiological assay (Snow and Manley-Harris, 2003).

Manuka honey possesses strong antibacterial activity with the MIC ranging from 3 % (Cooper *et al*., 2002) to 30 % (Mavric *et al*., 2008), while the range 10 to 15 % is the predominant MICs range (Basson *et al*., 2008; Lusby *et al*., 2005; Weston *et al*., 1998). In comparison to Manuka honey, other honeys tested were much weaker with MICs greater than 20 % (Lusby *et al*., 2005). In particular, the MIC of Argentine honey was as high as 45 % (Basualdo *et al*., 2007).

Table 2.1 The susceptibility studies of Manuka honey and other honeys

Honeys and related components	**MIC**	**Method (bacterial strains)**	**References**
Manuka honey (38-761 MGO mg/kg) MGO	15-30% 1.1mM	well diffusion (*E.coli* and *S. aureus*)	Mavric *et al*., 2008
Manuka honey	12.5-25%	broth dilution (oral micro-organisms, *E.coli* and *S. aureus*)	Basson *et al*.,2008
Argentina honeys	45%	well diffusion (*S. aureus*)	Basualdo *et al*.,2007
Jarraj honey Medihoney Comvita wound cream Artificial honey	15.4-18.55 % w/v 34-38 % 33-40 % 34-45 %	microdilution (fungal strains, *C. albicans*, *C. glabrata* and *C. dubliniensis*)	Irish *et al*., 2006
Rewa rewa honey Lavender honey Medihoney Red strubgy bark honey Manuka honey Paterson's curse	>20% >20% 10% 10-20% 10% >20%	agar dilution (*S. aureus* and *Salmonella spp*)	Lusby *et al*., 2005
Manuka honey pasture honey Artificial honey	3% 3% >30% v/v	agar dilution (MRSA)	Cooper *et al*.,2002
Manuka honey	125 g/L	Weill-diffusion method bioassays	Weston *et al*., 1999
Manuka honey	10-25% (11-17mm)	well diffusion (*S. aureus*)	Weston *et al*.,1998

Generally, hydrogen peroxide is the principal factor in honey, which is responsible for the antibacterial activity (Molan, 1992). By adding the enzyme catalase, the hydrogen peroxide in the aqueous solutions of honey is likely to be removed. A survey conducted by the Waikato group in 1991 (Allen *et al*., 1991) revealed that the greatest activity of Manuka honey was due to the non-hydrogen peroxide in the honey. The antibacterial activity of 345 New Zealand (includes Manuka honeys from *Leptospermum scoparium*) unpasteurised honeys from largely single floral sources were investigated. In this study, the agar well diffusion method was used to measure the antibacterial activity of the diluted honeys (with catalase solution for non-hydrogen peroxide-derived activity and without catalase solution for the determination of hydrogen peroxide derived activity). The results were compared to known phenol standards. The potency of antibacterial activity for New Zealand honeys ranged from an equivalent of < 2 % (w/v) phenol to 58 %, with significant differences between floral sources. Non-peroxide antimicrobial activity of honey was observed in 38 % of Manuka samples and 25 % of viper's bugloss samples.

The uniqueness of non-peroxide property of Manuka honey is not significantly affected by heat-treatment (Molan, 1992), and the honey is stable at alkaline pH. The two procedures mentioned here are likely to reduce or destroy the enzymatic activity of glucose oxidase in the honey. The additional evidence confirms that the antibacterial activity of Manuka honey is not only due to hydrogen peroxide in the honey, but other components in the honey also contribute to the antibacterial activity.

Since the discovery of the non-peroxide activity of Manuka honey, many researchers have been trying to distinguish the key chemical fractions in the honey, which is responsible for the uniqueness of Manuka honey. Allen (1991) attempted to isolate the non-peroxide portion of Manuka honey, resulting in a number of aromatic acid derivatives being suggested including syringic acid and phenyllactic acid (Russell *et al.*, 1990). Methyl syringate is abundant in rape and clover honey (Joerg and Sonntag, 1993). Furthermore, both methyl syringate and phenyllactic acids are the major acids in several European honeys (Steeg and Montag, 1987). Both of those components were proposed to be phytochemical markers, which could distinguish Manuka honey (Wilkin *et al.*, 1993). Although the isolated aromatic acid exhibited antibiotic activity towards a range of bacteria, but they are not be the only substances responsible for the high residual non-peroxide antibacterial activity characteristic of honey.

In another study, Weston *et al*. (1999) evaluated the antibacterial activity of phenolic fraction of Manuka honey individually and collectively. The major product in the phenolic extract of Manuka honey was identified using thin-layer chromatography and it was shown to be methyl syringate. Phenyllactic acid was the minor product in the phenolic acids of the Manuka honey. By conducting antibacterial screening using paper-disc-diffusion method on agar plates, methyl syringate did not deliver any inhibition ability towards the growth of the bacteria, and neither the phenyllactic acid could show significant antibacterial acidity towards the bacteria. As a whole, the phenolic fraction of Manuka honey showed antibacterial activity, but the degree of inhibition was similar for all the honeys (included both active and non-active honey). Therefore, the study by Weston *et al*. (1999) demonstrated that phenolic components of Manuka honey individually and collectively were antibacterial active, but it still could not be considered key parameters of the observed antibacterial activity of Manuka honey.

Another potential component of the non-peroxide activity of Manuka honey could be flavonoids. Evidence shows that some of the flavonoids found in the honey are the same as those indentified in the New Zealand propolis, and while the flavonoids have antibiotic properties (Weston *et al*., 1999). The amount of the flavonoids was far too low to contribute significantly to the antibacterial activity of Manuka honey (Weston *et al*., 1999). The Spanish group also showed that honey flavonoids are derived from propolis, since the flavonoids in the propolis have only weak antibacterial activity and they are 1000 times less abundant in honey than in propolis (Ferreres *et al*., 1992). Importantly, most European non-active honeys also contain these same flavonoids at similar concentrations (Weston *et al*., 2000).

In a recent study, Weigel *et al*. (2004) showed that honey contains variable amount of 1, 2-dicarbonyl compounds such as glyoxal (GO), methylglyoxal (MGO), and 3-deoxyglycosulose (3-DG) and 5 hydroxymethylfurfural. 1, 2-dicarbonyl is a product from Maillard reaction or caramelization of degradation products from reducing carbohydrates. Manuka honeys contain very high amounts of MGO ranging from 38 to 828 mg/kg, which is 1000-fold higher than conventional honey (Adams *et al*., 2008; Mavric *et al*., 2008). Mavric *et al.* (2008) for the first time demonstrated that MGO is directly responsible for the antibacterial activity of Manuka honey. Such high amount of MGO has not been reported in any other food. The MIC of MGO was 1.1 mM for both *E.coli* and *S. aureus* analyzed using agar well diffusion assay. It has been demonstrated that the bioactivity of MGO, at the levels at which it is present in the New Zealand Manuka honeys directly contributes to non-peroxide activity (Adam *et al*., 2008). The MGO can be converted from dihydroxyacetone (DHA) via a non-enzymatic activity (Stephen *et al*., 2009). DHA is found in the nectar of Manuka flowers in variable amounts. It is well known among beekeepers that the non-peroxide activity and MGO increase during storage (Adam *et al*., 2009). Fresh Manuka honey contains low levels of MGO and high levels of DHA. During storage at 37 °C, the concentration of MGO in Manuka honey increases and DHA decreases (Adam *et al.,* 2008; Adam *et al.,* 2009). Yahav *et al*. (2005) have also demonstrated that MGO levels in some plants increase significantly due to salinity, drought and cold stress conditions.

2.4.7 Kanuka honeys

Kanuka honey is sourced from the Kanuka tree flower. The Kanuka tree which starts off as a shrub, but grows to a tree of about 20 m tall and the trunk can reach more than 60 cm in diameter. Kanuka tree prefers dry, semi-fertile sites, with warm temperature in sub-alpine sites. The Kanuka leaves can be bunched or solitary and are of similar size to those of Manuka leaves. The Kanuka flowers are smaller than those of Manuka (3-5 mm in diameter) and occur in clusters, the seed capsules, which are 2-4 mm in diameter, are smaller and less persistence than those of Manuka (Douglas *et al.*, 2004). The Kanuka honey also possesses antibacterial activity against *Staphylococcus aureus* (Allen *et al.*, 1991). However, there is no published information on the non peroxide activities of Kanuka honey.

2.5 Bioactives and their antibacterial properties

Essential oils, plant extracts and honey products are natural bioactive ingredients such as Manuka tree oil (MTO), lavender essential oil (LO), green tea extract (GTE), olive leave extracts (OLE) and propolis, which have been used for centuries worldwide for medicinal, cosmetic, and spiritual purposes (Kassim, 2008; Hammer *et al.*, 1999). The antimicrobial activities of these bioactives were the main drivers to many of these applications (Hammers *et al.*, 1999). The characteristics of the bioactives are diverse and complex. By using analytical techniques (GC-MS and HPLC) and biological assays, the "traditional knowledge" about these bioactives could be studied and reported scientificallyy.

2.5.1 Manuka tree oil

Essential oil distilled from Manuka tree leaves (Figure 2.11) has been traditionally used in New Zealand Maori remedies as an external antiseptic (Douglas *et al.*, 2004). Manuka tree (*Leptospermum Scoparium*) is the most widely distributed, abundant, and environmentally-tolerated member of the woody plant in New Zealand (Perry *et al.*, 2006). It belongs to an indigenous native plant family of Myrtaceae, which is known by its richness in leaf oil (Figure 2.12 b) (Porter *et al.*, 2000). Manuka tree is a fast growing, conical-shaped bush that reaches about 4 m high, but local varieties vary in leaf form, flower size and colour.

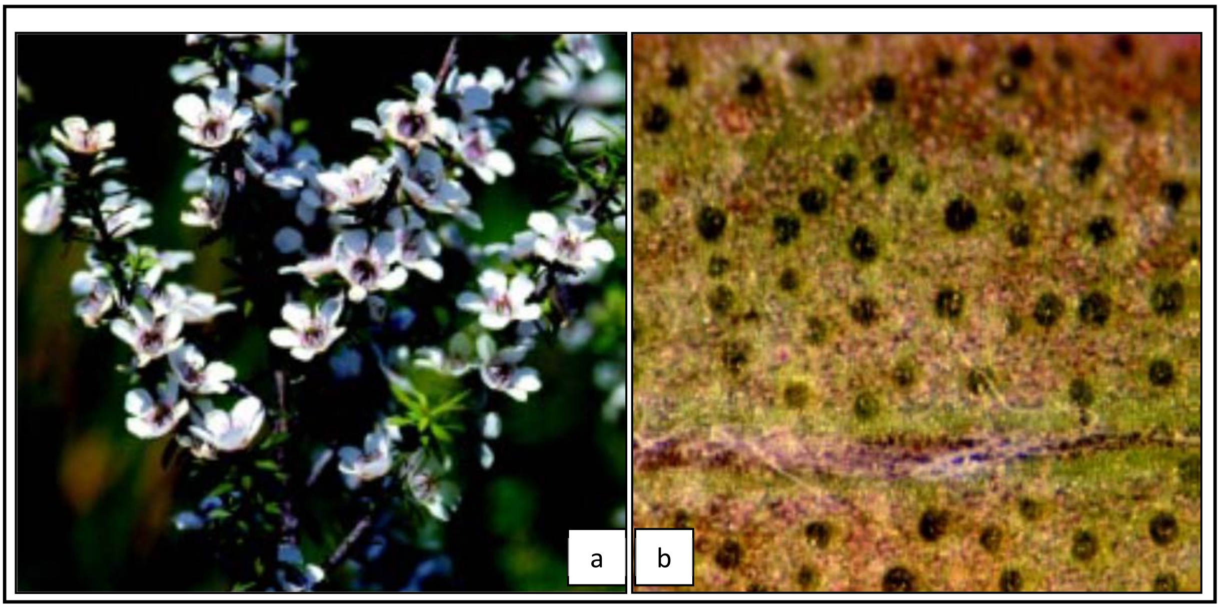


Figure 2.11 (a) Manuka flowers (b) oil glands in the Manuka leaf (Port *et al*., 2000)

In the last ten years, Manuka tree oil (MTO) has become commercially available to aromatherapists (Maddocks-Jennings *et al*., 2005). Currently, MTO has also been produced as a bioactive ingredient, which is used in cosmetics, hygiene and aromatherapy products, and herbal medicines (Perry *et al*., 2006). Full attention has been given to the antiseptic and antimicrobial actions of this oil. Many studies have showed that MTO has strong antibacterial activity against Gram-positive bacteria including antibiotic-resistant strains due to its β-triketone (Christoph *et al*., 2000; van Klink *et al.,* 2005; Maddocks-Jennings *et al*., 2005).

2.5.1.1 Chemical components of MTO

MTO is complex oil and the chemical composition of oil significantly varies based on demographical regions. In general, monoterpenes are present at low levels (≤ 3 %). Sesquiterpene hydrocarbons are predominant (≥ 60 %), oxygenated sesquiterpenes and triketones are also present (≤ 30 %) (Porter and Wilkins, 1998). According to the demographical variation, the chemical components of MTO were compared among three locations (North, East Cape and South) throughout New Zealand (Porter *et al.*, 2000). The chemotypes of MTO differ; MTO from the North is rich in monoterpenes (40 %) and sesquiterpenes (42 %), MTO from the East Cape contains a large proportion of sesquiterpenes (54 %) and a large amount of triketone (33 %), major chemotypes in the Southern area are sesquiterpenes (65 %). In another recent nationwide study conducted by Douglas *et al.* (2004), oils from 261 individual Manuka plants collected from 87 sites throughout New Zealand were analysed. A similar result was found that high triketone (> 20 %) chemotype was localised in the Eastern Cape (Figure 2.12)

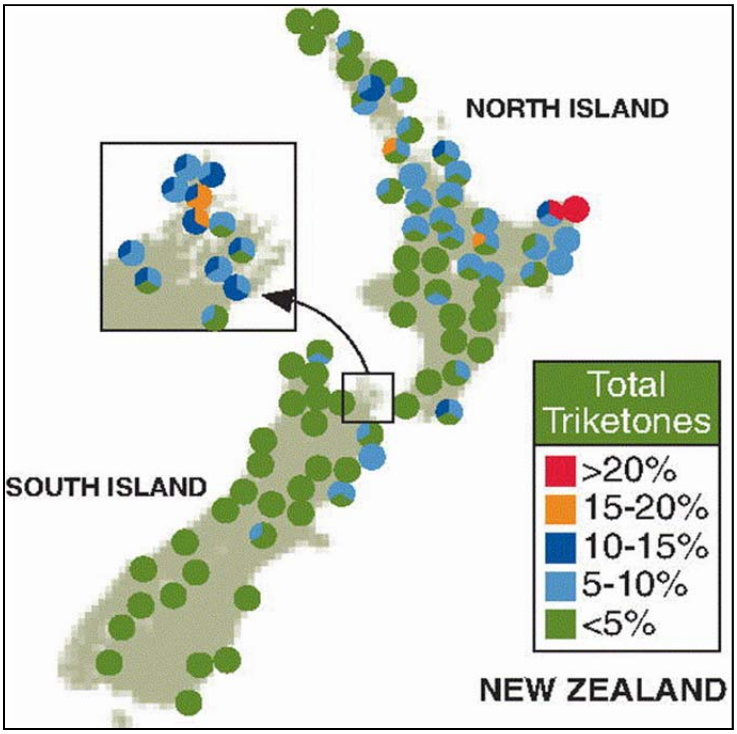


Figure 2.12 Sampling sites and triketone levels in New Zealand Manuka tree (Douglas *et al*., 2004)

2.5.1.2 Antibacterial activity of MTO

Based on recent reviews (Table 2.2) of the antibacterial activity of MTO, the oil exhibited a strong antibacterial activity against a wide range of Gram positive and Gram negative bacteria. Several studies have reviewed that MTO can inhibit the growth of *MRSA*, *E. coli*, *P. aeruginosa* and *C. albicans* at concentration ranges of 0.039-1.25 % (v/v) (Perry *et al.*, 1999; Porter and Wilkins, 1999; Lis-Balchin *et al*. 2000 and van Klink *et al*., 2005). However, there has been no study on the antibacterial activity of MTO against *P. acnes*. The triketone chemotype of MTO is the major chemical component responsible for its antimicrobial activities. The triketone may act by disrupting the cytoplasm membrane of bacterial cell due to their hydrophobic nature interfering with respiration and ATP synthesis, and stimulating oxygen consumption by resting cell suspensions (van Klink *et al*., 2005). MIC of triketone in the MTO levels has been reported at 120 - 1000 µg/mL against MRSA (van Klink *et al*., 2005).

Table 2.2 Antibacterial activities of Manuka tree oil

Antibacterial activity of MTO	**Method**	**References**
Inhibition towards MRSA, triketone with C_{12} about 1 ug/mL; naturally occurring triketone C_{4-12} of MTO (120-1000 ug/mL)	Disc diffusion method (60 µg/disc) Micro-dilution method (µg /mL)	van Klink *et al*., 2005
Iinhibited against 25 different species, and produced a mean of 15 mm (in diameter) zone of inhibition.	Well diffusion method (10µL/ well)	Lis-Balchin *et al*. 2000
Capable of killing bacteria at different MBC levels: *S. aureus*: 0.039 % (w/v), MRSA: 0.0195 % (w/v), *E. coli*: 1.25 % (w/v), *P. aeruginosa*: 1.25 % (w/v), *C. albicans*: 0.31 % (w/v).	Micro-dilution method to determine MIC and MBC	Porter *et al*, 1999
MTO from Eastern Cape was most active against *B. subtilis* with MIC of 150 µg per disc towards *B. subtilis*.	Disc diffusion method (30 µL/ disc)	Perry *et al*., 1996
MTO inhibited the growth of MRSA with an intermediate inhibition zones (35 mm inhibition zone)	Disc diffusion method (30 µL/disc)	Chao *et al*, 2006

2.5.2 Lavender oil

Lavender essential oil distilled from *Lavandula* species (Figure 2.13) is one of the most commonly used oil in aromatherapy (Buskle, 2003). *Lavendula* species are widely distributed in the Mediterranean region and cultivated throughout Europe, North America, and North Africa (Lawrence, 2004). There are three lavender species commonly used in cosmetics and therapeutic applications: *Lavandula latifolia*, a grass-like plant (common name spike lavender); *L. angustifolia*, a stocky bush with a full flower (also known as *L. vera*, *L. officinalis* or English lavender); and *L. Stoechas* (also known as French lavender in Europe and Spanish lavender in the United States) (Roller *et al*., 2009)

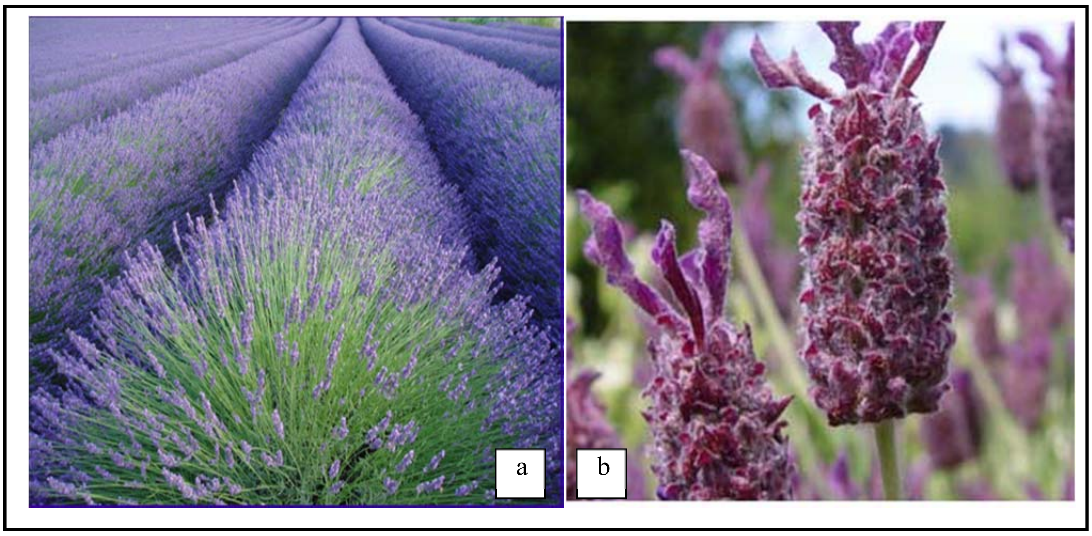


Figure 2.13 (a) Lavender fields (b) lavender flower http://www.google.co.nz/search?q=lavender&hl=en&prmd=ivns&tbm=isch&tbo=u&source=univ&sa=X&ei=QqHbTaqsIpC6vwPKjsGsDw&ved=0CDcQsAQ. Retrieved on 25 June 2011

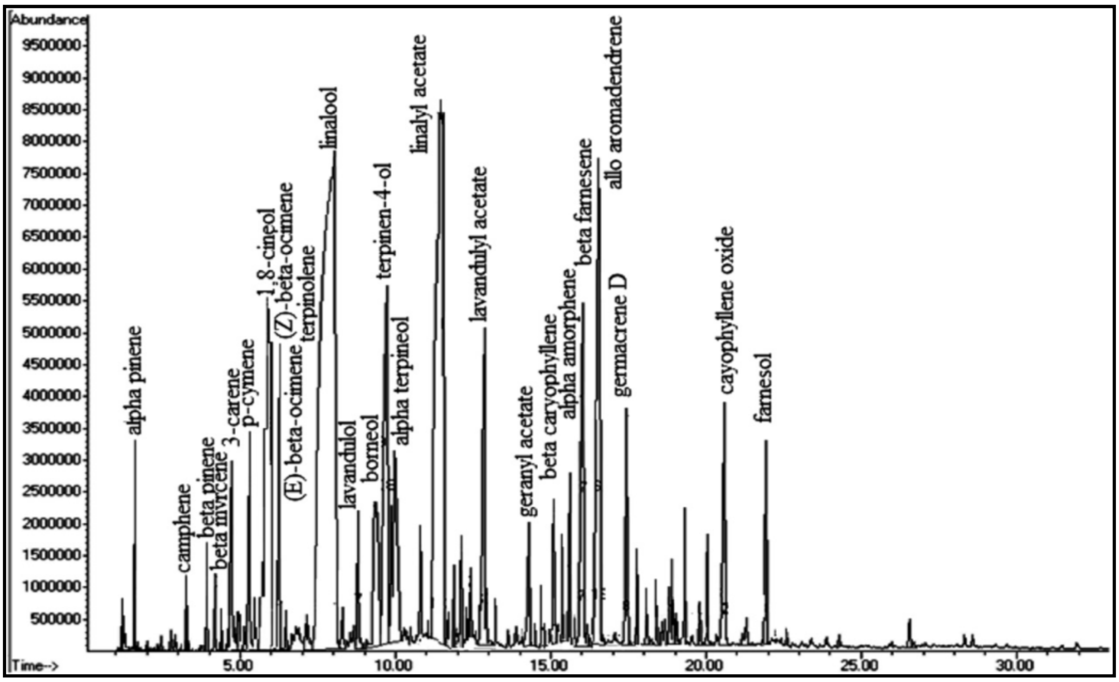


Figure 2.14 Typical GC-MS chromatogram of Lavandula angustifolia essential oil showing the separation of chemical components (Hussain *et al.*, 2010*)*

2.5.2.1 Chemical components of lavender essential oil

The chemical composition of the lavender essential oil is complex which can contain about 20 – 60 different chemical components (Figure 2.14) analyzed by GC-MS (Hussain *et al.*, 2010). In the study of Dadahoglu and Evrendilek (2004), 18 major constituents were identified in Spanish lavender (*L. latifolia*) essential oils; the major constituents being fenchone (55.79 %), camphor (18.18 %), 1,8-cineole (8.03 %) and myrtenyl acetate (6.25 %) (Table 2.3). Studies on *L. angustifolia* indicated that essential oils contained 20.2 % linalool, 18.6 % linalyl acetate, 16.0 % lavandulyl acetate, and 13.1 % 1,8 - cineole. The main components found in the *L. officinalis* were 45.6 % 1,8 - cineole, 4.9 % linalool, 17.9 % camphor (Mutima *et al.*, 2002).

Table 2.3 Essential oil constituents of Spanish lavender determined by GC-MS analysis (Dadahoglu and Evrendilek, 2004)

peak	constituent[a]	%	RT[b] (min)	identification method[c]
1	α-pinene	1.31	6.73	GC-MS
2	camphene	1.4	7.34	GC-MS
3	1,8-cineole	8.03	11.79	GC-MS
4	fenchone	55.79	15.90	GC-MS
5	linalool	0.29	16.98	GC-MS
6	camphor	18.18	19.14	GC-MS
7	myrtenal	0.25	22.09	GC-MS
8	fenchyl acetate	0.32	23.70	GC-MS
9	carvone	0.33	25.52	GC-MS
10	bornyl acetate	1.32	27.50	GC-MS
11	myrtenyl acetate	6.25	29.45	GC-MS
12	δ-cadinene	0.9	35.87	GC-MS
13	L-carveol	0.73	36.32	GC-MS
14	γ-cadinene	0.80	37.04	GC-MS
15	caryophyllene oxide	0.33	37.18	GC-MS
16	γ-selinene	2.54	37.67	GC-MS
17	aromadenderene	0.41	38.39	GC-MS
18	δ-cadinene	0.50	38.95	GC-MS

[a] All constituents were tentatively identified. [b] Retention time on HP-5MS column in minutes. [c] Gas chromatography–mass spectrophotometry (GC-MS).

2.5.2.2 Antibacterial activity of lavender oil

Lavender essential oils possess antimicrobial properties and scientific evidences (Table 2.4, 2.5 and 2.6) demonstrate antibacterial properties of different lavender oils were varied. Some studies showed that inhibitory activities of *L. langustifolia* oil were poor, and others reported that the oil has moderate inhibitory activity (Prabuseenivasan *et al*., 2006; Roller *et al*., 2009). A study by Hammer *et al*. (1999) suggested that the MICs of lavender essential oils largely differed among bacterial types; the MIC of 0.25 - 0.5 % (v/v) was effective against *E. coli*; for *S. aureus*, the MIC was 1 % (v/v) and MIC for *P. aeruginosa* was greater than 2 % (v/v). According to the study of Chao *et al.* (2006), lavender essential oils exhibited strong bactericidal activities; 0.25 % (v/v) lavender oil could completely kill *P. acnes* after 5 minutes (Chao *et al*., 2006). Some components found in lavender essential oil exhibit the antibacterial ability (Table 2.5). Linalool showed appreciable antibacterial activity with a MIC of 300 - 380 µL/mL against *S. aureus*, *B. cereus*, *B, subtilis*. The compound 1,8-cineol exhibited weaker antibacterial activity with a MIC of 900 µL/mL against *S. aureus*, *B. cereus*, *B, subtilis* and *P. aeruginosa* (Hussain *et al*., 2010).

Table 2.4 Antibacterial activity of *L. angustifolia* essential oils against selected strains of bacteria (Hussain *et al*., 2010)

Bacterial species	**Inhibition zone (mm)**	**MIC (ug/mL)**
S. aureus (NCTC 1803)	26.2	320.4
S. aureus (NCTC 6571)	24.1	310
B. cereus (ATCC 11778)	26.1	210.4
B. cereus (NCTC 10400)	24.3	80.4
Bacillus subtils (NCTC 10400)	22.2	70.3
Bacillus pumilis (wild type)	12.4	4700
Pseudomonas aeruginosa (NCTC 1662)	21	1040
Salmonella poona (NCTC 4840)	15.4	830.3
E. coli (ATCC 8739)	16.3	730.1
E. coli (NCTC 10418)	13.1	722.2

Notes: Diameter of inhibition zone (mm) including disc diameter of 6 mm, MIC=minimum inhibition concentration

Table 2.5 Minimum inhibitory concentration (MIC; μg/mL) of pure components (Hussain *et al*., 2010)

Tested Microorganisms	1,8-cineol	citronellal	linalool	thymol	Patchouli alcohol	Ciprofloxacin
Staphylococcus aureus (NCTC 1803)	930.0 ± 27.0^{f}	590.9 ± 16.7^{e}	380.0 ± 9.5^{b}	550.5 ± 13.5^{d}	490.3 ± 13.9^{c}	6.23 ± 0.17^{a}
S. aureus (NCTC 6571)	830.3 ± 20.2^{a}	490.0 ± 14.3^{b}	360.3 ± 9.9^{b}	$540.5 \pm 16.2c$	470.9 ± 13.5^{c}	15.60 ± 0.19^{a}
Bacillus cereus (ATCC 11778)	930.3 ± 26.6^{f}	398.5 ± 9.9^{e}	326.2 ± 9.8^{d}	310.4 ± 9.4^{c}	290.5 ± 7.9^{b}	6.66 ± 0.34^{a}
B. cereus (NCTC 7464)	910.7 ± 28.1^{e}	375.0 ± 12.3^{d}	300.5 ± 8.5^{c}	290.6 ± 9.3^{c}	200.3 ± 7.8^{b}	8.00 ± 0.47^{a}
Bacillus subtilis (NCTC 10400)	600.0 ± 20.3^{f}	385.6 ± 11.2^{e}	350.0 ± 11.2^{d}	250.1 ± 7.3^{c}	185.7 ± 4.4^{b}	4.47 ± 0.10^{a}
Bacillus pumilis (wild type)	980.2 ± 33.3^{f}	823.2 ± 24.9^{e}	790.0 ± 21.0^{d}	720.5 ± 19.4^{c}	340.0 ± 9.1^{b}	62.25 ± 1.70^{a}
Pseudomonas aeruginosa (NCTC1662)	> 1000	> 1000	> 1000	> 1000	> 1000	30.20 ± 1.70^{a}
Salmonella Poona (NCTC 4840)	> 1000	> 1000	> 1000	900.4 ± 27.4^{b}	> 1000	2.53 ± 0.13^{a}
Escherichia coli (ATCC 8739)	> 1000	> 1000	980.0 ± 30.0^{d}	910.3 ± 22.5^{c}	600.1 ± 19.7^{b}	4.48 ± 0.22^{a}
E. coli (NCTC 10418)[h]	> 1000	> 1000	> 1000	890.0 ± 28.5^{c}	635.5 ± 19.0^{b}	5.50 ± 0.21^{a}

[a–f]Means followed by different superscript alphabets (a–f) in the same rows present significant difference ($p < 0.05$).

[g] Values are mean ± standard deviation of three different samples of each Lamiaceae essential oils, analyzed individually in triplicate.

[h] Amphicilin resistant strain.

Table 2.6 Review on antibacterial activities of lavender essential oils

Inhibitory effects	**Method**	**Reference**
L. angustifoilia showed moderate to weak antibacterial activity to 10 food-borne pathogens with an inhibition zone of 13-26 mm in diameter. Linalool showed appreciable antibacterial activity with MIC of 300-380 µL/mL against *S. aureus*, *B. cereus*, *B, subtilis* and 790 µL/mL towards *P. aeroginosa*. 1,8-cineol exhibited weaker antibacterial activity 900 µL/mL against *S. aureus*, *B. cereus*, *B, subtilis* and *P. aeroginosa*;	Disc diffusion method (10 µL per disc) Microtiter-plate assay to determine MIC and MBC;	Hussain *et al.,* 2010
Four lavender oils tested showed inhibition growth MRSA. The inhibition zones ranged from 8-30 mm in diameter at oil doses;	Disc diffusion method: 1-20 µL of lavender oil per disc;	Roller *et al.*, 2009
L. langustifolis oils had no activity against *S. aureus*, *E.coli*, *K. pneumoniae* and poor antimicrobial properties against *B. subtilis*, and *P. aeruginosa*;	Disc diffusion method;	Prabuseenivasan *et al.*, 2006
Lavender essential oil showed stronger antibacterial effect on Gram-negative organism (*E. coli*) than Gram-positive bacteria (*S. aureus*). 10 % lavender essential oils inhibited the growth of *E. coli* by creating a 10.5 mm zone of inhibition. The antibacterial activity towards *S. aureus* was weaker, at 50 % lavender oil produced medium size zone of inhibition (9 mm);	Disc diffusion method: essential oils ranging from 10-100 %;	Lodhia *et al.*, 2009
Time-kill dynamic procedures showed that lavender oils exhibited strong bactericidal activities at a concentration of 0.25 % (v/v) and *P. acnes* was completely killed after 5 min.;	Time-to-kill method;	Zu *et al.*, 2010
Lavender (*Lavandula angustifolia*) essential oil gave 25 mm inhibition zone against MRSA;	Disc diffusion method (30 µL) against MRSA;	Chao *et al*,2006
Spanish Lavender (*Lavender stoechas*) showed strong antibacterial activity against the tested food-borne pathogens at dosages of 0-80uL/mL. About 30-40 uL/mL of essential oil doses reduced the initial bacteria of 3-4 log CFU/mL;	Determine the survival of viable cell under the treatment of essential oils at doses of 0, 5,10,20,30,40,50,80 µL /mL;	Dadalioglu *et al*., 2004
Lavender essential oil was tested against 11 common Gram positive and negative bacteria. Study results found that the MICs of lavender essential oils largely differed from bacteria, *E. coli* (0.25-0.5 %, v/v); *Staphylococcus aureus* (1 %, v/v); *Psudomonas aeruginosa* (> 2 %, v/v);	Agar dilution method and MICs	Hammer *et al*., 1999

2.5.3 Green tea extract

Tea is one of the most popular beverages consumed in the world. There are many types of tea, including green tea, black tea, and oolong tea, and each has several sub-classifications (Eden, 1976). The different types of tea are produced from the young leaves of *Camellia sinensis* (Figure 2.15) and its varieties. Teas also differ by the manufacturing processes. Among all, green teas are most widely consumed in Southeast Asia. Green teas are considered as not fermented and oxidized. Green tea is prepared from freshly harvested leaves and subsequently steamed to prevent fermentation and yielding a dry soluble green tea extract (Elsaie *et al*., 2009). Green tea is rich in antioxidants, it is also known for its ability to fight against diseases and maintaining healthy cell structure.

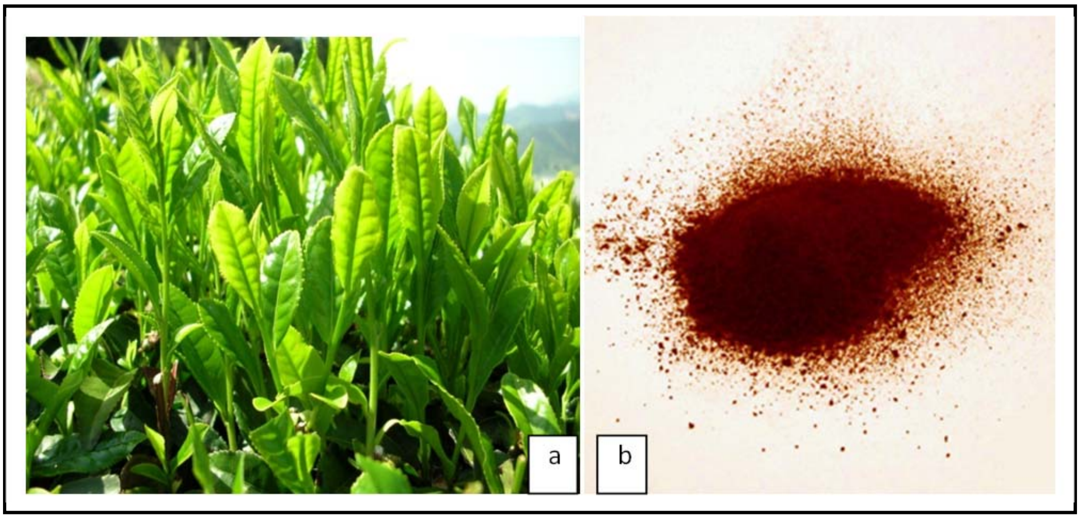


Figure 2.15 (a) Young tea leaves (Camellia sinensis) (b) green tea extract http://www.google.co.nz/search?hl=en&q=green%20tea&um=1&ie=UTF-8&tbm=isch&source=og&sa=N&tab=wi. Retrieved on 25 May 2011.

Antimicrobial activity of tea (an aqueous extract of *Camellia sinensis*) has been recognized for nearly a century (Yam *et al*., 1998). Modern clinical and experimental research on green tea has been done since 1957, followed by extensive studies since the late 1990s. In 1981, a clinical study showed that those who continuously drink a large amount of green tea have less tooth decay, after a year of continuous surveillance at elementary schools (Onishi *et al*., 1981). The anti-carcinogenic property of green tea suggested that tea has antibacterial activity against the bacteria resident in the mouth. In addition, more studies have shown that green tea extracts fight bacteria and reduces redness and inflammation as well as alter hormonal activity (Liao *et al*., 1999). The symptoms mentioned here are also involved in acne treatment. It has been long known that placing a warm tea bag on acne helps to draw the toxins out of the lesion, promoting quicker healing times (Soriya, 2007). The clinical study of Elsaie *et al.,* (2009) suggested that topical 2 % green tea lotion is an effective, cost-effective treatment for mid-to-moderate acne vulgaris.

2.5.3.1 Chemical components of green tea extract

The chemical component of green tea is complex. Understanding of the chemical components in the tea could help to explain the characteristic flavour and appearance of tea, subsequently achieving a comprehensive explanation of the antimicrobial and pharmacological active principles of tea.

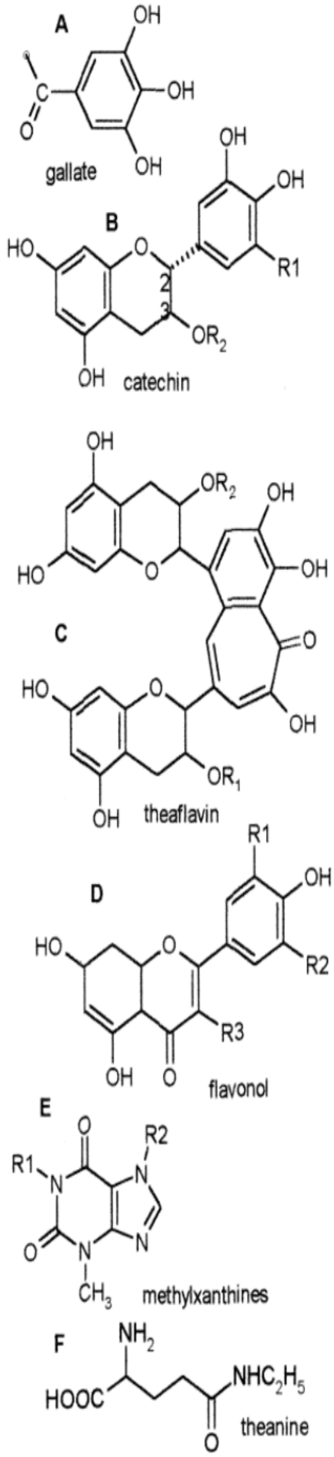
Green tea contains the best dietary source of catechins (10 %, w/w). Epigallocatechin gallate (EGCG) is the major catechin, which is present at concentrations of up to 1 mg/mL in a cup of tea (Hamilton-Miller, 1995). Green tea also contains small amounts of flavonols, such as quercetin, kaempferol and rutin are the most important flavonols (Hamilton-Miller, 1995). Tea contains phenolic acids; the main one being caffeic, quinic and gallic acids. Tea is also a good source of methylxanthines primarily in the form of caffeine (about one third the caffeine of coffee). Theanine is an amino acid found only in tea leaves (Dufresne and Farnworth, 2001). Tea also contains many flavour compounds (100 volatiles components), of which linalool is the most abundant amino acid (Kubo *et al*., 1992). The major constituents of green tea are summarised in Table 2.7

2.5.3.2 Antibacterial activity of green tea

The different varieties of green teas have strong antibacterial activities against a broad spectrum of microorganisms. Toda *et al.* (1991) found that extracts of green tea inhibited and killed *S. aureus*, *S. epidermidis*, *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella flexneri*, *Shigella dysenteriae* and *vibrio* spp. Toda *et al*. (1992) also reported that green tea inhibited the growth of MRSA at a 'cup-of-tea' level (3 mg /mL). In the study of Si *et al.* (2006), green tea extract (500 µg/mL) showed strong inhibition against growth of major food-borne pathogens (*E. coli*, *S. Typhimurium*, *L. monocytogenes*, *S. aureus*, and *B. cereus*). In the study of Tsai (2008), the methanolic extracts from crude green tea leaves were evaluated for growth inhibitory activity against cariogenic *S. mutans* and *S. sanguinis*. The methanolic extracts of tea showed antimicrobial activity against *S. sanguinis* with MIC of 4 mg/mL.

Table 2.7 The major constituents of green tea

	occurence (% dry weight) green tea	black tea	structure
Catechins	**30-42**	**10-12**	
epigallocatechin gallate	11		**B** (-)2,3-cis R1=OH R2=**A**
epicatechin gallate	2		**B** (-)2,3-cis R1=H R2=**A**
gallocatechin gallate	2		**B** (+)2,3-trans R1=OH R2=**A**
epicatechin	10		**B** (-)2,3-cis R1=R2=H
epigallocatechin			**B** (-)2,3-cis R1=OH R2=H
gallocatechin			**B** (+)2,3-trans R1=OH R2=H
catechin			**B** (+)2,3-trans R1=R2=H
Teaflavin		**3-6**	
theaflavin-3-gallate			**C** R1=OH R2=OH
theaflavin-3'-gallate			**C** R1=**A** R2=OH
theaflavin-3,3'-digallate			**C** R1=OH R2=**A**
Thearubigens		**12-18**	**C** R1=**A** R2=**A**
Theogallin	**2-3**		
Proanthoocyanidin			
Flavonols	**5-10**	**6-8**	
quercetin			**D** R1=OH R2=H R3=OH
kaempferol			**D** R1=R2=H R3=OH
rutin			**D** R1=OH R2=H R3=O-rutinose
Methylxanthines	**7-9**	**8-11**	
caffeine	3-5		**E** R1=R2=CH_3
theobromine	0.1		**E** R1=H R_2=CH_3
theophylline	0.02		**E** R= CH_3 R2=H
Amino acids			
theanine	4-6		**F**
Organic acids			
caffeic acid			
quinic acid	2		
gallic acid			
Volatiles			
linalool			
delta-cardinene			
geraniol			
nerolidol			
alpha-terpineol			
cis-jasmone			
indole			
beta-ionone			
1-octanal			
indole-3-carbinol			
beta-caryophyllene			



Sources: Dufresne and Farnworth (2001)

EGCG which is the most abundant component of green tea plays an important role in microbiological activity of the green tea at a 'cup-of-tea' level. Several studies have shown that the catechins (ECG and EGCG) fractions from the green inhibit the growth of many bacterial species. The bacterial effect of EGCG was attributed to membrane perturbation. In the study of Hamilton-Miller (1995), the MIC of EGCG was reported at 73 µg/mL and at 183 µg/mL for *E. coli*. In the study of Si *et al.* (2006), ECG and EGCG also proved to be the strongest antimicrobial components in the green tea. Using scanning electronic microscope, ECG and EGCG significantly changed the cell morphology of *S. Typhimurium* and *B. cereus* at 650 µL/mL (Figure 2.16). For instance, treatment of EGCG could have affected the bacterial cell division process; *S. typhimurium* cells form chains or clump of cells in the presence of EGCG due to incomplete separation of cells. For *B. cereus* cells using the same treatment of EGCG at 650 µL/mL, cells appeared to be locked in cellular division. Polyphenol treatment could have interfered with bacterial cell division, leading to cell deformation from the typical long rod shapes to short rods or coccus-like shapes. Similar results were observed with ECG-treated cells (Si *et al.*, 2006)

The flavonols of tea inhibited the growth of the Gram positive bacteria and fungi (Si *et al*., 2006) and for instance that, the MIC of quercetin was about 37µg/mL for *S. aureus*., which is a common flavonol derived from tea. Volatile flavour components make up a very small part of the flush and tea leaf, but the flavour components play very important roles in providing flavour and taste. Kubo *et al.* (1992) have reported that some of the flavour volatiles contain the microbiological properties. Most of the volatiles tested inhibited the growth of the cariogenic bacteria, *Streptococcus mutans* and acne-associated bacteria, *P. acne*s. In the study of Kudo *et al.* (1992), *P. acnes* was the most sensitive bacterium among the microorganisms tested with the MICs ranging between 3.13 and 400 µg/mL.

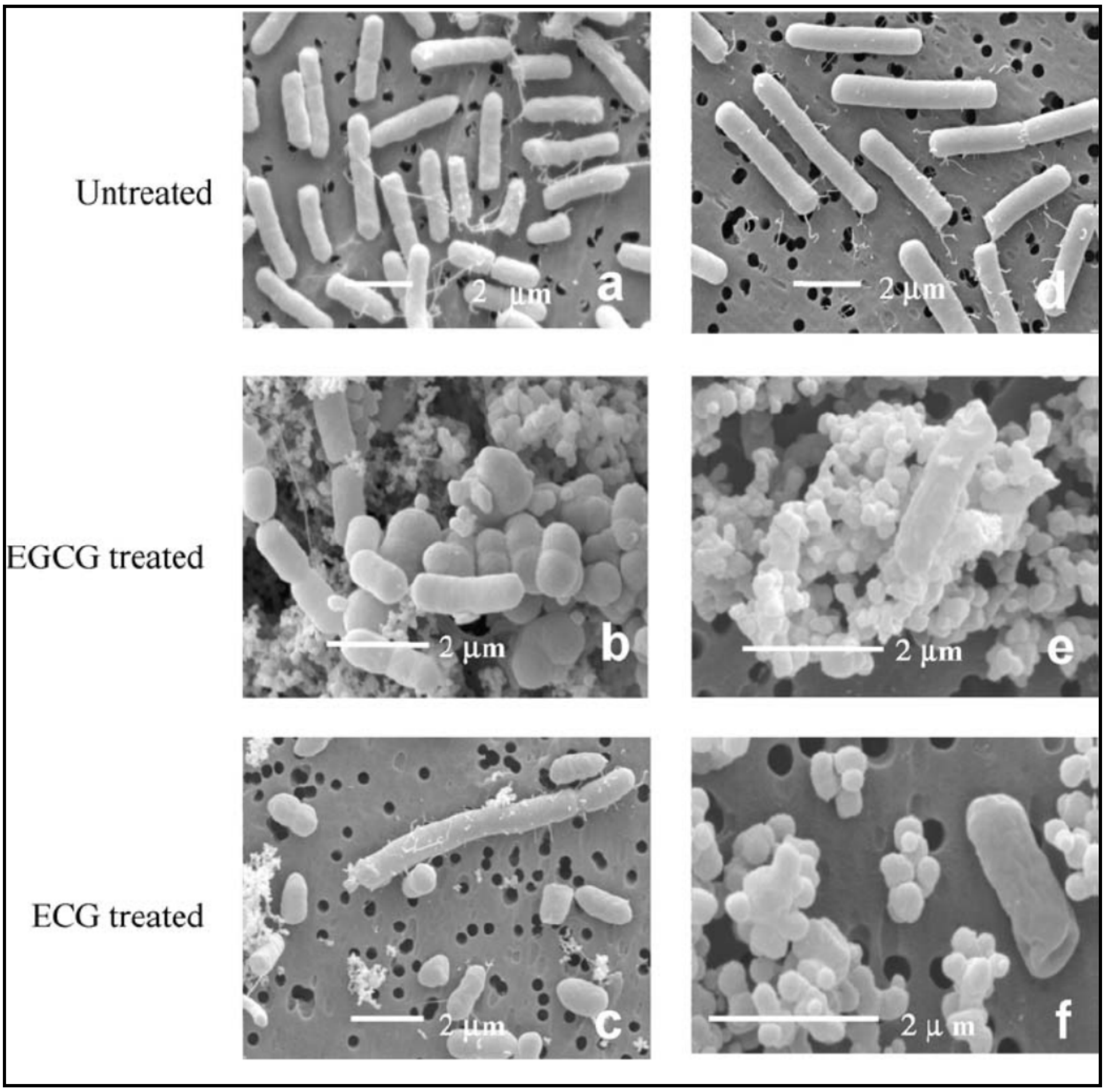


Figure 2.16 SEM images showing the effect of purified polyphenols on the cell morphology of bacterial pathogens. The pathogens were treated with EGCG (Panels b and e) and ECG (Panels c and f) for 16 h. The final concentrations of the polyphenols were 650 g/mL for both *S. Typhimurium* DT104 (Panels a, b, and c) and *B. cereus* (Panels d, e, and f). Panels a and b: untreated cells (Si *et al*., 2006).

2.5.4 Olive leaf extract and its antibacterial activity

Olive tree (Olea europaea L.) is one of the most important fruit trees in the Mediterranean countries (Privitera, 1996). The plant (Figure 2.17) has been used widely as a traditional medicine in countries such as Spain, Italy, France, Greece, Israel, Morocco, Tunisia and Turkey as well as the Mediterranean Islands. This olive tree was also mentioned in the bible, named as "the tea of life" dating back to the ancient times. In the biblical time (Ezekeil 47: 12), God spoke of a tree: "the fruit thereof shall be for meat, and the leaf thereof for medicine" (Privitera, 1996).

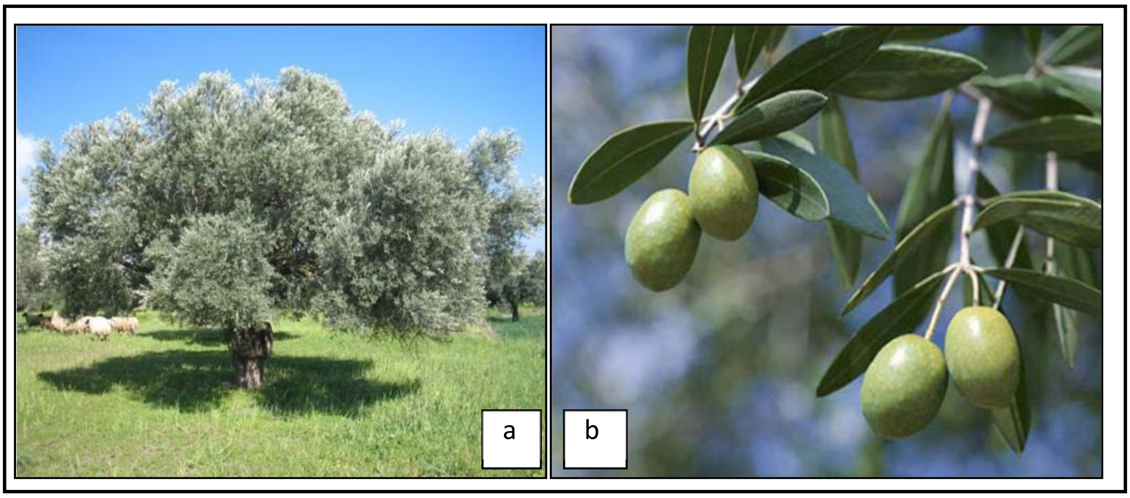


Figure 2.17 (a) Olive tree (b) olive tree leaves http://www.google.co.nz/search?q=olive+tree+leaf&hl=en&prmd=ivns&tbm=isch&tbo=u&source=univ&sa=X&ei=O7jcTYytEZHSuwOG8u2oDw&ved=0CCwQsAQ. Retrieved 25 May 2011

Historically, the olive leaf extracts (OLE) have been used for medicinal purposes for combating fevers and disease such as malaria (Bevavente-garcia *et al*., 2000). OLE is a dark brown, bitter-tasting liquid derived from the leaves of the olive tree. OLE is marketed as a natural medicine with wide-ranging health benefits (Sudjana *et al*., 2008). OLE is reported to aid the treatment of a broad range of infectious diseases if ingested (Sudjana *et al*., 2009).

2.5.4.1 Chemical components of olive leaf extract

OLE contains many different compounds collectively termed as olive bio-phenols (Figure 2.18), which are thought to give the extract its varied therapeutic properties. Oleuropein is the major bio-active polyphenolic component found in the OLE. Oleuropein has been reported to have potent antioxidant and anti-inflammatory activity (Bevavente-garcia *et al.*, 2000)

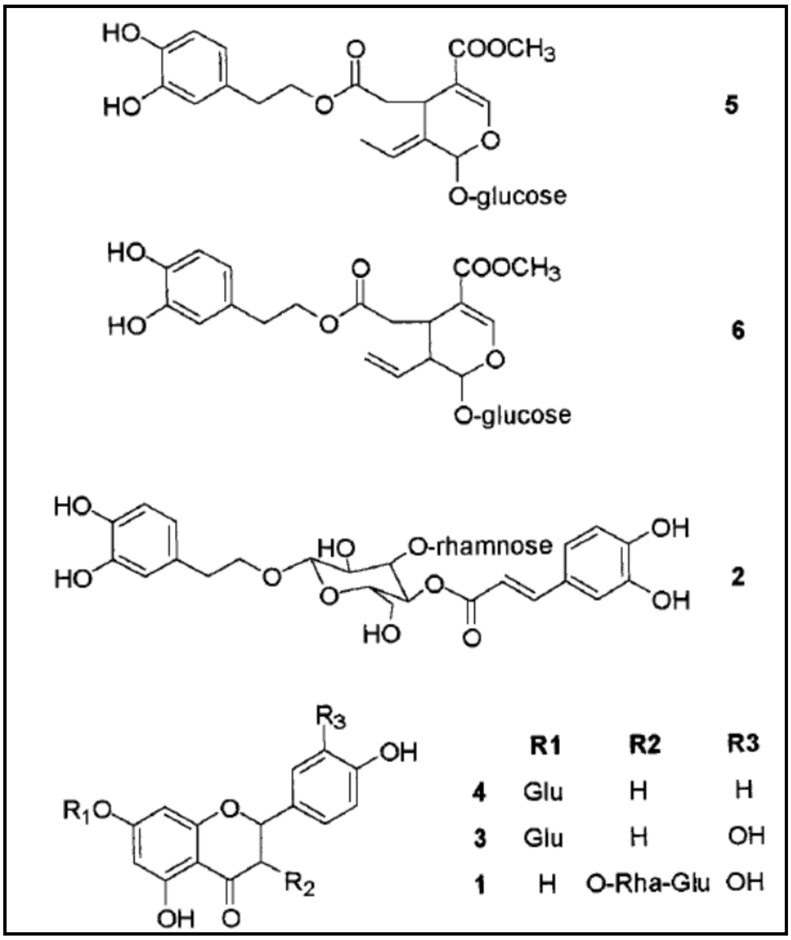


Figure 2.18 Structures of identified compounds in O. europaea L. leaves; 1 rutin; 2, verbascoside; 3, luteolin 7-glucoside; 4, apigenin; 5, oleuropein; 6, oleuroside. (Savournin *et al.*, 2001)

2.5.4.2 Antibacterial activity of olive leaf extract

Myers *et al*. (2006) conducted a series of biological experiments on the commercial olive leaf extract from Olive Products Australia Pty Ltd. The study determined the *in vitro* anti-inflammatory, anti-cancer, anti-bacterial and immunomodulatory properties of pure and high strength olive leaf extracts. The antimicrobial activity of the test samples was determined using the agar dilution method against a range of organisms comprising Gram-positive and Gram-negative bacteria and yeast. Results showed that MIC of both pure OLE was 16 - 0.063 mg/mL and high strength olive oil was 8 - 0.63 mg/mL. The highest inhibitory activities were observed in the two tested olive extracts against *S. aureus* and *P. vulgaris* with a MIC of 1 mg/mL.

Another study by Sudjana *et al*. (2009) used the same commercial OLE extract as Myers *et al*. (2006) to investigate the antibacterial activity of the OLE against a wide range of microorganisms (n = 122). The testing methods of Myers *et al*. (2006) were also applied in this study. Results (Table 2.4.6) suggested that OLE was most active against fastidious bacteria (*Campylobacter jejuni*, *Helicobacter pylori* and MRSA), with MICs as low as 0.31- 0.78 % (v/v). The OLE extract showed little activity against all other test organisms (n = 79), with MICs for most organisms ranging from 6.25 % to 50 % (v/v) (Table 2.8). It was found that the MICs of OLE was greater than 50 % for *B. subtilis*, *Candida* spp., *E. coli*, *Klebsiella pneumoniae*, *P. aeruginosa* and *Serratia marcescens*.

Table 2.8 Susceptibility data for microorganisms (n=122) tested against olive leaf extract (%, v/v)

Organism (*n*)	MIC_{range}	MIC_{90}	MBC/MFC_{range}	MBC_{90}/MFC_{90}
Acinetobacter calcoaceticus (2)	25		25	
Bacillus cereus (1)	12.5		12.5	
Bacillus subtilis (1)	50		50	
Campylobacter jejuni (10)	0.3–2.5	2.5		
Candida albicans (2)	50		>50	
Candida glabrata (2)	50		50 to >50	
Candida parapsilosis (2)	50		50 to >50	
Enterococcus faecalis (6)	25		50	
Escherichia coli (4)	25–50		25–50	
Helicobacter pylori (4)	0.6–1.2			
Klebsiella pneumoniae (3)	50		50	
Kocuria rhizophila (1)	12.5		50	
Lactobacillus acidophilus (1)	6.2		12.5	
Lactobacillus casei (3)	12.5–25		12.5–25	
Lactobacillus spp. (13)	12.5–25	25	25	25
Listeria innocua (1)	12.5		12.5	
Listeria monocytogenes (8)	25		25–50	
Micrococcus luteus (1)	6.2		25	
Pseudomonas aeruginosa (4)	25–50		50	
Salmonella enterica subsp. *enterica* (1)	25		25	
Serratia marcescens (3)	25–50		25–50	
MSSA (12)	0.8–6.2	6.2	0.8–6.2	6.2
MRSA (17)	0.8–12.5	12.5	0.8–12.5	12.5
Staphylococcus capitis (2)	3.1		3.1	
Staphylococcus epidermidis (4)	1.6–3.1		1.6–3.1	
Staphylococcus hominis (2)	6.2		6.2	
Staphylococcus xylosus (2)	6.2–25		6.2–25	
Streptococcus pyogenes (10)	3.1–25	25	6.2–50	50

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration; MSSA, meticillin-susceptible *Staphylococcus aureus*; MRSA, meticillin-resistant *S. aureus*.

Source: Sudjana *et al.*, (2009)

However, antibacterial activity of the OLE has not been consistently reported. A study by Klancnic *et al.* (2010) used the broth microdilution method to test the antibacterial activity of OLE. The results suggested that the MIC of OLE was about 20 mg/mL for *B. cereus*, 40 mg/mL against *S. aureus*, 10 mg/mL against *Campylobacter jejuni* and *Campylobacter coli*.

Hence, antibacterial activity of OLE was weaker against *S. aureus* than the study of Sudjana *et al.* (2009) with MIC of 1 mg/mL for *S. aureus*. The variation may be due to the chemotype of OLE. Unfortunately, none of these studies conducted a chemical analysis; the chemotype of the OLE cannot be compared among these studies.

2.5.5 Propolis

Propolis (sometimes also known as "bee glue") is the generic name for resinous substance collected by honeybees from different plant sources (Burdock, 1998). Propolis (Figure 2.19) is a strong adhesive, resinous substance collected, transformed and used by bees to seal holes in their honey combs, smooth out the internal walls and protect the entrance against intruders (Burdock, 1998). Generally, propolis contains 50 % resin and vegetable balsam, 30 % wax, 10 % essential and aromatic oils, as well as other substances (5 %), (Burdock, 1998).

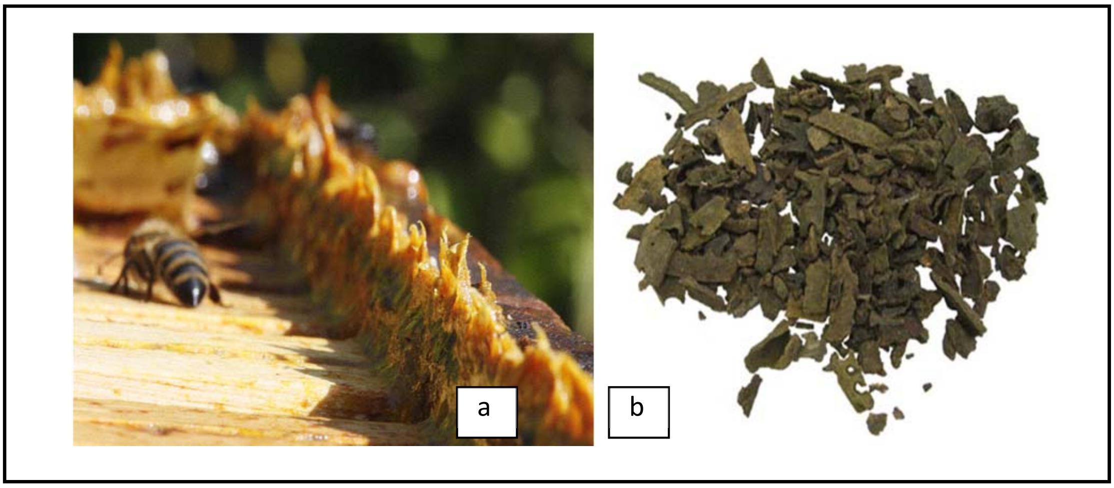


Figure 2.19 (a) Resins in hive, (b) raw propolis http://herbsknowledge.com/wp-content/uploads/2011/05/benefit-of-propolis-herb-propolis-propolis-madicine2.jpg. Retrieved on 16 August 2010

2.5.5.1 Chemical composition of propolis

The chemical composition of propolis is highly variable probably due to diverse plant sources. The variation in propolis is due to the propolis production, because bees use materials from different botanical processes in various parts of the plants. Within propolis, some substances were made from plants and substances exuded from wounds in plants (lipophilic materials on leaves and leaf buds, gums, resins, latics) (Bankova, 2005). Moreover, the chemical composition of propol is also depends on the specificity of the flora at the site of collection. Therefore, the geographic and climatic characteristic of the site leads to the diversity of chemical compositions of propolis.

The chemical characteristics of propolis are mainly analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (Bankova, 2005). More than 100 components have been detected, of which, flavonoid and phenolics are the most important. The flavonoids and phenolic acid esters (caffeates and ferulates) are known for their antibacterial, antibacterial and antioxidant activities (Pietta *et al.*, 2002).

A distinctive characteristic of the flavonoids in New Zealand propolis is the unusually high proportion (approximately 70 %) of dihydroflavonoids, e.g. pinocembrin, pinobanksin and pinobanksin 3-acetate (Markham, 1996). Non-flavonoids components analysed by GC-MS comprised a range of aromatic compounds (3 - 7.5 mg/mL), together with low levels of fatty acids (0.25 - 0.78 mg/mL). Furthermore, the New Zealand propolis also contains cinnamic acids and their esters, but also include the rare 5-phenyl-trans-trans-2,4-pentadienoic acid and the new natural product, 5-phenyl-trans-3-pentenoic acid (Markham, 1996). In a propolis sample from Egypt, along with poplar bud constituents, esters of caffeic acid with long-chain fatty alcohols (dodecanol, tetradecanol, tetradecenol, hexadecanol) were identified (Table 2.9, Bankova *et al.*, 2000).

Table 2.9 Characteristic compounds for propolis of different geographic origin

Geographical origin	Plant source	Typical bioactive constituent
Europe, Asia, North America	*Populus* spp.	Pinocembrin, pinobanksin, pinobanksin-3-O-acetate, chrysin, galangin, caffeates
Northern Russia	*Betula verrucosa*	Acacetin, apigenin, ermanin, rhamnocitrin, kaempferid, α-acetoxybetulenol
Brazil	*Baccahris* spp. *Araucaria* spp.	Prenylated p-coumaric acid, prenylated acetophenones, diterpenic acids
Canary Islands	Unknown	Furoruran lignans

Source: Bankova *et al*. (2000)

2.5.5.2 Antibacterial activity of propolis

Propolis has been commonly used in folk medicine for many years, and there is substantial evidence indicating that propolis has many biological activities, such as antiseptic, antifungal, antibacterial, antiviral, anti-inflammatory and antioxidant properties (Pietta, 2002). These biological activities are due to a large group of chemical components within the propolis (Table 2.10). Dermatological preparations are useful for wound-healing, treatment of burns, acne, herpes simplex, genitalis and neuro-dermatitis (Pietta, 2002). In addition, propolis is used in mouthwashes and toothpaste to prevent cancer and to treat gingivitis and stomatitis (Pietta, 2002).

Table 2.10 Compounds responsible for the biological activity of different types of propolis types

Propolis type	Antibacterial activity	Antiinflammatory activity	Antitumor activity	Hepatoprotective activity	Antioxidant activity	Allergenic action
European (poplar type)	Flavanones, flavones, phenolic acids and their esters (14)	Flavanones, flavones, phenolic acids and their esters (15)	Caffeic acid phenethyl ester (16)	Caffeic acid, ferulic acids acid, caffeic acid phenethyl ester (15)	Flavonoids, phenolic and their esters (15)	3,3-Dimethylallyl caffeate (14)
Brazilian (*Baccharis* type)	Prenylated *p*-coumaric acis, labdane diterpenes (15)	Unidentified (15)	Prenylated *p*-coumaric acids, clerodane diterpenes, benzofuranes (15)	Prenylated *p*-coumaric acis, flavonoids, lignans, caffeoyl quinic acids (15)	Prenylated *p*-coumaric acis, flavonoids (15)	Not tested
Cuban	Prenylated benzophenones (17)	Not tested	Prenylated benzophenones (13)	Unidentified (15)	Prenylated benzophenones (13)	Not tested
Taiwanese	Not tested	Not tested	Prenylated flavanones (42)	Not tested	Prenylated flavanones (42)	Not tested

Source: Bankova *et al*., (2005)

Propolis cannot be used as a raw material; it must be purified by extraction with solvents. Applying this process removes the inert material and preserves the polyphenolic fractions (Burdock, 1998). A multi-step extraction with ethanol is particularly suitable to obtain de-waxed propolis extracts rich in polyphenolic components:

a) Crude waxy propolis passes through the cold-water washing process where extrinsic wax will be removed;

b) Washed propolis is dissolved in 95 % ethanol and the remaining beeswax as well as bee parts and wood chips are removed;

c) Filtration is required to remove any remaining small particles of foreign materials in the propolis, creating propolis tincture, ‘propolis balsam’ or ethanol extract of propolis (EEP).

Antibacterial activity of propolis is bacteriostatic and can be bactericidal in high concentrations (Boyanova *et al*., 2006). Referring to published biological and chemical studies of propolis (Bankova *et al.*, 1999; Kusumoto *et al*., 2001; Miorin *et al.*, 2003; Gebara *et al*., 2002; Lu *et al*., 2004), the ethanol extraction method was used to dissolve raw crude propolis. In the study of Miorin *et al*. (2003), 30 g of crude propolis were dissolved in 100 mL of ethanol by shaking the solution at room temperature for 7 days; the solution was then filtered with Whatman filter paper no. 1 and placed in amber flasks. Propolis solutions were dried and weighed to obtain the correct concentration of each extract. The propolis solution was then used for determining MICs against the *S. aureus*. This was a well-defined method for preparing the ethanol extract of propolis. However, the concentration of the ethanol was not specified in the study. Ethanol (70 %) was commonly used for the preparation of the EEP. In the study of Kujumgiev *et al.* (1999), the antibacterial, antifungal and antiviral activity of propolis of different geographic origin were studied; the propolis was cut into small pieces and extracted with 70 % ethanol (1:10, w/v) for 24 h, the extracts were evaporated under vacuum to dryness. The yields of different samples of propolis ranged from 12 to 58 %.

The antibacterial activities of ethanol extract of propolis are normally screened by an agar-well or disk-diffusion method. A study by Kujumgiev *et al*. (1999) used 0.4 mg of ethanol extract of propolis against the growth of microorganisms (*S. aureus* and *E. coli*) using disk diffusion method. The results showed EEP lacked antibacterial activity against these bacteria with an inhibitory zone of less than 5 mm. Another study by Miorin *et al.* (2003) indicated that propolis samples had higher antibacterial activity against *S. aureus* in comparison to honey; the MIC of propolis ranged from 0.36 to 3.65 mg/mL, while the MIC of honey ranged from 126.23 to 185.70 mg/mL. In the study of Boyanova *et al*. (2006), the antibacterial activity of 30 % ethanolic extracts of Bulgarian propolis (30 μL and 90 μL, w/v) were investigated against 94 clinical anaerobic strains (including *P. acnes*) using both agar-well diffusion and disk-diffusion methods. Only 15 % of *Clostridium* spp., 3.3 % of other Gram-positive and 9.1 % of Gram-negative anaerobic strains were not inhibited by 30 µL propolis extract per well. Propolis extract was more active than the 95 % ethanol control at $P < 0.001$. This study was the first *in vitro* study that reported the antibacterial activity of propolis against *P. acnes*. Results of the study showed that 30 % propolis (90 µL) was highly active against all 13 strains of *P. acnes* with a large mean inhibitory zone diameter (17 mm). The study further showed that using 30 µL propolis (30 %) per well resulted in the inhibition of 92.3 % (12 of 13 strains) of *P. acnes* strains (mean inhibitory diameter of 12.6 mm) (Boyanova *et al*., 2006). The antibacterial activity of propolis is attributed to its ability to damage the cytoplasmic membrane and inhibition of bacterial motility and enzyme activity.

2.6 Susceptibility methods for *Propionibacterium acnes*

Antibacterial materials can affect the bacterial growth through a variety of mechanisms, which maybe bacteriostatic or bactericidal. Bacteriostatic means that the antibacterial material can have inhibitory effects on bacterial growth but does not necessarily kill the organisms, whereas, bactericidal materials have lethal effects on microorganisms (Kiser *et al*., 2010). Dilution (broth or agar) methods and agar diffusion methods used are the most common and reliable susceptibility testing methods to determine the antibacterial potency of bioactive materials (Murray *et al*., 2003).

2.6.1 Various susceptibility test methods

Dilution methods are used to determine the minimum concentration (measured in mg/mL) of antibacterial materials required to inhibit or kill a microorganism (Murray *et al*. 2003). Agar and broth-based dilution methods are the common susceptibility test procedures. The antibacterial materials are usually tested at two-fold serial dilutions;

the lowest concentration that inhibits the visible growth of an organism is regarded as the minimum inhibitory concentration (MIC) (Murray *et al*. 2003).

a) Agar dilution methods

Using the agar dilution method to obtain the MIC for one or more bacterial isolates, the antibacterial materials should be incorporated into liquefied agar medium at 45 ˚C to 50 ˚C, which is then mixed well, and poured into Petri plates, and allowed to solidify. The depth of the agar is critical for the susceptibility test and it is recommended that agar depth is about 4 mm (Lorain, 1991). About 36 different strains can be spot-incubated onto each plate. Most *in vitro* tests with antibacterial materials have been standardized with "overnight" incubation for 16-18 h. However, when testing microorganisms that fail to grow adequately after "overnight" incubation, the plates may be re-incubated for a further 24 hours. For anaerobic bacteria, agar plates should be incubated for 48 h in an anaerobic jar (Sutter *et al*, 1979).

Agar diffusion method was used by Wang *et al.* (1977) in the study of susceptibility of *P. acnes* to 17 antibiotics. In the study, brain heart diffusion agar (5 ug/mL hemin and 0.5 mg/mL vitamin K) was used. Stock solutions of the test drugs were prepared in sterile distilled water; further dilutions were made in brain heart infusion broth supplemented with hemin (5 µg/mL) and vitamin K1 (1.0 µg/mL) and added to similarly supplemented brain heart infusion agar at 46°C to yield final drug concentrations ranging from 0.1 to 50 µg/mL. The inoculum of *P. acnes* was prepared in brain heart infusion broth for 24 h, and provided an inoculum size of approximately 10^4 CFU/mL. After inoculation, all plates were incubated in ananaerobic chamber at 36 °C. After 48 h of incubation, the MIC values were recorded as the lowest concentrations permitting no growth, a barely visible fine haze, or not more than one discrete colony. The agar diffusion method has also been used by Odou *et al.* (2007) to determine the antimicrobial activity of retapamulin against a wide range 232 anaerobic clinical isolates of human origin, including the *P. acnes*. Hence, the agar diffusion method is suggested to be an efficient method to study antibacterial materials against the growth of *P. acnes* (Wang *et al.*, 1977; Odou *et al*., 2007).

b) Broth dilution methods

The broth dilution method uses two-fold serial dilutions in liquid medium for studying antimicrobial action of therapeutic agents, which includes macrodilution (Figure 2.20) and microdilution (Figure 2.21) (Lorian, 1991).Macrodilution broth uses broth volume for each antimicrobial concentration of 1-2 mL, while microdilution usually contains 0.1 ml volumes in wells of microdilution trays (Murrey *et al*., 2007). Broth dilution method may determine quantitatively and qualitatively the outcome of the interaction of microbe and antimicrobial materials. The distinctive advantage of the broth dilution methods permits the determination of the minimum bactacteriacidal concentration (MBC) endpoint, as well as MIC. The end-point of MIC is the lowest concentration of material at which the microorganism tested does not demonstrate visible growth. In some circumstances, 80-90 % decrease in growth compared with growth in the control can be also considered as the end-point for particular antibacterial materials (Lorian, 1991). In an early study by MacLowry *et al.,* (1970) showed that comparisons of results of broth and agar dilution methodologies were similar.

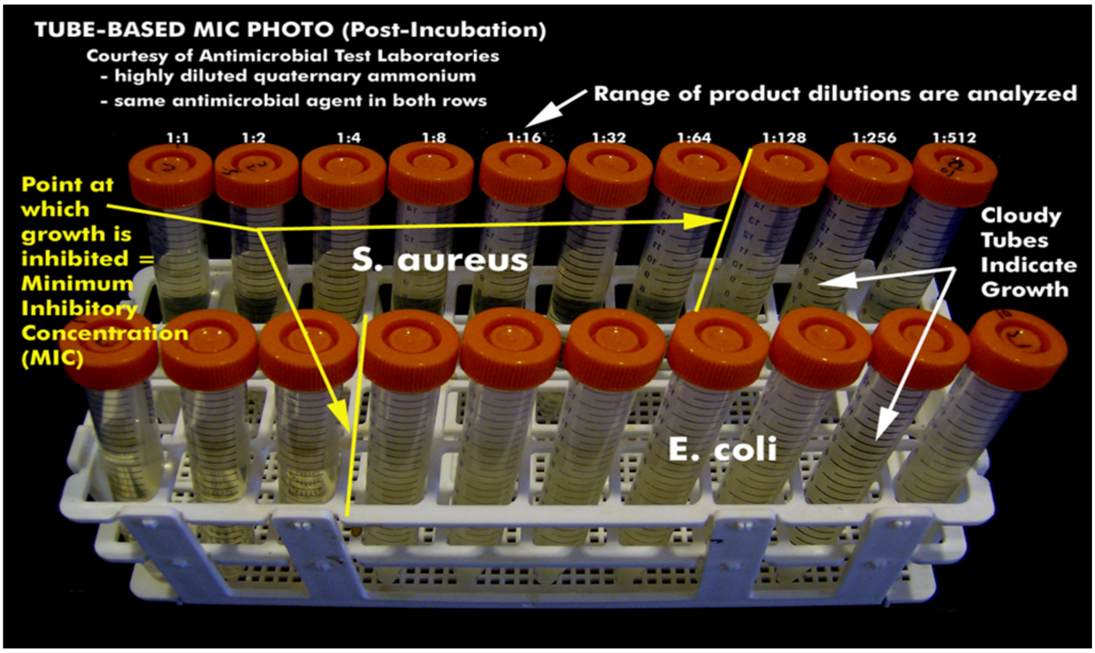


Figure 2.20 Standard MIC test using macrodilution method in the test tubes http://www.antimicrobialtestlaboratories.com/Minimum_Inhibitory_Concentration_Test_MIC.htm. Retrieved 11 December 2010

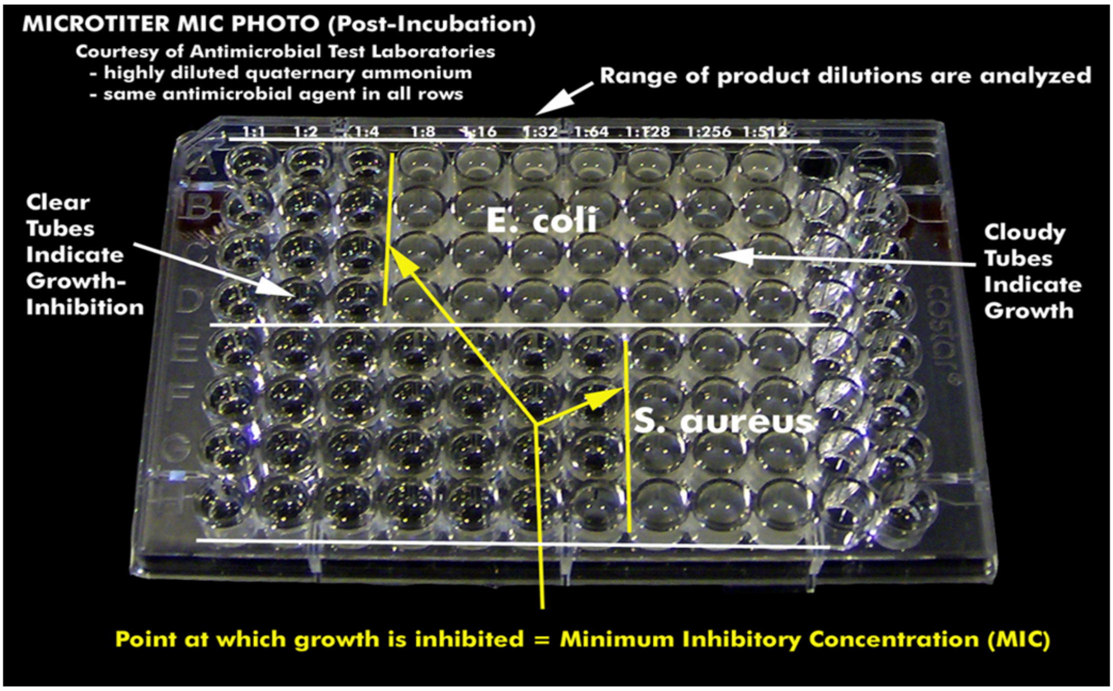


Figure 2.21 Standard MIC test using microdilution method in a microtiter plate. http://www.antimicrobialtestlaboratories.com/Minimum_Inhibitory_Concentration_Test_MIC.htm. Retrieved 11 December 2010

Several factors can influence the outcome and reproducibility of broth susceptibility test (Shimeld, 1999). The test microorganism is the most important factor affecting the test results, because the microorganism manifests its own genetic background, structure and metabolic behaviour (Shimeld, 1999). Then, the antibacterial material that possesses the characteristic can affect solubility, protein binding, distribution, absorption, stability and metabolite modification (Lorian, 1991).

The popularity of using microdilution method in susceptibility testing of antibacterial materials has rapidly grown. Furthermore, microdilution offers flexibility and convenience to the susceptibility testing procedure beyond the traditional macrodilution and agar dilution testing methods. Microdilution testing procedure has been extensively used in the antibacterial material study towards *P. acnes* (Tai *et al*., 2010; Nakatsuji *et al*., 2009; Docherty *et al*., 2007; Kumar *et al*., 2007; Chomnawang *et al*., 2005; Park *et al*., 2004; Eady *et al*., 1994; Himejima and Kubo, 1991). Among the studies mentioned here, the *P. acnes* (ATCC6919) has been commonly used as the testing culture (Tai *et al*., 2010; Nakatsuji *et al*., 2009; Docherty *et al*., 2007; Kumar *et al*., 2007, Chomnawang *et al*., 2005; Park *et al*., 2004).

The disc diffusion method of susceptibility testing allows the categorization of most bacterial isolates as susceptible, intermediate, or resistant to a variety of antimicrobial substances (Murrey *et al*., 2003). In order to perform this test, commercially prepared filter paper disc should be impregnated with a bioactive, and then, the paper disc is applied on the surface of the agar medium, which is inoculated with the test organism. Bioactives can diffuse through the agar. As the distance from the disc increases, the concentration of the antimicrobial agent decreases logarithmically, creating a gradient of drug concentrations in the agar medium surrounding each disk. Because of bacterial inhibition, a zone of inhibition around each paper disc is formed on the surface of the agar medium (Lorian, 1991; Shimeld, 1999).

According to Lorian (1991), the disk diffusion method can be used in susceptibility study of *P. acnes* and the results of inhibition zone can sufficiently present the antibacterial activity of bioactives of interests. In the study by Chomnawang *et al.* (2005), the *P. acnes* (ATCC 6919) was incubated in brain heart infusion medium with 1 % glucose for 72 h under anaerobic conditions and adjusted to yield ≈ 10^8 CFU/mL. Aliquots of molten BHI with glucose agar were used as an agar medium. This prepared inoculum was added to molten agar, mixed and poured over the surface of the agar base and left to solidity. A sterile paper disc (BD™, Ө=6mm) was impregnated with the test material and the disc was placed on the surface of the agar and the plates were then incubated at 37 °C for 72 h anaerobically. In the study on "Essential oil as cosmetic preservation" by Muyima *et al*. (2001), the agar diffusion assay was used to assess the antimicrobial activities of the oil. Antibiotic medium agar was used for both the base and seed layers. Six millimetre diameter discs were placed on the seeded agar and soaked with 15 µL of the test oil.

2.6.2 Preparation of bioactives in susceptibility testing of *Propionibacterium acnes*

Natural therapeutic agents from plate origins have different physical and chemical properties. They can be either water-, oil-based liquid or dry powder. They might be hydrophilic (plant extracts and honey) or hydrophobic (essential oil) or neither of these, for example propolis, a honey wax. The preparation of stock solution is the first essential step.

Many organic antimicrobial agents are hydrophobic in nature; hence, preparing a stable homogenized stock solution can be difficult. In the previous studies with antibacterial agents against *P. acnes*, dimethyl sulfoxide (DMSO) was used to prepare the stock solution of the antibacterial agent (Park *et al*., 2004; Kumar *et al.*,2007; Tsai *et al.*, 2010) and water-insoluble materials (Docherty *et al*., 2007). At room temperature, DMSO is a colour less organo-sulfur liquid. DMSO is an important polar aprotic solvent, which can dissolve either polar or non-polar compounds and is miscible with a wide range of organic solvents (Stoker, 2007).

The antibacterial activity of DMSO was studied by Basch and Gadebusch (1968). DMSO was found to exert a marked inhibitory effect on a wide range of bacteria and fungi (Table 2.11). The MIC of DMSO was 8 % in solution against *Corynebacteria* sp. (including *P. acnes*). In the susceptibility test of antibacterial materials, control solution of DMSO solution without any antibacterial agents should be used and compared with antibacterial agents against testing microorganisms. Hence, the finial concentration of DMSO should be in the range of 0.2 % to 5 %.

Table 2.11 Antibacterial activity of DMSO (Basch and Gadebusch, 1968)

Organism	Culture inoculum No.[a]	Culture inoculum Size[b]	MIC[c]	pH[d]	MMC[e]	pH[d]
Bacteria						
Staphylococcus aureus	1,276		8	7.6	30	7.8
S. aureus	1,276	(H)	20	6.8	40	8.2
S. aureus	2,406		30	7.9	40	8.3
S. pyogenes	3,862	(H)	10	7.3	30	7.8
S. faecalis	1,648	(H)	20	7.5	40	8.2
Diplococcus pneumoniae	5,479		20	7.6	40	8.1
Micrococcus lysodeikticus	2,529		10	7.5	30	7.8
Sarcina lutea	8,579	(H)	20	7.5	40	8.2
Corynebacterium sp.	8,621	(H)	8	7.3	10	7.5
Listeria monocytogenes	8,523		20	7.5	30	8.3
Mycobacterium tuberculosis var. BCG	5,516		8		10	
Clostridium pasterianum	1,759		5		10	7.1
Bacillus subtilis	3,777		20	7.6	30	8.7
Desulfovibrio desulfuricans	5,378		10		20	
Neisseria catarrhalis	5,348	(H)	20	7.6	40	8.2
Haemophilus influenzae	8,622		7	7.3	8	7.3
Pasteurella multocida	8,624	(H)	6	7.3	9	7.4
Escherichia coli	2,975	(H)	10	6.8	20	7.5
Aerobacter aerogenes	1,678	(H)	20	7.4	20	7.4
Klebsiella pneumoniae	1,565	(H)	9	7.0	30	7.9
Proteus vulgaris	3,855	(H)	20	7.6	20	7.6
P. mirabilis	3,873	(H)	20	7.5	20	7.5
Salmonella schottmuelleri	3,850	(H)	9	7.1	20	7.6
S. gallinarum	3,030	(H)	10	6.8	30	7.5
S. typhimurium	3,821	(H)	10	7.0	30	7.9
Shigella flexneri	1,461	(H)	10	7.1	30	7.8
Serratia marcesens	1,468	(H)	10	7.2	30	7.8
Herellea sp.	8,334		6	7.0	8	7.0
Pseudomonas aeruginosa	3,840	(H)	8	7.2	20	7.5
Fungi						
Aspergillus niger	2,828		9	7.3	30	8.0
A. fumigatus	2,100		10	7.4	30	7.9
Candida albicans	5,314		8	6.7	30	7.6
C. krusei	2,616		9	7.1	20	7.5
Saccharomyces cerevisiae	1,600		8	7.1	20	7.5
Geotrichum candidum	8,623		8	6.8	20	7.5
Nocardia asteroides	2,626		20	7.6	40	8.1
Cladosporium resinae	5,476		7	6.2	30	6.7
Trichophyton mentagrophytes	2,637		8	7.2	30	8.0
Penicillium notatum	2,122		10	7.2	30	8.1
Fusarium bulbigenum	5,273		20	7.7	30	8.0
Microsporum audouini	5,282		7	7.2	8	7.2
Pullularia pullulans	2,599		9	6.8	20	7.7
Parasite						
Trichomonas vaginalis	8,560		4		NT[f]	

[a] Squibb stock culture number.
[b] (H) is equal to 10^6 cells/ml; for all other bacteria, *Nocardia*, and yeasts, the inoculum was 10^3 cells/ml; for filamentous fungi, the inoculum was 2 ml of a 1:50 dilution of mat or spore suspension derived from a 48-hr growth (25 C) of the organism on 100 ml of Penassay agar in a 250-ml Erlenmeyer flask.
[c] Minimal inhibitory concentration in DMSO (%).
[d] After incubation.
[e] Minimal microbicidal concentration in DMSO (%).
[f] Not tested.

3.0 Materials and methods

3.1. The *in vitro* study of *P. acnes*

3.1.1 Bacterial strain and media

Frozen dried culture of *Propionibacterium acnes* ATCC6919 was obtained from the Environmental Science and Research Institute (ESRI) in New Zealand. All experiments were conducted under strict aseptic conditions.

Brain Heart infusion (BHI) (Merck, Fort Richard NZ) was used as the cultivation broth for the *P. acnes*. BHI is a general-purpose liquid medium used for cultivation of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria. The medium contains infusions of brain and heart tissue and peptones to supply protein and other nutrients necessary to support the growth of fastidious and non-fastidious microorganisms.

Fastidious anaerobe agar (FA agar) was used for purity confirmation of the *P. acnes* by streaking on agar plates and for standard plate counts to test its growth during the study. Sterilized defibrinated horse blood was supplied by Fort Richard NZ.

3.1.2 Preparation of media

Double strength BHI: Of the BHI broth powder (Merck, Fort Richard NZ) 22.2 g was dissolved in 300 mL of distilled water. The mixture was heated on a hot plate with constant stirring to dissolve, followed by autoclaving at 121 °C for 5 min.

Single strength BHI: Of the BHI broth powder, 22.2 g was dissolved in 600 mL of distilled water. The mixture was heated on a hot plate with constant stirring to dissolve, followed by autoclaving at 121 °C for 5 min.

Fastidious anaerobic agar (FA agar) (containing 5 % horse blood) was prepared by dissolving 46 g of powder and added to 1 L of deionised water. The suspension was allowed to soak for 10 minutes, swirled to mix then sterilised by autoclaving at 121 °C for 15 minutes. The solution was cooled to 47 °C and then sterile defibrinated horse blood was added into the molten agar, mixed well and poured onto Petri dishes.

3.1.3 Preparation of the broth culture of *P. acnes*

3.1.3.1 Activation of *P. acnes*

The freeze dried *P. acnes* was supplied in a double-layered vacuum glass tube. Once the seal of the vacuum tube was broken, 1 mL BHI broth was added into the glass tube. The glass container was swirled until the dried *P. acnes* cells were fully dissolved in the BHI nutrient broth. The culture was purified by streaking on FA agar and incubated at 37 °C for 3 days under anaerobic condition using GasPak™ anaerobic sachets (BD BBL™, US).

3.1.3.2 Preparation of routine cell culture

For routine antibacterial activity of testing solution against *P. acnes,* a loop-full of isolated pure colonies of *P.* acnes were removed and inoculated into 20 mL BHI nutrient broth in a universal bottle. The broth culture was then vigorously mixed. Two sets of broth cultures and blank BHI broth (20 mL) were prepared and incubated at 37 °C for 18 h anaerobically. After incubation, positive bacterial growth could be observed as colour changes of BHI broth from clear to cloudy. The absorbance of broth cell culture was recorded at 595 nm using a spectrophotometer (Nova spec ® II, Biochrom, UK). The absorbance of the blank solution of BHI was recorded as the blank reference. The growth of *P. acnes* was recorded as the difference between the absorbance of overnight cultured BHI broth and blank reference. A standard cell density (Abs_{595nm} = 0.2) was required for further antibacterial activity work. If the cell density of *P. acnes* in the BHI broth was above the standard cell density, the blank BHI nutrient broth was used to dilute the standard cell density to about Abs_{595nm} = 0.2.

3.1.3.3 Storage of *P. acnes*

The pure culture of *P. acnes* on the FA agar plates was kept in the refrigeration for 7 days, after which a fresh culture was prepared. To prepare a fresh pure culture, a single colony of bacterial cell culture from the previous 7-day old FA agar plate was streaked over the FA agar plate. A set of three agar plates were prepared and these plates were incubated anaerobically at 37 °C for 3 days.

3.1.4 Analyzing the growth of *P. acnes* using microtiter plate reader

The 18 h culture of *P. acnes* (100 µL) was transferred onto the 96-well microtiter plate reader (Fluostar Optima, BMG Labtech, UK). To each of the microtiter well containing a culture of *P. acnes,* 100 uL BHI nutrient broth were added. The microtiter plate was incubated in the microtiter plate reader at 37 °C, both aerobically and anaerobically for 48 h. The growth of *P. acnes* was recorded as increase of absorbance at 595 nm.

3.2 The antibacterial activities of Manuka honeys and Kanuka honey

3.2.1 Honey samples

Four honey samples were sourced from Comvita New Zealand Limited to investigate their antibacterial activity against the growth of *P. acnes*. The honey samples were Manuka honey 20+ UMF, Manuka honey 15+ UMF, Manuka honey 10+ UMF and Kanuka honey. All the honey samples were stored at 4 °C and protected from light to prevent enzymatic reaction and photo degradation, which may cause glucose oxidase metabolise glucose to hydrogen peroxide in honey. Honey solutions were handled aseptically. The testing solution was prepared just before inoculation to ensure there was no loss of hydrogen peroxide activity in the testing solution.

Chemical analyses of honey showed the Manuka honey 20+ UMF contained 834 mg/kg MGO and 4410 mg/kg DHA; Manuka honey 15 + UMF contained 656 mg/kg MGO and 3580 mg/kg DHA; Manuka honey 10+ UMF contained 439 mg/kg of MGO and 1020 mg/kg DHA (Comvita NZ Limited, unpublished data). Kanuka honey had similar phenolic acids in both quantity and variety with the three different Manuka honeys, except that, the methyl syringate was more than double the amount in the Manuka honeys (Appendix C). The amount of MGO found in the Kanuka honey was about 9.4 mg/kg and the amount of DHA was less than 10 mg/kg.

Artificial honey was prepared by dissolving 1.5 g sucrose, 7.5 g maltose, 40.5 g fructose and 33.5 g glucose in 17 mL sterile deionised water as described by Cooper *et al*., (2002). The sugar in the artificial honey represents the proportions of the four predominant sugars in the natural honey samples.

3.2.2 Spectrophotometric assay using microplate reader

Overnight bacterial liquid culture of *P. ac*nes was prepared by aseptically removing half loop-full of *P. acnes* into sterile BHI (20 mL) nutrient broth into a universal tube. The inoculated nutrient broth was then incubated at 37 °C for 18 h anaerobically. The absorbance of the bacterial culture was recorded at 595 nm using a spectrophotometer (Nova Spec II, Biochrom, UK) after incubation. The inoculated broth culture was adjusted to $Abs_{565\ nm}$ of the 0.2 ± 0.01 using BHI nutrient broth.

To prepare 50 % stock honey solution, 2 g of honey were weighed and dissolved in sterile deionised water. The final volume was made up to 4 mL. To prepare stock honey solution without catalase, catalase (Sigma NZ, 1 unit decomposes 1 mmol H_2O_2/min, PH 7, 25 °C) was used to remove hydrogen peroxide from diluted honey solution. Catalase solution was prepared by dissolving catalase (0.02 g) in sterile deionised water (10 mL), which was prepared according to Snow (2004). Stock honey solution (50 %, w/v) with catalase (2 mg/mL) was made by diluted 2 g of honey using the catalase solution to a final volume of 4 mL.

The honey stock solutions were immediately used for preparing a series of honey concentrations ranging from 12.5 % to 0.5 % (w/v) with a 2.5 % interval. The final range of concentrations of honey was sequentially prepared over 3 steps of dilutions. The first step was to prepare 4 times honey concentrations (50 % to 2 %, w/v) of the final honey solutions (12.5 % to 0.5 %, w/v). This was obtained by adding sterile deionised water to 50 % (w/v) stock solution. The concentrations of honey were further diluted to twice the concentrations (25 % to 1 %, w/v) of the final concentrations in the second step by mixing 1 mL (4 x concentrations) honey solution with 1 mL sterile double-strength BHI nutrient broth. In the last step of the preparation, a 96-well flat bottom microtiter plate (with lid) was used to determinate the antibacterial activity of honeys. One hundred uL of the tested honey samples at 2 times the final concentrations were pipetted into each well of the microtiter plate. The final honey concentration (25 % to 1 %, w/v) was achieved by mixing 100 uL of the overnight culture of *P. acnes* inoculated into honey samples in each well. Within each microtiter plate, a set of positive and negative growth controls were prepared. Positive growth control was prepared by mixing 100 uL of single strength of BHI nutrient broth with 100 uL inoculated culture solution of *P. acnes*. The negative growth control contained BHI nutrient broth only without *P. acnes* culture.

Once honey samples and controls were loaded, the microtiter plate was then loaded into the chamber of the plate reader (Fluostar Optima, BMG Labteck, UK). The plate reader was set at 37 °C for 48 hour with the absorbance at 595 nm. During incubation, the microtiter plate was scanned horizontally and absorbance values of each microtiter were recorded hourly. Changes in absorbance values were indicative of bacterial growth during incubation, which can be expressed as ΔAbs 595 nm = Abs: T_n - Abs: T_0, (Abs: T_n stands for Abs_{595nm} at a given time hourly during incubation; Abs: T_0refers to the initial value of Abs_{595nm}). Absorbance values obtained were used to generate growth curves using MS Excel 2007 (Massey University, New Zealand). The growth inhibition for the test well at each honey dilution was determined by the formula: percent inhibition = [1-(Abs_{595nm} test well / $Abs_{595nm}$of the corresponding control well)] x 100%.

An anaerobic environment was setup in the incubation chamber of plate reader by modifying the composition of inlet air pumped into the chamber. CO_2 gas (100 % food grade, BOC NZ) was pumped at inlet pressure of 4 bars into the incubation chamber of the plate reader through the inlet air valves (CO_2 Beverage Regulator, Cigweld, BOC NZ). An anaerobic environment was maintained by pumping CO_2 to wastage (Brian Hight, personal communication on 14 June 2010, Alphatech NZ)

3.3 Screening natural bioactives

Antibacterial activities of bioactives were tested by modified methods, which are based on the spectrophotometric assays and paper disc diffusion methods described by Chomnawang *et al*., (2005), Muyima *et al*., (2001) and Lorian (1991) and modified by the National Committee of Clinical Laboratory Standards Guidelines (2001).

3.3.1 Preparation of stock solutions of bioactives

3.3.1.1 Propolis

Propolis (80 %, NZ Propolis) obtained in powder form was mixed with tricalcium phosphate (15 %) and silicon dioxide (5%). The propolis (0.3 g) was dissolved in 1 mL of dimethyl sulfoxide (DMSO), (Sigma-Aldrich, NZ). The suspension was centrifuged at 10,000 rpm for 5 min using a high speed centrifuge (Eppendorf, Germany). Tricalcium phosphate and silicon dioxide were precipitated. The supernatant of the aqueous solution was transferred into a sterilized tube. Tween-20 (0.5 mL) (Sigma-Aldrich NZ) was added into the supernatant, followed by the addition of 49 mL de-ionized and sterilized water. The final solution contained 2.4 mg/mL (0.24 %, v/v) propolis in 50 mL.

3.3.1.2 Propolis control solution

DMSO (0.5 mL) was mixed with Tween 20 (0.5 mL) in a falcon tube, followed by the addition of 49 mL de-ionized and sterilized water. The final concentration of the stock control solution contained 1 % DMSO (10 mg/mL) and 1 % Tween-20 in 50mL.

3.3.1.3 Manuka tree oil test solution

Manuka tree oil (100 %, Tairawhiti Manuka Oil – Manex Manuka Oil®, Tairawhiti Pharmaceuticas Ltd NZ) is a clear yellow aerometric essential oil with a density of 0.99 g/mL and it contains 29.8 % of triketone (Appendix C2-4). Manuka oil is extracted from the Manuka tree leaves (*Leptospermum scoparium*). The stock solution of MTO was prepared by mixing 0.1 mL MTO with 0.05 mL DMSO. De-ionised and sterilized water (4.85 mL) was added and made up to 5 mL. The final solution contained 20 mg/mL (2 %, v/v) MTO and 1 % DMSO (10 mg/mL) in 5 mL solution.

3.3.1.4 Manuka tree oil control solution

DMSO (0.05 mL) was mixed with 4.95 mL de-ionized and sterilized water. The final concentration of the stock control solution contained 1 % DMSO (10 mg/mL) in 5 mL.

3.3.1.5 Lavender oil solution

Lavender oil (Lavender oil with product code of 38/40 natural, Australian botanical products Pty Ltd) has a colourless to pale yellow colour with a density of 0.888 g/mL at 20 °C (Appendix C2-3). The stock solution of lavender oil was prepared by mixing 1 mL lavender oil with 0.5 mL DMSO. Once the oil and DMSO were completely mixed, 4.85 mL sterilized de-ionized water were added). The final solution contained 88 mg/mL (10 %, v/v) lavender and 5 % (v/v) DMSO (50 mg/mL) in 5 mL solution.

3.3.1.6 Lavender control solution

DMSO (0.5 mL) was mixed with 4.5 mL de-ionized and sterilized water, giving a final concentration of 5 % DMSO in 5 mL.

3.3.1.7 Olive leaf extract test solution

Olive leaf extract (100 %, Olive Leaf Extract – Pure 1:1®from Olive Leaf Australia Pty Ltd) is a dark-brown herbal liquid containing 2000 mg/mL fresh olive leaf, 1000 mg/mL leaf dried and 8 mg/mL oleuropein (Appendix C2-2). The stock solution was prepared by adding 1.6 mL OLE to 3.4 mL distilled water. The final solution contained 480 mg/mL (32 %, v/v) OLE in 5 mL solution.

3.3.1.8 Green tea extract solution

At the industrial scale, green tea extract (Greentea®, Platextrakt Germany) is enriched with catechins during spray-drying. The powder contains 60 % catechins, 5 % caffeine and 30 % epigallocatechingallate (EGCG). To prepare stock solution, 0.1 g powder was weighed into 5 mL deionised water. The final solution of GTE contained 2 g/mL (2 %, w/v) in 5 mL solution.

3.3.2 Disc diffusion method

Screening of bioactives for antibacterial activity was done by the disc diffusion method, which is normally used as preliminary check and to select the most active bacterial materials (Zu *et al.,* 2010; Prabuseenivasan *et al*., 2006)

3.3.2.1 Preparation of bioactive sample solutions

A series of 2-fold dilutions were prepared from stock solutions of bioactives (Table 3.1). A concentration of stock solution (1:1) was prepared by using 0.5 mL stock solution and mixed with 0.5 mL BHI broth (0.5 mL). Subsequently, a concentration of

1:2 solution was prepared by mixing 0.5 mL of 1:1 stock solution with 0.5 mL BHI. All solutions of bioactives were sterilized by filtration using a 0.45 µm membrane filter (MS®CA syringe filter, US) before loading onto the discs.

Table 3.1 Bioactives used in the disc diffusion method

Bioactive samples in aqueous solution	Undiluted mg/mL (% w/v)	1:1 mg/mL (% w/v)	1:2 mg/mL (% w/v)
GTE	20 (2)	10 (1)	5 (0.5)
LO	88 (10)	44 (5)	22 (2.5)
MTO	20 (2)	10 (1)	5 (0.5)
OLE	480 (32)	240 (16)	120 (8)
Propolis	2.4 (0.2)	1.2 (0.12)	0.6 (0.06)

Notes: GTE=Greet tea extract; LO= Lavender oil; MTO=Manuka tree oil; OLE=Olive leaf extract

3.3.2.2 Preparation of fastidious anaerobic agar

The depth of the FA agar medium is important for the disc diffusion test (Lorian, 1991). The depth of FAA agar medium was poured to about4 mm, which does not significantly affect the test results.

3.3.2.3 Preparation of *P. acnes* inoculum

About 10 isolated and purified colonies of *P. acnes* were aseptically picked using a sterile loop from the surface of the FAA agar and added into BHI broth (20 mL) in a universal bottle. The bacterial cell suspension was vigorously mixed using a Vortex (Wise Mix®, Korea) at maximum speed for a minute. Once the cell suspension was well-mixed, it was then incubated for 18 h at 37 °C. After incubation, the cell culture suspension was diluted approximately to 10^7 to 10^8 CFU/mL ($Abs_{595nm} \approx 0.2$).

3.3.2.4 Streaking of FA agar plates

The surface of the plates must be inoculated evenly. A sterilized cotton swab was dipped in the *P. acnes* inoculum and the excess liquid was removed by rotating the swab several times against the inside wall of the tube above the fluid level in the universal tube. To ensure an even distribution of the *P. acnes* inoculum, FA agar plate was streaked 4 times. After each streaking, the FA agar plate was rotated 45 degree clockwise.

3.3.2.5 Preparation of disc loading with bioactives

Within 15 minutes after the inoculation of the plates, the 6 mm diameter discs (BD Sensi-Disc™, US) were applied on the FA agar plate using a pair of sterilized forceps. The test solution (20 uL) (Table 3.1) was loaded onto each disc using a pipette (100 µL) (Eppendorf, Germany). Discs must be arranged at least 15 mm from the edge of the plate and about 18 mm from each other to reduce the likelihood of overlapping zones (Lorian, 1991). Discs with solutions were allowed to dry on the plate up right for 15 minutes. The amounts of bioactives loaded on each disc were compared as shown in the Table 3.2.

Table 3.2 Two-fold dilution series of bioactives (mg per disc) loaded on each disc

Bioactive and control samples	**Amount of bioactives (mg) per disc**		
	Undiluted	1:1	1:2
Green tea extract	0.4	0.2	0.1
Lavender oil (LO)	1.8	0.89	0.44
Manuka tree oil (MTO)	0.4	0.2	0.1
Olive leave extract	9.6	4.8	2.4
Propolis	0.048	0.024	0.012
5% DMSO (control of LO)	1	0.5	0.25
1% DMSO(control of MTO)	0.2	0.1	0.05
1% DMSO & 1 % Tween-20 (control of Propolis)	0.2	0.1	0.05

3.3.2.6 Incubation condition

After placing the discs on the FAA agar plates, the plates were left for 30 minutes first at room temperature (20 °C ± 2 °C) to allow the diffusion of the bioactive material into the agar. The plates were then incubated anearobically in an inverted position at 37 °C for 48 h.

3.3.2.7 Interpretation of results

The diameter of zone of inhibition was measured in mm by a pair of callipers (Fuller, NZ) after incubation. Any zone of inhibition above 7 mm in diameter was considered as positive growth (Prabuseenivasan *et al.*, 2006). The disc diffusion method was conducted 3 times to screen the antibacterial activity of the bioactives (Table 3.2). Zone of inhibition was recorded as the average diameter of the inhibition zone ± standard error of mean, which were obtained using MS Excel 2007 (Massey University, New Zealand).

3.3.3 Spectrophotometric assay

3.3.3.1 Preparation of *P. acnes* inoculum

About 10 isolated colonies of *P. acnes* were aseptically picked from FA agar using a sterile loop and added into BHI broth (20 mL) in a universal bottle. The bacterial cell suspension was vigorously mixed using Vortex (Wise Mix®, Korea) at the maximum speed for a minute. Once the cell suspension was well mixed, it was then incubated for 18 h at 37 °C. After the incubation, cell density was recorded by a spectrophotometer (Biochrom Novaspec® II, UK). Subsequently, the bacterial cell density was diluted to ABS_{595nm} of 0.2 ± 0.01 using sterile BHI broth.

3.3.3.2 Preparation of solutions of bioactives

A series of 2-fold dilutions were prepared from stock solutions of bioactives by using BHI broth at 1: 1 ratio showed in Table 3.3

3.3.3.3 Setup of microtiter plates

One hundred (100) uL of bioactive (Table 3.3) plus the control solution (100) uL) were transferred into each well of the microtiter plate (Biofill®, Jet Tissue Culture plate 96 wells flat bottom, Japan) in duplication. 100 uL of overnight *P. acnes* (see section 4.2.3.1) was added into each well and fairly mixed with bioactives. The bioactive solution and cell culture we remixed at 1:1 ratio, thus, the final concentrations of bioactives and cell density of *P. acnes* were reduced by half (Figure 4.35). In two of the microtiter wells, BHI broths without antimicrobial materials were inoculated as control for viability (growth control) and a second non-inoculated well served as sterility controls.

Table 3.3 Solutions of bioactives

Samples of bioactives	Concentration of bioactives, mg/mL(% w/w)				
Green tea extract	20 (2)	10 (1)	5 (0.5)	2.5 (0.25)	1.25 (0.125)
Lavender oil	88 (10)	44 (5)	22 (2.5)	11 (1.25)	5.5 (2.75)
Manuka tree oil	20 (2)	10 (1)	5 (0.5)	2.5 (0.25)	1.25 (0.125)
Olive leaf extract	480 (32)	240 (16)	120 (8)	60 (4)	30 (2)
Propolis	2.4 (0.2)	1.2 (0.12)	0.6 (0.06)	0.3 (0.03)	0.15 (0.015)
Control					
5% DMSO (control of LO)	50 (5)	25 (2.5)	12.5 (1.25)	6.25 (0.625)	3.125 (0.3125)
1% DMSO (Control of MTO)	10 (1)	5(0.5)	2.5(0.25)	1.25(0.125)	0.625 (0.0625)
1% DMSO & 1 % Tween-20 (control of Propolis)	10 (1)	5(0.5)	2.5(0.25)	1.25(0.125)	0.625 (0.0625)

Note: Concentration values in (...) are expressed in percentages (%)

3.3.3.4 Determination of antibacterial activity of bioactives using microtiter plate reader

The microtiter plate reader (Fluostar Optima BMG Labteck, UK) was used to measure absorbance of incubated broth culture at 595 nm. The microtiter plate was incubated at 37 °C anearobically for 24 h. The absorbance measurements of the microtiter plate were recorded at start and after 24 h of incubation. The changes in absorbance during the incubation period were directed related to the growth of *P. acnes* in each treatment sample of bioactives.

The MICs were determined as the lowest concentration of bioactives at which *P. acnes* failed to grow in broth (no significant change in turbidity compared to the positive growth control: *P. acnes* only). Experiments on the evaluation of MICs were conducted in duplicate or triplicate.

3.3.3.5 Determination of the minimum bactericidal concentration

Minimum bactericidal concentration was measured as the lowest concentration at which the bacterium failed to growth in BHI broth (Rota *et al*., 2004). One hundred uL were withdrawn from microtiter plate wells with no growth. A series of 10-fold dilution were prepared from 10^{-2} to 10^{-5} on the FA agar plate. The end-point of MBC would be determined as the concentration leading to colony forming units of less than 100 CFU/mL. The evaluation of the MIC and the MBC was carried out in duplicate or triplicate.

3.4 Effects of bioactives and Manuka honey on killing rates of *P. acnes*

The microtiter plate was used to aid the study of the killing rate of Manuka honey 10+ UMF and bioactives (MTO and GTE). The test samples included 20 % 10+ UMF honey (200 mg/mL), 1 % GTE (10 mg/mL), 1 % MTO (10 mg/mL), plus combination #1 containing 20 % honey (200 mg/mL) and 1 % GTE (10 mg/mL) and combination #2 containing 20 % honey (200 mg/mL) and 1 % MTO (10 mg/mL). The samples were prepared aseptically. Of the test samples, 100 uL were transferred into the wells of the microtiter plate. An overnight culture of *P. acnes* (100 uL) was added to each well. The mixture was mixed by squirting gently for 20 times using a 300-uL Eppendorf multichannel pipette. A positive control sample was prepared using 100 uL *P. acnes* with BHI solution (100 uL) without any addition of antibacterial substances. One percent (1 %) DMSO solution (100 uL) mixed with overnight *P. acnes* culture (100 uL) was used as the blank control of MTO solution for combination #2 (honey and MTO). Three replications were prepared for each test sample. The samples were incubated aerobically in a plate reader for 24 h. Viable cell counts of *P. acnes* in each test samples were conducted using the FA agar plate at different times (0, 6, 24 and 48 h) throughout the assay period and total viable cell counts were determined using the spread plate method. The samples (100 uL) were diluted using 900 uL distilled water into a series of concentrations (10^{-2} to 10^{-8}). Data obtained were plotted on the graphs to determine killing rates of *P. acnes* in the test samples.

3.5 Evaluating the antibacterial activities of emulsion creams containing honey and bioactives

3.5.1 Preparation of *P. acnes* inoculum

Few isolated pure colonies of *P. acnes* were picked using a sterile loop and added into sterile BHI broth (20 mL) in a universal bottle. The mixture was mixed by votexing for a few seconds and then incubated overnight (18 h). Increase in turbidity measured at 595 nm in a spectrophotometer was indicative of the growth of *P. acnes*. The cell density was standardised to an ABS_{595} of 0.2 ± 0.01 by diluting the overnight cell inoculum with sterile BHI broth.

3.5.2 Preparation of cream containing honey and bioactives separately

Ingredients were measured as shown in Table 3.4. Manuka honey 10+ UMF and GTE were dissolved in warm distilled water (65 °C) for 1 minute. An emulsifier (Olivem® 1000, Italy, Appendix C6) was transferred into a universal glass tube and placed in the water bath at 65 °C until it was fully melted. The molten emulsifier was then mixed with glycerine. For the MTO emulsion, essential oil was first dissolved in the molten emulsifier with glycerine and then distilled water added slowly into the mixture while it was kept warm in the water bath. For honey and the GTE cream, honey and GTE, sterile water was steadily added to the molten emulsifier while being continuously mixed in the water bath (60 °C). Immediately after mixing, the cream solution was vortexed (WiseMix VM10, Korea) vigorously for 1 minute to mix.

Table 3.4 Preparation of emulsion samples

Emulsion samples (%, w/w)	Concentration of ingredients					
	Glycerine (g)	Emulsifier (g)	Honey (g)	MTO (uL)	GTE (g)	Water (mL)
Positive control	0	0	0	0	0	1
Emulsion control	0.5	0.5	0	0	0	4
30% Honey	0.5	0.5	3	0	0	1
Honey control	0.5	0.5	1	0	0	3
1.0% GTE	0.5	0.5	0	0	0.1	3.9
0.5% GTE	0.5	0.5	0	0	0.05	3.95
0.25% GTE	0.5	0.5	0	0	0.025	3.975
0.125% GTE	0.5	0.5	0	0	0.0125	3.9875
0.5% MTO	0.5	0.5	0	50	0	3.95
0.25% MTO	0.5	0.5	0	25	0	3.975
0.125% MTO	0.5	0.5	0	12.5	0	3.9875
0.25% MTO+ 10% honey	0.5	0.5	1	25	0	2.975
0.125% MTO + 10% honey	0.5	0.5	1	12.5	0	2.9875
0.5% GTE+ 10% honey	0.5	0.5	1	0	0.05	2.950
1.0% GTE+ 10% honey	0.5	0.5	1	0	0.1	2.900
10% Honey + 0.125% MTO +1% GTE	0.5	0.5	1	12.5	0.1g	2.8875

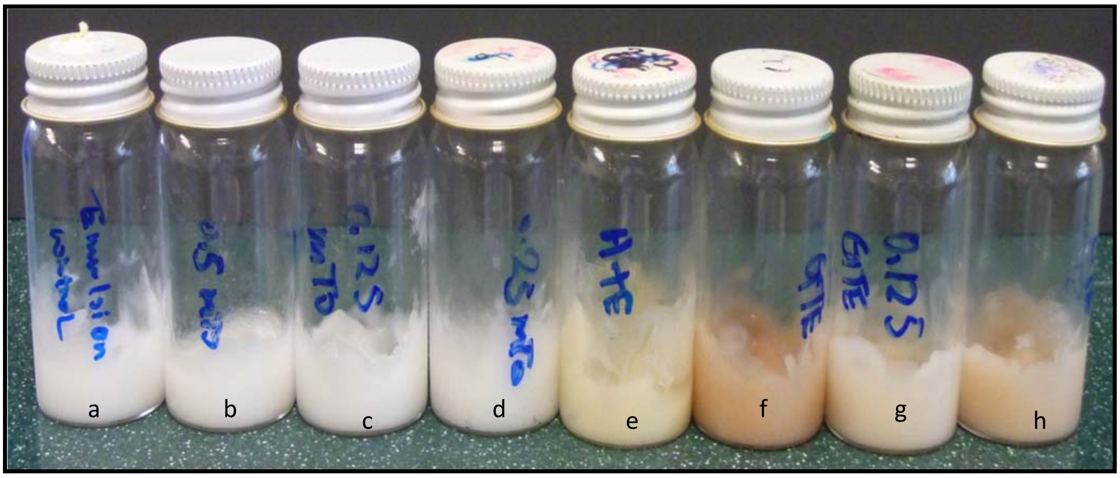


Figure 3.1 Cream samples; (a) emulsion control, (b) 0.5 % MTO, (c) 0.125 % MTO, (d) 0.25 % MTO, (e) 10 % honey, (f) 1 % GTE, (g) 0.125 % GTE, (h) 0.5 % GTE. Images were captured by a digital camera (Lumix DMC-FP3, Panasonic, China)

3.5.3 Preparation of cream samples containing honey and bioactives cooperatively

The cream sample containing 10 % honey and 1 % GTE in the emulsion cream base was prepared as follows: GTE powder, honey, emulsifier and glycerine were measured as shown in Table 3.4. The GTE powder was firstly dissolved in the water at 65 °C in a water bath, and honey wasthen added into the GTE solution. The emulsifier and glycerine were melted upon heating in the 65 °C water bath for 2 minutes. Once the mixture of emulsifier and glycerine turned into transparent liquid, honey and GTE aqueous solution were then slowly added into the molten emulsifier. A bench-top vortex was used to mix the honey and GTE cream solution.

For the preparation of honey and MTO cream, the emulsifier was firstly melted with glycerine in the universal bottle (25mL) in a 65 °C water bath for 5 minutes. Once the emulsifier was melted and became liquid, MTO was then added to the emulsifier. Later, honey was dissolved into the water and quickly warmed up to 65 °C in the water bath. The honey solution was then slowly added into molten emulsifier while the bottle was kept inside the water bath. The emulsion mixture was then mixed using a bench-top vortex (WiseMix VM10, Korea).

Final cream sample containing honey, MTO and GTE were prepared in three stages. The first stage involved the preparation of the emulsion solution by mixing the emulsifier and glycerine. This mixture was then heated up to 65 °C in a water bath; then the MTO oil was dissolved slowly into the molten liquid emulsifier. The second stage involved the preparation of the aqueous solution; honey and green tea extract powder were transferred into universal bottles, water wasthen added into the bottle. The honey and GTE powder were then well-dispersed into the water and warmed up to 65 °C in a water bath. In the third and final stage, the honey aqueous solution was mixed into the MTO emulsion solution. In order to obtain a stable emulsion, a bench-top vortex (WiseMix VM10, Korea) was used at high shear power to the emulsion mixtures.

3.5.4 Evaluation of antibacterial ability of creams with honey and bioactives

Emulsion samples (1 g) were transferred into sterilized universal bottles. A standardised 18 h broth culture of *P. acnes* (1 mL) was added into the emulsion samples and vortexed for 1 minute. The mixture was then incubated anaerobically at 37 °C for 24 h. After 24 h incubation, a series of 10-fold dilutions (10^2 to 10^9) were prepared for each sample. Of each diluted sample, 100 µL was withdrawn and streaked over FA plate (containing 5 % horse blood). Each treatment was prepared in duplicate. The plates were then incubated anaerobically at 37 °C for 72 h. Developed colonies were enumerated and analysed as CFU/g. In order to confirm reproducibility and repeatability of the experimental method, the experiments were repeated twice.

3.6 Statistical analysis

Data were subjected to statistical analysis using Minitab 15 (Minitab Inc, Pennsylvania State University, USA). Correlation coefficients, regression correlation, and ANOVA with Tukey's post hoc analysis were used to analyse the data at 95 % confidence interval ($\alpha = 0.05$). Bacterial growth curves and viable cell counts were plotted using MS Excel 2007 (Massey University, New Zealand).

4.0 Results

4.1 The *in vitro* study of *P. acnes*

4.1.1 Characterisation of *P. acnes* when grown on the Fastidious Anaerobic agar plate

In this study, *P. acnes* was able to grow aerobically and anaerobically at 37 °C for 3 days. Growth of the bacterium under anaerobic environment was more pronounced. It seemed that *P. acnes* grows better under an anaerobic environment. *P. acnes* grew rather poorly on the FA agar plate under an aerobic environment (Figure 4.1 a). Bacterial colonies could not develop fully on the agar plate under an aerobic environment. Under an anaerobic environment, the *P. acnes* showed better growth on the FA agar as shown in Figure 4.1 (b).

During incubation of the *P. acnes* under both environments, the bacterium produced a smelly acid with a characteristic French cheese odour. Under an anaerobic environment, surface colonial morphology on streaked plates were circular, raised, smooth, glistening and had an entire edge with a average size of 1 mm in diameter. The colonies appeared creamy white and opaque.

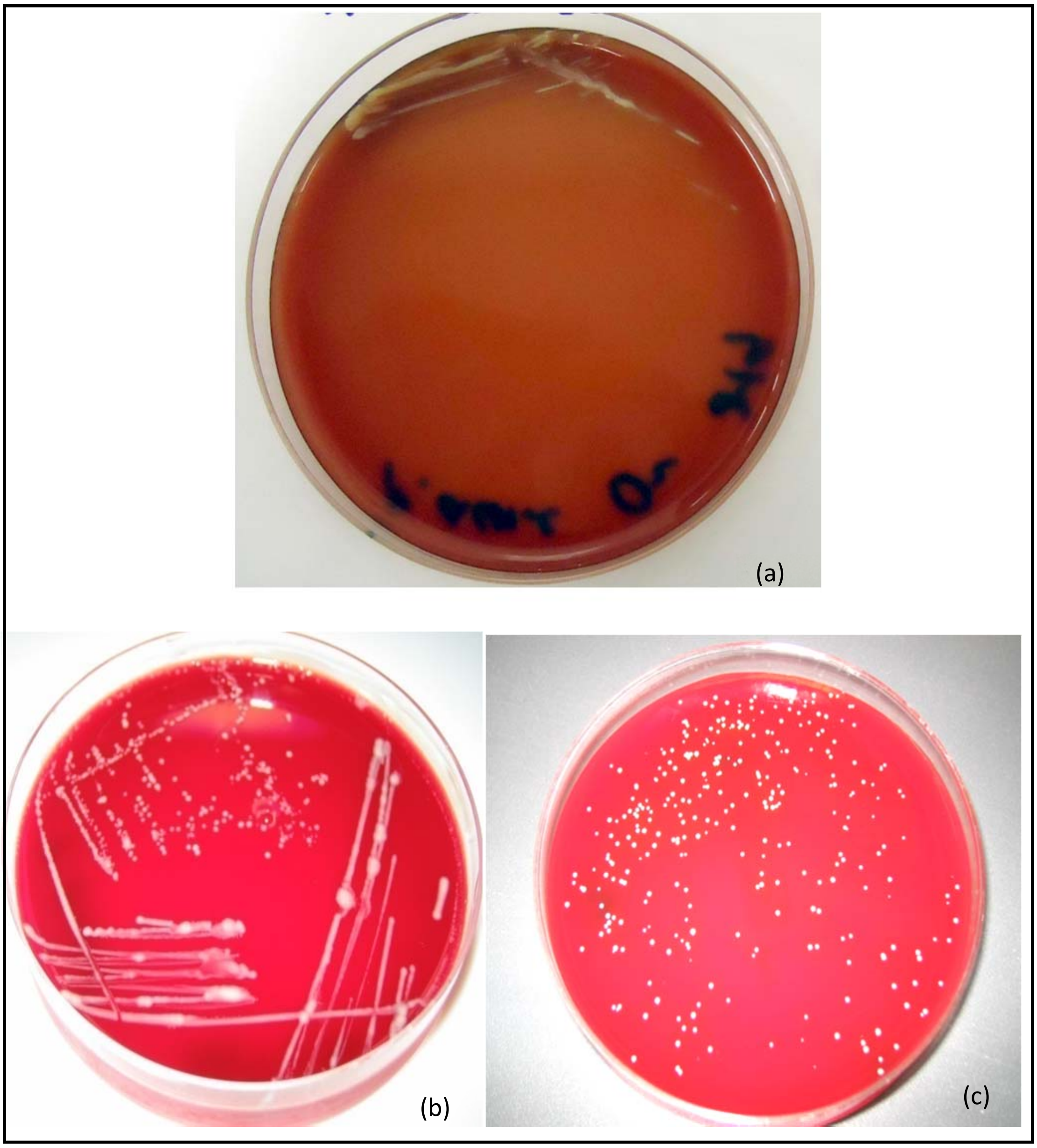


Figure 4.1 Images of *P. acnes* inoculated on FA agar containing 5 % horse blood incubated under aerobic and anaerobic environments. (a) Streaked plate incubated aerobically; (b) streaked plate incubated anaerobically; (c) spread plate incubated anaerobically. Images were captured by a Panasonic Digital Camera (Lumix DMC-FP3, China)

4.1.2 Growth of *P. acnes* in BHI broth

A loopful of *P. acnes* colonies was removed from FA agar plate and cultivated in BHI liquid broth under anaerobic and aerobic conditions (Figure 4.2). The growth of *P. acnes*in BHI broth was confirmed by recording absorbance values at 595 nm. During 24 h incubation, the bacterial growth was recorded as increase of absorbance or turbidity of the BHI nutrient broth. Absorbance values were observed similarly under both aerobic and anaerobic environments and no marked differences were observed between both conditions.

Figure 4.2 The growth of *P. acnes* in the BHI broth, from left to right: blank control, the growth of *P. acnes* under aerobic condition and growth of *P.acnes* anaerobically. Images were captured by a Panasonic Digital Camera (Lumix DMC-FP3, China)

4.1.3 Gram stain of *P. acnes*

Gram stain of *P. acnes* from the blood culture showed that the bacterium was Gram-positive (Figure 4.3), non-spore-forming and non-motile. The *P. acnes* is a pleomorphic rod; the organism ranges from small, plump rods which tend to occur in pairs with the cells joined at a slight angle, swollen or even club-shaped. Some bacterial cells appear to be unevenly stained, which may be also called Gram-variable rods. The size of the individual cells in Gram stains is approximately 0.03 - 0.06 by 0.05 - 0.3 μm.

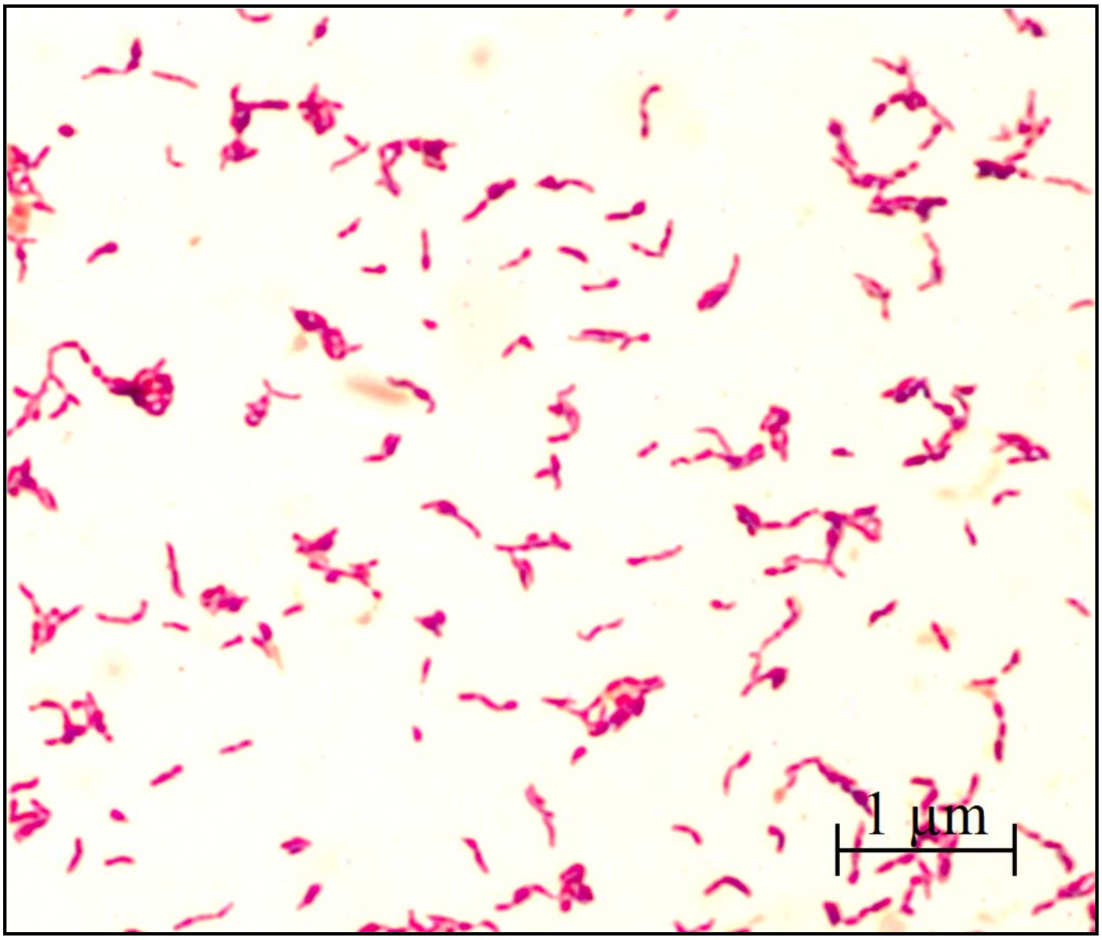


Figure 4.3-Gram stain of *Propionibacterium acnes* from blood culture. Amplification: x 1000 (Transmission light microscope, Carl Zees-Model HBO 50/AC, Germany)

4.1.4 Growth of *P. acnes* measured by microtiter plate reader

Microbial kinetic growths were automatically recorded on a micro-plate reader (BMG Labtech, UK). The wavelength was set at 595 nm and the absorbance was recorded hourly. The microtiter plates were shaken for 30 seconds prior to measurement to achieve homogeneous suspension. The growth curves were plotted as averages of three parallel wells over different experiments.

Figure 4.4 shows that the *P. acnes* could effectively grow in the BHI nutrient broth incubated in the chamber of the microtiter plate reader aerobically and anaerobically. There was no significant difference ($p > 0.05$) between two dynamic growth curves of *P. acnes* under both environmental conditions. Under optimal growth conditions, the lag phase of the bacterium was about 1 h, which was followed by a rapid bacterial growth in the exponential phase and then a steady growth in the stationary phase. The exponential phase of kinetic growth curves under an anaerobic environment was up to 25 h with a growth rate of about 0.029 h^{-1}. When grown aerobically, the exponential phase of the bacterium was about 35 h with a growth rate of about 0.024 h^{-1}. Towards the end of incubation, the maximum bacterial growth was about the same under both growth conditions. Hence, the *P. acnes* grew better anaerobically, but differences in bacterial growth between both conditions were not significantly different ($p>0.05$).

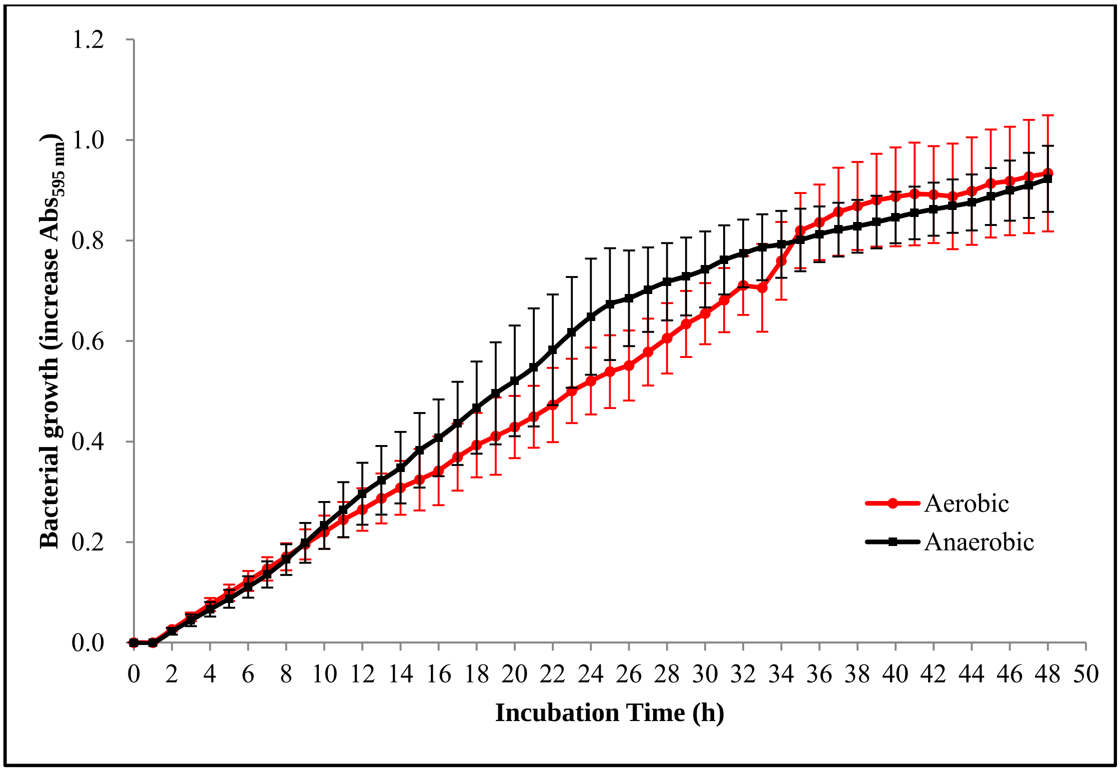


Figure 4.4 The growth of *P. acnes* over 48 h at 37 °C aerobically and anaerobically

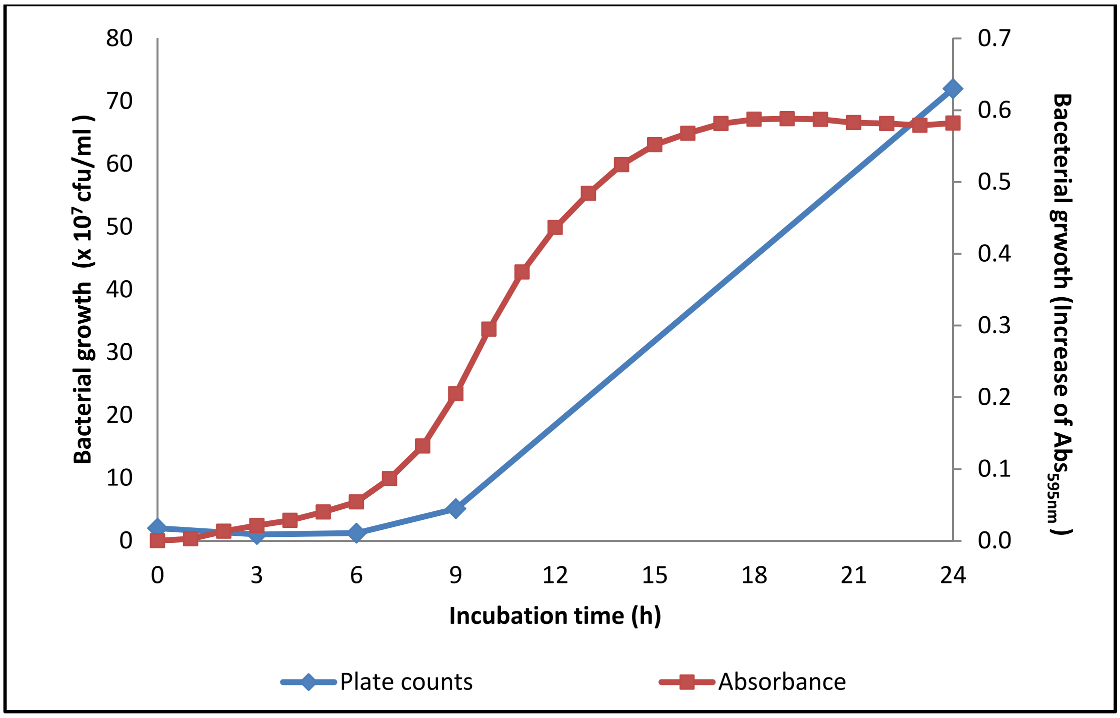


Figure 4.5 The growth curve of *P. acnes* measured by absorbance and plate counts in 10 % Manuka honey 10+ UMF

4.1.5 Correlation between the standard plate count and absorbance readings

Work done by Lorian (2005) and Murray (2007) recommended that abs_{595nm} measurements require calibration with viable cell counts obtained from standard plate counts. In the present study, this was achieved by comparing the results from viable cell counts obtained from the standard plate counts method and the absorbance values as an estimate of cell growth of *P. acnes* obtained from microtiter plate reader method.

Figure 4.5 show that *P. acnes* was capable of growing in the Manuka honey 10+ UMF solution (10 %) in the incubation chamber of plate reader under an aerobic environment at 37 °C for 24 h. The bacterial growth curves were plotted as increase of viable cell counts and increase of absorbance hourly during incubation. Both bacterial growth curves showed similar increasing patterns. In the honey solution, there was approximately 2 x 10^7 CFU/mL of *P. acnes* inoculated initially. In the first stage, the bacterial growth (increase of $Abs_{595\ nm}$) was initially very slow in the first 8 h of incubation, which could be considered as the lag phase. During the first initial incubation period, there was no marked change in viable cell counts (CFU/mL). In the second stage of bacterial growth, the absorbance of bacterial cell culture increased rapidly from 8 to 16 h of incubation. Meanwhile, the viable cell counts increased rapidly from 2 x 10^7 CFU/mL to 7 x 10^8 CFU/mL. In the last bacterial growth phase (16 to 24 h), there were no marked changes in the absorbance values. At 24 h of incubation, the final viable cell count was about 7.2 x 10^8CFU/mL. Hence, bacterial growth shown as increase in absorbance was suggested to be closely correlated with increase of viable cell counts throughout the bioassay. The results showed that the micro-plate reader is a suitable method to investigate the growth patterns of *P. acnes* in the Manuka honey solution.

4.2 The antibacterial activities of Manuka honey and Kanuka honey

A 48h spectrophotometric assay using a 96-well microtiter plate was used to study the antibacterial activity of the honey samples against the growth of *P. acnes* at 37 °C; the bio-assay was recommended as a sensitive assay with good reproducibility and repeatability (Patton *et al*., 2006). Dose-dependent growth curves were constructed to show the inhibitory effect of *P. acnes* by increasing the concentrations of Manuka honey samples and Kanuka honey which ranged from 0.5 to 12.5 % (w/v). Based on dose-dependent regression curves, the MIC_{50}, MIC90, MIC_{100} of honeys were calculated.

4.2.1 Growth kinetics of *P. acnes* in Manuka and Kanuka honeys under aerobic environment

4.2.1.1 Growth (aerobic) of *P. acnes* in Manuka honey 15 + and 20 + UMF

Manuka honey samples 20+ UMF and 15+ UMF exhibited strong antibacterial activities against the growth of *P. acnes* at concentrations of 2.5 % (w/v) and above (Figures 4.6 and 4.7).Manuka honey samples ranging from 2.5 % to 12.5 % (w/v) clearly showed antibacterial effects on the lag phase of the growth curves. At 2.5 % honey (w/v), the bacterial growth curves of *P. acnes* were clearly shifted downwards away from the positive control, suggesting partial inhibition. From 5 % to 12.5 % honey (w/v), the bacterial growth curves were close to the x-axis and the increases of Abs_{595nm} were between 0 – 0.1 towards the end of the incubation. Thus, Manuka honey samples 15+ and 20+ UMF with concentration ranging from 5 to 12.5 % (w/v) completely inhibited the growth of *P. acnes*.

In contrast, no inhibitory effect was observed in the 0.5 % and 1 % (w/v) honey samples (Figures 4.6 and 4.7): the bacterial growth curves of *P. acnes* were close to the positive control; there were no significant differences between the treatment growth curves and the positive control ($p > 0.05$).

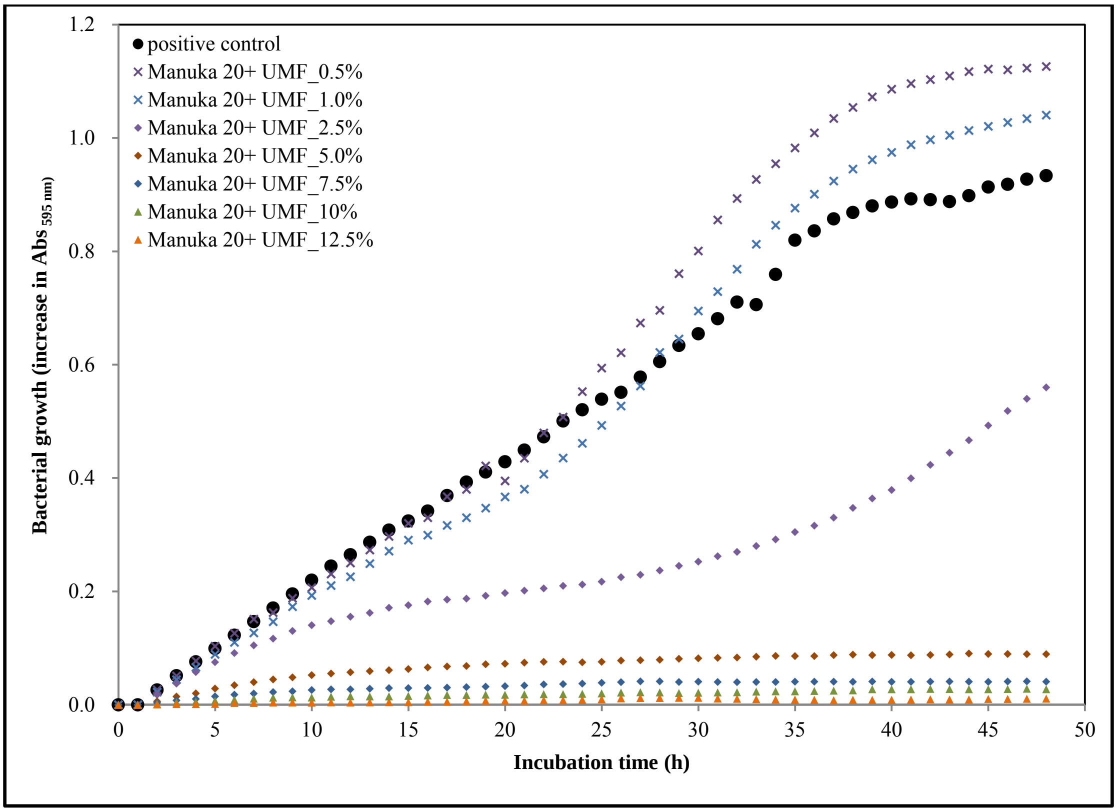


Figure 4.6 Growth (aerobic) of *P. acnes* in various concentrations of Manuka 20+ UMF honey samples at 37 °C for 48 h

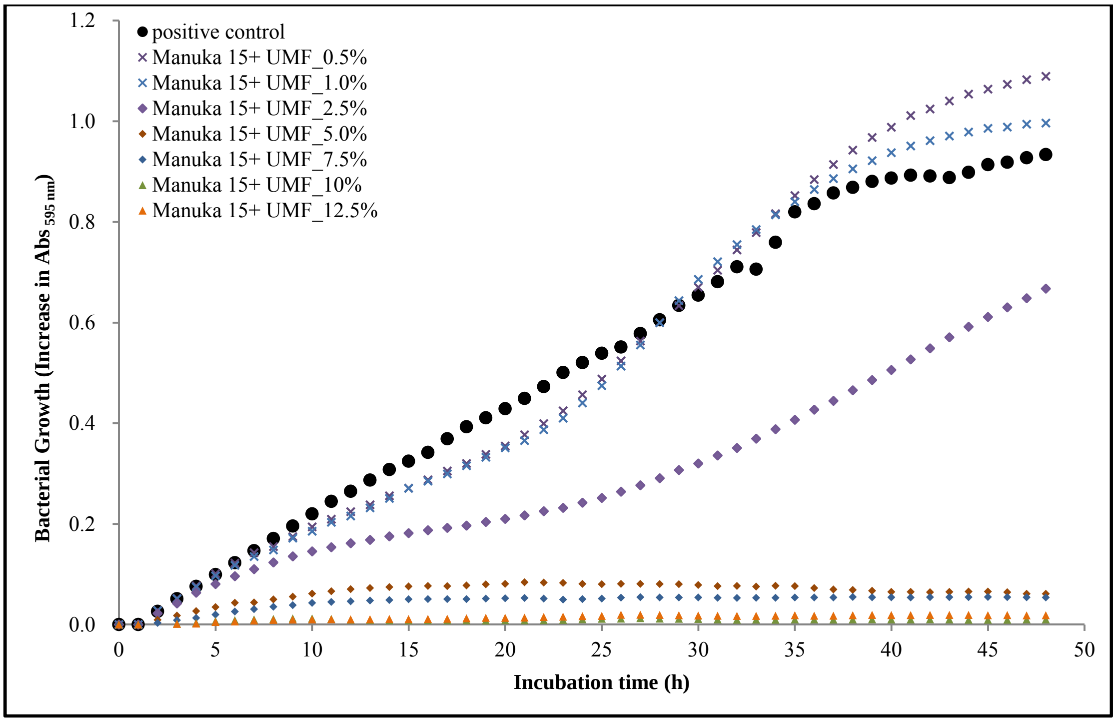


Figure 4.7 Growth (aerobic) of *P. acnes* in various concentrations of Manuka 15+ UMF samples at 37 °C for 48 h

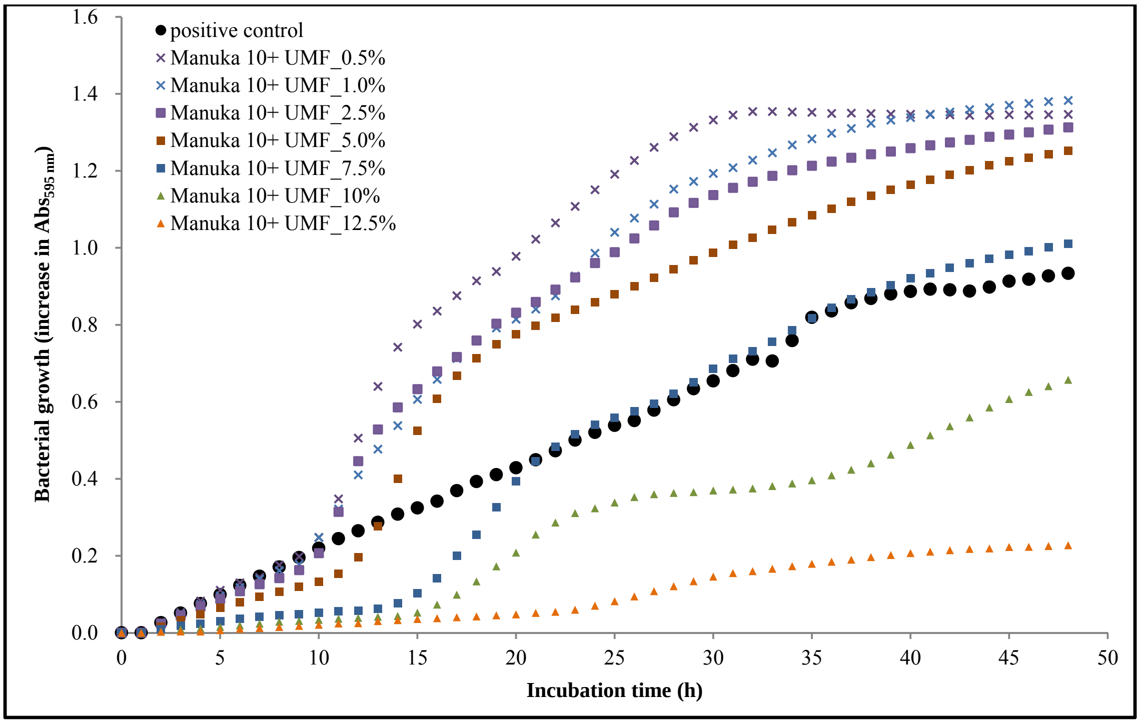


Figure 4.8 Growth (aerobic) of *P. acnes* in various concentrations of Manuka 10+ UMF samples at 37 °C for 48 h

4.2.1.2 Growth (aerobic) of *P. acnes* in Manuka honey 10 + UMF

Figure 4.8 shows that the growth of *P. acnes* was inhibited by Manuka honey 10+ UMF at 7.5 % (w/v) and above under aerobic environment. The growth of *P. acnes* was partially inhibited by 7.5 % (w/v) Manuka 10+ UMF resulting in an extended lag phase; no bacterial growth was observed in the first 15 h. The inhibitory effect was increased in the 10 % and 12.5 % (w/v) Manuka 10+ UMF honey samples: the growth curves of *P. acnes* clearly moved away from the positive control. This movement of the bacterial growth curves resulted an extended lag phase of 15 and 25 h. In the 10 % honey solution, the bacterial growth (Abs_{595nm}) was only half of the maximum growth (Abs_{595nm}) in the positive control, and the bacterial growth (Abs_{595nm}) in the 12.5 % honey solution was about a quarter of the maximum of growth towards the end of incubation. On the other hand, it was observed that honey concentrations ranging from 0.5 % to 5 % (w/v) supported the growth of *P. acnes*. The growth of *P. acnes* was similar to the positive control initially. After the first 10 h of incubation, the growth of *P. acnes* increased rapidly. Towards the end of incubation, the bacterial growth curves (0.5 – 5 % honey, w/v) were above the positive control. The shift of growth curves of the bacterium in the samples above the positive control suggested that the honey had stimulatory effects on the growth of *P. acnes*.

4.2.1.3 Growth (aerobic) of *P. acnes* in Kanuka honey

Figure 4.9 shows that the growth of *P. acnes* was markedly inhibited by Kanuka honey at the concentration of 0.5 % (w/v) and above under aerobic environment. *P. acnes* partially grew in the Kanuka honey solutions ranging from 0.5 – 7.5 % (w/v). For instance, the growth of *P. acnes* was slow during the first 24 h of incubation in 0.5 - 2.5 % Kanuka honey solution, but bacterial growth then increased rapidly towards the end of incubation, eventually, the bacterial growth curves merged with the positive control. Moverover, 5 % and 7.5 % Kanuka honey samples also caused partial inhibition of *P. acnes*: the lag phases (no growth) were extended to 15 and 20 h; after the lag phase, the bacterial growth was increased exponentially in the next 5 h; the total bacterial growth curves were remained below the positive growth curves throughout the incubation period. The inhibitory effect was markedly increased in the 7.5 % and above: with a slight horizontal shift to the right, the bacterial growth curve in 7.5 % (w/v) honey solution was parallel to the growth of *P. acnes* in 5 % (w/v) honey solution; there was no bacterial growth throughout the assay period for 10 and 12.5 % (w/v) honey solutions.

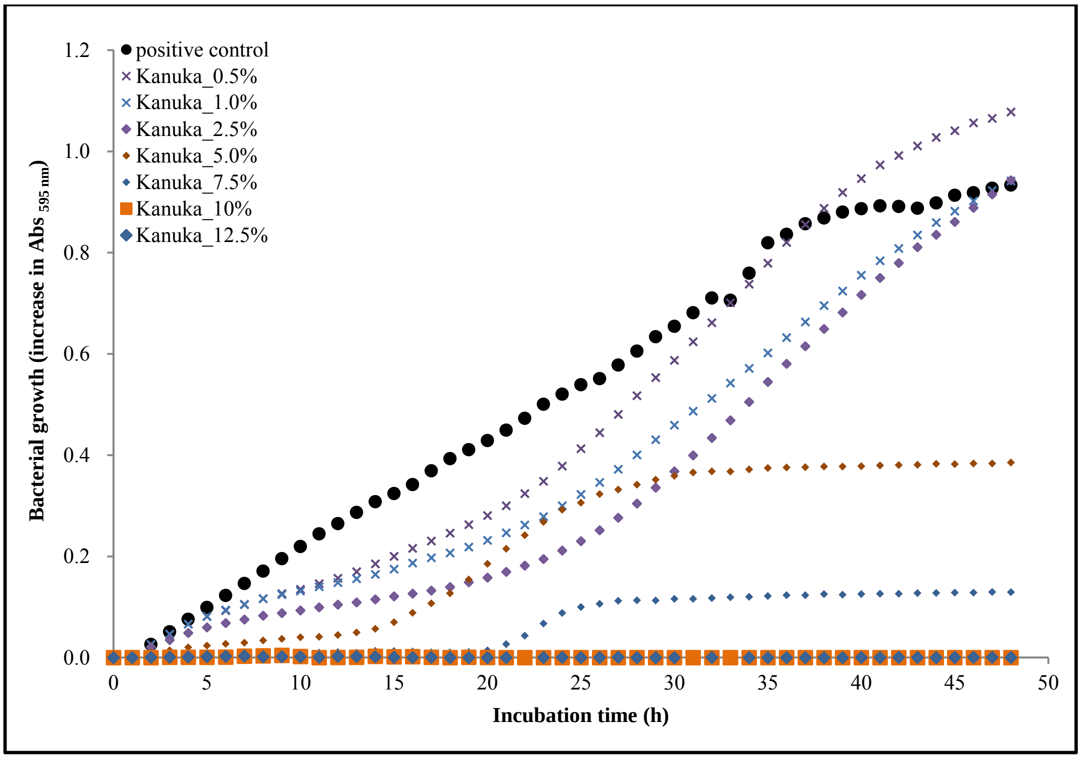


Figure 4.9 Growth (aerobic) of *P. acnes* in various concentrations of Kanuka samples at 37 °C for 48 h

4.2.2 Growth kinetics of *P. acnes* in the Manuka honey and Kanuka honey samples with catalase (non-peroxide) under aerobic environment

Hydrogen peroxide in the diluted honey solutions was eliminated by adding catalase (Weston, 2000). Figures 4.10 to 4.13 show the effects of non-peroxide activity of Manuka honey and Kanuka honey samples against the growth of *P. acnes* under aerobic environment. The three Manuka honey samples (Manuka honey 10+, 15+ and 20+ UMF) exhibited non-peroxide activity against the growth of *P. acnes* with the addition of catalase. The strength of the antibacterial potency was related to the UMF rating of Manuka honey samples and the honey concentrations used. Meanwhile, Kanuka honey samples did not show non-peroxide activity against *P. acnes*; the Kanuka honey possessed no antibacterial activity with the addition of catalase.

4.2.2.1 Growth (aerobic) of *P. acnes* in Manuka honey 15+ and 20+ UMF with catalase

Figures 4.10 and 4.11 show that Manuka honey samples 20+ and 15+ UMF had strong antibacterial activities against the growth of *P. acnes* at 0.5 % honey (w/v) and above even in the presence of catalase. From 0.5 - 5 % honey solutions, there was partial inhibition of *P. acnes* initially in the Manuka honey 20+ UMF sample (Figure 4.10); the growth curves were below the positive control curves initially, but towards the end of incubation (27 h), the curves increased rapidly and subsequently the bacterial curves were higher than the positive control curves. A similar growth pattern was observed for Manuka honey 15+ UMF samples ranging from 0.5 – 5 % (Figure 4.11). Unlike Manuka honey 20+, the bacterial growth curves in Manuka honey 15+ UMF merged with the positive control curves within a short time (24 h) and the curves subsequently went above the positive control curves (Figure 4.11). This observation suggests that 0.5 – 5 % Manuka honey 20+ and 15+ UMF samples have exhibited temporary inhibitory effects (Figures 4.10 and 4.11). However, at 7.5 % and above, Manuka honey 15+ and 20+ UMF samples with catalase were below the positive control curves during the incubation period (48 h) under aerobic environment (Figures 4.10 and 4.11). This suggests that the partial inhibitory effects of the honey samples against *P. acnes* were active in both Manuka honey samples at 7.5 % (w/v) and above.

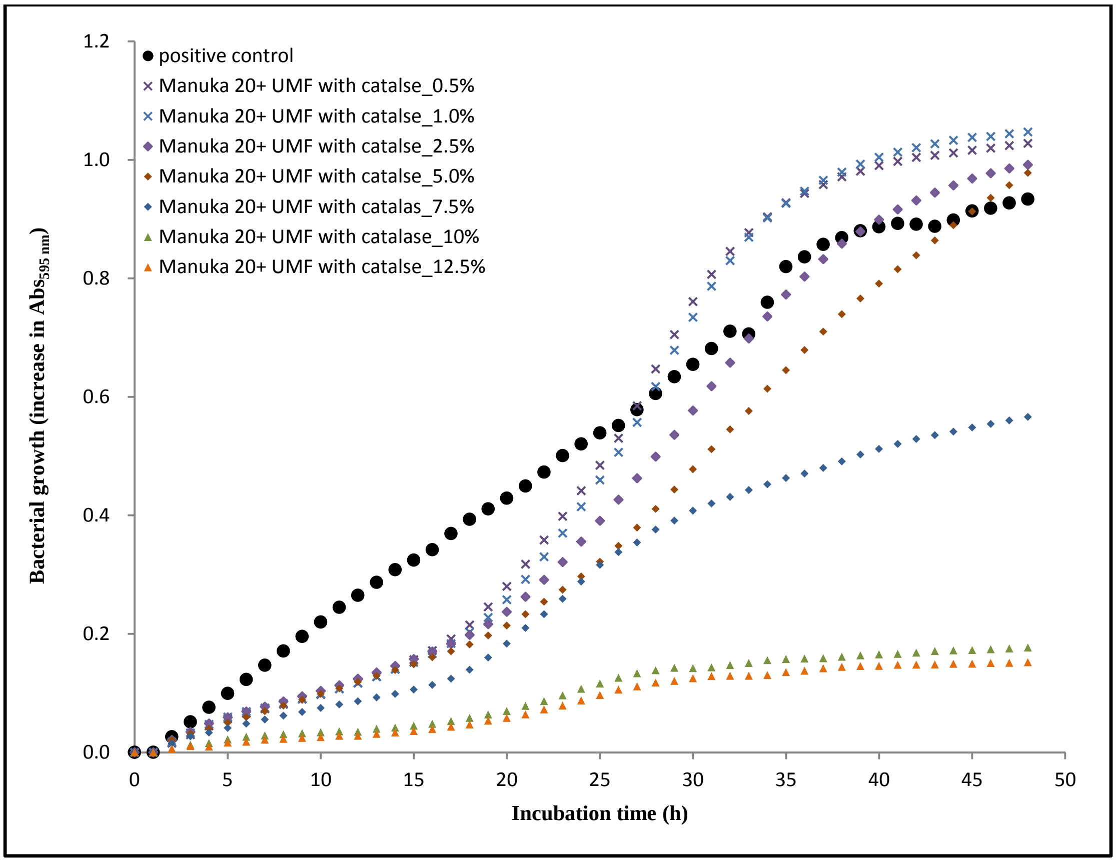


Figure 4.10 Growth (aerobic) of *P. acnes* in various concentrations of Manuka UMF 20+ samples with catalase at 37 °C for 48 h

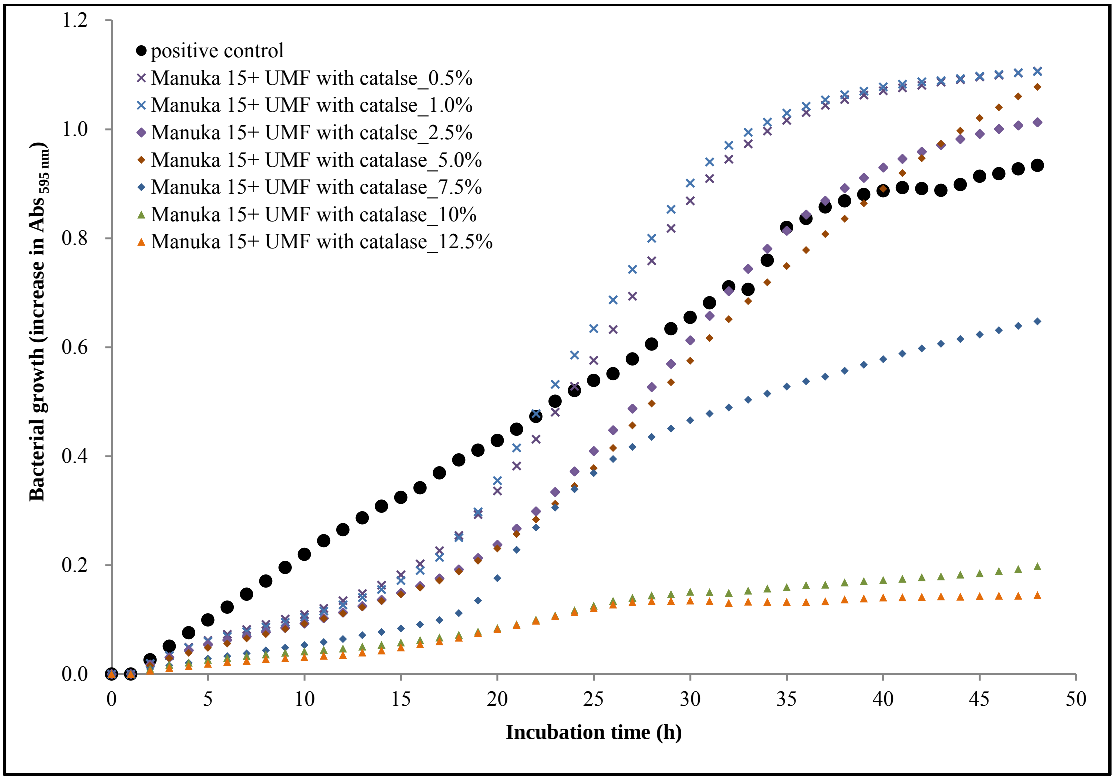


Figure 4.11 Growth (aerobic) of *P. acnes* in various concentrations of Manuka UMF 15+ samples with catalase at 37 °C for 48 h

4.2.2.2 Growth (aerobic) of *P. acnes* in Manuka honey 10+ UMF with catalase

Figure 4.12 shows that the growth of *P. acnes* was inhibited by Manuka honey 10+ UMF sample with catalase under aerobic environment. The growth curves of *P. acnes* shifted to the right with a steady clockwise turning towards the horizontal direction, when the amount of honey was increased in the treatment solutions. Figure 4.12 shows that the temporary inhibitory effect of Manuka honey was observed in honey concentrations ranging from 0.5 – 10 %. At the lowest honey concentration (0.5 %), the lag phase increased to about 10 h and at the highest concentration (12.5 %), the lag phase was 25 h. The growth of *P. acnes* rapidly increased after the lag phase. Within the next 3 to 5 h, the bacterial growth in the honey treatments (0.5 % to 7.5 %) was markedly higher than the positive control. The inhibitory effect of 10 % honey was not apparently evident after 24 h of incubation. Only 12.5 % of Manuka honey 10+ UMF sample with catalase could maintain its antibacterial effect throughout the bioassay (Figure 4.12). By the end of incubation, about 50 % the growth of *P. acnes* had been reduced in comparison to the maximum bacterial growth observed in the positive control.

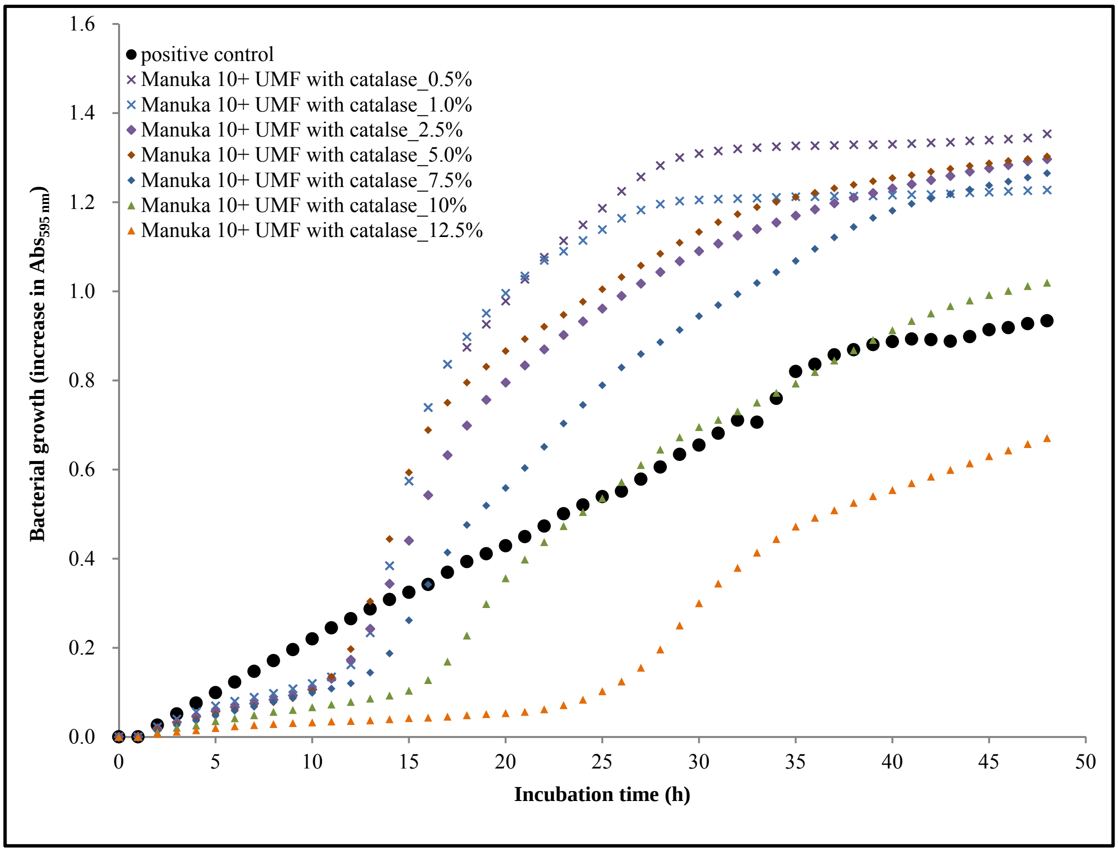


Figure 4.12 Growth (aerobic) of *P. acnes* in various concentrations of Manuka UMF 10+ samples with catalase at 37 °C for 48 h

4.2.2.3 Growth (aerobic) of *P. acnes* in Kanuka honey with catalase

Figure 4.13 shows that Kanuka honey lost the inhibitory effect against the growth of *P. acnes* after the addition of catalase in the honey solution. This indicates that hydrogen peroxide present in Kanuka honey was the most important component responsible for the inhibition of *P. acnes.*

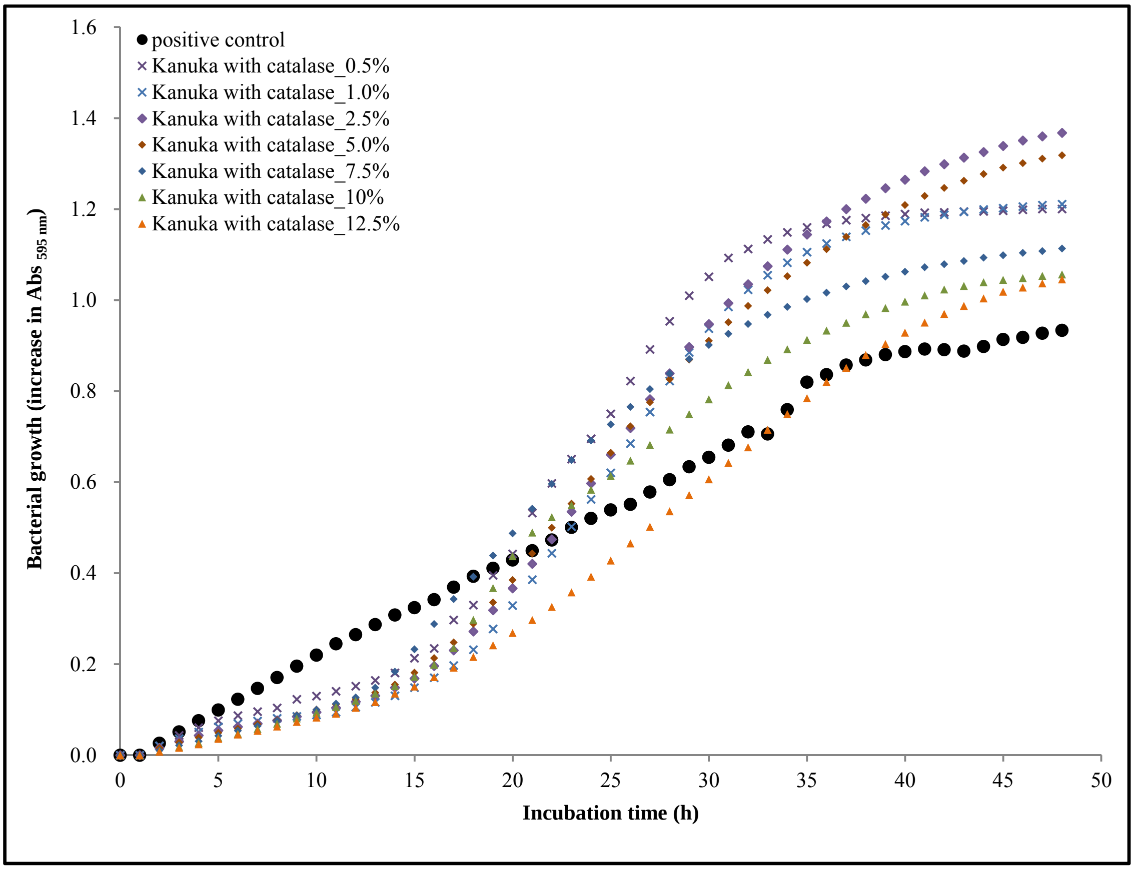


Figure 4.13 Growth (aerobic) of *P. acnes* in various concentrations of Kanuka honey samples with catalase at 37 °C for 48 h

4.2.3 Growth kinetics of *P. acnes* in Manuka and Kanuka honey samples

4.2.3.1 Growth (anaerobic) of *P. acnes* in Manuka honey15+ and 20+ UMF samples

Under anaerobic environment, Figures 4.14 and 4.15 show that Manuka honey samples 20+ and 15+ UMF inhibited the growth of *P. acnes* at 0.5 % (w/v). The antibacterial activity of the two Manuka honey samples was similar. The growth of *P. acnes* was inhibited by both types of honey samples at 0.5 % and 1 % (w/v) for up to 20 h of incubation; the growth rates of the *P. acnes* in the honey solution were below the positive control during the lag and exponential phases up to 20 h of incubation, followed by a rapid growth in the next 5 to 8 h. The growth of *P. acnes* was similar to the positive control towards the end of incubation. Nonetheless, in 1 % (w/v) Manuka honey 20+ UMF sample, the bacterial growth (Abs_{595nm}) was about 70 % of the maximum positive control. At honey concentrations of 2.5 % and 5 % (w/v), the bacterial growth increased during the incubation time (the growth rate did not change). It was therefore difficult to describe the growth curves: the lag phase, exponential phase, and stationary phase along the growth curves due to the linear growth (Figures 4.14, 4.15 and 4.16). The growth rates of *P. acnes* in 2.5 and 5 % (w/v) honey solutions were lower than the positive control and the final bacterial growth were about 60 % and 30 % of the maximum bacterial growth of the positive control towards the end of the incubation periods. By increasing the honey concentration, the bacterial growth curves were almost horizontal at 7.5 % (w/v) and above. The total bacterial growth in this honey solution was about 10 % (w/v) of the maximum bacterial growth in the positive control. Further, there was no bacterial growth during the assay for both Manuka honey samples at 10 % and 12.5 % (w/v).

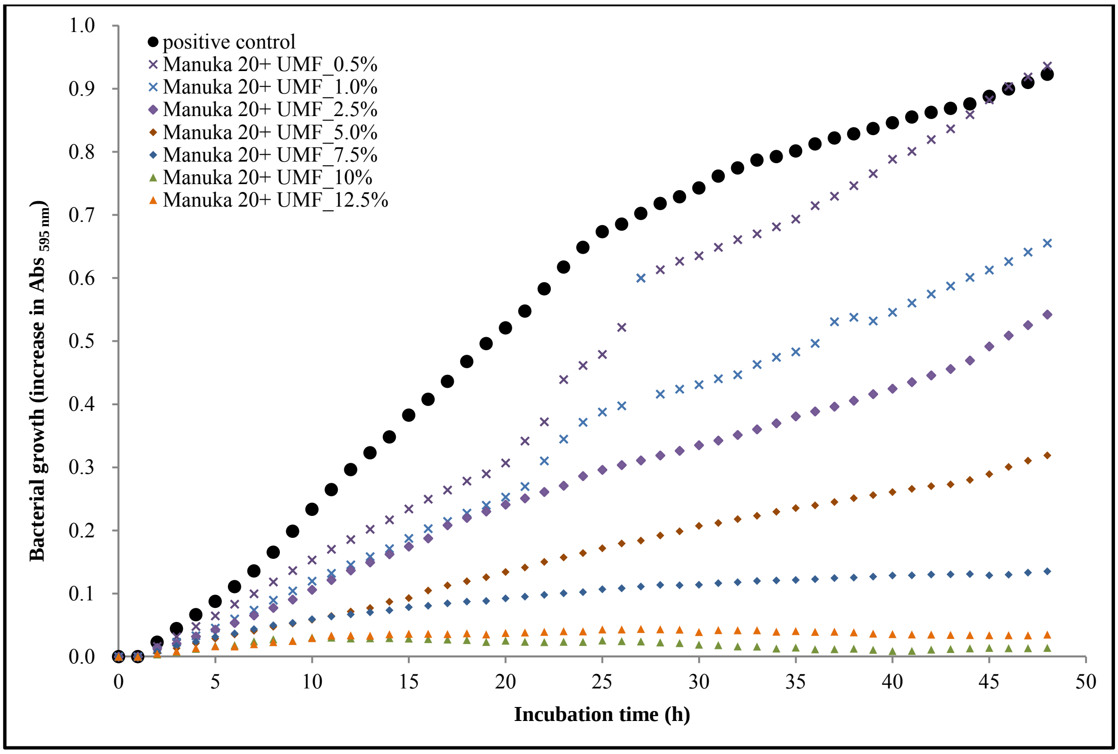


Figure 4.14 Growth (anaerobic) of *P. acnes* in various concentrations of Manuka honey 20+ UMF samples at 37 °C for 48 h

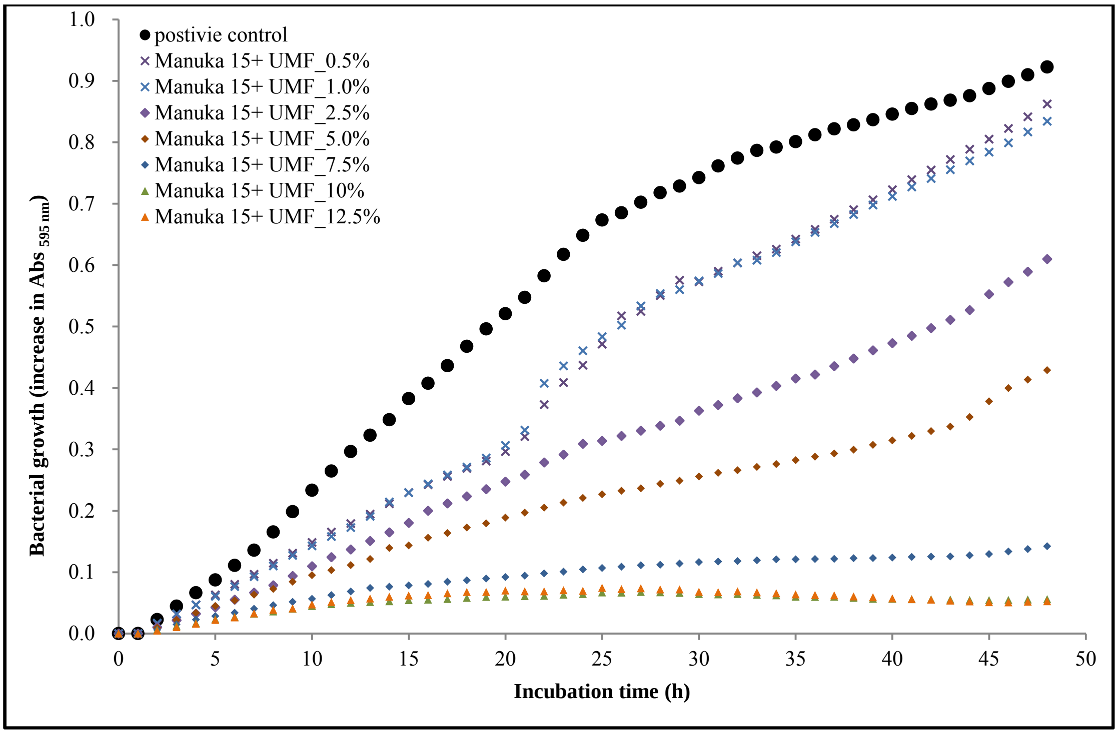


Figure 4.15 Growth (anaerobic) of *P. acnes* in various concentrations of Manuka honey 15+ UMF samples at 37 °C for 48 h

4.2.3.2 Growth (anaerobic) of *P. acnes* in Manuka honey 10+ UMF samples

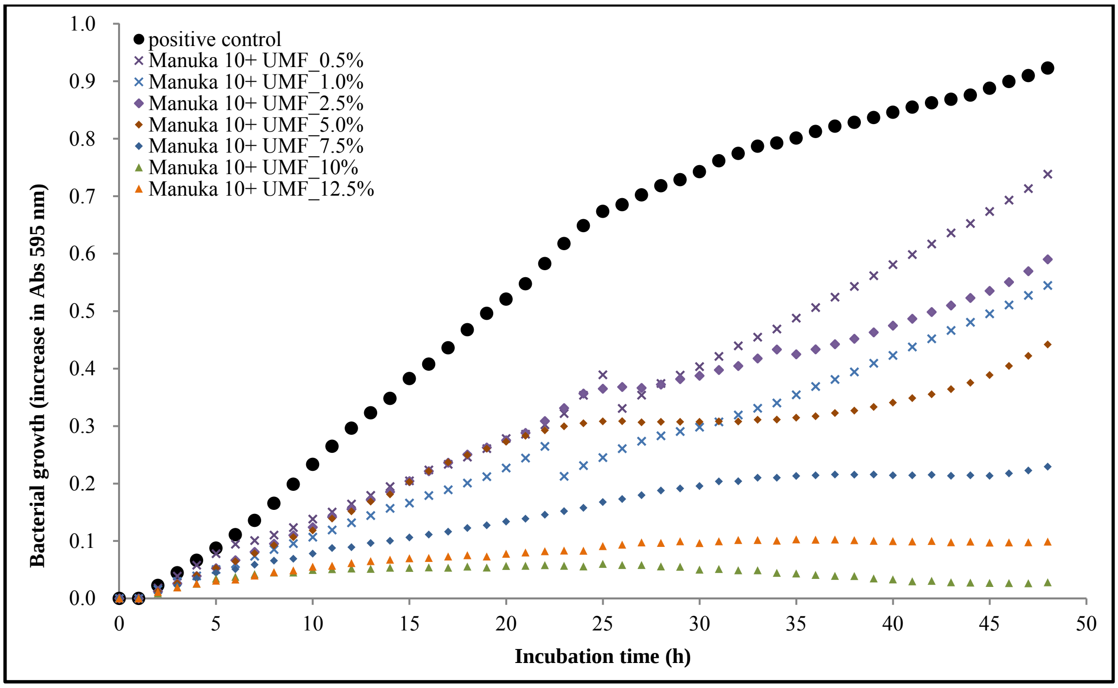


Figure 4.16 Growth (anaerobic) of *P. acnes* in various concentrations of Manuka honey 10+ UMF samples at 37 °C for 48 h

Figure 4.16 indicates that Manuka honey 10+ UMF samples showed inhibitory activity at honey concentrations ranging from 0.5 to 12.5 % under anaerobic environment. The bacterial growth patterns were similar in the honey solutions ranging from 0.5 to 5 % (w/v) up to 24 h of incubation. The bacterial growth increased with different rates which caused the curves to separate from each other. Bacterial growth was the highest in the 0.5 % (w/v) honey solution, which was about 90 % of the maximum bacterial growth of the positive control; bacterial growth was the lowest in the 5 % honey solution, which gave about 50 % of the maximum bacterial growth; the other two honey concentrations (1 % and 2.5 %, w/v) had similar bacterial growth between them, which were about 60 % maximum bacterial growth of the positive control. Inhibitory effect of Manuka honey 10+ UMF samples clearly increased in the honey solutions with concentrations ranging from 7.5 % to 12.5 %. The bacterial growth increased slowly in the first 5 h, followed by no growth until the end of incubation, especially for the 12.5 % (w/v) honey solution under anaerobic environment.

4.2.3.3 Growth (anaerobic) of *P. acnes* in Kanuka honey

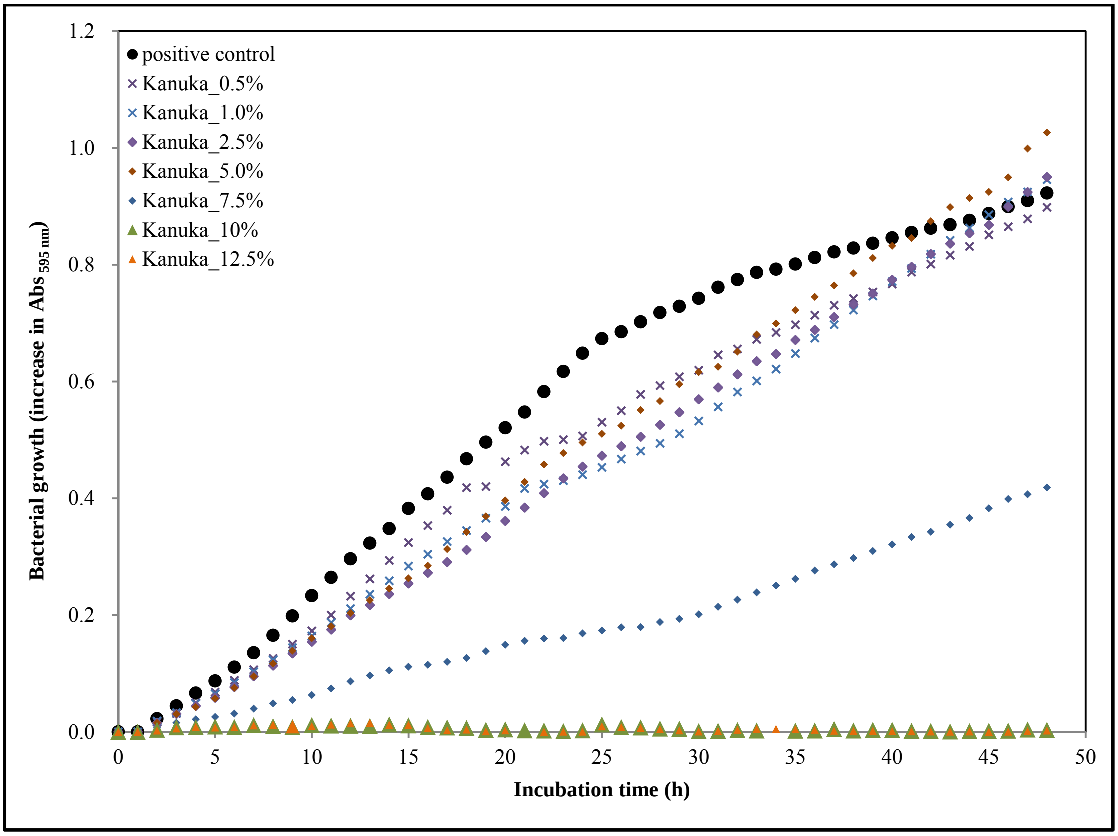


Figure 4.17 Growth (anaerobic) of *P. acnes* in various concentrations of Kanuka honey samples at 37 °C for 48 h

Figure 4.17 shows that Kanuka honey has antibacterial activity at 7.5 % (w/v) and above. From 0.5 - 5 % honey samples (w/v), the bacterial growth curves were similar to the positive control; antibacterial activities were therefore not observed. In the 7.5 % honey solution, the growth rate of *P. acnes* was below the positive control curves and the total bacterial growth of *P. acnes* was about 50 % of the maximum growth of positive control at the end of incubation. There was no bacterial growth in 10 % and 12.5 % (w/v) honey solutions throughout the assay.

4.2.4 Growth (anaerobic) kinetics of *P. acnes* in the Manuka and Kanuka honey samples with catalase

4.2.4.1 Growth (anaerobic) of *P. acnes* in Manuka honey 20+ UMF with catalase samples

Figure 4.18 shows that Manuka honey 20+UMF samples had antibacterial activity against the growth of *P. acnes* at 0.5 % (w/v) and above, even with the addition of catalase. Manuka honey 20+ UMF samples ranging from 0.5 % to 2.5 % (w/v) could only partially inhibit the growth of the *P. acnes* throughout the assay. The bacterial growth curves were lower than the positive control curves during the initial 30 to 40 h of incubation. Manuka honey 20+ UMF at 5 % and 7.5 % (w/v) showed marked reductions in the growth of *P. acnes*, since the bacterial growth curves were well below the positive control. Towards the end of incubation, the final Abs_{595nm} in 5 % and 7.5 % (w/v) honey solution were about 70 % and 30 % of the maximum bacterial growth (Abs_{595nm}) at the end of incubation (T = 48 h). Similarly, in 10 % and 12.5 % honey samples (w/v), the bacterial growth was very close to the x-axes and inhibition of the bacterial growth was nearly 100 %.

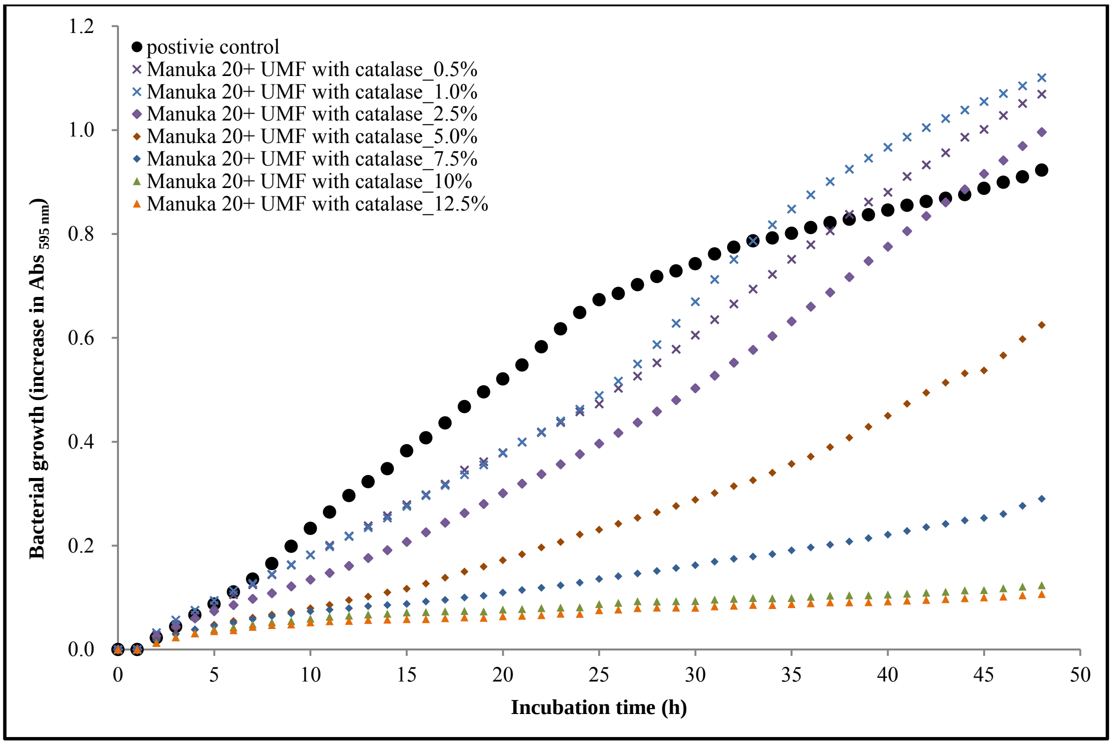


Figure 4.18 Growth (anaerobic) of *P. acnes* in various concentrations of Manuka honey 20+ UMF with catalase at 37 °C for 48 h

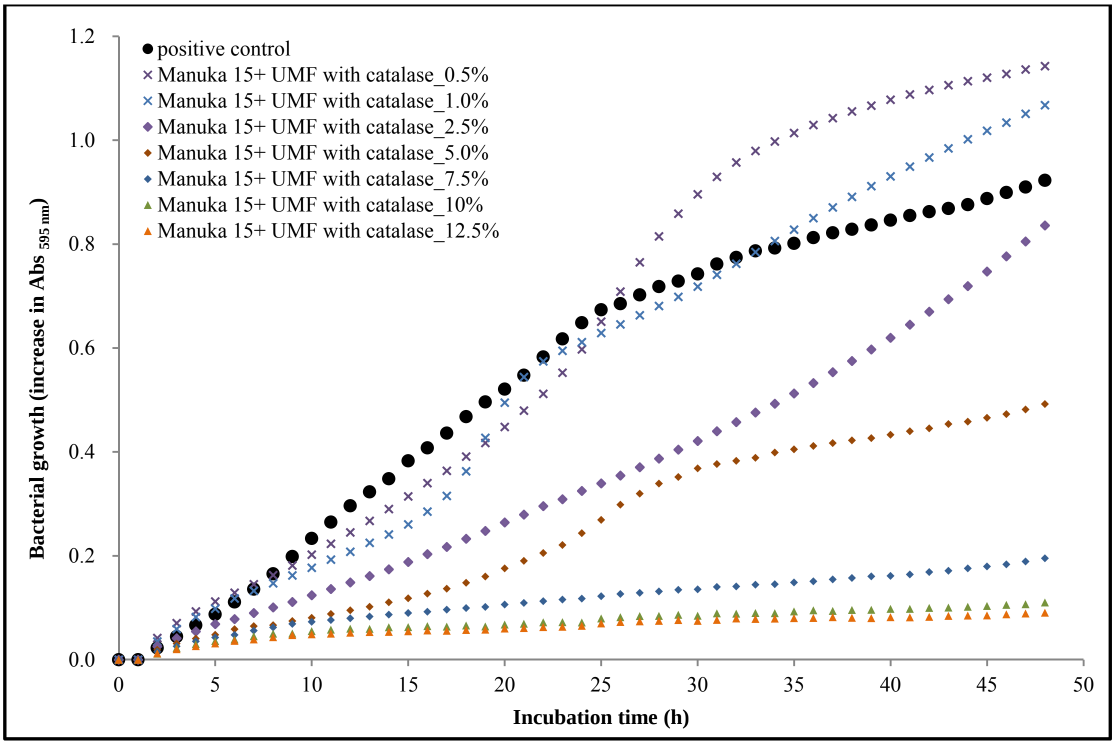


Figure 4.19 Growth (anaerobic) of *P. acnes* in various concentrations of Manuka honey 15+ UMF samples with catalase at 37 °C for 48 h

4.2.4.2 Growth (anaerobic) of *P. acnes* in Manuka honey 15+ UMF samples with catalase

Manuka honey 15 + UMF exhibited antibacterial activity with the addition of catalase under anaerobic environment at honey concentrations of 2.5 % (w/v) and above (Figure 4.19). In 0.5 and 1 % (w/v) honey samples, there were no inhibitory effects against the growth of *P. acnes*; the bacterial growth curves were initially close to the positive control curves. However, after 30 h of incubation, the growth curves of *P. acnes* in the honey solutions shifted and elevated above the positive control. Figure 4.19 shows that at 2.5 % (w/v), the inhibitory activity of Manuka honey 15 + UMF had increased. Based on the growth pattern, the inhibitory ability of honey had become weaker towards the end of incubation.

Figure 4.19 also shows that the antibacterial activity of the honey was enhanced by the 5 % (w/v) honey solution; the lag phase of the bacterial growth increased to about 20 h. The growth rate of *P. acnes* increased between 20 and 30 h; the growth curve entered the stationary phase at 30 h until the end of incubation, during which the bacterial growth was about 50 % of the bacterial growth of the positive control. In the 7.5 % honey sample (w/v) and above, there was no clear bacterial growth throughout the entire incubation period (Figure 4.19). The bacterial growth values (Abs_{595nm}) were around 10 % of the maximum bacterial growth (Abs_{595nm}) of the positive control.

4.2.4.3 Growth (anaerobic) of *P. acnes* in Manuka honey 10+ UMF samples with catalase

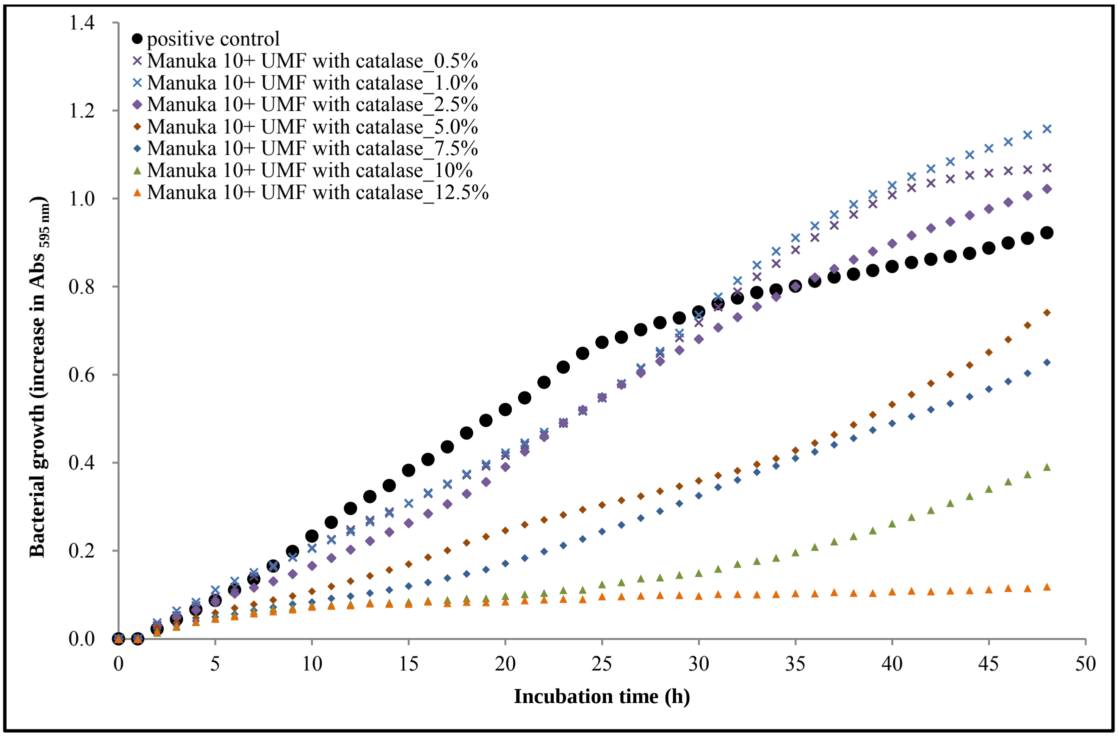


Figure 4.20 Growth (anaerobic) of *P. acnes* in various concentrations of Manuka honey 10+ UMF with catalase at 37 °C for 48 h

Figure 4.20 shows that the growth of *P. acnes* was inhibited by Manuka honey 10+ UMF with catalase at 5 % (w/v) and above under anaerobic environment. No inhibitory activity against the growth of *P. acnes* was observed in honey concentrations ranging from 0.5 % to 2.5 % (w/v). At honey concentrations of 5 % and 7.5 %, the antibacterial activities of Manuka honey were markedly increased; the bacterial growth curves were lower than the positive control with final Abs_{595nm} values (48 h) of 80 % and 70 % of the maximum growth of the positive control. For 10 % honey solution, no bacterial growth was observed up to 30 h and the final Abs_{595nm} value (48 h) was about 40 % of the maximum bacterial growth (Abs_{595nm}) of the positive control. Meanwhile, 12.5 % (w/v) Manuka honey 10+ UMF with catalase could efficiently inhibit the growth of *P. acnes* and no bacterial growth was observed during 48 h of incubation under anaerobic environment.

4.2.4.3 Growth (anaerobic) of *P. acnes* in Kanuka honey samples with catalase

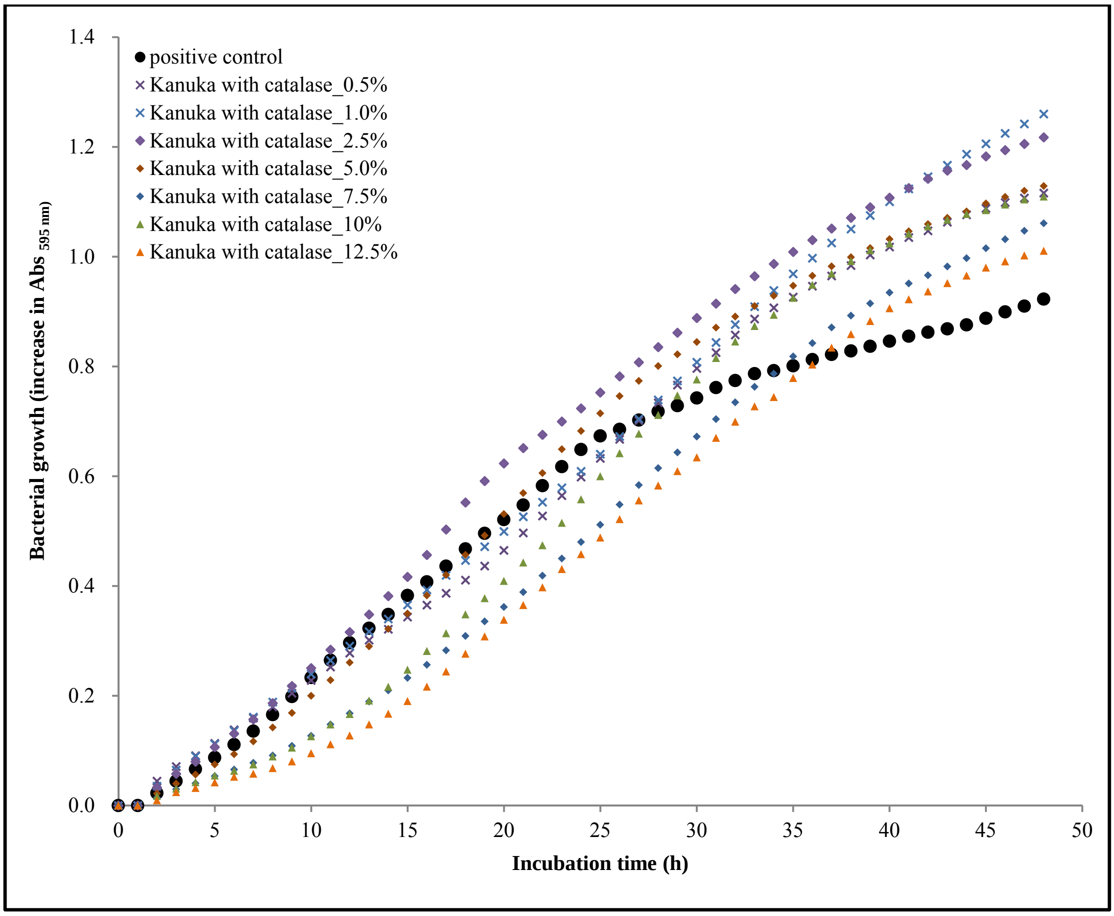


Figure 4.21 Growth (anaerobic) of *P. acnes* in various concentrations of Kanuka honey samples with catalase at 37 °C for 48 h

Figure 4.21 shows that, with the addition of catalase, Kanuka honey did not exhibit antibacterial activity against *P. acnes* under anaerobic environment. The bacterial growth curves were very close to the positive control and there was no significant difference ($p > 0.05$) between the growth of *P. acnes* in the Kanuka honey samples and the positive control.

4.2.5 Growth of *P. acnes* in the artificial honey solution

The *P. acnes* was capable of growing in the artificial honey solutions ranging from 7.5-12.5 % (w/v) with or without catalase under both aerobic and anaerobic environment. The bacterial growth patterns in the honey solutions with or without catalase were close to the positive control (Figure 4.22 - 4.25).

Generally, the rates of bacterial growth were slower than the positive control in the artificial honey solutions. No significant differences ($p > 0.05$) in bacterial growth were observed between 7.5 and 10 % artificial honey solutions and positive growth. The bacterial growth in the 12.5 % artificial honey solution was however different from the positive control during incubation under both aerobic and anaerobic environment. This indicated that artificial honey at 12.5 % (w/v) had antibacterial activity against the growth of *P. acnes.*

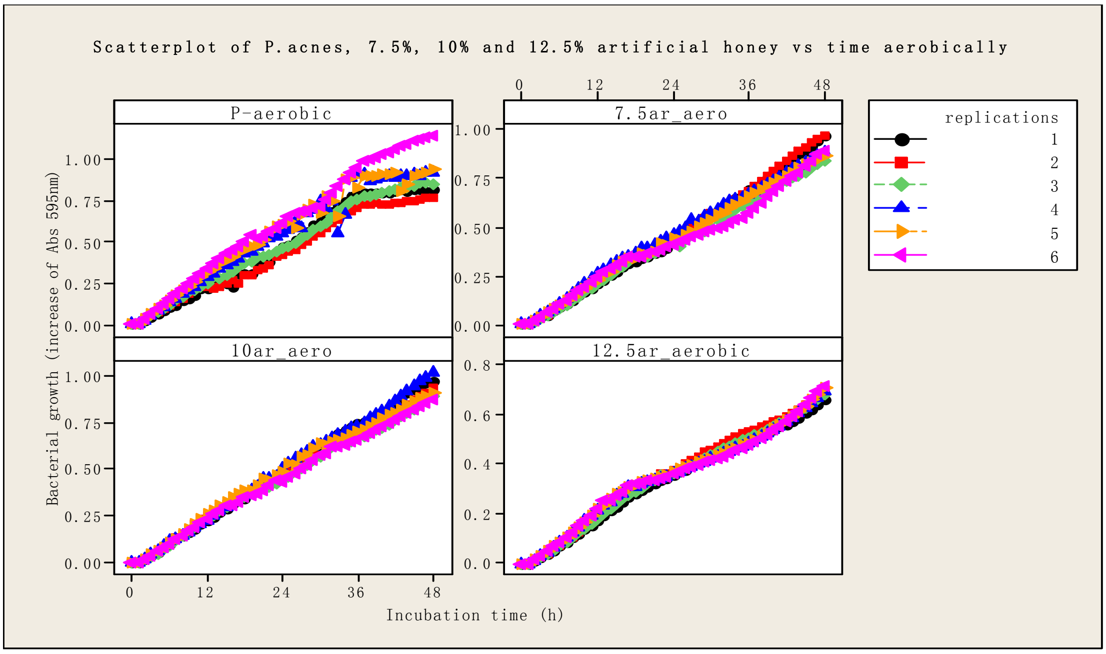


Figure 4.22 Growth of *P. acnes* in various concentrations of artificial honey under aerobic environment at 37 °C for 48 h. Each experiment was done in triplicate and on at least two separate occasions. P-aerobic: positive control under aerobic environment; 7.5ar-aero: 7.5% artificial honey, 10ar_aero: 10% artificial honey, 12.5ar_aero: 12.5% artificial honey

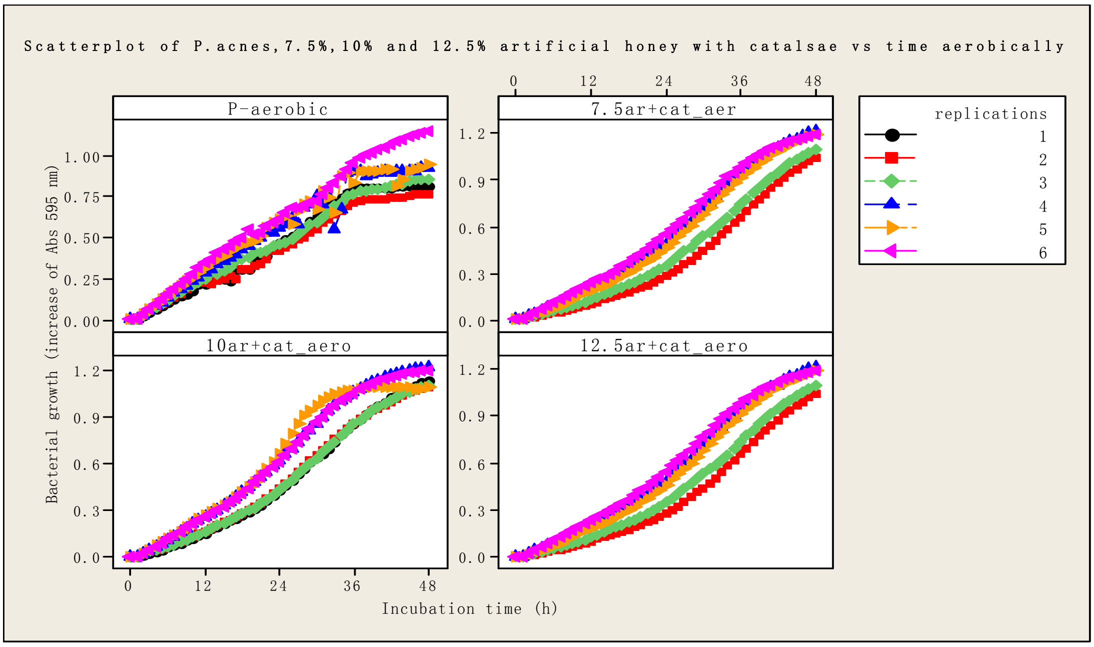


Figure 4.23 Growth of *P. acnes* in various concentrations of artificial honey with catalase under aerobic environment at 37 °C for 48 h. Each experiment was done in triplicate and on at least two separate occasions. P-aerobic: positive control under aerobic environment; 7.5ar-aero: 7.5% artificial honey, 10ar_aero: 10% artificial honey, 12.5ar_aero: 12.5% artificial honey

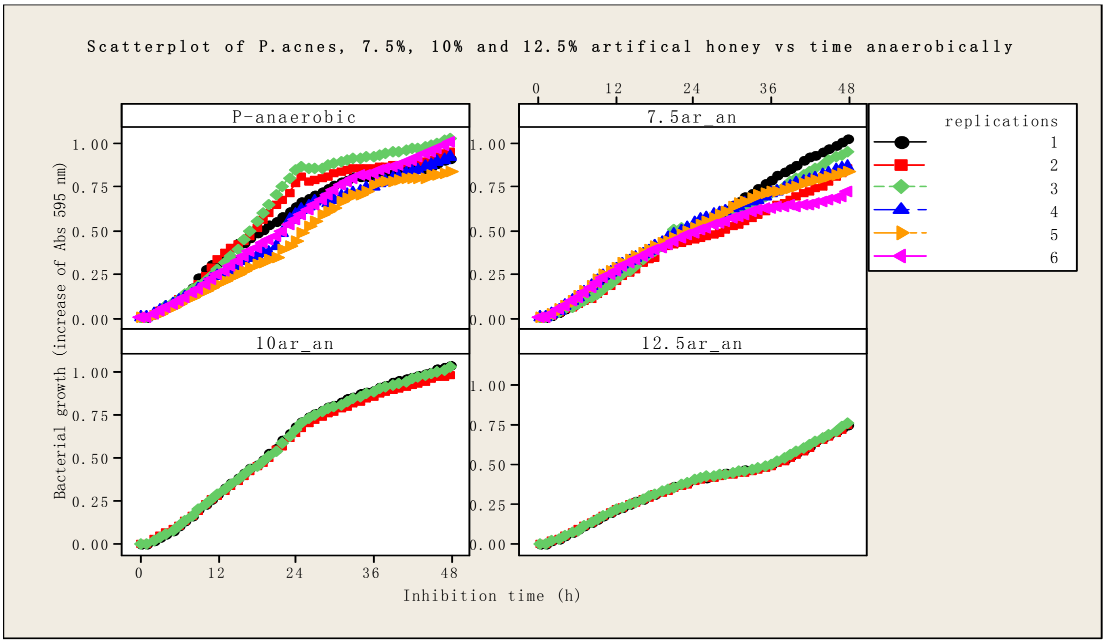


Figure 4.24 Growth of *P. acnes* in various concentrations of artificial honey under anaerobic environment at 37 °C for 48 h. Each experiment was done in triplicate and on at least two separate occasions. P-anaerobic: positive control under anaerobic environment, 7.5ar-aero: 7.5% artificial honey, 10ar_aero: 10% artificial honey, 12.5ar_aero: 12.5% artificial honey

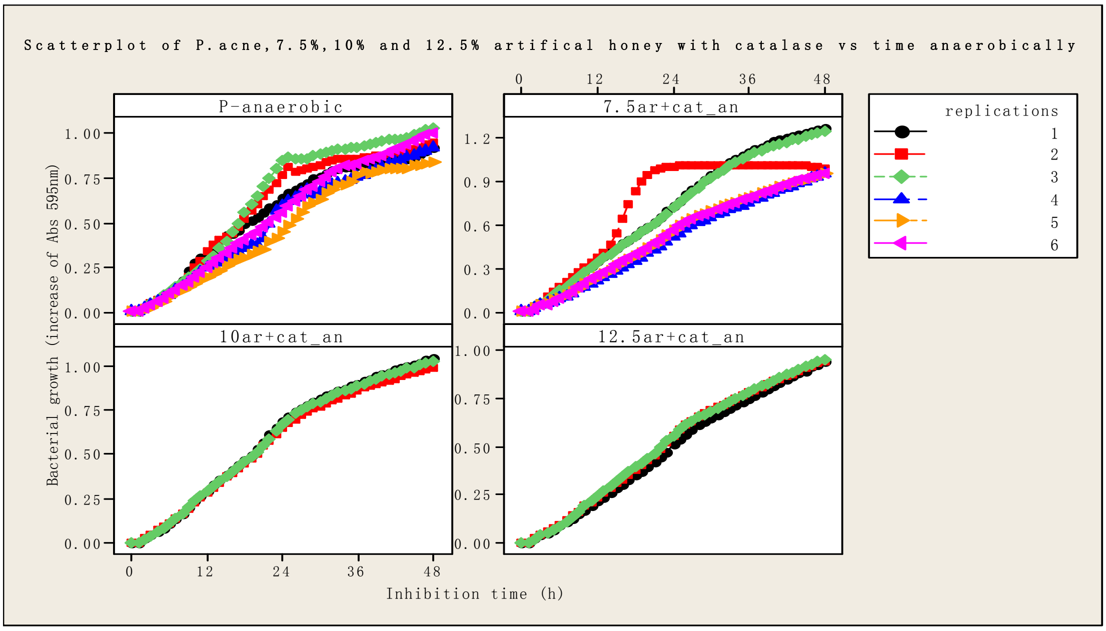


Figure 4.25 Growth of *P. acnes* in various concentrations of artificial honey under anaerobic environment. Each experiment was done in triplicate and on at least two separate occasions at 37 °C for 48 h. P-anaerobic: positive control under anaerobic environment, 7.5ar-aero: 7.5% artificial honey, 10ar_aero: 10% artificial honey, 12.5ar_aero: 12.5% artificial honey

4.2.6 Dose-response curves of *P. acnes* in Manuka and Kanuka honey samples

Dose-response curves for the antibacterial activity of Manuka honey samples against *P. acnes* under different environmental conditions (Figure 4.26 to 4.29) showed the inhibitory activities of three Manuka honey samples against the growth of *P. acnes.* The percentage of inhibition showed positive correlations with honey concentrations ($p \leq 0.05$), suggesting that higher concentrations of honey samples would lead to higher percentages of inhibition against the growth of *P. acnes*. For Manuka honey 15+ and 20+ UMF samples (Figures 4.26 to 4.29), the percentages of inhibitions increased exponentially with increase in honey concentrations, with the exception of Figure 4.27, which showed a linear relationship between two parameters.

In the case of the antibacterial activity of Manuka 10+ UMF against *P. acnes*, dose-response curves (Figure 4.26 to 4.29) show that the percentage of inhibition had a linear relationship with the honey concentrations. With the exception of Manuka honey 10+ UMF with catalase under anaerobic environment (Figure 4.29), it was observed that Manuka 10+ UMF sample had a weak antibacterial activity compared to Manuka honey 15+ and 20+, even at the highest test concentration (12.5 %).

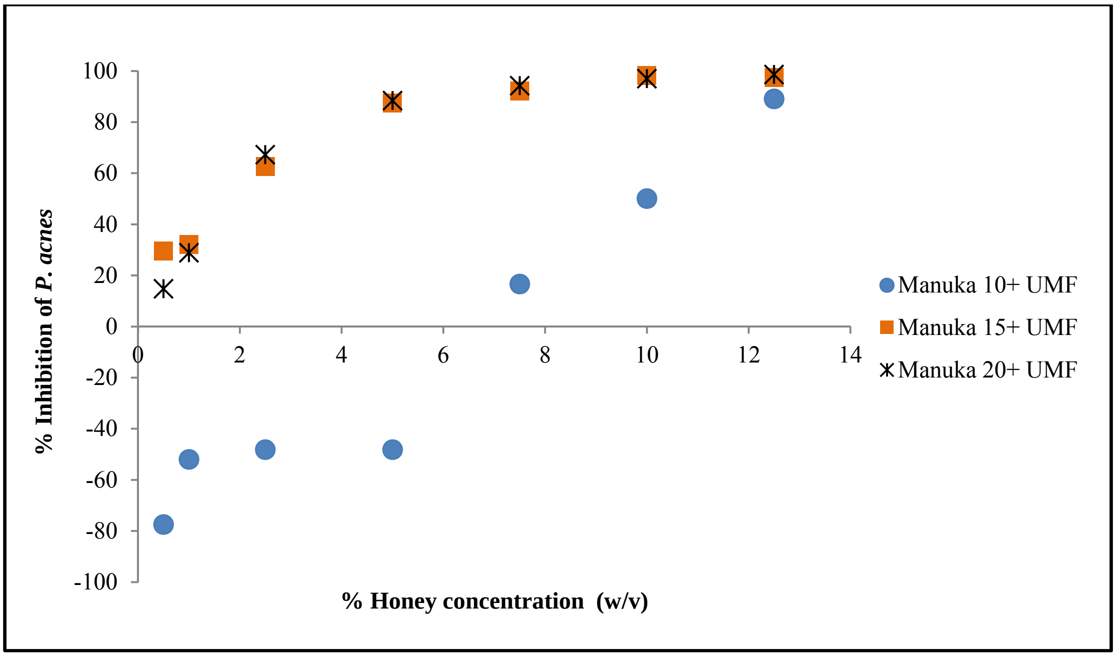


Figure 4.26 Dose-response curves for the antibacterial activity of Manuka honey against *P. acnes* under aerobic environment at 37 °C. The activity is expressed as percentage inhibition of growth of the culture, with inhibition being determined by comparison to the control after 24 h incubation

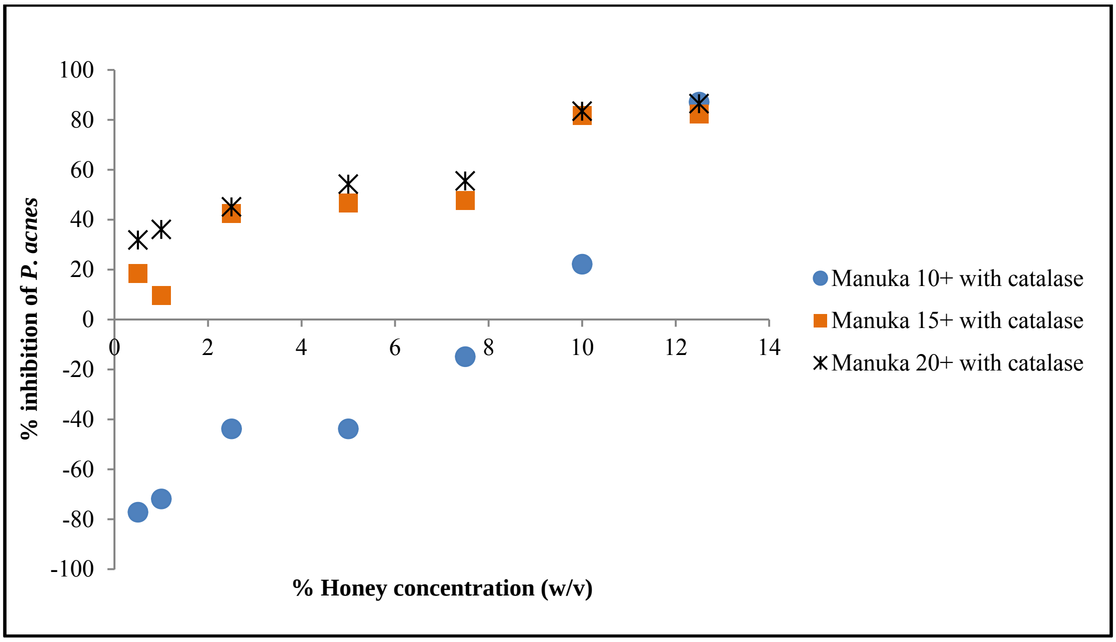


Figure 4.27 Dose-response curves for the antibacterial activity of Manuka honey with catalase against *P. acnes* under aerobic environment at 37 °C. The activity is expressed as the percentage inhibition of growth of the culture, with inhibition being determined by comparison to the control during 24 h of incubation

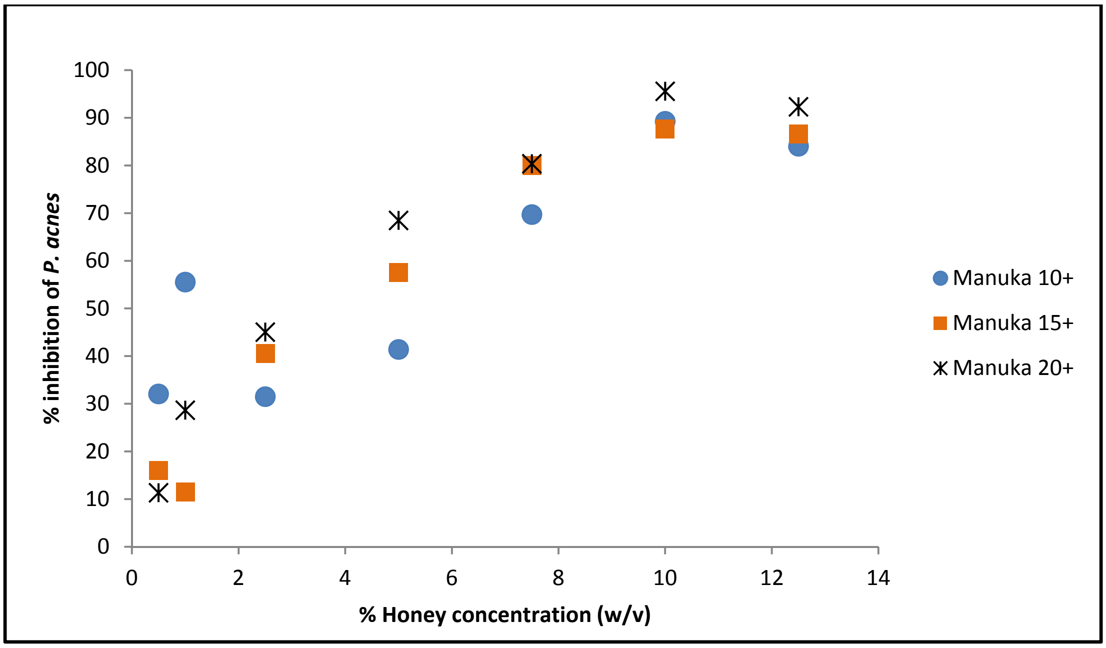


Figure 4.28 Dose-response curves for the antibacterial activity of Manuka honey against *P. acnes* under anaerobic environment at 37 °C. The activity is expressed as the percentage inhibition of growth of the culture, with inhibition being determined by comparison to the control during 24 h of incubation

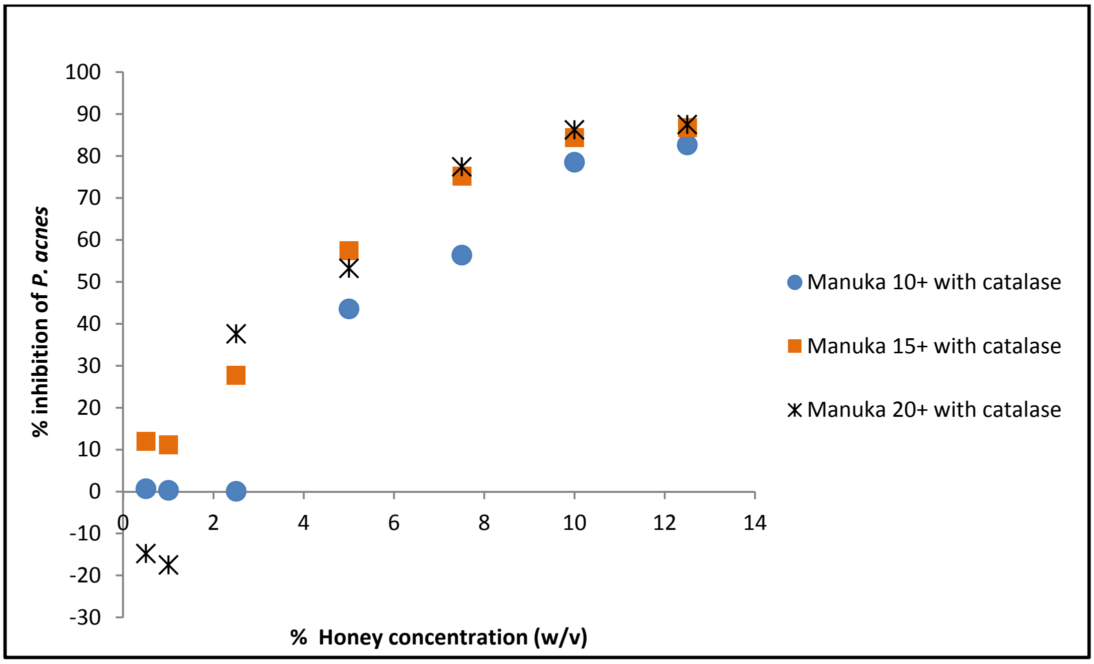


Figure 4.29 Dose-response curves for the antibacterial activity of Manuka honey with catalase against *P. acnes* under anaerobic environment at 37 °C. The activity is expressed as the percentage inhibition of growth of the culture, with inhibition being determined by comparison to the control during 24 h of incubation

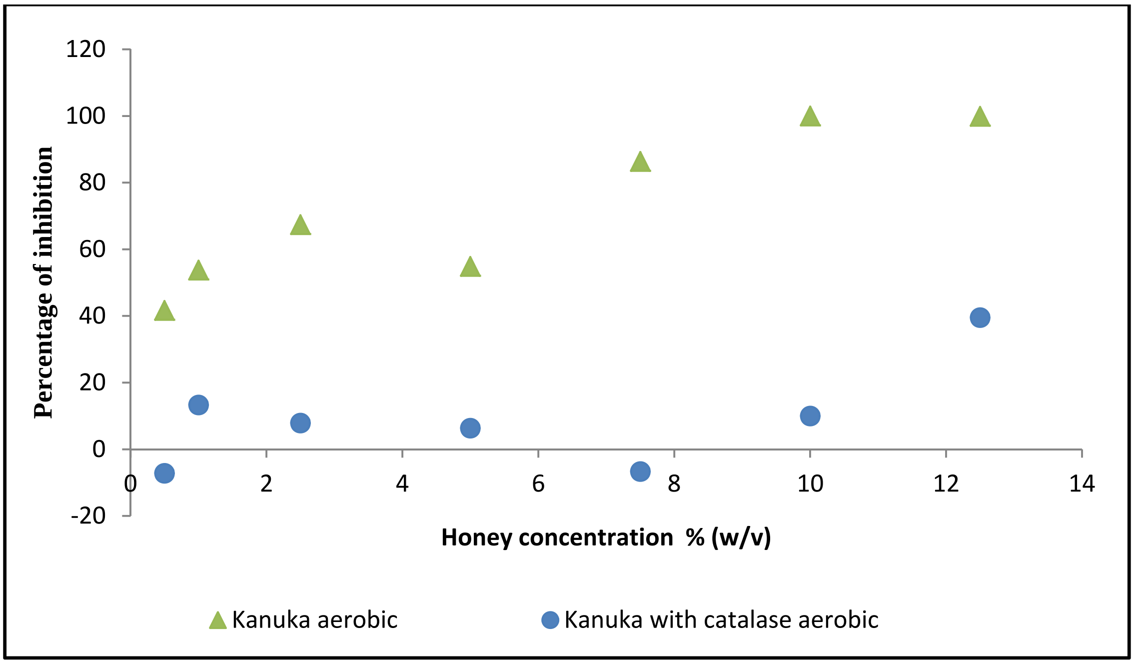


Figure 4.30 Dose-response curves for the antibacterial activity of Kanuka honey with and without catalase against *P. acnes* under aerobic environment. The activity is expressed as the percentage inhibition of growth of the culture, with inhibition being determined by comparison to control well during 24 h of incubation

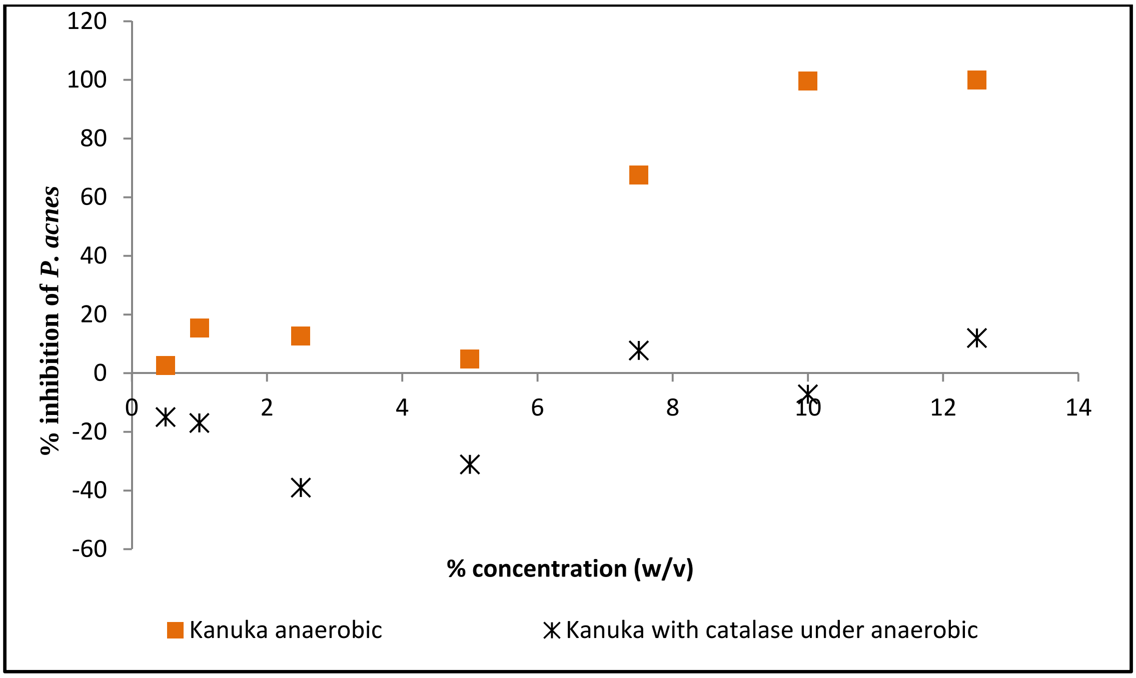


Figure 4.31 Dose-response curves for the antibacterial activity of Kanuka honey with and without catalase against *P. acnes* under aerobic environment at 37 °C. The activity is expressed as the percentage inhibition of growth of the culture, with inhibition being determined by comparison to the control during 24 h of incubation

Kanuka honey has strong antibacterial activity against the growth of *P. acnes* without catalase under both aerobic and anaerobic environment (Figure 4.30 and 4.31). About 40 % inhibition of *P. acnes* was observed at 0.5 % Kanuka honey under aerobic environment; however, under an anaerobic environment there was no antibacterial activity against the bacterial growth. With the addition of catalase in the Kanuka honey samples; there was weak inhibition against the growth of *P. acnes* under aerobic and anaerobic environment. However, there was a week correlation between the Kanuka honey concentration and the percentage of inhibition against the growth of *P. acnes* due to the horizontal linear trend lines (Figure 4.30).

4.2.7 Determination of the MICs of Manuka and Kanuka honey samples

Does-dependent curves showed that the growth of *P. acnes* and the percentages of inhibition were positively correlated with the honey (Manuka and Kanuka) concentrations (section 4.2.6). The relationship between the percentage of inhibition and honey concentration was analysed by regression correlation equations using Minitab version 15 (Minitab Inc, Pennsylvania State University, USA). Based on the statistical equations, the MICs of honey were determined and expressed in three commonly used terms of MICs: MIC_{50}, MIC_{90} and MIC_{100},referring to the minimal inhibition concentrations required to obtain 50 %, 90 % and 100 % inhibition of the bacterial growth. The MICs values are presented in Table 4.1 and Figure 4.32.

Table 4.1 The MICs of three Manuka honey samples and Kanuka honey

Honey samples	**MIC_{50} (mg/mL)**	**MIC_{90} (mg/mL)**	**MIC_{100} (mg/mL)**
Manuka 20	30.14	44.18	148.90
Manuka 20_cat	81.89	142.92	156.69
Manuka 20*	26.78	103.35	112.59
Manuka 20_cat*	30.88	123.31	133.31
Manuka 15	28.57	68.49	125.81
Manuka 15_cat	61.92	155.34	178.33
Manuka 15*	14.65	83.00	116.37
Manuka 15_cat*	28.12	120.46	145.74
Manuka 10	118.77	139.29	144.43
Manuka 10_cat	120.08	129.07	131.13
Manuka 10*	21.60	119.93	144.50
Manuka 10_cat*	47.55	132.43	198.34
Kanuka	21.49	58.15	123.28
Kanuka _cat	285.08	496.38	549.21
Kanuka *	50.69	105.86	119.66
Kanuka _cat*	285.07	496.38	549.20

* Experiments were conducted under anaerobic environment; otherwise, it was under aerobic environment; _cat refers to honey sample-containing catalase, otherwise, honey samples contained no catalase

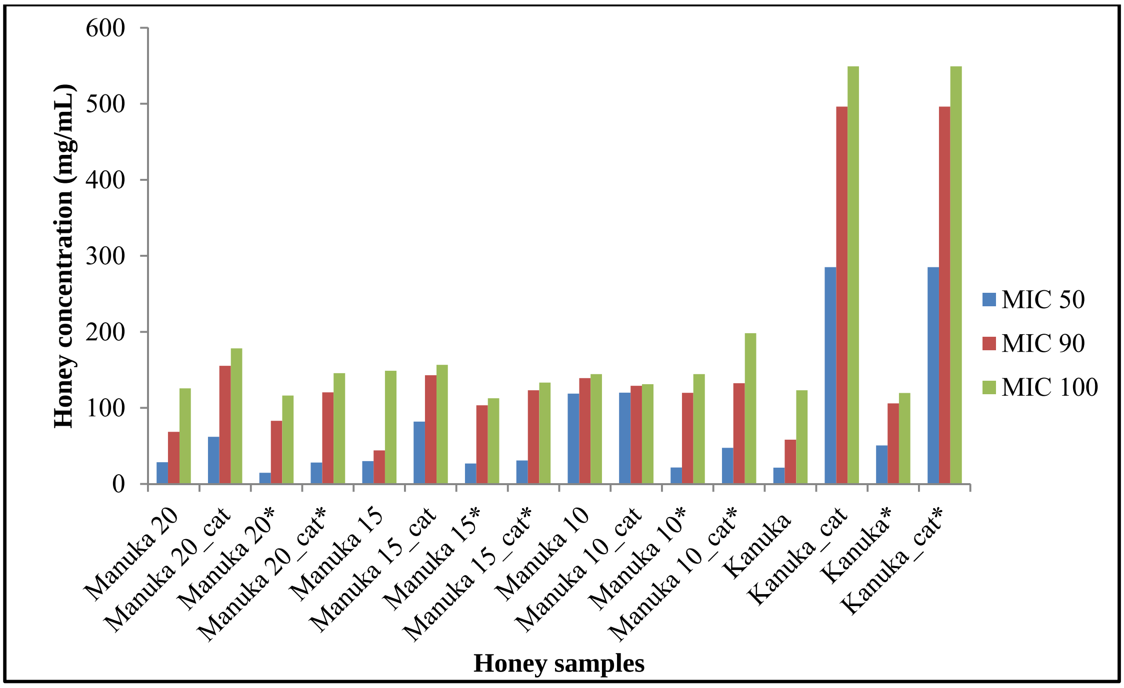


Figure 4.32 Minimal inhibition concentration (MIC) of Manuka and Kanuka honey samples against the growth of *P. acnes*

Table 4.1 and Figure 4.32 clearly show that Manuka honey samples (Manuka 10+, 15+ and 20+ UMF) had strong antibacterial activities with MIC_{50} of 14.65 to 118.77 mg/mL, MIC_{90} of 44.18 to 155.34 mg/mL and MIC_{100} of 116.37 to 198.34 mg/mL. In general, MICs values of Manuka honey 20+ UMF samples were similar to MICs values of Manuka 15+ UMF. Both of these MICs values were lower than the MICs of Manuka 10+ UMF with or without catalase in the honey samples under aerobic and anaerobic environment. For Manuka honey, the MICs values of honey without catalase were on average lower than the MICs of Manuka honey with catalase; the two treatments (with and without catalase) were not significantly different ($p > 0.05$).

Meanwhile, Kanuka honey samples distinctly exhibited strong antibacterial activity with MIC_{50} of 21.49 mg/mL, MIC_{90} of 58.15 mg/mL and MIC_{100} of 123.28 mg/mL (Table 4.1), suggesting that Kanuka had comparable antibacterial activity to Manuka 20+ and 15+ UMF under both incubation environments. However, the addition of catalase in the Kanuka honey solution increased the MIC values by nearly 10 times (MIC_{50} of 285.07 mg/mL, MIC_{90} of 496.38 mg/mL and MIC_{100} of 549.20 mg/mL). This clearly showed that the antibacterial activity of the Kanuka honey was linked to the peroxide activity of the honey.

4.3 Screening natural bioactives

4.3.1 Disc diffusion method

A preliminary screening of the *in vitro* antimicrobial activities of five natural bio-active agents was studied against *P. acnes* using the disc diffusion method. Five natural organic bioactives were screened by disc diffusion assays; results were shown as zones of inhibition against the growth of *P. acnes*. The zones of inhibition were measured in mm (12-16 mm, 17-21 mm, 22-30 mm for weak, moderate and highly active inhibitory zones respectively) (Bhovi and Gadafinamath, 2005)

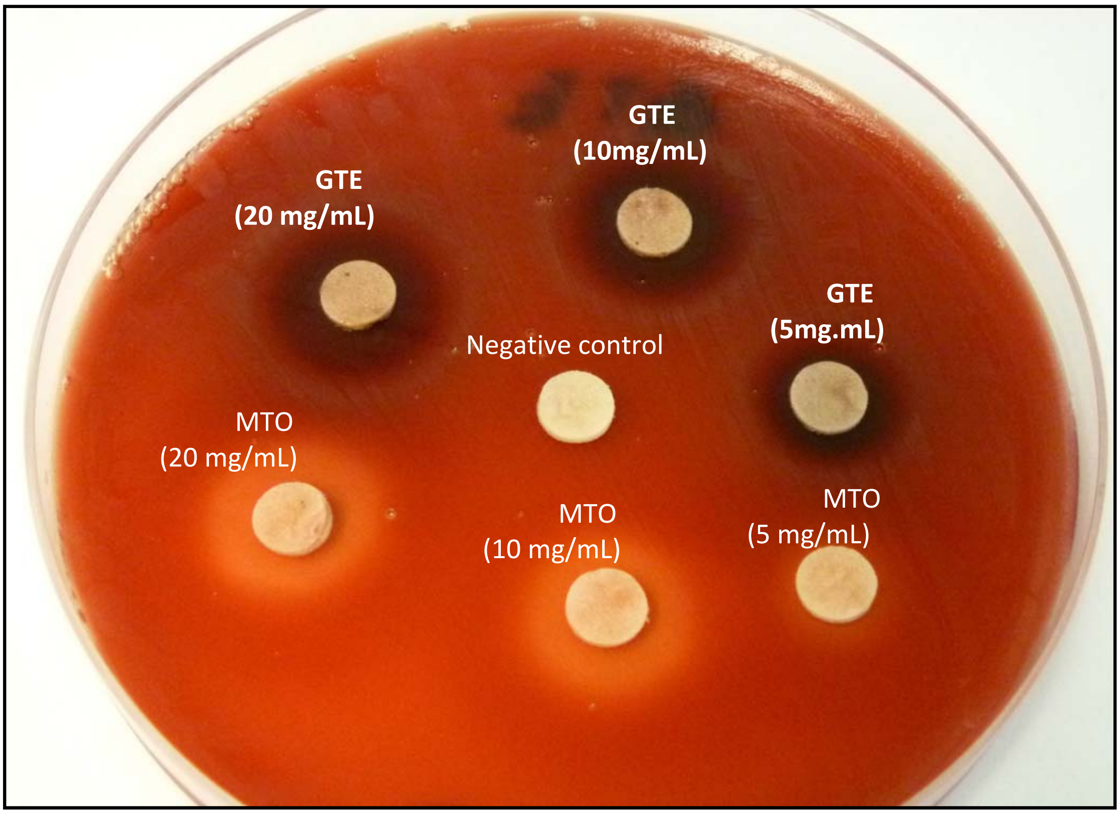


Figure 4.33 Zones of inhibition produced by MTO and GTE at 20, 10, 5 mg/mL. MTO= Manuka tree oil; GTE=green tea extract; (Image captured by a Panasonic Digital Camera, DMC-FP3, China)

Results revealed that green tea extract and Manuka tree oil had moderate inhibitory activities against the growth of *P. acnes*. Green tea extracts (0.1 mg/ disc to 0.4mg/ disc or 5 mg/mL to 20 mg/mL) showed a zone of inhibition of 9.2 mm to 17.5 mm for *P. acnes* (Figure 4.33). Samples of MTO (0.1 mg/ disc to 0.4mg/ disc or 5 mg/mL to 20 mg/mL) produced zones of inhibitions ranging from 6mm - 21.2mm for the bacterium (Figure 4.34).

Further comparisons between MTO and GTE at different concentrations were done by ANOVA and Tukey's test. The inhibition activities of GTE towards *P. acnes* were significantly different ($p \leq 0.05$) among three different testing concentrations. There was a significant difference ($p \leq 0.05$) of the inhibition zones among three MTO concentrations. According to the Tukey's test, the zone of inhibition produced by the highest concentration of MTO (20 mg/mL) was significantly different from the MTO (10 mg/mL) and MTO (5 mg/mL). Meanwhile, there was no sufficient evidence to show any statistical difference ($p > 0.05$) between zones of inhibition for MTO (10 mg/mL) and MTO (5 mg/mL). Furthermore, comparing between MTO and GTE at various concentrations, no evidence was found to suggest that the zones of inhibition produced by MTO was significantly different ($p \leq 0.05$) from the GTE at three concentrations (20 mg/mL, 10 mg/mL and 5 mg/mL). Therefore, it is reasonable to state that the MTO and GTE had similar inhibitory abilities against the growth of *P. acnes*.

Table 4.2 shows that lavender oil; olive leaf extract and propolis produced no clear zones of inhibition around the sample discs. Therefore, no inhibitory activities against the growth of *P. acnes* were detected from lavender oil, olive leaf extract and propolis at the given concentration ranges. In addition, the control plates with DMSO (1 % and 5 %) also showed non-inhibitory activities against *P. acnes*.

Table 4.2 Antimicrobial activity of 2-fold dilutions of the bioactives expressed as diameter of zone of inhibition in millimetres (mm) against *P. acnes*

Samples	Diameter of zone of inhibition (mm) (mg per disc)					
	Undiluted		1:1		1:2	
GTE	17.5±0.9	(0.4)[a]	12.9±1.8	(0.2)[b]	9.2 ± 0.7	(0.1)[c]
LO	ND	(1.8)	ND	(0.89)	ND	(0.44)
MTO	21.2 ± 4.0	(0.4)[a]	12.5 ± 3.0	(0.2)[bc]	6.0± 0.0	(0.1)[c]
OLE	ND	(9.6)	ND	(4.8)	ND	(2.4)
Propolis	ND	(0.048)	ND	(0.024)	ND	(0.012)

Each disc (ϴ = 6 mm) was loaded with 20 uL of bioactive solution at 3 different concentrations. The values are averages of replications ±SE. GTE=green tea extracts; LO=lavender essential oil; MTO=Manuka tree oil. OLE refers to olive leaf extract. [a b c] means of the difference of inhibitory zones by each bioactive dilution were found statistically significant ($p \leq 0.05$).

No zones of inhibition were produced by propolis, OLE and LO, suggesting these three bioactives were either inactive towards *P. acnes* or the disc diffusion method was not the ideal susceptibility testing method to determine the bacterial activity of the materials against the growth of the bacterium.

The potency of the antibacterial activities of MTO and GTE had positive correlations at the level of concentrations used. Pearson’s correlation coefficient between the zone of inhibition and MTO concentration was 0.902 ($p \leq 0.05$) and between the zone of inhibition and GTE was 0.921 ($p \leq 0.05$). Berg and Latin (2008) reported that if Pearson’s correlation coefficient is greater than 0.76, then, the relationship between the variables is highly correlated; hence, the relationship between zone of inhibition and concentration of these bioactives was very high.

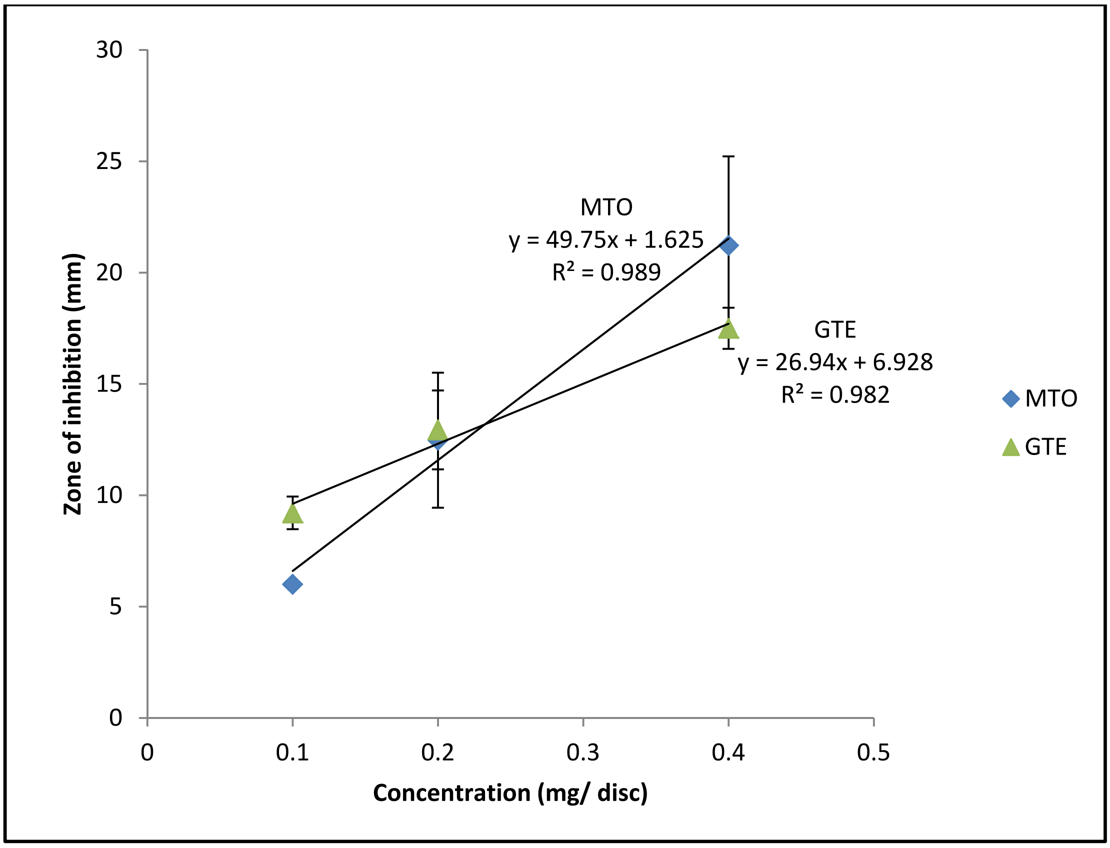


Figure 4.34 Relationship between antibacterial activities of bioactives (Manuka tree oil and Green tea extract) expressed as zones of inhibitions (mm) and testing concentrations. MTO = Manuka tree essential oil; GTE = Green tea extract

Based on the correlation coefficients, a simple linear regression was subsequently used to explain the changes in the zones of inhibitions. Positive linear trends were obtained as shown in Figure 4.34. The rate of increase suggests the inhibitory ability of MTO and GTE against the growth of *P. acnes*; the higher the rates mean that the higher susceptibility of the bioactives is toward the *P. acnes*. Two different rates of increase were observed. The inhibitory rate of MTO was 49.75 mm for any marginal increase of concentration per disc. The rate of MTO was about 1.8 times higher than the rate of GTE (rate =26.94). Comparing MTO and GTE, this difference in inhibition rate of *P. acnes* suggest that this bacterium was more susceptible to MTO than GTE at any concentration.

4.3.2 Inhibition potential of bioactive against *P. acnes* using the spectrophotometer method

The spectrophotometer method using the plate reader can determine the bacteriostatic (MIC) and bactericidal concentration (MBC) of testing materials. In this study, a positive bacterial growth could be observed as clear transparent solution became turbidly. A clear solution after incubation, suggests little to no bacterial growth during incubation. In each experimental trial, a standard positive control of *P. acnes* (without any antibacterial materials or any limiting factors) and a negative control (BHI without addition of *P. acnes*) were set up in parallel with other treatments for each microtiter plate. The turbidity of the testing solutions with *P. acnes* was compared to the positive growth of *P. acnes* and negative control. For example, Figure 4.35 showed that some wells were turbid and others remained transparent after incubation under anaerobic environment.

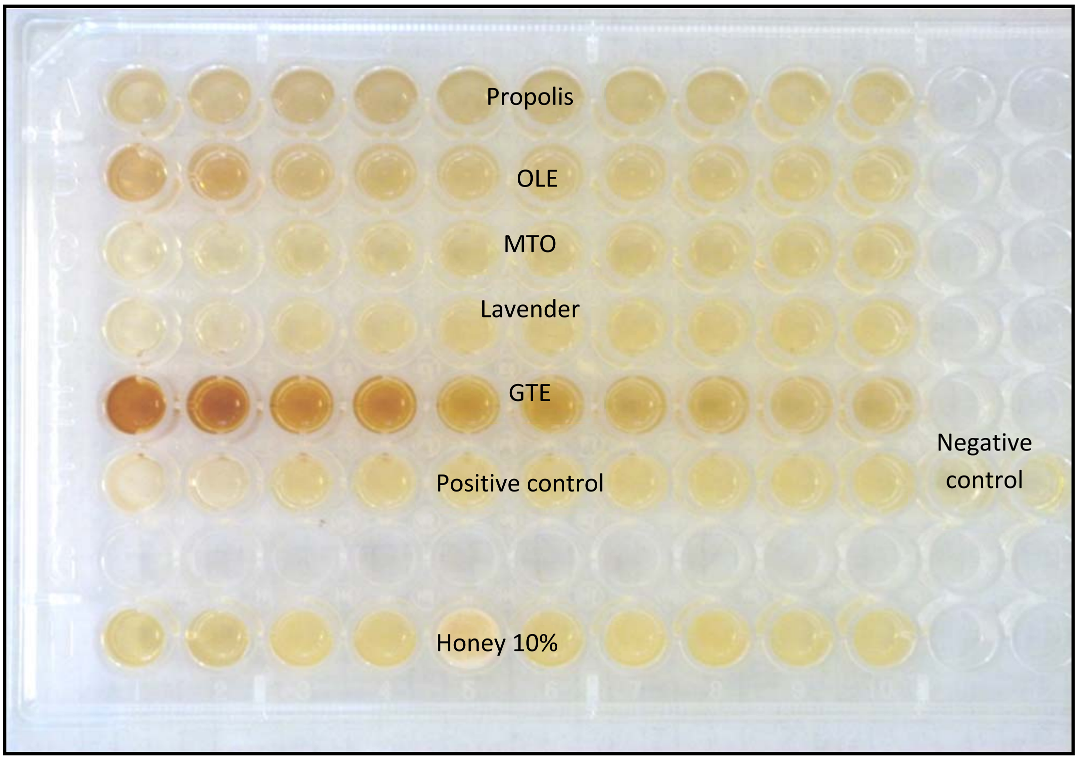


Figure 4.35 Image of microtiter plate with testing materials after 48 h of incubation (Images captured with a Panasonic Digital Camera, DMC-FP3, China).

4.3.2.1 MIC determination by visual observation

The turbidity of each solution was also assessed visually with positive and negative control solutions. Qualitative assessment of bacterial growth in each bioactive treatment was rated on a 0 – 4 (Table 4.3). Preliminary tests showed that the microdilution method was not a suitable susceptibility method for GTE, because the colour of GTE solution was dark with poor transparency and a brown precipitate collected at the bottom of the well after incubation.

Table 4.3 Visual assessment of cell growth (turbidity) in the microtiter plate

Sample	**Stock concentration mg/mL (% w/w)**	**Concentrations (2-fold dilution)**									
		1:1	1:1	1:2	1:2	1:4	1:4	1:8	1:8	1:16	1:16
Propolis	2.4 (0.24)	2	2	2	2	2	2	2	2	3	3
Olive leaf extract	480 (32)	0	0	1	1	2	2	2	2	3	3
Lavender oil	88.5 (10)	2	2	2	2	2	2	3	3	4	4
Manuka tree oil	20 (2)	0	0	1	1	1	1	2	2	2	2
Green tea extract	20 (2)	N.D.								3*	3*
Propolis (control)	1% DMSO and 1 % Tween-20	2	2	2	2	3	3	3	3	4	4
Manuka tree oil (control)	1% DMSO	2	2	2	2	3	3	3	3	4	4
Lavender oil (control)	5% DMSO	2	2	2	2	3	3	3	3	4	4

Note: 0=no growth; 1=light growth (very little turbidity compared to *P. acnes*); 2=medium growth (slightly turbid than *P. acnes*); 3=normal growth (turbidity similar to *P. acnes* broth culture); 4=heavy growth (more turbid than *P. acnes*). N.D. = the growth of *P. acnes* was not observed.

MICs were determined as the lowest concentration of bioactives at which the bacterium was unable to grow in the solution. According to Table 4.3, the scale of 1 means there is little to no change in turbidity compared to the positive growth control. Therefore, based on the visual assessment, MIC of OLE was about 120 mg/mL (8 %, w/v) and MTO was about 2.5 mg/mL (0.25 %, w/v). The propolis OLE and LOW were scaled more than 1 at their highest concentration, hence, the MICs of propolis, OLE and LO could not be obtained using visual assessments.

4.3.2.2 MIC determination using the microtiter plate

The bacterial growth (increase of absorbance at 595 nm) was automatically recorded hourly during incubation in the plate reader at 37 °C for 24 h under aerobic environment. The MIC at 90 % (MIC_{90}) of inhibition was used to express strength of antibacterial potencies. MIC_{90} can be defined as the minimum inhibitory concentration required inhibiting the growth of 90 % of organisms, which was calculated graphically and expressed in mg/mL.

a. Propolis

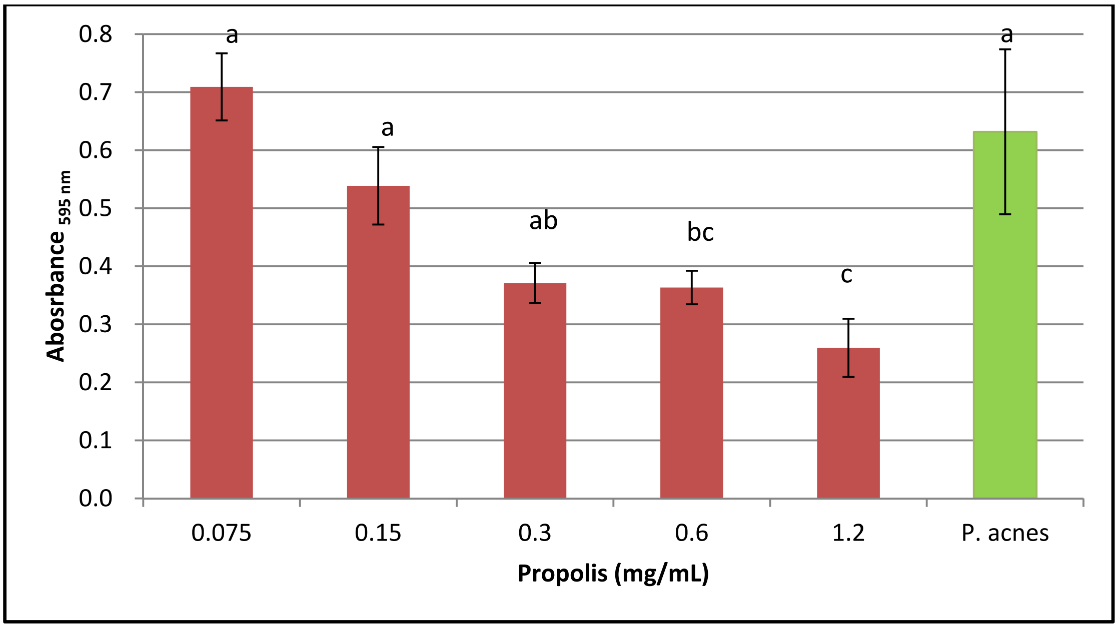


Figure 4.36 Aerobic growth of *P. acnes* in propolis solution when incubated at 37°Cfor 24 h in a plate reader; Bars with the same letters[a-c] were not significantly different ($p > 0.05$). Error bar is the standard error of the mean

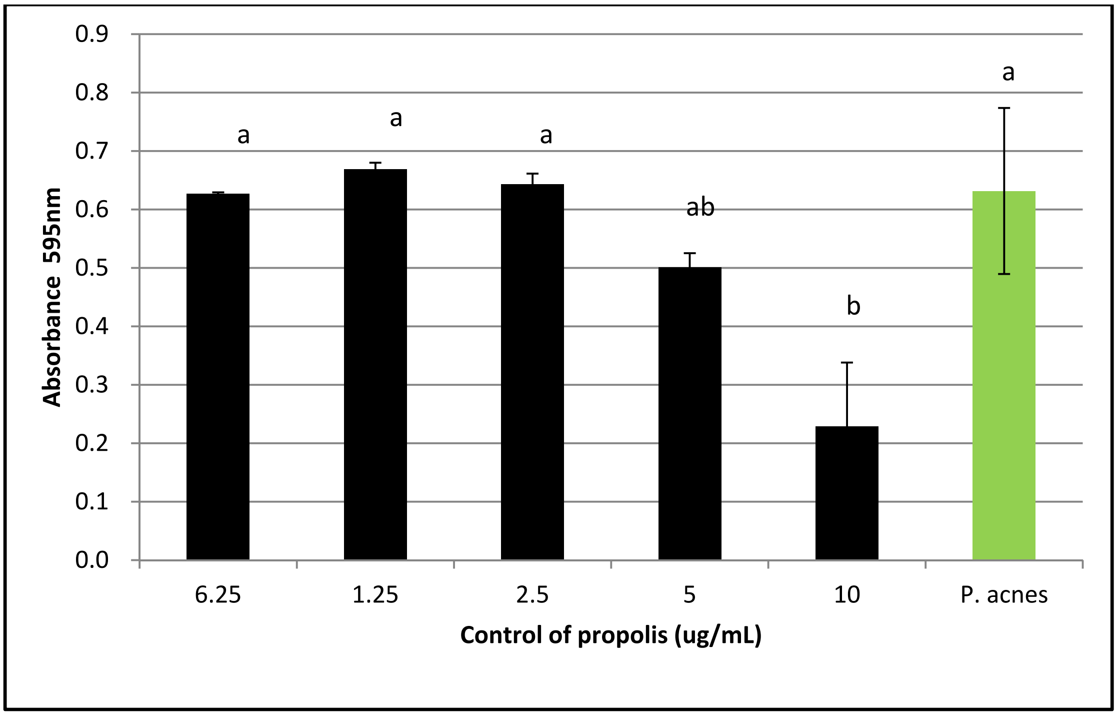


Figure 4.37 Aerobic growth of *P. acnes* in the control solution of propolis when incubated at 37 °C for 24 h in a plate reader; Bars with the same letters[a-c] were not significantly different ($p > 0.05$). Error bar is the standard error of the mean

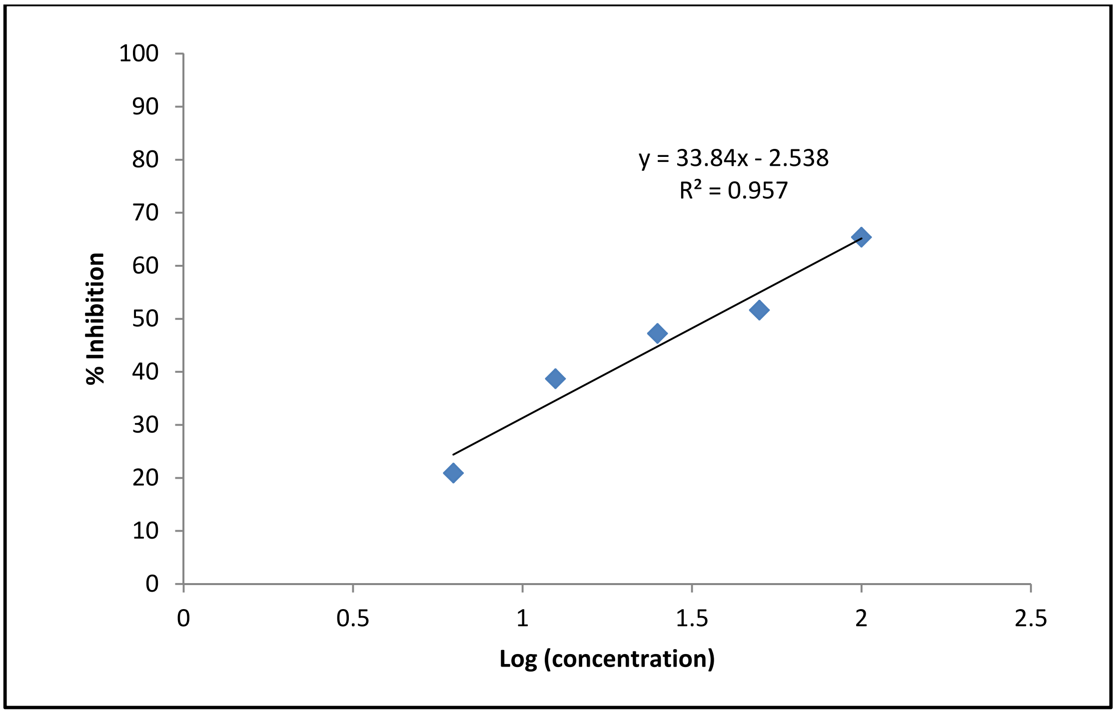


Figure 4.38 Inhibition curves of propolis against the aerobic growth of *P. acnes* after 24 h incubation at 37 °C

Figure 4.36 showed that the growth of *P. acnes* was inhibited by propolis solution ranging from 0.075 - 1.2 mg/mL. Inhibition was observed from 10 % to 70 % propolis, which suggested this propolis solution was poor against *P. acnes* within the test concentration range. The percentage of inhibition had a positive linear relationship with propolis concentration in the logarithmic phase ($p \leq 0.05$, $r > 0.75$). Although MIC_{90} was not determined in the test concentration range, it can be however predicted by using the linear regression equation. The MIC_{90} of *P. acnes* was about 550 mg/mL. Figure 4.37 shows that the control solution of propolis has no antibacterial activity until the concentration was about 10 ug/mL.

b. Olive leaf extract

The antibacterial activity of OLE is dosage-dependent; a higher concentration of the OLE results in a lower bacterial growth ($Abs_{595\ nm}$) (Figure 4.39). The growth of *P. acnes* was completely inhibited by OLE (240 mg/mL). A partial inhibition effect against the bacterium was observed at a concentration with a range of 15 – 120 mg/mL. The percentage of inhibition of the bacterium had a positive linear relationship with the concentration of OLE in the logarithmic phase (Figure 4.40). Based on the regression equation of the percentage of inhibition against the amount of OLE, the MIC_{90} of OLE was 179.17 mg/mL.

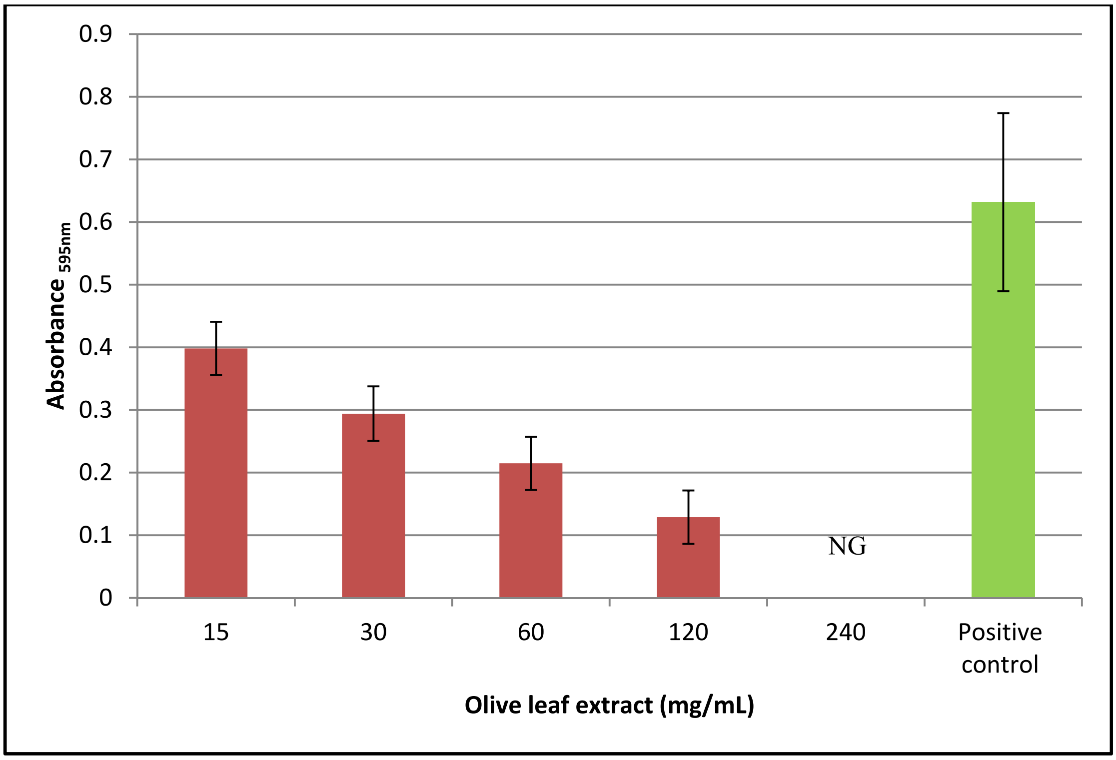


Figure 4.39 Aerobic growth of *P. acnes* in olive leaf extract solution compared to the positive control when incubated at 37 °C for 24 h in a plate reader. (NG: No growth). Bars with the same letters[a-c] were not significantly different ($p > 0.05$). Error bar is the standard error of the mean

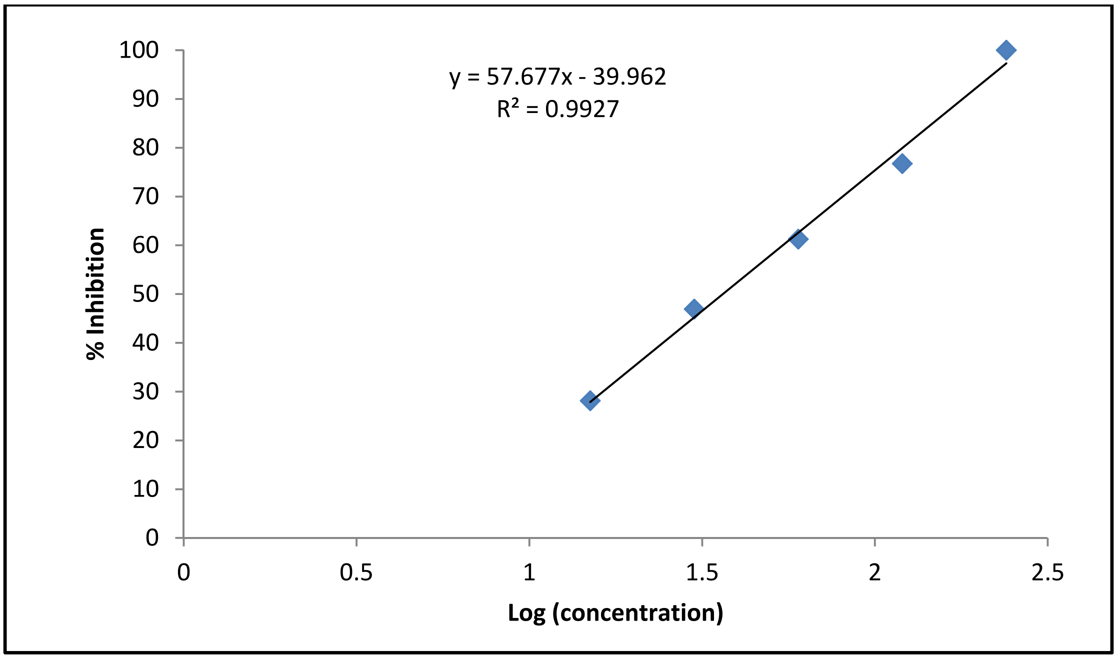


Figure 4.40 Inhibition curves of olive leaf extract against the aerobic growth of *P. acnes* incubated at 37 °C for 24 h in plate reader

c. Manuka tree oil

The MTO had the strongest antibacterial activity against the growth of *P. acnes*.The growth of *P. acnes* was completely inhibited at 2.5 mg/mL and above of MTO. A partial inhibition was noted at concentrations of 0.625 mg/mL and 1.25 mg/mL (Figure 4.41). Based on the polynomial regression equation shown in Figure 4.42, MIC_{90} can be calculated as2.11 mg/mL of MTO in the solution. In addition, a control solution of MTO (DMSO, 0.03125-5 µg/mL) showed no significant bacteriostatic effect towards *P. acnes*. In this experiment, 1 % DMSO was used to aid the preparation of MTO stock solution. Results showed that the DMSO control solution had weak antibacterial activity against the growth of *P. acnes* (Figure 4.43). There was no significant difference in the inhibitory effect of DMSO (0.3125 – 5 ug/mL) against the growth of *P. acnes*.

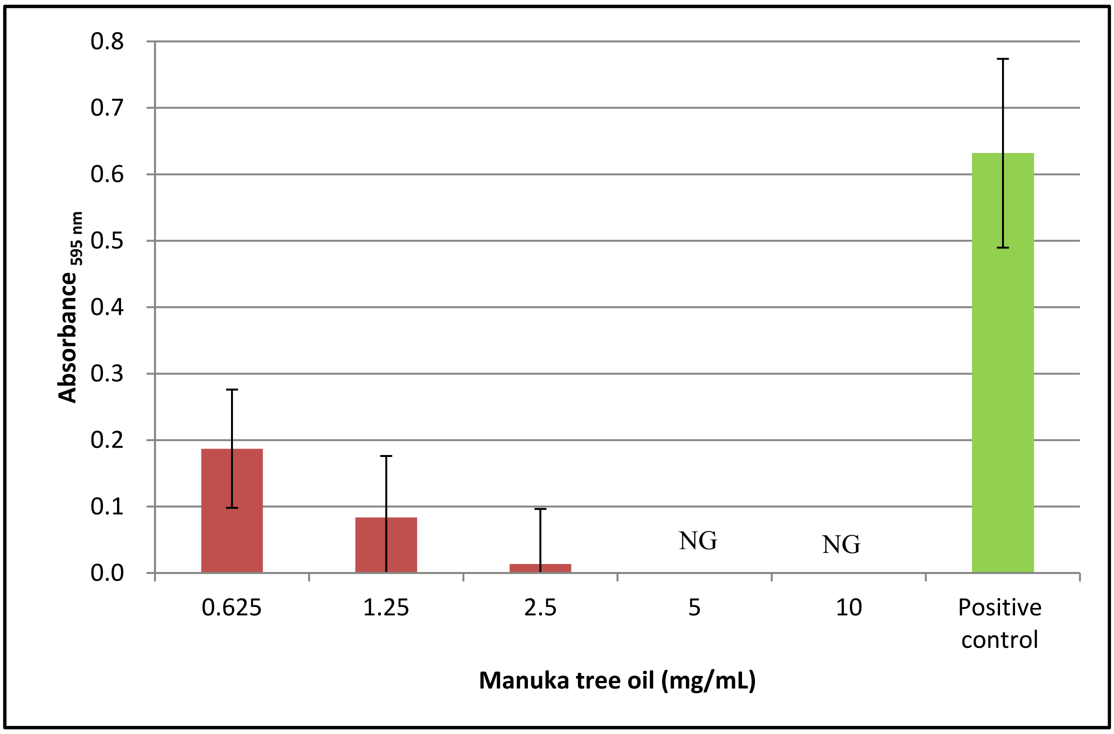


Figure 4.41 Aerobic growths of *P. acnes* in MTO solution when incubated at 37 °C for 24 h in a plate reader. (NG: No growth). Error bar is the standard error of the mean

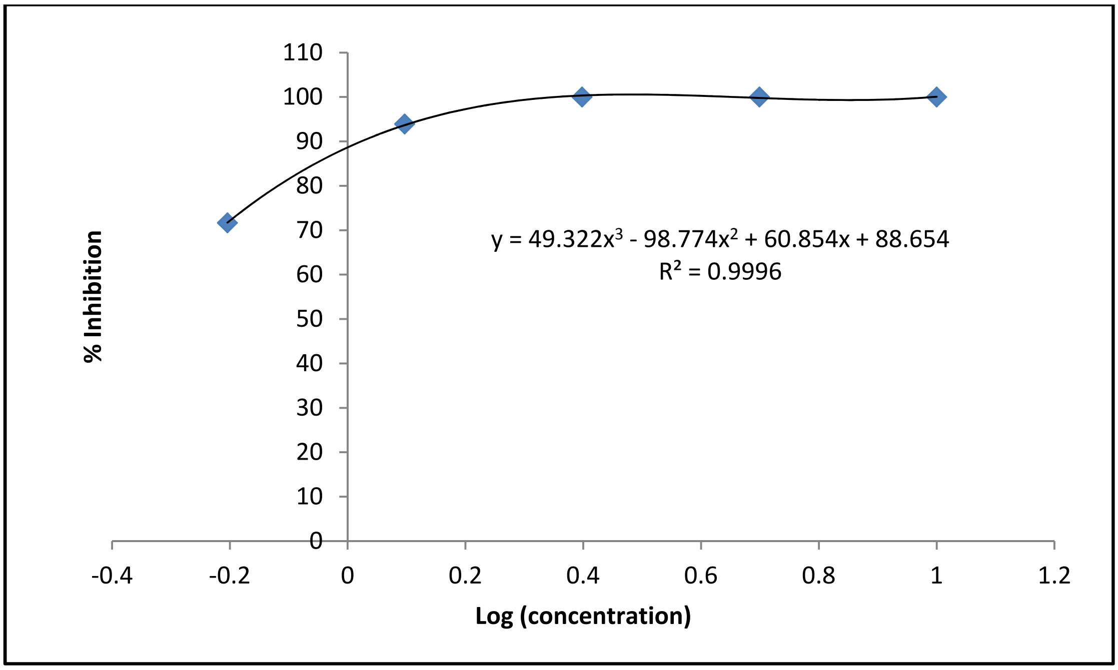


Figure 4.42 Inhibition curve of MTO against the aerobic growth of *P. acnes* incubated at 37 °C for 24 h in a plate reader

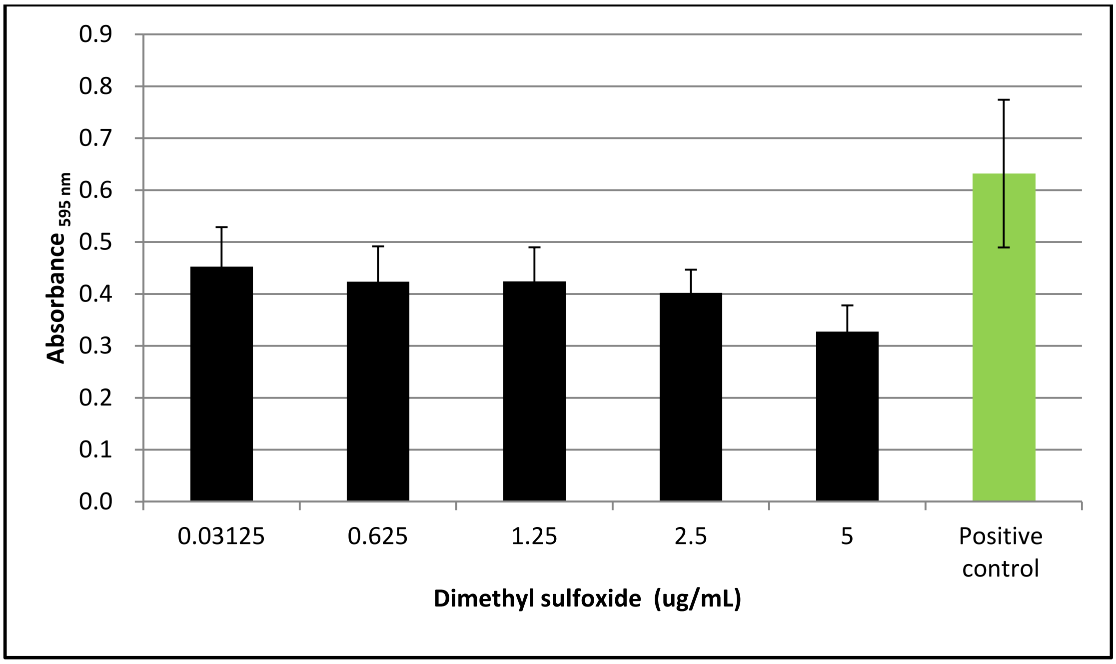


Figure 4.43 The aerobic growth of *P. acnes* in the MTO control solution with DMSO (0.03125-5 ug/mL) when incubated at 37 °C for 24 h in a plate reader. Error bar is the standard error of the mean

d. Lavender oil

LO solutions (1.56-25 mg/mL) showed weak to moderate antibacterial potencies against the growth of *P. acnes* (Figure 4.44). With increasing concentrations of LO, the inhibition ability increased markedly. Although the highest inhibition observed was 85 %, the MIC $_{90}$ of LO was calculated as 54.66 mg/mL based on the linear regression equation of percentage of inhibition and concentration. The control solution for lavender oil was DMSO solution ranging from 1.56 to 25 ug/mL (Figure 4.45). The antibacterial activity of DMSO was weak between1.56 to 6.25 mg/mL, and the antibacterial activity of DMSO was markedly increased at 25 ug/mL.

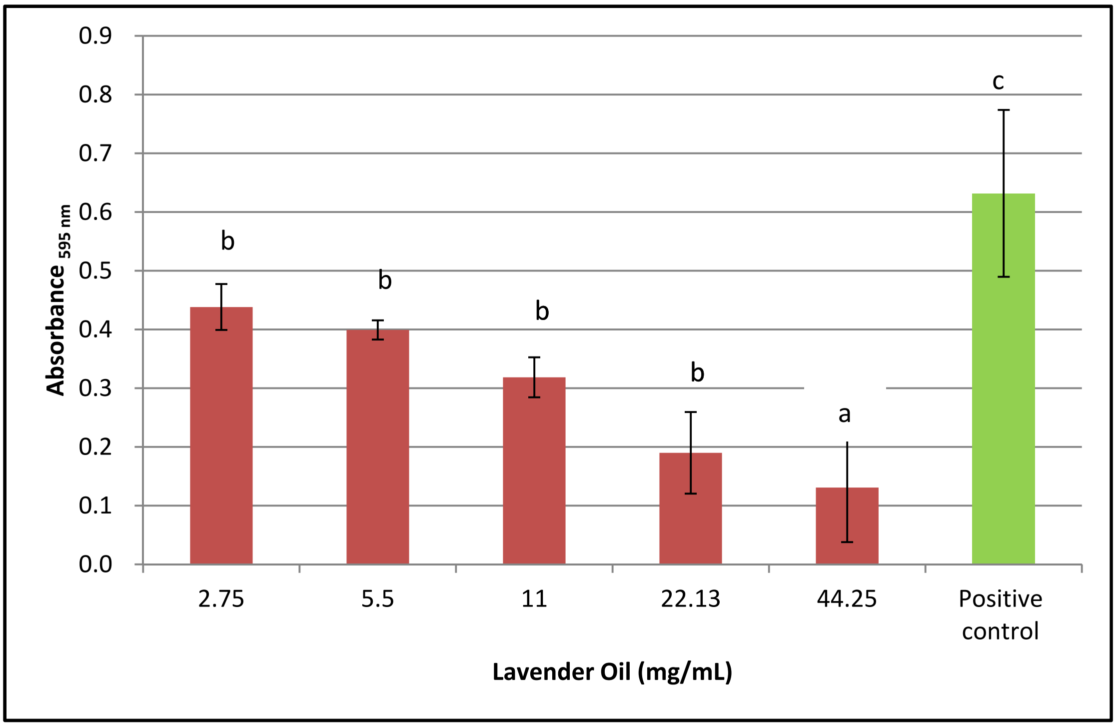


Figure 4.44 The aerobic growth of *P. acnes* in the lavender oil solution when incubated at 37 °C for 24 h in a plate reader. Bars with the same letters[a-c] were not significantly different ($p > 0.05$). Error bar is the standard error of the mean

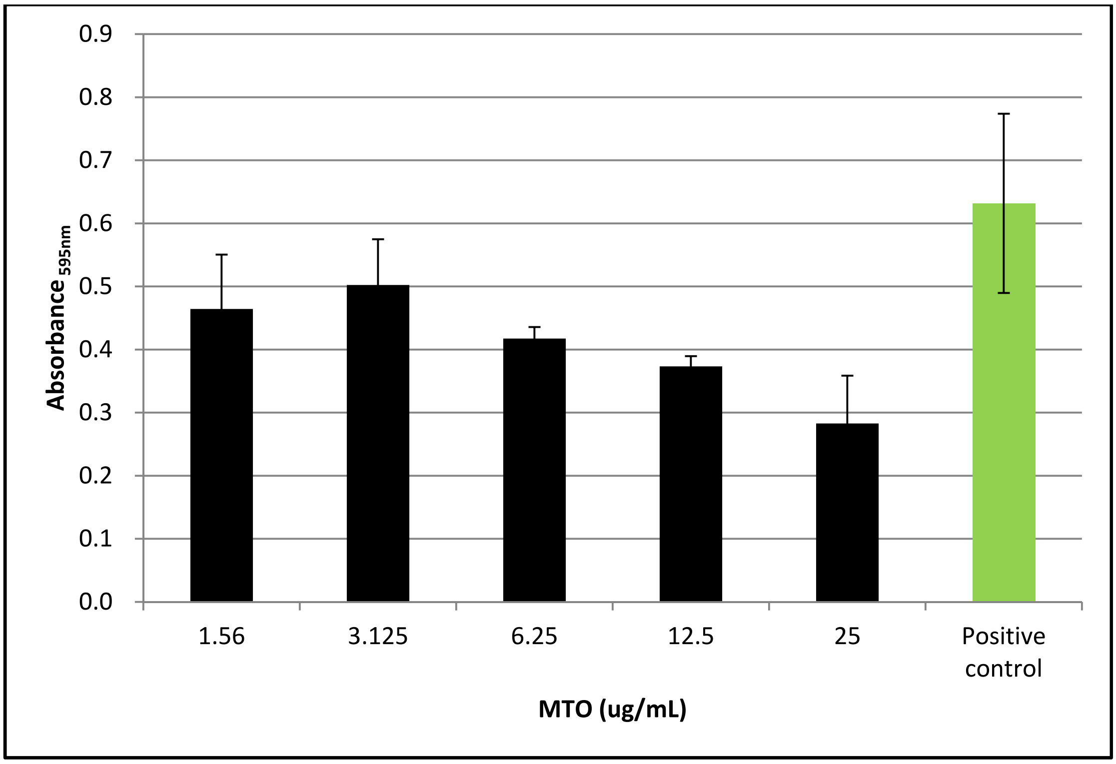


Figure 4.45 The aerobic growth of *P. acnes* in the MTO control solution with DMSO (1.56-25 ug/mL) when incubated at 37 °C for 48 h in a plate reader. Error bar is the standard error of the mean

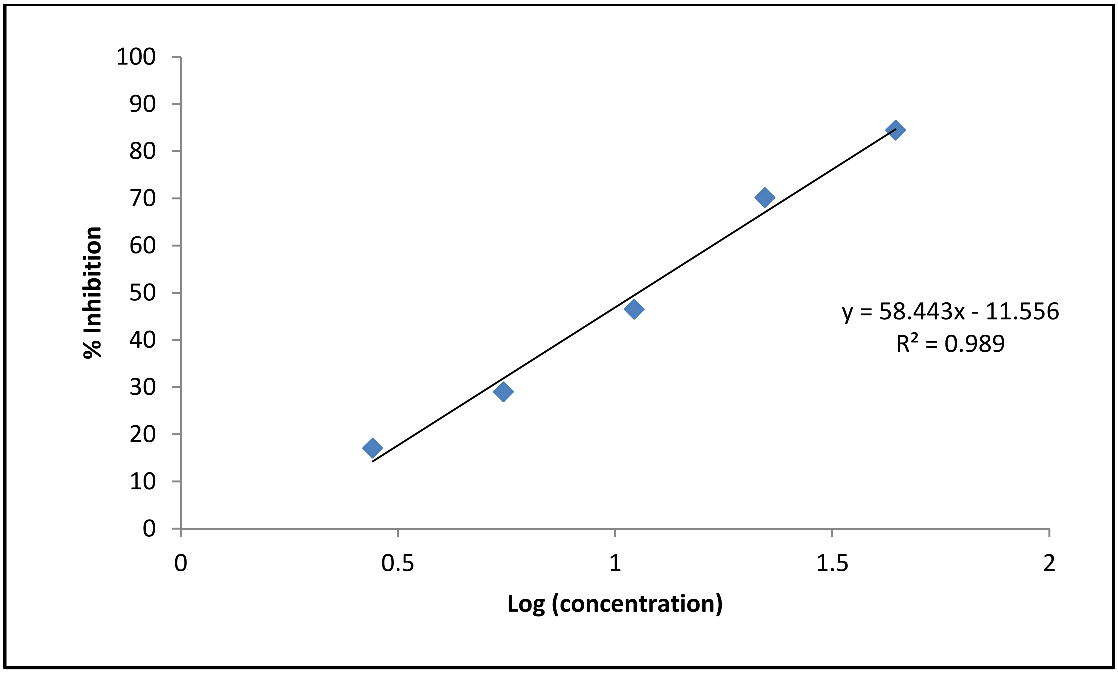


Figure 4.46 Inhibition curve of LO against the aerobic growth of *P. acnes* incubated at 37 °C for 24 h in a plate reader

4.3.3 Antibacterial activity of five bioactives determined by the minimum bactericidal concentration

The minimum bactericidal concentration (MBC) is defined as the lowest concentration at which bacteria unable to grow in BHI broth (Rota *et al*., 2004); the FA agar was used to enumerate the microorganism. In this study, the MBC was demonstrated when a particular concentration of bioactives reduced the viable cell counts from the initial 10^8 CFU/mL to less than (or equal to) 10^2 CFU/mL.

Table 4.4 shows that MBCs of propolis and lavender oil could not be determined in test ranges used in this study. However, the MBCs of MTO and GTE were about 2.5 mg/mL, which was the smallest value (MBC) among the five samples. OLE also demonstrated its killing ability of *P. acnes* at 120 mg/mL, of which is about 6 times higher than both MTO and GTE. However, the MBC of propolis and lavender essential oil could not be determined at the highest concentration used in the study.

Table 4.4 The bacteriostatic and bactericidal concentrations of bioactives

Bioactives	Concentration (mg/mL)		
	$MIC_{\text{visual assessment}}$	$MIC_{90,\text{ calculated}}$	MBC
Propolis	>1.2	550*	>1.2
MTO	2.5	2.11**	2.5
LO	>44.25	54.66*	>44.25
GTE	ND	ND	2.5
OLE	120	179.14**	240

ND: not defined; *predicted MIC_{90} based on the regression curve (not within given bioactives concentration range used), **calculated MIC_{90} based on the regression equation (within the given bioactives concentration range).

Propolis, MTO, LO and OLE have marked difference in their bacteriostatic and bactericidal abilities. Combined results from both MIC_{90} and MBC suggested that MTO and GTE had similar antibacterial activities against the growth of *P. acnes*. MTO and GTE showed the strongest antibacterial activities among the five samples used in this study. LO and OLE had the weakest antibacterial activity against *P. acnes*. It would be therefore of interest to investigate the killing rates of MTO and GTE.

4.3.4 Time-to-kill curves of GTE and MTO

Data obtained were plotted on the graphs to determine killing rates of test samples of *P. acnes*. The antibacterial concentrations were used to determine the killing rates of various bioactives and honey against *P. acnes*. In this study, bactericidal concentrations of Manuka tree oil and green tea extract were used at 5 mg/mL. Figure 4.47 shows that the growth of *P. acnes* in the test solutions (honey and bioactive) were lower than the positive control of *P. acnes* throughout the assay. Manuka honey 10+ UMF inhibited the growth of *P. acnes* at 10 %; the total bacterial growth in the honey treatment was lower than the positive control and the bacterial growth of *P. acnes* was greater than the initial inoculated viable cell counts of *P. acnes*. Hence, the killing curves suggest that Manuka honey 10+ UMF was bacteriostatic against the growth of *P. acnes*. Furthermore, the viable cell counts of *P. acnes* in 0.5 % GTE and MTO were markedly lower than the inoculated viable cell counts of *P. acnes*. The reduction in the viable cell numbers of the bacterium suggested that both bioactives (GTE and MTO) were capable of killing *P. acnes*. The bactericidal activities of GTE and MTO were different from the killing rates. Killing curves (Figure 4.47) showed that the total viable cell counts were reduced from 1.5 x 10^8 CFU/mL to less than 100 CFU/mL up to 24 h of incubation for GTE. The killing rate of GTE solution (0.5 %) against *P. acnes* was about 10^4 CFU/mL per hour. For MTO (0.5 %), the killing rate was about 10 CFU/mL per h. The difference in the killing rates of both GTE and MTO indicated that the GTE had stronger bactericidal ability against the growth of *P. acnes*.

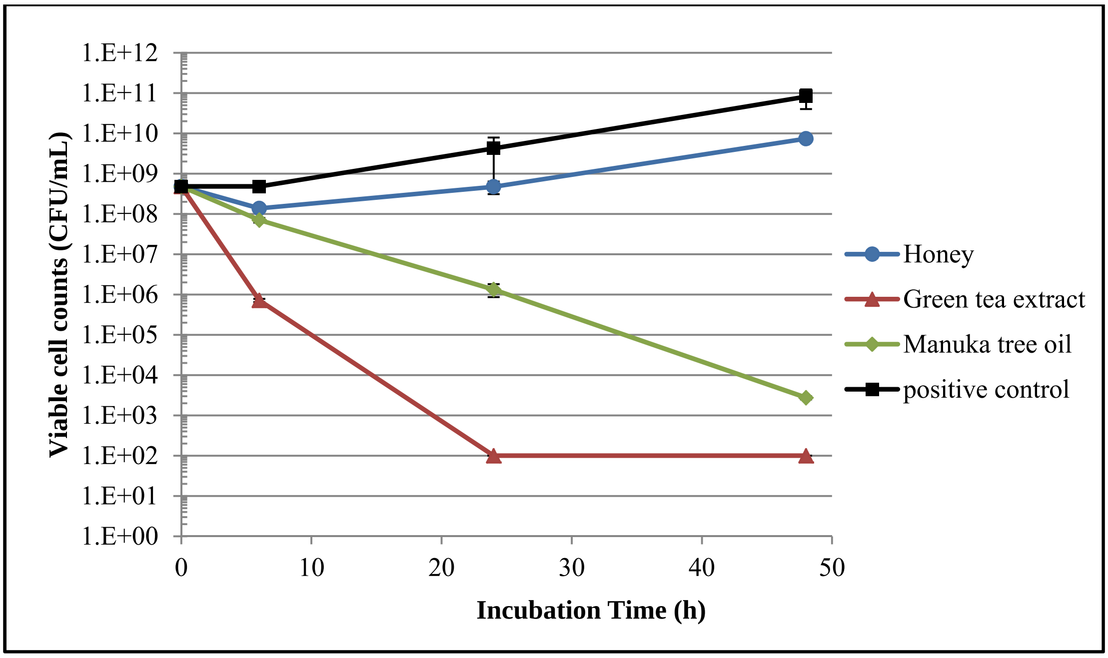


Figure 4.47 Killing curves of honey and bioactives of *P. acnes* under aerobic environment for 48 h at 37 °C. The viable cell counts of *P. acnes* were plotted on a logarithmic scale. The lowest concentration of the test solutions were conducted at 10^2 dilutions. Error bars indicate the standard deviations of the mean

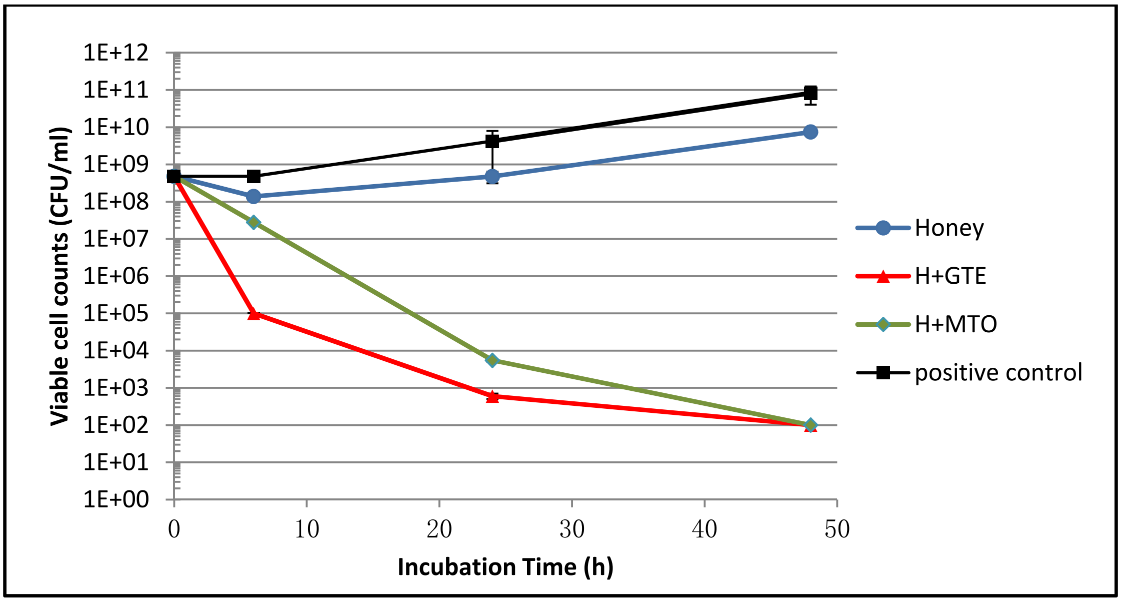


Figure 4.48 Killing curves of honey and two different combinations: honey with GTE and honey with MTO under aerobic environment for 48 h. The viable cell counts of *P. acnes* were plotted on a logarithmic scale. The lowest concentration of the test solution was conducted at10^2 dilutions. Error bars indicate the standard deviations. H+GTE=10% honey plus 0.5% green tea extract; H+MTO= 10% honey plus 0.5% Manuka tree oil

The killing curve shown in Figure 4.48 shows the synergistic effects of honey with two different bioactives against the growth of *P. acnes.* The viable cell counts of *P. acnes* when grown in honey with GTE, and honey with MTO were much lower than the initial viable cell counts of the bacterium. The reduction in cell numbers demonstrated that the two types of combinations were capable of killing the *P. acnes.* The killing curves of honey combined with GTE were lower than the curve of the honey combined with MTO. In both combinations, the number of viable cells rapidly decreased in the first 24 h of incubation followed by slower reduction rate up to 48 h. For the combination of honey with GTE, there was about3.5 log reductions in the number of viable cells during the first 6 h of incubation and the reduction rate gradually decreased until the end of incubation. Meanwhile, there was only one log reduction in the bacterial cell numbers observed in the combination of honey with MTO up to 6 h, which was followed by a similar reduction rate up to 24 h. Based on the killing curves in the first 24 h, killing rates were about 10^3 CFU/mL for the combination of honey with MTO, and 10^4 CFU/mL per h for the combination of honey with GTE.

The synergistic effect of honey with GTE and MTO was clearly demonstrated in Figures 4.47 and 4.48. The bactericidal ability of the four treatments was weighted as combination #1 (honey and GTE) ≈GTE > combination #2 (honey and MTO) >MTO. It therefore seems that combination 1 had a similar killing ability with the GTE, but, combination #1 (honey with GTE) resulted in3.5 log reductions, and for GTE alone, it was about 3 log reductions in the first 6 h. Therefore, a combination of honey with bioactives showed stronger antibacterial ability than any of the single treatments (honey, GTE or MTO).

4.4 Evaluating the antibacterial activities of formulated emulsion creams containing honey and bioactives

The emulsion cream contained Manuka honey 10+ UMF showed excellent bacteriostatic activity against the growth of *P. acnes*. GTE and MTO also exhibited good bactericidal abilities by reducing the number of cells inoculated in the emulsion cream. The antibacterial activity of Manuka honey 10+ UMF was markedly enhanced by various combinations with bioactives in the cream base.

4.4.1 Antibacterial activity of cream containing honey MTO, GTE separately

On average, 8 x 10^7 CFU/mL of *P. acnes* were inoculated into different emulsion samples containing honey and bioactives alone. After 24 h incubation at 37 °C, the numbers of viable bacterial cells were enumerated and recorded in various samples (Figure 4.50). The growth of *P. acnes* in incubated water without any limiting factors was referred as the positive control, which was about 2.7 x 10^8 CFU/g. The growth of *P. acnes* was also studied in the emulsion base; the number of viable cell counts was 2.65 x 10^8 CFU/ g in the emulsion. There was no significant difference ($p \leq 0.05$) in viable cell counts obtained in the emulsion control and positive control. This indicated that the emulsion base had no inhibitory components against the growth of *P. acnes*.

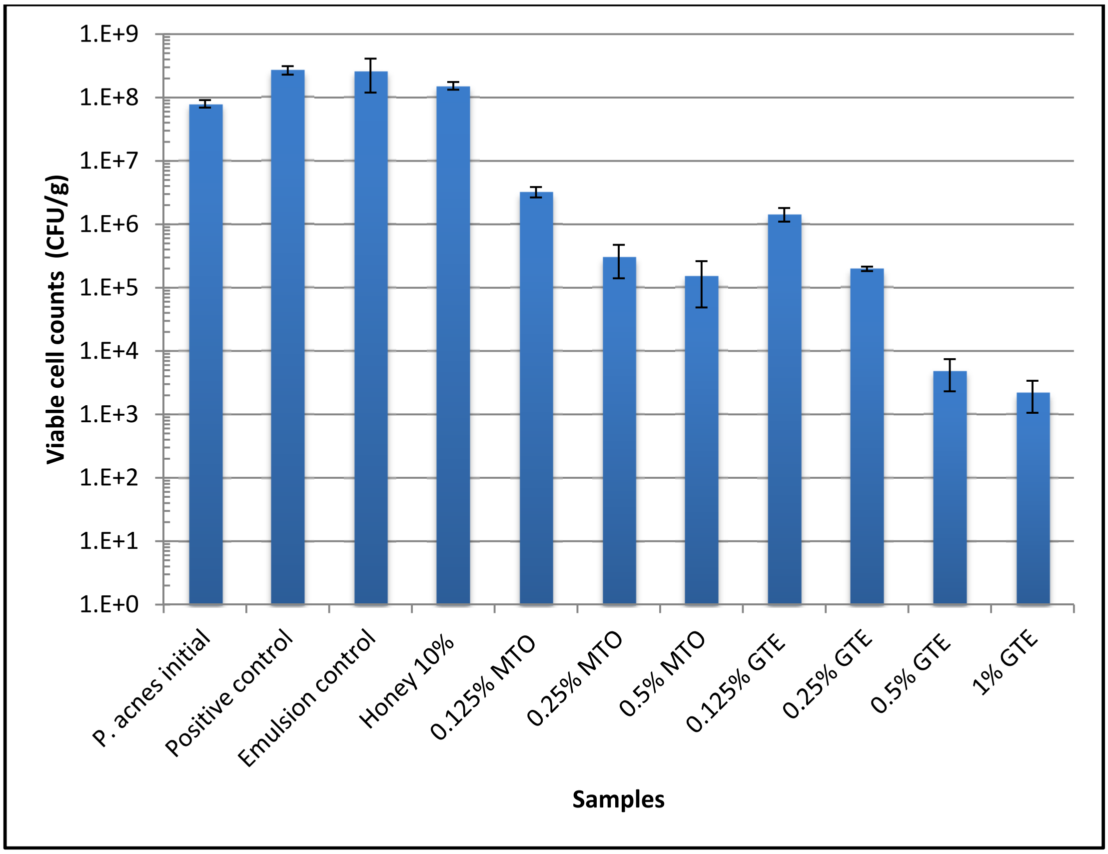


Figure 4.49 Viable cell counts of *P. acnes* in emulsion cream samples containing single bioactives: Manuka honey 10+ UMF, GTE and MTO after 24 h incubation under anaerobic environment. Each sample was done in duplicate and the experiment was repeated two times; error bars are the standard deviations of the 4 results. Notes: Honey: Manuka honey 10+ UMF (10%); MTO: Manuka tree oil, GTE: green tea leave extract.

Figure 4.49 shows that the viable cell counts were reduced to 1.55 x 10^8 CFU/g in the 10 % Manuka honey 10+ UMF. The number of viable cell counts present in the honey emulsion was higher than the initial inoculated *P. acnes*, but it was lower than the positive control. This suggested that 10 % Manuka honey 10+ UMF had bacteriostatic ability and this cream formula could inhibit the growth of *P. acnes*.

The cream formula containing MTO and GTE had bactericidal ability against the growth of *P. acnes*. The number of viable cell counts ranged from 1.55 x 10^5 to 3.27 x 10^6 CFU/g in the cream formula containing MTO (0.125 % to 0.5 %, w/v). The emulsion formula containing MTO was capable of reducing the initial number of viable cell counts of *P. acnes* inoculated.

Furthermore, there were approximately 1.5 to 2.5 log reductions in bacterial cell numbers compared to the positive control. The viable cell counts present in the MTO emulsion samples had negative correlations with the amount of MTO used in the cream. Result showed that 0.125 % MTO has highest number of bacterial cell and 0.5 % MTO has the lowest the number of bacterial cell. The cream formula containing GTE showed that the killing effect was concentration-dependent. The emulsion cream containing GTE (0.125 % to 1 %, w/w) varied from 2.23 x 10^3 to 1.45 x 10^6 CFU/g. There were about 1.5 to 4.5 log reductions in viable cell counts compared to the positive control. As shown in Figure 4.50, the bactericidal activity could be weighted in the following order: GTE (1 %)> GTE (0.5 %)> GTE (0.25 %) ≈ MTO (0.5 %) > MTO (0.25 %) > GTE (0.125 %) > MTO (0.125 %).

4.4.2 Antibacterial activity of honey cream formula with and without bioactives

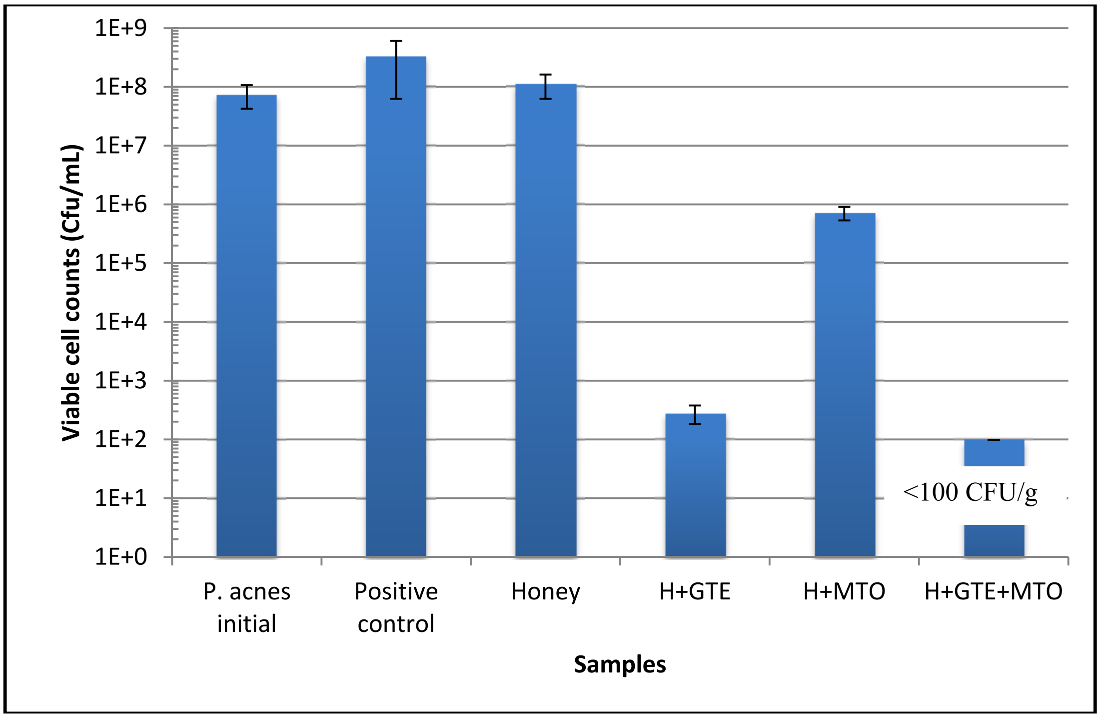


Figure 4.50 Viable cell counts of *P. acnes* in four different honey treatments singly and in combination with GTE and MTO using survival rates after 24 h anaerobic incubation at 37 °C. Each sample was tested in duplicate and the experiment was repeated three times; error bars indicate the standard deviations of the 6 results. Notes: Honey =Manuka honey 10+ UMF (10%); H+GTE = 10% honey and 1% GTE; H+MTO = 10% honey and 0.125% MTO; H+GTE+MTO = 10% honey and 1% GTE and 0.125% MTO

Figure 4.50 shows that antibacterial activity of Manuka honey 10+ UMF (10 %) was enhanced by a combination of honey with MTO or GTE. The viable cell counts found in the respective combinations were markedly lower than the initial inoculum of *P. acnes*. The reduction of the viable cell numbers indicated that honey combined with either GTE or MTO could kill *P. acnes* inoculated at the start of the experiment. The viable cell counts were 2.8 x 10^2 CFU/g presenting the cream formula containing honey (10 %, w/v) and GTE (1 %, w/v), and there was 5.5 log reduction compared to the positive control. For the emulsion formula containing honey (10 %) and MTO (0.125 %), there was about 2 log reductions compared to the positive control and the viable counts were 7.22 x 10^5 CFU/g. The final formula containing three different active components comprising 10 % honey, 1 % GTE and 0.125 % MTO. There was no bacterial growth in the lowest dilution plate (10^2 dilution), which suggested that there were less than 100 CFU/g. There were more than 6 log reductions in cell numbers compared to the positive control in the final cream formula.

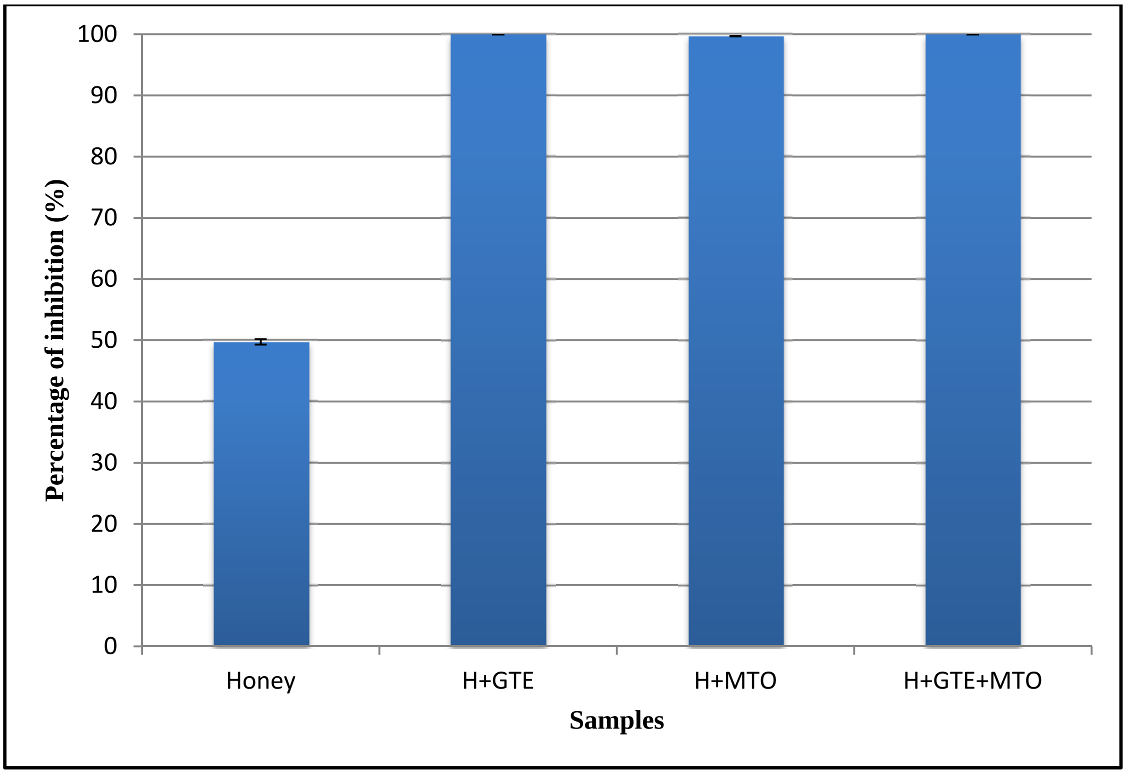


Figure 4.51 Percentage of inhibition of the growth of *P. acnes* in various honey formula incubated at 37 °C for 24 h

Figure 4.51 suggests that synergistic effect of honey with different bioactives was significantly enhanced in the cream formula. Based on the percentages of inhibition, there was about 50 % inhibition against the growth of *P. acnes*. The percentage of inhibition increased to 100 % in the honey cream containing either MTO or GTE, and honey combined with both MTO and GTE.

4.4.3 Comparison of the antibacterial activity of honey formula with bioactives and commercial products

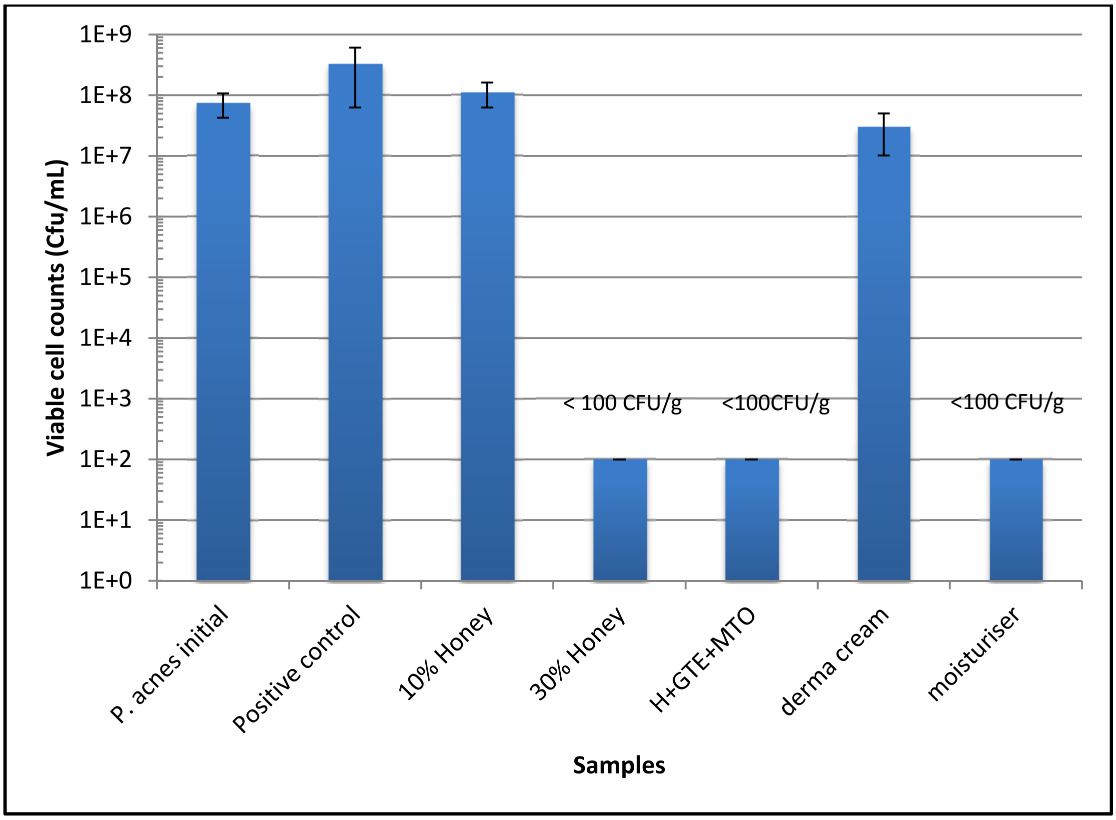


Figure 4.52 Viable cell counts in 10 % and 30 % honey cream formula alone, combined formula and commercial products

Figure 4.52 shows that Manuka honey exhibited inhibitory ability against the growth of *P. acnes* at 10 % honey. Cells of *P. acnes* were completely killed in the cream containing 30 % honey. No bacterial growth was observed in honey combinations with GTE and MTO. The commercial product Derma™ cream showed weak inhibitory ability against the growth of *P. acnes*. The viable cell counts in the Derma™ cream were lower than the positive control and there was about 50 % inhibition against the bacterial growth. There was less than 100 CFU/mL found in the moisturiser.

5.0 Discussion

5.1 The *in vitro* study of *P. acnes*

In this study, it was shown that *P. acnes* can grow under both anaerobic and aerobic environments; the microorganism is therefore a facultative bacterium, although *P. acnes* has been described as anaerobic in very early studies by Douglase and Gunter (1946). The aerobic growth of *P. acnes* has been also reported in previous studies (Evans *et al.,* 1979; Cove *et al.*, 1983; Esteban *et al.*1996; Lambert, 2006; Poppert *et al.*, 2009). Vries *et al*. (1972) reported that the organism was not very sensitive to oxygen. The presence of oxygen is not lethal to the organism and *P. acnes* can tolerate oxygen concentration up to 100 % saturation but grows at reduced rates (Cove, 1983). The study of Gribbon *et al.* (1994) suggested *P. acnes* to be micro-aerophilic. In the present study, it was observed that the bacterium grew better under anaerobic environment when streaked on agar plate and in BHI liquid broth; however, the growth curves of *P. acnes* were not significantly different between anaerobic and aerobic environments.

5.1.1 Colonial and bacterial cell morphology

The colonies and bacterial cell morphology were similar to earlier reports of *P. acnes* (Poppert *et al.*, 2009; Perry and Lambert, 2006; Esteban *et al.*, 1996). In this study, the surface colonies of the *P. acnes* were cream white with a 1 mm diameter after 3 days incubation at 37 °C under anaerobic environment. With extended incubation, the colonies changed from the initial small white colonies to large and dark yellow or pink colonies (Anderson *et al.*, 1999). The surfaces of colonies varied from 1.5 to 4.0 mm in diameter, and the colour of colonies was pink after 4 - 5 days under anaerobic incubation (Douglase and Gunter, 1946).

Individual cells observed in this present study were non-spore-forming, Gram positive, anaerobic, pleomorphic rods, mixed with cocco-bacilli and club-shaped cells. In terms of quantitative comparisons, it seemed that the numbers of cocco-bacilli cells were far less than the long rod and club-shaped cells.

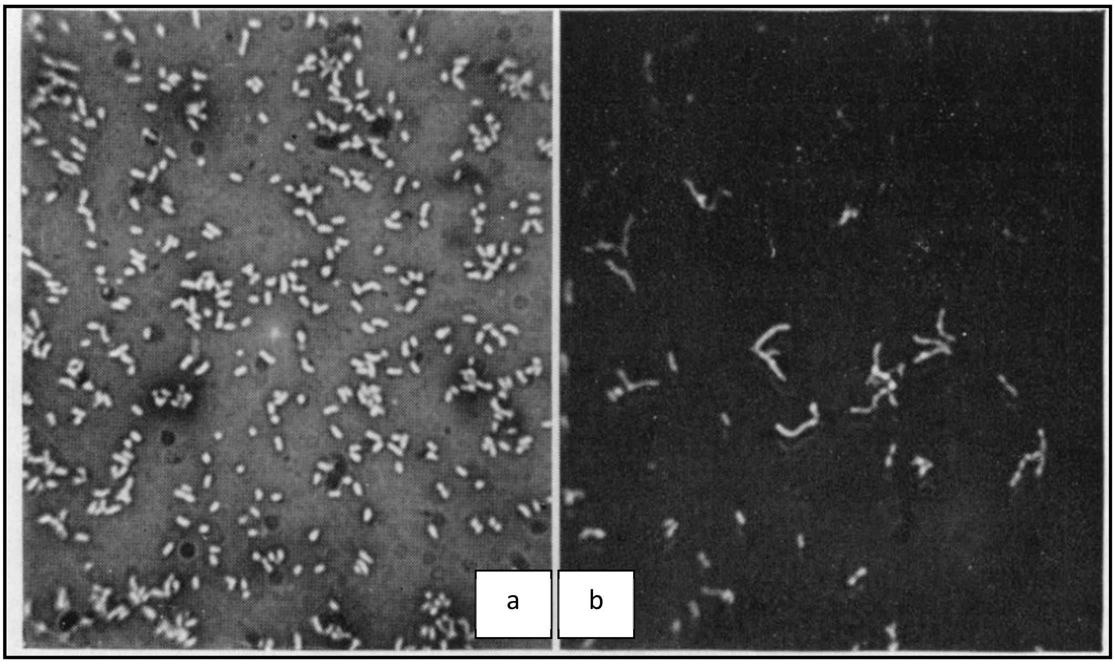


Figure 5.1 Gram stain of four day old slant culture incubated (a) anaerobically (b) aerobically (Gouglas and Gunter, 1946)

The study by Douglas and Gunter (1946) reported that small rods were dominant in the anaerobic slant culture (Figures 5.1 a). Long, swollen and club-shaped cells appeared to be the dominant cells in the aerobic culture (Figure 5.1 b). The change in cellular morphology could be a sign of cell stress, metabolic and genetic changes. *P. acnes* with long and club-shape cells were dominant under aerobic environment. It seems this type of cellular morphology is more suitable under aerobic environment and these types of cells have better chances of survival under oxygen. In this study, pure cell culture of *P. acnes* was dominant with rod-shaped cells, which suggested that the strain of *P. acnes* used in this study could have high tolerant to oxygen.

5.1.2 Biochemical characteristics of *P. acnes* grown under both aerobic and anaerobic environment

P. acnes can grow under both aerobic and anaerobic environment and release volatile acids (Cove *et al*., 1983). The biochemical reactions of *P. acnes* were suggested to be different with and without the presence of oxygen (Cove *et al*., 1983). Therefore, different incubation conditions of *P. acnes* can produce different bio-wastes, metabolites, mediator and extracellular enzymes.

Historically, *P. acnes* have been classified as an anaerobe; deriving its energy from the fermentation of lactate and carbohydrate to propionate, acetate and carbon dioxide in a ratio of 2:1:1 (Ye *et al*., 1999). Moreover, vitamin B_{12} accumulates intracellularly under anaerobic environment (Allen *et al*., 1964). In contrast, more than one mechanism is operative in stimulating the aerobic growth of *P. acnes* (Evan and Mattern, 1979). It appears that *P. acnes* may carry out aerobic respiration through the formation of succinate by transmitting electrons from cytochromes *b* and *c* to fumarate when oxygen serves as a final electron acceptor (Evan and Mattern, 1979). The *P. acnes* contains the enzymes of the tricarboxylic acid (TCA) cycle and can therefore utilize this pathway to oxidize some intermediates (Ye *et al.,* 1999).

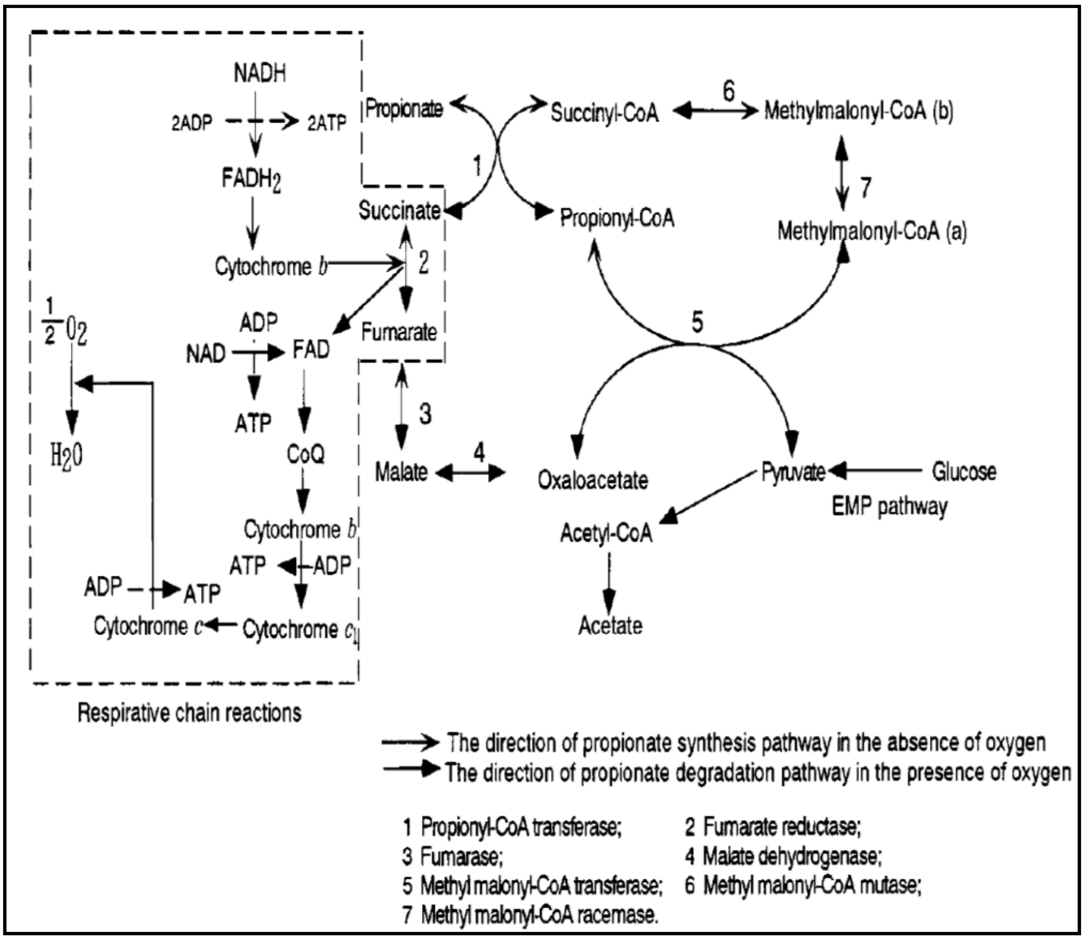


Figure 5.2 Metabolic pathway of *P. acnes*growing under aerobic condition (Ye *et al*., 1999)

The presence of oxygen causes the decrease in the activities of fumarate reductase, malate dehydrogenase, and fumarate reductase. Further, the synthesis of cytochrome *b* is also affected by the presence of oxygen (Vries *et al*., 1997). This probably explains the sensitivity of *P. acnes* to excess oxygen. Therefore, the cell growth significantly reduces with prolonged exposure to oxygen. It is however suggested that the growth of *P. acnes* is promoted by the utilization of molecular oxygen at low concentrations (Gribbon *et al*., 1983)

5.2 The antibacterial activity of Manuka honey and Kanuka honey

Manuka honey has been widely researched and its antibacterial potential is renowned worldwide (Badet and Quero, 2011; Mandal *et al*., 2010; Sherlock *et al*., 2010). Similar to Manuka honey, Kanuka honey is also readily available in New Zealand. However, the quality and floral origin of Kanuka honey have yet to be determined and standardized. Manuka honey with UMF greater than 10 has been recommended for wound care (Tan *et al.*, 2009). Three Manuka honeys with unique Manuka factors of 10+, 15+ and 20+ were used in this study.

5.2.1 Spectrophotometric assay to determine the antibacterial activity of Manuka and Kanuka honeys

When determining antibacterial activity of inhibitory compounds, the selection and use of suitable methods are important. The choice of the methods ranges from agar diffusion, disc diffusion, broth diffusion, or variants of these methods. More recently, there has been an increased interest in the spectrophotometric assay. The spectrophotometric method was used to determine the antibacterial activity of honey and bioactives in this present study and the experimental protocols were adapted from the study of Patton *et al.* (2006). Patton *et al.* (2006) published the first report that compared the spectrophotometric assay using the 96-well microtiter plate to strand methods of well/disc diffusion for the evaluation of the antibacterial properties of Manuka honey. The spectrophotometric assay was reported to be more sensitive and amenable to statistical analysis than the disc/well diffusion method. The bioassay method was therefore recommended as a simple and rapid assay, which permits extensive kinetic studies even in the presence of low honey concentrations (Sherlock *et* *al*., 2010). In addition, using this microdilution method, the MIC values determined were lower (indicating higher activity) than those obtained using agar well-diffusion method, as diffusion rates of active constituents in agar may be slower than those in broth (Tan *et al.*, 2009).

5.2.2 Dose-response curves and MIC for determining the antibacterial activity of Manuka and Kanuka honeys

Observing and comparing dynamic movement of bacterial growth curves alone may be considered subjective and qualitative in determining the antibacterial activity of the honeys. The does-response curves obtained from plotting the concentration of honey against the percentage of inhibition of bacterial growth could also determine the antibacterial activity of honey in terms of the MIC value. However, MIC provides little information on the kinetic changes in bacterial growth when an antibacterial agent is present (Othman *et al*., 2011). Hence, combining both dynamic bacterial growth curves and the MIC values could generate more quantitative results and is more amendable to statistical analysis. Moreover, the MIC is commonly expressed as MIC_{50} (concentration of test material which results in 50 % inhibition of growth; also known as the median response), MIC_{90} and MIC_{100} (the lowest concentration of test material which results in 90 % and 100 % inhibition of growth respectively). In this study, the antibacterial activity of Manuka honey and Kanuka honey were evaluated and expressed as MIC_{50}, MIC_{90} and MIC_{100}.

5.2.3 The kinetic bacterial growth of *P. acne*s in Manuka honey and Kanuka honey

The dynamic movement of bacterial growth curves from left to right, thus moving away from the positive control were recognized as growth retardation (Figures 4.6 - 4.21). Once honey concentration or the antibacterial activities of honeys was increased, the bacterial growth curves were more likely to be driven further away from the positive control curves. The distance between the bacterial growth curves and the positive control curves can be a measured parameter of defining the antibacterial ability of the honey samples.

According to our knowledge, the current study is the first to evaluate and compare the antibacterial activity of Manuka honey and Kanuka honey against the growth of *P. acnes in vitro*. The bacterial growth curves (Figures 4.6 – 4.9) obtained from the spectrophotometric assays showed that Manuka honey and Kanuka honey possess strong antibacterial activity against the growth of *P. acnes* at concentrations of 2.5 % (w/v). Meanwhile, a weaker inhibitory effect was observed for Manuka honey 10+ UMF with a minimum concentration of 5 % (w/v) required to inhibit the growth of *P. acnes.* At honey concentration of 7.5 % and above, four samples of honey (Manuka honey and Kanuka honey) caused a significant delay of the bacterial growth. In particular, there were no bacterial growths throughout the incubation period for 7.5 % Manuka honey 20+ and 15+ UMF.

5.2.4 The antibacterial activity of Manuka honey and Kanuka honey

Significant antibiotic resistance and multiple drug resistance have been identified for *P. acnes* strains from acne patients with long-term antibiotic treatments (Nakatsuji *et al*., 2009). Moreover, Biofilm formation by *P. acnes* increases the resistance against antimicrobial agents (Coenye *et al*., 2007). Manuka honey, however, has been shown to exhibit antibacterial activity against MRSA (Tan *et al*., 2009; Sherlock *et al*., 2010). In addition, bactericidal effects of Manuka honeys were equally observed on both plank-tonic and Biofilm-formed bacteria (Badet and Quero, 2011). Thus, Manuka honey may have potential properties to be used as effective antibacterial treatments for antibiotic-refractory acne.

The MICs data obtained in this study revealed that Kanuka honey (MIC_{100} of 123.28 mg/mL or 12.33 %) had similar inhibitory activity to Manuka honeys 20+ (MIC_{100} of 148.90 mg/mL or 14.90 %) and 15+ UMF (MIC_{100} of 125.81 mg/mL or 12.58 %). The three honeys had much stronger activities than Manuka honey 10+ UMF (144.43 mg/mL or 14.43 %) against the *P. acnes*. The results of the present study are in resonance with previous studies despite the different bacteria used (Sherlock *et al*. 2010; Mavric *et al*., 2008; Cooper *et al*., 2002). In general, Manuka honeys possess strong antibacterial activities with MIC ranging from 3 % (Cooper *et al*., 2002) to 30 % (Mavric *et al*., 2008), whereas, concentrations of 10 to 15 % were reported as predominant MICs ranges (Basson *et al*., 2008; Lusby *et al*., 2005; Weston *et al*., 1998). Similar spectrophotometric assays were used; the MIC of Manuka honeys was 12.5 % (v/v) for MRSA isolates (Sherlock *et al*., 2010), 12.5 % (v/v) for *E. coli*, 6.25 % for *S. aureus* and 3.125 % for *B. cereus* (Patton *et al*., 2006). The study of Tan *et al.* (2009) reported that the MIC of Manuka honey was 11.25 % for 13 different wound and enteric microorganisms.

In comparison to Manuka honey, most other honeys have weaker antibacterial activities, with MICs greater than 20 % (Lusby *et al*., 2005). In particular, the MIC of Argentine honey was as high as 45 % (Basualdo *et al*., 2007). Like Kanuka honey, Ulmo 90 honey (from the Ulmo tree native to Chile) had superior antibacterial activity against all 13 clinical bacterial isolates compared to Manuka honey with a lower MIC of 3.1 % - 6.3 % (Sherlock *et al.*, 2010). Although data from our study showed that Kanuka honey possesses strong antibacterial activity, it has not been reported elsewhere.

5.2.5 Non-peroxide activity of Manuka and Kanuka honeys

Unlike Manuka honey, both Kanuka honey and Ulmo honey do not have non-peroxide activity (Sherlock *et al.*, 2010). Upon diluting the honey, the hydrogen peroxide is produced from glucose via enzymatic reaction using glucose oxidase. Since the presence of hydrogen peroxide in honey is responsible for their antibacterial activities, we tested the non-peroxide activity of both Manuka honey and Kanuka honey after the addition of catalase. A study by Snow and Harris (2003) showed that 2 mg/mL of catalase in the test solution was effective for eliminating hydrogen peroxide from honey solution.

As predicted, the antibacterial activities of Manuka honeys persisted after the addition of catalase. However, after removing the hydrogen peroxide the bacterial growth curves of Manuka honeys were slightly moved below the aerobic growth curve of positive control. MIC values of Manuka honey samples also increased but the changes were not significant ($p > 0.05$). Results showed that after removing the hydrogen peroxide, MIC_{100} values of Manuka 20+ UMF increased from 148.90 to 156.69 mg/mL, MIC_{100} of Manuka honey 15+ UMF increased from 125.81 to 178.33 mg/mL and for Manuka 10+ UMF, the MIC_{100} increased from 144.43 to 131.13 mg/mL. Therefore, the initial presence of hydrogen peroxide in the honey solution could have contributed to the antibacterial activity of Manuka honey. Irrespective of this, Manuka honey has been previously shown that its antibacterial activity is attributed mainly from the non-peroxide components such as MGO (Mavric *et al*., 2008).

In contrast, the removal of hydrogen peroxide from Kanuka honey reduced its antibacterial activity almost completely (Figures 4.13 and 4.21). Bacterial growth curves in the Kanuka honey with catalase were close to the positive control curves (Figures 4.13 and 4.21). The MIC values of the Kanuka honey increased five times from 123.28 to 549.21 mg/mL after the addition of catalase. As predicted, the changes in the bacterial growth in the Kanuka honey after the addition of catalase were significant ($p \leq 0.05$). The changes suggested that the bacterial inhibition of Kanuka honey in the present experiments were mainly due to the presence of hydrogen peroxide.

5.2.6 Effect of honey concentrations on the antibacterial activity of Manuka honey and Kanuka honey

Although the presence of hydrogen peroxide contributes to the antibacterial activities of honey, the high osmotic pressure or high sugar concentrations also influence the inhibitory effects of honey against bacteria. In our study, the antibacterial activity of laboratory prepared artificial honey was evaluated and compared to the positive control. Bacterial growth curves showed that the sugar contents had no effect on the growth of *P. acnes* at sugar concentrations of 7.5 % and 10 % (w/v) (Figures 4.22 to 4.25). However, the bacterial growth in the 12.5 % sugar solution was lower than the positive control and there was significant difference among the three honey solutions ($p \leq 0.05$). This suggested that the high sugar content could have an inhibitory effect on the growth of *P. acnes*. In the study of Sherlock *et al*. (2010), the inhibitory effect of high sugar was also obtained with artificial honey (MIC of 50 %) against *E. coli* and *Pseudomonas* species.

Dose-response curves of honey showed that Manuka honey 10+ (with and without catalase) and Kanuka honey with catalase at lower than 6 % (w/v) yielded a negative value in the percentage of inhibition. This observation suggested that honey at low concentration could promote the growth of *P. acnes*. The positive percentages of inhibitions were obtained when the concentration of honey was increased above 7.5 %.

The stimulatory effect of honey was likely to be a result of excess carbon nutrient available for the growth of *P. acnes* (Gribbon *et al*., 1994). The additional amount of nutrient could be beneficial and it is likely to increase the bacterial metabolism rate especially during long periods of incubation (48 h). Interestingly, the suspected stimulatory effect of honey at low concentration on bacterial growth has not been reported elsewhere and it is difficult to explain without further study on the biochemical behaviour of *P. acnes*. The stimulatory effect in bacterial growth caused by the low concentration of honey was not covered in this project. This finding could also lead to a complete new area of microbiological study of the *P. acnes*.

5.3 Screening natural bioactives for antibacterial activities

Essential oils, plant extracts and honey products are typical natural bioactives. Most of these products have exhibited strong antibacterial activity against a wide range of skin associated pathogens (Kassim, 2008; Hammer *et al.,* 1999). In this study, the antibacterial activity of Manuka tree oil (MTO), lavender essential oil (LO), green tea extract (GTE), olive leave extract (OLE) and propolis were studied in terms of MIC_{90} and MBC using the disc diffusion and the microdilution methods against the growth of *P. acnes*. The microtiter plate reader was used in these experiments.

Various reports have documented the antibacterial activity of essential oils and plant extracts (Kassim, 2008; Douglas *et al.*, 2004; Perry *et al.*, 2006; Hammer *et al.,* 1999). The bioactives tested in this study (MTO, GTE, propolis, OLE) have not been investigated *in vitro* against the growth of *P. acnes* using a combination of traditional methods and modern susceptibility testing techniques. The results could contribute towards better understanding of antibacterial effects of the bioactives against *P. acnes* with the aim of producing a potential acne-treatment product.

5.3.1 The antibacterial activity of Manuka tree oil

Manuka tree oil has been traditionally used in New Zealand Maori remedies as an external antiseptic (Douglas *et al.*, 2004). Manuka tree is the most widely distributed and environmentally-tolerant member of the woody plants in New Zealand (Perry *et al.*, 2006). It was therefore of interest to investigate the antibacterial activities of the bioactive against the common skin pathogen, *P. acnes*. This information would add value to the local economy and also bring high commercial value to develop potential acne products. To date, there is no published scientific study on the antibacterial activity of MTO against *P. acnes*. Hence, the antibacterial activity of MTO towards this bacterium reported in this study could not be compared to any previous published scientific research. Nevertheless, species of *P. acnes* are known as small Gram positive rods. Gram-positive microorganisms share similar cellular membrane structure. Therefore, the antibacterial activities of MTO against other Gram-positive bacteria were compared with the results obtained from this study. MTO has been reported to possess strong antibacterial activity against Gram-positive bacteria (Christoph *et al.*, 2000; van Klink *et al.*, 2005; Maddocks-Jennings *et al.*, 2005). MTO can inhibit the growth of MRSA, *E. coli*, *P. aeruginosa* and *C. albicans* at concentrations ranging from 0.039 - 1.25 % (v/v) (Perry *et al.*, 1999, Porter and Wilkins, 1999, Lis-Balchin *et al.*, 2000 and van Klink *et al.*, 2005).

In this study, MTO showed strong antibacterial activity against *P. acnes* (Figure 4.33 and Table 4.4). A disc (θ = 6 mm) loaded with 200 μg MTO produced an inhibition zone with θ of 12.5 mm. A disc loaded with 400 μg MTO generated a larger inhibition zone (θ = 21.2 mm). The results obtained in this study were similar to the study of Perry *et al.* (1999). MTO from East Cape used by Perry *et al*. (1999) was most active against *Bacillus subtilis* with MIC of 150 μg per disc giving an inhibition zone of 7 mm; MTO from north of New Zealand had an MIC of 600 mg disc with 7 mm inhibition zone; MTO from south of the country showed no inhibitory effects. The antibacterial activity of MTO towards *P. acnes* showed some similarity with MTO towards *B. subtilis* sourced from East Cape of New Zealand. In addition, *P. acnes* and *B. subtilis* are Gram positive, rod shaped, and can tolerate oxygen, but unlike *P. acnes*, *B. subtilis* has the ability to form a tough, protective endospore and it could be classified as obligate aerobe (Sonenshein *et al.*, 2002). By comparing both results, the disc diffusion method is effective to investigate the antibacterial activity of *P. acnes*.

In this study, the spectrophotometric assay using the microtiter plate proved to be a quick and efficient method to analyse the antibacterial activity of MTO against the *P. acnes* with MIC of 2.11 mg/mL (or 0.211 % w/v) and MBC of 2.5 mg/mL (or 0.25 % w/v). Porter *et al*. (1999) showed that the MBC of Gram positive bacteria were about 0.039 % for *S. aureus* and 0.0195 % for MRSA.

Time-to-kill curves (Figure 4.47) indicated that MTO is capable of killing *P. acnes* during incubation, although the killing rate was not as rapid as that of the green tea solution against the bacterium. There is however limited information about how the MTO kills *P. acnes* at different concentrations; hence no direct comparison with published results can be done.

The results from this study showed that MTO has strong bacteriostatic and bactericidal activity against the growth of *P. acnes* at relatively low concentrations. During this study, it was noted that MTO had a strong fragrance and the colour of the essential oil was very dark. The smell of MTO was over-powdering and rather unpleasant even at 0.25 %. It may therefore be necessary to reduce the concentration of the MTO if it is applied in a commercial product. Therefore, use of 0.125 % MTO is recommended in the cream emulsion instead of 0.25 % MTO.

5.3.2 The antibacterial activity of lavender oil

Unlike MTO, the lavender oil used in our study produces an enchanted, soothing, calming and relaxing fragrance. The essential oil is therefore suitable to formulate into commercial cosmetic products. Lavender oil was capable of inhibiting the growth of *P. acnes*. In comparison to the MTO, the antibacterial activity of LO was much weaker with an MIC_{90} of 54.66 mg/mL (≈5.5 % v/v); the paper disc absorbed LO and therefore could not produce a zone of inhibition on the plate fully cultivated with *P. acnes.* Moreover, LO did not have killing ability towards *P. acnes* showed in this study.

The weak antibacterial activity of LO was not only against the growth of *P. acnes,* but LO also appeared to have weak inhibitory effect on many other microorganisms (Roller *et al*., 2009). Some studies showed that inhibitory activities of lavender oil was poor but other reports have indicated moderate inhibitory activity (Chao *et al*., 2006, Prabuseenivasan *et al*., 2006; Roller *et al*., 2009).

MICs of LO largely differ among the types of susceptibility methods due to variations in dissolving methods, bacterial species and lavender oils. Using the microdilution method, MIC of 0.25 - 0.5 % (v/v) was effective against *E. coli*; for *S. aureus*, the MIC was 1 % (v/v) and MIC for *P. aeruginosa* was greater than 2 % (v/v) (Hammer *et al*., 1999). LO has showed a stronger antibacterial effect on *E. coli* (Gram negative organism) than *S. aureus* (Gram positive bacteria). LO (10 %) inhibited the growth of *E. coli* by creating a weak zone of inhibition (θ = 10.5 mm). Therefore, 50 % of LO was required to show antibacterial activity towards *S. aureus* with a medium zone of inhibition (9 mm). Furthermore, the study of Chao *et al.* (2006) showed that LO (*Lavandula stoechas*) had strong antibacterial activity with both MIC and MBC value of 0.125 % (v/v). LO at 0.25 % could completely kill *P. acnes* after 5 min. (Zu *et al*., 2010). Due to the large differences in published data, it is therefore difficult to make consistent comparisons with our results.

5.3.3 The antibacterial activity of green tea extract

Tea is one of the most popular beverages consumed worldwide. Many authors have reported that green tea has demonstrated high antibacterial activity against a wide range of pathogens since early 1957 (Sakanaka *et al*., 1989; Kubo *et al*. 1992; Lee *et al*., 2006). These findings were confirmed in our investigation.

In the study of Kudo *et al*., (1992), GTE showed bacteriostatic ability against the growth of *P. acnes* with MICs between 3.13 and 400 μg/mL. This antibacterial activity was confirmed by Lee *et al*., (2006), who showed that the MIC was in the range of 63 to 130 μg/mL. The data from our study also showed that green tea extract contained bacteriostatic and bactericidal ability towards *P. acnes*. A higher concentration was used against the growth of *P. acnes*. Using paper discs containing 100 μg GTE could produce clear zones of inhibition. The solution of GTE was very dark. Brown precipitates were observed at the bottom of the microtiter plate during incubation. Therefore, the spectrophotometric assay using the microtiter plate reader was not a suitable susceptible testing method for detecting the antibacterial activity of GTE.

In the present study, GTE solution at 2.5 mg/mL was capable of killing the *P. acnes* after 24 h. The time-to-kill curves illustrated that the GTE could kill *P. acnes* faster than the MTO (Figure 4.47). According to our knowledge, this study represents the first report to reveal the killing effect of GTE against *P. acnes in vitro*. Moreover, Toda *et al.* (1991) found that GTE could kill *S. aureus*, *S. epidermidis*, *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella flexneri*, *Shigella dysenteriae* and *Vibrio* spp. Later, Toda *et al*. (1992) also reported that green tea inhibited the growth of MRSA at 'cup-of-tea' level (3 mg /mL).

The distinctive antibacterial activity of GTE was due to the presence of catechins (ECG and EGCG) in the tea solution. Si *et al.* (2006) showed that treatment of EGCG could affect the bacterial cell division process. *S typhimurium* cells form chains or clump of cells in the presence of EGCG due to incomplete separation of cells. For *B. cereus* cells using the same treatment of EGCG at 650 μL/mL, cells appeared to be locked in cellular division. GTE solution can interfere with bacterial cell division, leading to cell deformation from the typical long rod shapes to short rods or coccus like shapes. Similar results were observed with ECG-treated cells (Si *et al*., 2006).

5.3.4 The antibacterial activity of olive leaf extract

The OLE have been traditionally used for medicinal purposes for combating fevers and disease such as malaria (Bevavente-garcia *et al.*, 2000). OLE is reported to aid the treatment of a broad range of infectious diseases if ingested (Sudjana *et al*., 2009). The antibacterial activity of the OLE was studied extensively against a wide range of microorganisms (n > 100) (Myers *et al*., 2006). Results from Myers *et al*. (2006) suggested that the OLE possessed a weak antibacterial activity, among all the microorganisms tested, it was most active against fastidious bacteria *Campylobacter jejuni* (MIC of 0.3-2.5 %, v/v), *Helicobacter pylori* (MIC of 0.6-1.2 %, v/v) and MRSA (MIC of 0.8-12.5 %, v/v). The OLE showed little activity against other test organisms (n = 79), with MICs ranging from 6.25 % to 50 % (v/v). Furthermore, the OLE showed poor activity against *B. subtitils*, Gram negative bacteria and fungi with MICs greater than 50 %. A study of Klancnic *et al.* (2010) suggested that the MIC of OLE was about 20 mg/mL for *B. cereus*, 40 mg/mL against *S. aureus*, 10 mg/mL against *Campylobacter jejuni* and *Campylobacter coli*.

Although the antibacterial activity of OLE was reported differently due to the diversity of the chemotype, the antibacterial activity results from our present study confirmed the results obtained by Myers *et al*., (2006). Our results showed that the OLE possessed weak bacteriostatic activity against the growth of *P. acnes* with predicted MIC_{90} of 179.14 mg/mL (equivalent to 24 %, v/v). None of the published reports mentioned the killing ability of OLE against the microorganisms. Our study, therefore, could be the first to report about the bactericidal ability of OLE towards *P. acnes* with a MBC of 240 mg/mL.

According to the results obtained in this study, the OLE exhibited bacteriostatic and bactericidal ability against *P. acnes*, but in comparison to the MTO and GTE, OLE has much weaker antibacterial activity than the other bioactives. Hence, OLE may not be recommended as suitable for the development of potential anti-acne products in the form used in this study.

5.3.5 The antibacterial activity of Propolis

Like most natural bioactives, propolis possesses complex chemical profiles (Markham *et al*., 1996). More than 100 components have been detected in propolis, of which, only flavonoids and phenolics are known for their antibacterial and antioxidant activities (Pietta *et al*., 2002). Antibacterial activity of propolis is bacteriostatic and can be bactericidal in high concentrations (Boyanova *et al*., 2006).

In general, propolis cannot be used as a raw material; it must be purified by extraction with solvents. A multi-step extraction with ethanol is particularly suitable to obtain de-waxed propolis extracts rich in polyphenolic components, which are commonly known as the ethanol extract of propolis (EEP) (Burdock, 1998). The presence of ethanol could play a part in detecting antibacterial activity of the EEP and the true bioactive action of propolis may not be revealed. Moreover, it is not desirable to include ethanol in the potential acne produce.

Unlike the dissolving method of propolis mentioned previously in the studies of Pietta *et al.* (2002) and Boyanova *et al.* (2006), the propolis was dissolved in the aqueous solution containing 1 % Tween 20 and DMSO in this study. The advantage of this method was to avoid using ethanol during the preparation. Hence, ethanol could not have affected the results during the investigation of the antibacterial activity. The disadvantages of this practice were that, firstly, little amount of propolis could be solubilised in the aqueous solution; secondly, the chemical materials with bioactive properties could not be released into the aqueous solution and hence, limited amount of polyphenolic fractions of propolis was dissolved in the solution. Therefore, it is assumed that based on this present solubilisation method of propolis, some active components might be not as active as shown in the EEP solution tested elsewhere.

The antibacterial activities of propolis are normally screened by an agar-well or disc-diffusion method (Weston *et al*., 2000; Nagai *et al*., 2001; Nasuti *et al*., 2006). In our study, there was no zone of inhibition found around the paper disc loaded with 0.048 to 0.012 mg of propolis per disc. However, in the study of Boyanova *et al*. (2006), 27 mg propolis (90 µL, 30 % propolis) was shown to be highly active against all 13 strains of *P. acnes* with a large mean inhibitory zone diameter (17 mm). The antibacterial activity of propolis at very high dosage (9-27 mg per well) was suggested to damage the cytoplasmic membrane and inhibition of bacterial motility and enzyme activity of *P. acnes*. Although paper disc diffusion was not capable of detecting the antibacterial activity of propolis, the microdilution method predicted the MIC_{90}atabout 550 mg/mL.

Comparison of the results obtained in this study with previous published results is always problematic for natural bioactives. The composition of plant oils and extracts are known to vary according to local climatic and environmental conditions (Steinberg *et al*., 1996). Some bioactive materials with similar names may be largely different in chemical profile and plant species. Secondly, the methods used to assess antimicrobial activity, and the choice of test organisms vary between publications. The disc diffusion method is frequently used as a preliminary test to screen the plant extracts and plant essential oils.

However, most plant essential oils and extracts are hydrophobic in nature, which prevent the uniform diffusion of these substances through the agar medium (Hammer *et al.*, 1999). The broth dilution method is also commonly used and many factors could affect the results. The factors include differences in microbial growth, exposure of micro-organisms to plant oil, the solubility of oil or oil components and the use and quantity of emulsifier. These and other elements may account for the large differences in MIC obtained by the broth dilution method in this study.

5.4 Combined effects of honey and bioactives

Results obtained in this study have shown that MTO and GTE exhibited bacteriostatic and bacteriocidal ability against the growth of *P. acnes* with an average MIC value of 0.25 % (w/v). However, only Manuka honey 10+ UMF demonstrated bacteriostatic ability against *P. acnes* (Figure 4.47). Furthermore, the growth of *P. acnes* was significantly reduced in 10 % Manuka honey 10+ UMF and there was no bacterial growth up to 15 h. Moreover, the MIC value of Manuka honey 10+ was approximately 12.5 % (w/v). Based on the time-to-kill curves (Figure 4.48), the initial bacterial number (CFU/mL) decreased by half in the Manuka honey during the first 6 h, which was followed by a steady bacterial growth. It seems that Manuka honey 10+ has weak and instant bactericidal ability, and the killing effect was only noticeable in the first few hours (< 6 h).

By combining honey with GTE and MTO, a synergistic effect was observed (Figures 4.48). The killing effects of these honey combinations were much stronger than the killing rates measured in GTE, MTO or honey solution alone. Hence, the potential anti-acne products should contain honey and bioactives (GTE, MTO or both GTE and MTO). Based on this formulation, various formulations were made in the cream base.

5.5 Evaluation of the antibacterial activity of formulated emulsion creams

The emulsion creams were prepared by mixing 10 % Manuka honey and other active ingredients together in the emulsion base. In order to maintain their antibacterial concentrations, caution must be exercised during sample preparation.

Heat was applied to melt the emulsifier. Cream base was prepared by mixing the melted emulsifier with glycerine. The melting temperature of emulsifier was around 65 °C and the solidified temperature was approximately 40 °C (Appendix C6). Many chemical components found in the honey, MTO and GTE may be sensitive to heat. Hence, it is important to avoid mixing honey, MTO and GTE in hot emulsion cream bases at 65 °C and above. The preferable temperature could be 45 – 50 °C. Once the temperature had dropped below 40 °C, the liquid mixture of emulsifier and glycerine became very thick, hence, the honey and MTO were unable to be evenly mixed in the cream base. A water bath was therefore used to control the temperature of the emulsifier. In addition, it is also important to dissolve the GTE powder in the warm water (45 – 50 °C) first before mixing with the cream base.

The antibacterial activities of the creams containing honey, MTO, GTE single components were evaluated first. It was found that 10 % honey in the cream was capable of inhibiting the growth of *P. acnes*. Creams containing MTO (0.125 % to 0.5 %) and GTE (0.125 % to 1 %) possessed strong bactericidal ability, which caused 90-99.9 % reduction in bacterial numbers. The killing effects were clearly strengthened when honey was combined with GTE or both GTE and MTO; results showed that there were no bacterial cells in these creams and 100 % of inhibition was achieved. When comparing the antibacterial activity of the newly developed honey creams with the existing commercial products (Derma™ cream and a daily 'moisturiser' from Comvita® NZ Ltd), our developed cream containing 10 % honey, 1 % GTE and 0.125 % MTO exhibited much stronger killing ability than Derma™ cream, but surprisingly, the 'moisturiser' also had equivalent killing ability as our newly developed cream. Therefore, it would be of interest to investigate the unknown ingredients in the 'moisturizer', which showed promising killing ability against the *P. acnes*.

6.0 Conclusion

The growth of *P. acnes* was evaluated under both aerobic and anaerobic environment. *P. acnes* was capable of growing under both incubation conditions. It seemed that *P. acnes* grows better under anaerobic environment. The presence of oxygen showed negative impact on the growth of *P. acnes* on the FA agar under aerobic environment, but the gas did not affect the growth of *P. acnes* when incubated in nutrient broth. When grown on surface agar, *P. acnes* produced circular raised colonies which were creamy, smooth, glistening and had an entire edge with an average 1 mm diameter colony. *P. acnes* cells were Gram positive, non-spore forming and non-motile. The cellular morphology was rods in shape and the size ranged from 0.03-0.06 by 0.05 to 0.3 μm.

The growth of *P.acnes* in the Manuka honey (Manuka honey 10+, 15+ and 20+ UMF) and Kanuka honey were investigated and compared with the growth of *P. acnes* in the nutrient BHI alone as positive control. The non-peroxide activities of the honeys were also studied under both aerobic and anaerobic environment. Manuka honey and Kanuka honey exhibited antibacterial activity against the growth of *P. acnes*.

Results also showed that Kanuka honey had similar antibacterial activity as Manuka honey 15+ UMF and Manuka honey 20+ UMF. The growth curves of *P. acnes* were markedly below the positive control; the inhibitory effects of honey samples were observed at 2.5 % for Manuka honey 15+ UMF, Manuka honey 20+ UMF and Kanuka honey. Nevertheless, it required Manuka honey 10+ UMF at 7.5 % or higher concentration in order to express their inhibitory effect against *P. acnes.*

Dose-response curves of honey samples showed that the percentage of inhibition had positive correlation with the concentrations of Manuka honey and Kanuka honey without catalase. Based on regression relationships, the MIC_{100} of Manuka honey 20+ UMF was 148.90 mg/mL (aerobic condition) and 112.59 mg/mL (anaerobic condition).TheMIC_{100} of Manuka honey 15+ UMF was 125.81 mg/mL (aerobic) and 116 mg/mL (anaerobic); the MIC_{100} of Manuka honey 10+ UMF was 144.43 mg/mL (aerobic) and 144.50 mg/mL (anaerobic); the MIC_{100} of Kanuka honey was 123.28 mg/mL (aerobic) and 119.66 mg/mL (anaerobic). With the addition of catalase, Manuka honey samples demonstrated non-peroxide activity and the antibacterial activity of Kanuka honey significantly ($p \leq 0.05$) decreased after the exclusion of hydrogen peroxide with MIC_{100} of 549.21 mg/mL (aerobic) and 549.20 mg/mL (anaerobic). Artificial honey did not show any antibacterial activity against *P. acnes* at lower than 12.5 %.

Five bioactives (Manuka tree oil, lavender essential oil, green tea extract, olive leaves extracts and propolis) were screened using a combination of methods. All the bioactives demonstrated antibacterial activity against the growth of *P. acnes* and only green tea extract and Manuka tree oil had bactericidal ability to kill the bacterium (Figure 4.47).

The killing rate of GTE was greater than MTO. When GTE was combined with honey, and MTO was combined with honey, the killing effects against *P. acnes* were higher than the bioactives used singly.

The antibacterial activity of formulated creams containing honey, MTO and GTE were studied separately and in combination. The creams containing 10 % honey demonstrated bacteriostatic ability. Creams containing MTO (0.125 - 0.5 %) and GTE (0.125– 1 %) exhibited bactericidal ability against *P. acnes* and the cell counts decreased rapidly. Cream containing 10 % honey and 1 % GTE caused approximately 5 log reductions in cell numbers; cream containing 10 % honey and 0.125 % MTO caused about 2 log reductions in cell numbers. No bacterial cells were detected at 10^{-2} dilution were found in the cream containing honey (10 %), MTO (0.125 %) and GTE (1 %). It is therefore evident that the honey containing both MTO and GTE had the most bactericidal ability against *P. acnes.*

7.0 Recommendations

Based on the experimental results and discussion, a few recommendations can be made for the further work:

1. Some studies showed that honey and GTE could damage the integrity the cell membrane of Gram positive cells (Henriques *et al*. 2006; Si *et al*. 2006). Scanning electron microscope (SEM) could be used to study the cellular components in greater detail; any change in the cellular integrity due to addition of honey and GTE in the treatments may be revealed. It therefore could be of interest to study the changes of cellular morphology of *P. acnes* when the bacterium is subjected to Manuka honey and honey creams containing MTO and GTE.

2. Study of the biochemical characteristics of *P. acnes* is suggested. The current study observed that the growth of *P. acnes* was stimulated by 10 % Manuka honey 10+ (with and without catalase) and Kanuka honey (with catalase) at 6 % and below.

3. It is also of interest to determine, if the Manuka honey inhibits *P. acnes* induced secretion of preinflammatory factors in skin follicles using a cell culture based method

4. The honey-based creams containing MTO and GTO exhibited strong antibacterial activities against the growth of *P. acnes in vitro*. The promising antibacterial activities of the formulations could be conducted with human trials.

5. It would be desirable to investigate the acceptability or likeness of the honey creams in terms of texture, appearance, fragrance and skin-feels after application using appropriate consumer panels.

6. Further analysis of the commercial product 'Moisturizer' from Comvita is recommended, because it showed bactericidal ability against *P. acnes* in this study. The antibacterial activity could be related to the presence of ZnO in the product. Raghupathi *et al*. (2011) suggested that ZnO nano-particles could be developed as antibacterial agents against a wide range of microorganisms. Gordon *et al*. (2011) also suggested ZnO nano-particles exhibit the high bactericidal activity; the compound can kill *S. aureus* and *E. coli* at 0.3 %.

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APPENDICES

Appendix A: Raw data of spectrophotometric assay

A.1 The growth of *Propionibacterium acnes* (Abs $_{595nm}$)

Table A1-1 Measuring the growth of *P. acnes* under aerobic condition recorded as increase of Abs$_{595nm}$ from time 0 – 24 hours

Time (hr)	Replications							
	1	2	3	4	5	6	7	8
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0.025	0.0282	0.0257	0.0268	0.0291	0.0331	0.0218	0.0203
3	0.0445	0.0503	0.0523	0.0555	0.06	0.0666	0.0427	0.0387
4	0.0668	0.0746	0.0749	0.0811	0.091	0.0982	0.0644	0.0562
5	0.0832	0.0938	0.0948	0.1074	0.1199	0.1275	0.0888	0.0787
6	0.1005	0.1158	0.1155	0.134	0.1477	0.1568	0.1121	0.1002
7	0.1188	0.1379	0.1407	0.1596	0.175	0.1869	0.1349	0.1205
8	0.1413	0.1545	0.1684	0.1836	0.2034	0.218	0.1582	0.1396
9	0.1585	0.1826	0.1917	0.209	0.2305	0.2492	0.1816	0.1616
10	0.1783	0.2192	0.2044	0.232	0.2575	0.2793	0.2063	0.1812
11	0.2161	0.229	0.2242	0.2578	0.2878	0.311	0.2279	0.2037
12	0.2192	0.229	0.2466	0.2857	0.3161	0.3422	0.2531	0.2271
13	0.235	0.2183	0.2886	0.3095	0.3441	0.3704	0.28	0.2492
14	0.2639	0.2317	0.2794	0.3375	0.368	0.4021	0.3071	0.2753
15	0.2479	0.2433	0.2961	0.3581	0.3893	0.4279	0.3337	0.299
16	0.2318	0.2638	0.3142	0.379	0.4152	0.4475	0.3591	0.325
17	0.3072	0.2497	0.3522	0.402	0.4373	0.4732	0.3848	0.3471
18	0.3088	0.3034	0.3694	0.4267	0.4568	0.499	0.4096	0.3708
19	0.3104	0.3017	0.379	0.4457	0.4708	0.5469	0.4366	0.3955
20	0.3576	0.3252	0.4062	0.4735	0.481	0.5224	0.4531	0.4121
21	0.401	0.3389	0.4072	0.4959	0.5106	0.5334	0.4761	0.432
22	0.3831	0.3618	0.4187	0.5263	0.5611	0.5684	0.4967	0.4665
23	0.438	0.4124	0.4434	0.5321	0.5919	0.5891	0.5165	0.482
24	0.4694	0.4208	0.4569	0.5554	0.6079	0.6125	0.5478	0.4928

Table A1-2 Measuring the growth of *P. acnes* under aerobic condition recorded as increase of Abs_{595nm} from time 25 – 48 hours

Time (hr)	Replications							
	1	2	3	4	5	6	7	8
25	0.4845	0.4378	0.4701	0.5788	0.6272	0.6518	0.5616	0.501
26	0.5106	0.4538	0.4881	0.6235	0.58	0.6762	0.5702	0.5085
27	0.5294	0.4846	0.5221	0.5839	0.6616	0.6856	0.6151	0.5435
28	0.5623	0.5014	0.5439	0.6782	0.6969	0.691	0.6068	0.564
29	0.6004	0.5293	0.5751	0.6962	0.7238	0.7086	0.6409	0.5972
30	0.6256	0.5521	0.5905	0.7523	0.6735	0.7185	0.666	0.6581
31	0.6605	0.5856	0.617	0.7021	0.7786	0.7601	0.7094	0.6371
32	0.6895	0.6112	0.6599	0.7521	0.7451	0.8009	0.7548	0.6716
33	0.7155	0.6376	0.6904	0.5529	0.652	0.8347	0.8097	0.755
34	0.7463	0.661	0.716	0.6679	0.8755	0.8768	0.7794	0.7527
35	0.7739	0.6858	0.752	0.8906	0.8897	0.9185	0.8368	0.8105
36	0.7887	0.7106	0.7611	0.9078	0.8329	0.9558	0.8762	0.8563
37	0.8007	0.7256	0.7781	0.9082	0.9019	0.9885	0.9543	0.8009
38	0.803	0.7301	0.7816	0.87	0.8996	1.002	0.9739	0.8886
39	0.8021	0.7329	0.7899	0.8822	0.9026	1.0137	0.9871	0.9312
40	0.8013	0.7302	0.7954	0.894	0.9079	1.0322	1.0033	0.9308
41	0.7978	0.7299	0.8054	0.9003	0.9118	1.0456	1.0137	0.9373
42	0.8041	0.7365	0.8142	0.8955	0.9176	1.0617	0.9619	0.9381
43	0.8145	0.7388	0.8266	0.8995	0.8114	1.0847	0.985	0.942
44	0.8119	0.7461	0.8256	0.8986	0.8499	1.0973	0.9996	0.9576
45	0.8155	0.7516	0.8437	0.9074	0.8996	1.1117	1.0064	0.9727
46	0.8171	0.7592	0.856	0.9039	0.912	1.1254	1.0112	0.9622
47	0.8137	0.7634	0.8552	0.9193	0.9244	1.1392	1.0194	0.9838
48	0.8172	0.7663	0.8522	0.9197	0.9452	1.1467	1.0287	0.9935

Table A1-3 Measuring the growth of *P. acnes* under anaerobic condition recorded as increase of Abs_{595nm} from time 0 – 24 hours

	replications							
Time (hr)	1	2	3	4	5	6	7	8
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0.0202	0.0222	0.022	0.0197	0.0121	0.0192	0.0327	0.0332
3	0.0399	0.0443	0.0466	0.0379	0.0246	0.0391	0.0623	0.0608
4	0.0606	0.0644	0.0677	0.0587	0.0438	0.0585	0.0886	0.0887
5	0.0791	0.0823	0.0907	0.0764	0.0608	0.0791	0.1172	0.1131
6	0.1019	0.1052	0.1129	0.0956	0.0821	0.0997	0.1461	0.1436
7	0.1261	0.1308	0.1379	0.1174	0.0977	0.1217	0.1794	0.1737
8	0.1713	0.1573	0.1639	0.1416	0.119	0.1464	0.2132	0.2102
9	0.2332	0.1911	0.1889	0.1672	0.1365	0.1698	0.2536	0.248
10	0.2763	0.2442	0.2154	0.1953	0.1569	0.1969	0.2937	0.2874
11	0.3056	0.2879	0.2397	0.2214	0.1749	0.2213	0.3375	0.3287
12	0.3217	0.3389	0.2795	0.2448	0.1927	0.2464	0.3776	0.3687
13	0.3319	0.3786	0.315	0.2589	0.21	0.2734	0.4135	0.4029
14	0.3416	0.4036	0.358	0.274	0.2323	0.2998	0.4426	0.4329
15	0.4294	0.4217	0.3953	0.3024	0.2527	0.3286	0.4699	0.4612
16	0.4346	0.4402	0.4475	0.3226	0.2716	0.3566	0.4979	0.4893
17	0.4594	0.4727	0.4978	0.341	0.2887	0.3817	0.5283	0.5193
18	0.4889	0.5202	0.5545	0.3589	0.3056	0.4066	0.5569	0.549
19	0.5106	0.5634	0.6043	0.3725	0.3204	0.4286	0.5873	0.5805
20	0.5338	0.6029	0.6515	0.3768	0.3388	0.452	0.6086	0.602
21	0.5557	0.6489	0.7043	0.4078	0.349	0.4723	0.626	0.6159
22	0.5813	0.68	0.7526	0.4902	0.3892	0.4985	0.6397	0.6296
23	0.6066	0.7197	0.8023	0.5677	0.4159	0.5306	0.6532	0.6424
24	0.6389	0.7677	0.8479	0.5961	0.4442	0.5607	0.6729	0.6595

Table A1-4 Measuring the growth of *P. acnes* under anaerobic condition recorded as increase of Abs_{595nm} from time 25 – 48 hours

Time (hr)	replications							
	1	2	3	4	5	6	7	8
25	0.665	0.8093	0.8628	0.6212	0.4852	0.5937	0.6801	0.6698
26	0.6839	0.7817	0.8594	0.6429	0.5212	0.6218	0.6901	0.6805
27	0.7021	0.7896	0.8563	0.67	0.5562	0.6513	0.7002	0.6919
28	0.7244	0.8014	0.8609	0.6808	0.5889	0.6815	0.7058	0.7007
29	0.7446	0.8139	0.8722	0.6611	0.6101	0.7061	0.7129	0.7077
30	0.7611	0.8243	0.8827	0.6787	0.6263	0.7341	0.7177	0.715
31	0.7792	0.8362	0.8921	0.6945	0.6698	0.7602	0.7289	0.7304
32	0.7951	0.8475	0.9007	0.7078	0.6885	0.7805	0.7363	0.7381
33	0.806	0.855	0.9096	0.7176	0.7096	0.8034	0.7452	0.747
34	0.8098	0.8575	0.9157	0.7283	0.7078	0.8194	0.7472	0.7523
35	0.8142	0.8607	0.9212	0.7404	0.7283	0.8226	0.758	0.7636
36	0.8172	0.8613	0.9255	0.7535	0.7661	0.8317	0.7684	0.7748
37	0.8212	0.8643	0.934	0.7685	0.782	0.844	0.7766	0.7831
38	0.8256	0.8677	0.9396	0.7837	0.7793	0.8537	0.7862	0.7908
39	0.8308	0.8734	0.9477	0.7963	0.7847	0.8649	0.7954	0.8006
40	0.8377	0.8793	0.9536	0.8105	0.7957	0.8798	0.8028	0.8073
41	0.8464	0.8873	0.9635	0.8269	0.8021	0.8933	0.8064	0.8132
42	0.8521	0.8915	0.9678	0.8395	0.7999	0.9087	0.8171	0.8218
43	0.8553	0.8952	0.9684	0.8522	0.801	0.9253	0.8239	0.827
44	0.8568	0.8959	0.9778	0.8653	0.8044	0.9425	0.83	0.8334
45	0.8706	0.9082	0.9917	0.8778	0.8238	0.9561	0.8342	0.8383
46	0.888	0.9252	1.0027	0.8932	0.823	0.9728	0.8426	0.8469
47	0.9022	0.9382	1.0182	0.9057	0.8257	0.9899	0.846	0.8519
48	0.9174	0.9515	1.0305	0.9219	0.8388	1.0045	0.8556	0.861

A.2 The growth of *P.acnes* in honeys (Abs $_{595nm}$)

Table A2-1 Growth of *P. acnes* (Abs $_{595nm}$) in 0.5 % honeys under aerobic condition

Rep	time	A0.5	AC0.5	B0.5	BC0.5	C0.5	CC0.5	K0.5	KC0.5
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.02	0.02	0.03	0.02	0.03	0.02	0.03	0.02
1	3	0.05	0.03	0.06	0.03	0.06	0.04	0.04	0.04
1	4	0.07	0.05	0.09	0.05	0.08	0.06	0.06	0.05
1	5	0.10	0.06	0.12	0.06	0.11	0.07	0.08	0.06
1	6	0.12	0.07	0.14	0.07	0.13	0.08	0.09	0.07
1	7	0.15	0.08	0.16	0.08	0.14	0.09	0.10	0.08
1	8	0.17	0.08	0.19	0.09	0.18	0.10	0.11	0.09
1	9	0.20	0.09	0.20	0.10	0.21	0.11	0.12	0.10
1	10	0.21	0.10	0.22	0.11	0.30	0.12	0.14	0.10
1	11	0.23	0.11	0.24	0.12	0.48	0.14	0.15	0.12
1	12	0.25	0.12	0.26	0.14	0.66	0.17	0.16	0.13
1	13	0.26	0.13	0.27	0.15	0.76	0.25	0.17	0.15
1	14	0.29	0.15	0.30	0.16	0.83	0.37	0.19	0.17
1	15	0.30	0.16	0.31	0.18	0.88	0.52	0.21	0.19
1	16	0.30	0.18	0.33	0.20	0.89	0.69	0.23	0.21
1	17	0.32	0.20	0.34	0.22	0.93	0.81	0.24	0.25
1	18	0.33	0.22	0.36	0.25	0.96	0.82	0.26	0.29
1	19	0.37	0.25	0.38	0.29	1.00	0.88	0.28	0.34
1	20	0.35	0.28	0.39	0.33	1.03	0.94	0.30	0.40
1	21	0.38	0.32	0.42	0.37	1.07	0.98	0.32	0.46
1	22	0.43	0.36	0.45	0.42	1.11	1.04	0.35	0.53
1	23	0.45	0.40	0.48	0.47	1.15	1.09	0.37	0.59
1	24	0.51	0.44	0.51	0.52	1.19	1.13	0.40	0.64
1	25	0.54	0.48	0.55	0.56	1.22	1.17	0.44	0.70
1	26	0.54	0.53	0.59	0.62	1.25	1.20	0.47	0.77
1	27	0.60	0.58	0.64	0.68	1.28	1.24	0.51	0.85
1	28	0.58	0.64	0.68	0.75	1.29	1.27	0.55	0.91
1	29	0.67	0.70	0.70	0.81	1.30	1.30	0.59	0.97
1	30	0.71	0.76	0.74	0.86	1.30	1.32	0.62	1.02
1	31	0.80	0.81	0.78	0.91	1.31	1.33	0.66	1.05
1	32	0.85	0.85	0.83	0.94	1.32	1.33	0.69	1.08
1	33	0.89	0.88	0.88	0.97	1.32	1.34	0.73	1.10
1	34	0.91	0.91	0.92	1.00	1.32	1.34	0.76	1.13
1	35	0.94	0.93	0.97	1.02	1.32	1.34	0.80	1.14
1	36	0.98	0.95	1.01	1.03	1.32	1.34	0.85	1.15
1	37	1.01	0.96	1.05	1.05	1.32	1.34	0.88	1.17
1	38	1.03	0.98	1.09	1.06	1.32	1.34	0.92	1.18

Table A2-1 $_{cont.}$ Growth of *P. acnes* (Abs $_{595nm}$) in 0.5 % honeys under aerobic condition

Rep	time	A0.5	AC0.5	B0.5	BC0.5	C0.5	CC0.5	K0.5	KC0.5
1	39	1.06	0.99	1.12	1.07	1.32	1.34	0.95	1.18
1	40	1.08	1.00	1.15	1.07	1.32	1.34	0.99	1.19
1	41	1.09	1.01	1.17	1.08	1.32	1.35	1.02	1.20
1	42	1.11	1.01	1.19	1.08	1.32	1.35	1.04	1.20
1	43	1.12	1.02	1.21	1.09	1.32	1.35	1.06	1.20
1	44	1.13	1.02	1.23	1.09	1.32	1.35	1.09	1.21
1	45	1.13	1.03	1.24	1.10	1.32	1.35	1.10	1.21
1	46	1.12	1.03	1.25	1.10	1.32	1.35	1.12	1.21
1	47	1.13	1.04	1.27	1.11	1.32	1.36	1.13	1.22
1	48	1.13	1.04	1.28	1.11	1.32	1.38	1.15	1.22
2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.03	0.02	0.03	0.02	0.02	0.02	0.03	0.02
2	3	0.06	0.03	0.05	0.03	0.05	0.04	0.05	0.05
2	4	0.08	0.05	0.08	0.05	0.08	0.06	0.07	0.07
2	5	0.11	0.06	0.10	0.06	0.10	0.07	0.09	0.08
2	6	0.14	0.07	0.12	0.07	0.12	0.08	0.10	0.09
2	7	0.17	0.07	0.14	0.08	0.13	0.09	0.12	0.10
2	8	0.17	0.08	0.15	0.09	0.17	0.10	0.13	0.11
2	9	0.21	0.09	0.16	0.10	0.19	0.11	0.14	0.15
2	10	0.22	0.10	0.18	0.11	0.22	0.13	0.15	0.16
2	11	0.25	0.11	0.20	0.12	0.26	0.14	0.17	0.16
2	12	0.26	0.12	0.21	0.13	0.34	0.15	0.18	0.17
2	13	0.29	0.13	0.22	0.14	0.45	0.18	0.20	0.17
2	14	0.32	0.14	0.24	0.16	0.60	0.27	0.21	0.18
2	15	0.34	0.16	0.25	0.18	0.68	0.48	0.23	0.23
2	16	0.39	0.17	0.26	0.20	0.74	0.70	0.25	0.24
2	17	0.45	0.19	0.27	0.22	0.80	0.82	0.26	0.35
2	18	0.49	0.22	0.29	0.25	0.85	0.88	0.28	0.36
2	19	0.51	0.25	0.30	0.29	0.85	0.94	0.30	0.44
2	20	0.47	0.28	0.32	0.33	0.89	0.98	0.31	0.46
2	21	0.56	0.32	0.34	0.38	0.95	1.04	0.34	0.60
2	22	0.60	0.36	0.36	0.43	1.01	1.09	0.36	0.67
2	23	0.63	0.41	0.38	0.48	1.06	1.13	0.39	0.70
2	24	0.66	0.45	0.41	0.52	1.11	1.17	0.42	0.72
2	25	0.71	0.49	0.44	0.57	1.16	1.20	0.46	0.78
2	26	0.75	0.54	0.47	0.63	1.20	1.24	0.49	0.85
2	27	0.79	0.59	0.51	0.69	1.24	1.27	0.53	0.91
2	28	0.84	0.65	0.53	0.75	1.28	1.30	0.56	0.97
2	29	0.90	0.71	0.57	0.81	1.32	1.32	0.60	1.03

Table A2-1cont. Growth of *P. acnes* (Abs $_{595nm}$) in 0.5 % honeys under aerobic condition

Rep	time	A0.5	AC0.5	B0.5	BC0.5	C0.5	CC0.5	K0.5	KC0.5
2	30	0.95	0.76	0.60	0.86	1.35	1.33	0.63	1.07
2	31	0.99	0.81	0.63	0.90	1.38	1.33	0.66	1.12
2	32	1.02	0.85	0.67	0.94	1.39	1.34	0.70	1.13
2	33	1.06	0.88	0.70	0.96	1.40	1.34	0.74	1.15
2	34	1.08	0.90	0.74	0.99	1.40	1.34	0.77	1.16
2	35	1.11	0.93	0.77	1.01	1.39	1.34	0.81	1.17
2	36	1.13	0.95	0.80	1.02	1.39	1.34	0.85	1.18
2	37	1.15	0.96	0.83	1.04	1.39	1.34	0.88	1.18
2	38	1.17	0.97	0.86	1.04	1.39	1.34	0.91	1.18
2	39	1.18	0.98	0.89	1.05	1.39	1.34	0.93	1.19
2	40	1.19	0.99	0.91	1.06	1.39	1.35	0.96	1.19
2	41	1.19	1.00	0.94	1.06	1.39	1.35	0.98	1.19
2	42	1.19	1.00	0.95	1.07	1.39	1.35	0.99	1.19
2	43	1.20	1.01	0.97	1.08	1.39	1.35	1.01	1.19
2	44	1.20	1.01	0.99	1.08	1.38	1.35	1.02	1.19
2	45	1.21	1.02	1.00	1.09	1.39	1.35	1.03	1.19
2	46	1.21	1.02	1.00	1.09	1.39	1.36	1.05	1.20
2	47	1.21	1.02	1.01	1.09	1.39	1.36	1.05	1.20
2	48	1.21	1.03	1.01	1.10	1.39	1.36	1.06	1.20
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.03	0.01	0.03	0.02	0.03	0.02	0.03	0.02
3	3	0.05	0.03	0.05	0.04	0.06	0.04	0.05	0.05
3	4	0.07	0.04	0.07	0.05	0.09	0.05	0.06	0.07
3	5	0.10	0.05	0.09	0.07	0.12	0.07	0.08	0.08
3	6	0.11	0.06	0.11	0.08	0.14	0.08	0.09	0.09
3	7	0.14	0.07	0.13	0.09	0.16	0.08	0.10	0.10
3	8	0.14	0.08	0.13	0.10	0.19	0.09	0.11	0.11
3	9	0.16	0.08	0.16	0.10	0.20	0.10	0.11	0.12
3	10	0.20	0.09	0.18	0.11	0.23	0.11	0.12	0.13
3	11	0.21	0.10	0.19	0.13	0.30	0.13	0.12	0.14
3	12	0.24	0.11	0.21	0.14	0.51	0.16	0.13	0.16
3	13	0.27	0.12	0.22	0.15	0.71	0.27	0.14	0.18
3	14	0.28	0.14	0.24	0.17	0.80	0.51	0.15	0.20
3	15	0.33	0.15	0.26	0.19	0.85	0.72	0.16	0.22
3	16	0.30	0.17	0.27	0.21	0.88	0.83	0.17	0.25
3	17	0.33	0.19	0.30	0.23	0.90	0.88	0.19	0.29
3	18	0.32	0.21	0.31	0.27	0.93	0.93	0.20	0.34
3	19	0.38	0.24	0.33	0.31	0.97	0.97	0.21	0.40
3	20	0.36	0.27	0.35	0.35	1.01	1.01	0.23	0.46

Table A2-1$_{cont.}$Growth of *P. acnes* (Abs $_{595nm}$) in 0.5 % honeys under aerobic condition

Rep	time	A0.5	AC0.5	B0.5	BC0.5	C0.5	CC0.5	K0.5	KC0.5
3	21	0.36	0.31	0.37	0.40	1.04	1.06	0.24	0.53
3	22	0.41	0.35	0.39	0.45	1.08	1.10	0.26	0.60
3	23	0.44	0.39	0.41	0.49	1.12	1.12	0.29	0.66
3	24	0.49	0.44	0.44	0.54	1.16	1.15	0.31	0.72
3	25	0.53	0.48	0.48	0.59	1.19	1.19	0.34	0.78
3	26	0.58	0.53	0.51	0.65	1.23	1.23	0.37	0.85
3	27	0.63	0.58	0.55	0.71	1.26	1.26	0.40	0.91
3	28	0.67	0.64	0.59	0.77	1.29	1.27	0.44	0.97
3	29	0.71	0.70	0.63	0.83	1.32	1.28	0.48	1.03
3	30	0.75	0.75	0.67	0.88	1.34	1.28	0.51	1.07
3	31	0.77	0.80	0.70	0.92	1.35	1.28	0.55	1.10
3	32	0.80	0.84	0.73	0.96	1.35	1.29	0.59	1.13
3	33	0.84	0.87	0.76	0.98	1.35	1.29	0.64	1.15
3	34	0.87	0.90	0.79	1.01	1.34	1.29	0.68	1.16
3	35	0.89	0.92	0.82	1.02	1.34	1.30	0.72	1.17
3	36	0.92	0.94	0.84	1.04	1.34	1.30	0.77	1.18
3	37	0.95	0.95	0.86	1.05	1.34	1.30	0.80	1.18
3	38	0.96	0.97	0.88	1.06	1.34	1.30	0.84	1.18
3	39	0.98	0.97	0.90	1.07	1.34	1.30	0.87	1.19
3	40	0.99	0.98	0.91	1.08	1.33	1.30	0.90	1.19
3	41	1.00	0.99	0.92	1.08	1.33	1.30	0.92	1.19
3	42	1.01	1.00	0.93	1.09	1.33	1.30	0.94	1.19
3	43	1.01	1.00	0.94	1.09	1.33	1.30	0.96	1.19
3	44	1.02	1.00	0.95	1.10	1.33	1.31	0.97	1.19
3	45	1.03	1.01	0.96	1.10	1.33	1.31	0.99	1.19
3	46	1.03	1.01	0.96	1.11	1.33	1.31	1.00	1.19
3	47	1.04	1.02	0.97	1.11	1.33	1.32	1.01	1.19
3	48	1.04	1.02	0.98	1.11	1.33	1.32	1.02	1.18

Notes: A0.5=0.5 % Manuka honey 20+ UMF; AC0.5=0.5 % Manuka honey 20+ UMF with catalase; B0.5=0.5 % Manuka honey 15+ UMF; BC0.5=0.5 % Manuka honey 15+ UMF with catalase; C0.5=0.5 % Manuka honey 10+ UMF; CC0.5=0.5 % Manuka honey 10+ UMF with catalase; K0.5=0.5 % Kanuka honey; KC0.5=0.5 % Kanuka honey with catalase

Table A2-2 Growth of *P. acnes* (Abs $_{595nm}$) in 0.5 % honeys under anaerobic condition

Rep	time	A0.5	AC0.5	B0.5	BC0.5	C0.5	CC0.5	K0.5	KC0.5
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.02	0.04	0.02	0.03	0.03	0.03	0.02	0.03
1	3	0.04	0.07	0.04	0.05	0.06	0.05	0.03	0.06
1	4	0.06	0.09	0.06	0.07	0.09	0.07	0.05	0.08
1	5	0.07	0.11	0.07	0.08	0.11	0.10	0.07	0.10
1	6	0.10	0.13	0.09	0.10	0.13	0.12	0.09	0.12
1	7	0.11	0.15	0.11	0.12	0.11	0.14	0.11	0.15
1	8	0.13	0.17	0.13	0.14	0.10	0.16	0.13	0.17
1	9	0.15	0.19	0.15	0.16	0.11	0.18	0.15	0.19
1	10	0.17	0.21	0.17	0.18	0.11	0.20	0.18	0.21
1	11	0.19	0.23	0.18	0.20	0.11	0.22	0.20	0.24
1	12	0.20	0.25	0.20	0.22	0.12	0.24	0.24	0.26
1	13	0.22	0.27	0.21	0.24	0.12	0.26	0.27	0.28
1	14	0.24	0.30	0.23	0.26	0.12	0.28	0.30	0.31
1	15	0.26	0.32	0.25	0.28	0.12	0.30	0.33	0.33
1	16	0.27	0.34	0.26	0.30	0.13	0.32	0.35	0.36
1	17	0.29	0.37	0.28	0.32	0.13	0.34	0.38	0.38
1	18	0.30	0.39	0.29	0.36	0.14	0.36	0.44	0.41
1	19	0.32	0.41	0.30	0.37	0.15	0.38	0.40	0.44
1	20	0.33	0.44	0.32	0.38	0.16	0.40	0.48	0.49
1	21	0.34	0.46	0.34	0.40	0.16	0.42	0.49	0.53
1	22	0.42	0.48	0.44	0.42	0.19	0.44	0.50	0.58
1	23	0.49	0.51	0.49	0.44	0.23	0.46	0.50	0.63
1	24	0.53	0.53	0.52	0.46	0.28	0.48	0.50	0.67
1	25	0.56	0.56	0.55	0.46	0.42	0.51	0.53	0.72
1	26	0.59	0.59	0.58	0.51	0.20	0.53	0.55	0.76
1	27	0.61	0.62	0.61	0.53	0.23	0.56	0.59	0.80
1	28	0.59	0.65	0.63	0.55	0.26	0.59	0.60	0.84
1	29	0.61	0.69	0.68	0.58	0.28	0.62	0.62	0.87
1	30	0.62	0.72	0.64	0.61	0.29	0.65	0.62	0.91
1	31	0.64	0.76	0.66	0.64	0.31	0.69	0.67	0.93
1	32	0.65	0.80	0.68	0.67	0.33	0.73	0.67	0.96
1	33	0.67	0.83	0.70	0.70	0.34	0.76	0.70	0.97
1	34	0.68	0.86	0.72	0.73	0.36	0.79	0.72	0.99
1	35	0.69	0.89	0.74	0.75	0.38	0.82	0.72	1.00
1	36	0.71	0.93	0.76	0.78	0.40	0.86	0.75	1.01
1	37	0.73	0.95	0.78	0.81	0.42	0.89	0.76	1.02
1	38	0.75	0.98	0.80	0.85	0.44	0.91	0.76	1.02
1	39	0.76	1.01	0.82	0.87	0.46	0.94	0.77	1.02

Table A2-2 $_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 0.5 % honeys under anaerobic condition

Rep	time	A0.5	AC0.5	B0.5	BC0.5	C0.5	CC0.5	K0.5	KC0.5
1	40	0.78	1.04	0.84	0.88	0.48	0.97	0.78	1.03
1	41	0.80	1.06	0.86	0.92	0.50	0.99	0.80	1.03
1	42	0.82	1.09	0.88	0.94	0.52	1.01	0.81	1.02
1	43	0.84	1.11	0.90	0.97	0.54	1.04	0.82	1.02
1	44	0.85	1.13	0.92	1.01	0.56	1.05	0.84	1.02
1	45	0.87	1.15	0.94	1.02	0.58	1.07	0.86	1.02
1	46	0.89	1.17	0.96	1.05	0.60	1.08	0.87	1.02
1	47	0.91	1.19	0.98	1.08	0.63	1.09	0.88	1.02
1	48	0.93	1.21	1.01	1.09	0.65	1.09	0.90	1.02
2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.02	0.04	0.01	0.03	0.01	0.03	0.02	0.05
2	3	0.04	0.07	0.03	0.05	0.03	0.05	0.03	0.07
2	4	0.06	0.09	0.04	0.07	0.05	0.08	0.05	0.09
2	5	0.07	0.11	0.06	0.08	0.06	0.09	0.07	0.11
2	6	0.10	0.13	0.07	0.10	0.08	0.12	0.09	0.13
2	7	0.11	0.14	0.09	0.12	0.10	0.14	0.11	0.15
2	8	0.13	0.16	0.11	0.14	0.12	0.16	0.13	0.18
2	9	0.15	0.18	0.12	0.16	0.13	0.19	0.15	0.20
2	10	0.17	0.20	0.14	0.18	0.15	0.21	0.18	0.22
2	11	0.19	0.22	0.16	0.20	0.17	0.23	0.20	0.25
2	12	0.20	0.24	0.17	0.22	0.19	0.25	0.24	0.27
2	13	0.22	0.27	0.18	0.24	0.21	0.27	0.27	0.29
2	14	0.24	0.29	0.20	0.26	0.24	0.29	0.30	0.31
2	15	0.26	0.31	0.21	0.28	0.26	0.31	0.33	0.34
2	16	0.27	0.34	0.23	0.30	0.28	0.33	0.35	0.36
2	17	0.29	0.36	0.24	0.32	0.30	0.35	0.38	0.38
2	18	0.30	0.38	0.25	0.34	0.31	0.38	0.40	0.40
2	19	0.32	0.41	0.26	0.36	0.34	0.40	0.43	0.42
2	20	0.33	0.44	0.27	0.38	0.36	0.42	0.46	0.44
2	21	0.40	0.48	0.28	0.40	0.36	0.45	0.48	0.47
2	22	0.42	0.51	0.31	0.42	0.36	0.47	0.49	0.49
2	23	0.54	0.56	0.33	0.44	0.37	0.50	0.49	0.52
2	24	0.55	0.60	0.36	0.46	0.36	0.53	0.51	0.55
2	25	0.56	0.66	0.39	0.48	0.38	0.57	0.53	0.58
2	26	0.63	0.72	0.42	0.51	0.40	0.60	0.55	0.61
2	27	0.65	0.79	0.44	0.53	0.42	0.64	0.57	0.64
2	28	0.66	0.85	0.48	0.55	0.44	0.68	0.59	0.67
2	29	0.66	0.90	0.50	0.58	0.45	0.71	0.61	0.70
2	30	0.66	0.95	0.52	0.61	0.46	0.75	0.63	0.74

Table A2-2 $_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 0.5 % honeys under anaerobic condition

Rep	time	A0.5	AC0.5	B0.5	BC0.5	C0.5	CC0.5	K0.5	KC0.5
2	31	0.67	0.99	0.55	0.64	0.48	0.78	0.65	0.77
2	32	0.68	1.02	0.56	0.67	0.50	0.82	0.66	0.81
2	33	0.68	1.05	0.56	0.70	0.52	0.85	0.67	0.84
2	34	0.69	1.07	0.57	0.73	0.53	0.88	0.67	0.87
2	35	0.69	1.08	0.59	0.75	0.55	0.91	0.69	0.90
2	36	0.72	1.10	0.60	0.78	0.57	0.94	0.70	0.92
2	37	0.73	1.11	0.61	0.81	0.59	0.97	0.72	0.95
2	38	0.75	1.12	0.63	0.84	0.60	0.99	0.74	0.98
2	39	0.77	1.12	0.64	0.86	0.62	1.01	0.76	1.01
2	40	0.80	1.13	0.66	0.89	0.64	1.03	0.77	1.03
2	41	0.80	1.13	0.67	0.91	0.65	1.04	0.79	1.05
2	42	0.82	1.14	0.69	0.93	0.67	1.05	0.81	1.08
2	43	0.84	1.14	0.70	0.96	0.69	1.05	0.82	1.10
2	44	0.87	1.14	0.72	0.98	0.70	1.06	0.84	1.12
2	45	0.90	1.14	0.73	1.00	0.72	1.06	0.86	1.14
2	46	0.93	1.14	0.75	1.02	0.74	1.06	0.87	1.15
2	47	0.93	1.14	0.77	1.04	0.76	1.06	0.88	1.16
2	48	0.94	1.15	0.79	1.06	0.79	1.06	0.90	1.16
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.01	0.04	0.01	0.03	0.01	0.03	0.01	0.06
3	3	0.02	0.07	0.03	0.05	0.03	0.05	0.03	0.09
3	4	0.03	0.09	0.04	0.07	0.04	0.08	0.04	0.11
3	5	0.05	0.11	0.06	0.09	0.06	0.09	0.06	0.13
3	6	0.06	0.13	0.07	0.11	0.07	0.12	0.08	0.16
3	7	0.07	0.14	0.09	0.13	0.09	0.14	0.10	0.18
3	8	0.09	0.16	0.11	0.15	0.11	0.16	0.12	0.19
3	9	0.11	0.18	0.12	0.16	0.13	0.19	0.14	0.22
3	10	0.12	0.20	0.14	0.18	0.15	0.21	0.16	0.25
3	11	0.13	0.22	0.16	0.20	0.17	0.23	0.19	0.27
3	12	0.15	0.24	0.17	0.22	0.19	0.25	0.23	0.30
3	13	0.16	0.26	0.19	0.24	0.20	0.27	0.25	0.33
3	14	0.18	0.29	0.21	0.26	0.22	0.29	0.29	0.34
3	15	0.19	0.31	0.23	0.28	0.24	0.31	0.32	0.36
3	16	0.20	0.34	0.24	0.30	0.26	0.33	0.35	0.38
3	17	0.22	0.37	0.25	0.32	0.27	0.35	0.38	0.40
3	18	0.23	0.40	0.27	0.34	0.29	0.38	0.41	0.42
3	19	0.24	0.43	0.28	0.36	0.30	0.40	0.43	0.44
3	20	0.26	0.46	0.30	0.38	0.32	0.42	0.45	0.47

Table A2-2 $_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 0.5 % honeys under anaerobic condition

Rep	time	A0.5	AC0.5	B0.5	BC0.5	C0.5	CC0.5	K0.5	KC0.5
3	21	0.28	0.50	0.35	0.40	0.34	0.45	0.48	0.49
3	22	0.28	0.54	0.37	0.42	0.36	0.47	0.51	0.51
3	23	0.29	0.59	0.40	0.43	0.37	0.50	0.51	0.55
3	24	0.30	0.66	0.43	0.45	0.41	0.53	0.51	0.58
3	25	0.32	0.73	0.47	0.48	0.37	0.57	0.53	0.60
3	26	0.34	0.81	0.55	0.50	0.39	0.60	0.55	0.63
3	27	0.61	0.88	0.53	0.52	0.41	0.64	0.57	0.66
3	28	0.59	0.94	0.54	0.55	0.43	0.68	0.59	0.69
3	29	0.61	0.98	0.55	0.57	0.44	0.71	0.59	0.72
3	30	0.62	1.01	0.56	0.60	0.45	0.75	0.61	0.75
3	31	0.64	1.04	0.56	0.63	0.47	0.78	0.63	0.77
3	32	0.65	1.05	0.57	0.66	0.49	0.82	0.64	0.81
3	33	0.67	1.06	0.58	0.69	0.50	0.85	0.65	0.84
3	34	0.68	1.06	0.59	0.72	0.52	0.88	0.66	0.87
3	35	0.69	1.06	0.60	0.75	0.53	0.91	0.68	0.88
3	36	0.71	1.06	0.62	0.77	0.55	0.94	0.69	0.91
3	37	0.73	1.06	0.63	0.80	0.57	0.97	0.71	0.93
3	38	0.75	1.06	0.64	0.83	0.59	0.99	0.72	0.95
3	39	0.76	1.06	0.66	0.85	0.61	1.01	0.73	0.98
3	40	0.78	1.07	0.67	0.88	0.63	1.03	0.75	1.00
3	41	0.80	1.07	0.69	0.90	0.64	1.04	0.77	1.02
3	42	0.82	1.07	0.70	0.92	0.66	1.04	0.79	1.04
3	43	0.84	1.07	0.71	0.95	0.68	1.04	0.80	1.07
3	44	0.85	1.07	0.73	0.97	0.70	1.05	0.82	1.09
3	45	0.87	1.07	0.74	0.99	0.72	1.05	0.84	1.11
3	46	0.89	1.07	0.76	1.01	0.74	1.05	0.85	1.13
3	47	0.91	1.07	0.78	1.03	0.76	1.05	0.87	1.14
3	48	0.93	1.08	0.79	1.05	0.78	1.06	0.90	1.17

Notes: A0.5=0.5 % Manuka honey 20+ UMF; AC0.5=0.5 % Manuka honey 20+ UMF with catalase;B0.5=0.5 % Manuka honey 15+ UMF; BC0.5=0.5 % Manuka honey 15+ UMF with catalase; C0.5=0.5 % Manuka honey 10+ UMF; CC0.5=0.5 % Manuka honey 10+ UMF with catalase; K0.5=0.5 % Kanuka honey; KC0.5=0.5 % Kanuka honey with catalase

Table A2-3 Growth of *P. acnes* (Abs $_{595nm}$) in 1 % honeys under aerobic condition

Rep	Time (h)	A1	AC1	B1	BC1	C1	CC1	K1	KC1
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.02	0.02	0.03	0.02	0.03	0.02	0.02	0.02
1	3	0.04	0.03	0.05	0.03	0.05	0.04	0.05	0.04
1	4	0.06	0.05	0.07	0.05	0.07	0.06	0.07	0.05
1	5	0.08	0.06	0.09	0.06	0.09	0.07	0.09	0.06
1	6	0.10	0.07	0.11	0.07	0.12	0.08	0.10	0.06
1	7	0.12	0.08	0.13	0.08	0.14	0.09	0.12	0.07
1	8	0.14	0.08	0.15	0.09	0.16	0.10	0.13	0.07
1	9	0.17	0.09	0.17	0.10	0.16	0.11	0.14	0.08
1	10	0.18	0.10	0.18	0.11	0.15	0.12	0.15	0.08
1	11	0.20	0.11	0.20	0.12	0.17	0.14	0.17	0.08
1	12	0.21	0.12	0.21	0.13	0.19	0.17	0.18	0.09
1	13	0.23	0.13	0.23	0.14	0.19	0.25	0.19	0.10
1	14	0.24	0.14	0.25	0.16	0.21	0.37	0.20	0.12
1	15	0.26	0.15	0.27	0.17	0.23	0.52	0.21	0.13
1	16	0.27	0.17	0.28	0.19	0.24	0.69	0.23	0.15
1	17	0.29	0.19	0.29	0.21	0.25	0.81	0.24	0.18
1	18	0.29	0.21	0.31	0.24	0.27	0.89	0.26	0.21
1	19	0.31	0.23	0.32	0.27	0.29	0.95	0.27	0.25
1	20	0.32	0.26	0.34	0.31	0.30	0.99	0.29	0.30
1	21	0.34	0.30	0.35	0.35	0.33	1.01	0.31	0.35
1	22	0.35	0.34	0.38	0.40	0.38	1.02	0.33	0.41
1	23	0.37	0.38	0.40	0.44	0.50	1.02	0.35	0.47
1	24	0.39	0.42	0.42	0.49	0.63	1.02	0.38	0.53
1	25	0.43	0.46	0.45	0.54	0.75	1.02	0.41	0.58
1	26	0.45	0.50	0.49	0.59	0.83	1.02	0.44	0.64
1	27	0.48	0.55	0.53	0.65	0.90	1.02	0.48	0.71
1	28	0.51	0.60	0.57	0.71	0.99	1.01	0.51	0.78
1	29	0.54	0.66	0.61	0.77	1.03	1.01	0.55	0.84
1	30	0.59	0.72	0.64	0.82	1.07	1.01	0.58	0.90
1	31	0.63	0.77	0.68	0.87	1.10	1.00	0.62	0.95
1	32	0.67	0.81	0.71	0.90	1.14	1.00	0.65	0.99
1	33	0.75	0.85	0.75	0.93	1.18	1.00	0.68	1.02
1	34	0.78	0.89	0.78	0.95	1.22	1.00	0.71	1.05
1	35	0.81	0.92	0.81	0.98	1.26	1.00	0.73	1.08
1	36	0.84	0.94	0.84	0.99	1.29	1.00	0.77	1.10
1	37	0.86	0.96	0.86	1.01	1.32	1.00	0.79	1.12
1	38	0.89	0.97	0.89	1.02	1.35	1.00	0.82	1.13
1	39	0.91	0.99	0.91	1.03	1.37	1.00	0.84	1.15

Table A2-3$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 1 % honeys under aerobic condition

Rep	Time (h)	A1	AC1	B1	BC1	C1	CC1	K1	KC1
1	40	0.93	1.00	0.93	1.04	1.38	1.00	0.86	1.16
1	41	0.95	1.01	0.95	1.04	1.40	1.00	0.89	1.17
1	42	0.97	1.02	0.97	1.05	1.41	1.00	0.91	1.17
1	43	0.98	1.03	0.98	1.05	1.42	1.00	0.93	1.18
1	44	0.99	1.03	0.99	1.06	1.43	1.00	0.95	1.19
1	45	1.00	1.04	1.00	1.06	1.44	1.00	0.97	1.19
1	46	1.01	1.04	1.01	1.07	1.45	1.00	0.98	1.19
1	47	1.02	1.05	1.02	1.07	1.46	1.00	0.99	1.20
1	48	1.03	1.05	1.03	1.07	1.46	1.00	1.01	1.20
2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.02	0.02	0.03	0.02	0.03	0.02	0.03	0.02
2	3	0.04	0.03	0.05	0.03	0.05	0.04	0.05	0.04
2	4	0.07	0.05	0.08	0.04	0.08	0.06	0.07	0.05
2	5	0.09	0.06	0.10	0.06	0.10	0.07	0.08	0.06
2	6	0.11	0.07	0.12	0.06	0.12	0.08	0.09	0.07
2	7	0.12	0.07	0.14	0.07	0.14	0.09	0.10	0.07
2	8	0.14	0.08	0.15	0.08	0.17	0.10	0.11	0.08
2	9	0.17	0.09	0.17	0.09	0.22	0.11	0.12	0.08
2	10	0.19	0.09	0.18	0.10	0.41	0.13	0.13	0.08
2	11	0.20	0.10	0.20	0.11	0.59	0.14	0.13	0.09
2	12	0.23	0.11	0.21	0.12	0.75	0.15	0.14	0.10
2	13	0.24	0.12	0.23	0.13	0.83	0.18	0.15	0.11
2	14	0.29	0.13	0.24	0.15	0.90	0.27	0.15	0.12
2	15	0.29	0.15	0.26	0.17	0.94	0.48	0.16	0.14
2	16	0.30	0.16	0.28	0.19	0.98	0.70	0.17	0.16
2	17	0.31	0.18	0.30	0.22	1.00	0.82	0.18	0.18
2	18	0.34	0.19	0.31	0.27	1.04	0.88	0.19	0.21
2	19	0.35	0.22	0.33	0.35	1.06	0.94	0.20	0.25
2	20	0.37	0.25	0.35	0.44	1.09	0.98	0.21	0.30
2	21	0.39	0.28	0.36	0.54	1.11	1.04	0.22	0.36
2	22	0.42	0.31	0.39	0.63	1.13	1.09	0.24	0.41
2	23	0.47	0.35	0.41	0.70	1.15	1.13	0.25	0.46
2	24	0.48	0.39	0.43	0.77	1.18	1.17	0.27	0.52
2	25	0.51	0.43	0.47	0.82	1.22	1.20	0.29	0.59
2	26	0.55	0.47	0.50	0.87	1.24	1.24	0.31	0.65
2	27	0.59	0.52	0.55	0.92	1.27	1.27	0.33	0.71
2	28	0.64	0.57	0.59	0.96	1.30	1.30	0.36	0.78
2	29	0.68	0.63	0.63	1.01	1.32	1.32	0.38	0.84
2	30	0.73	0.68	0.67	1.04	1.34	1.33	0.41	0.89

Table A2-3$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 1 % honeys under aerobic condition

Rep	Time (h)	A1	AC1	B1	BC1	C1	CC1	K1	KC1
2	31	0.76	0.74	0.71	1.08	1.35	1.33	0.44	0.94
2	32	0.80	0.78	0.75	1.10	1.37	1.34	0.46	0.98
2	33	0.83	0.82	0.78	1.11	1.38	1.34	0.49	1.02
2	34	0.87	0.85	0.81	1.12	1.40	1.34	0.52	1.05
2	35	0.90	0.88	0.83	1.13	1.41	1.34	0.55	1.07
2	36	0.92	0.90	0.86	1.13	1.42	1.34	0.58	1.09
2	37	0.94	0.92	0.88	1.14	1.44	1.34	0.61	1.10
2	38	0.96	0.94	0.90	1.14	1.45	1.34	0.64	1.12
2	39	0.98	0.96	0.91	1.15	1.46	1.34	0.67	1.13
2	40	0.99	0.97	0.92	1.15	1.47	1.35	0.71	1.14
2	41	1.00	0.98	0.94	1.15	1.48	1.35	0.74	1.15
2	42	1.00	0.99	0.95	1.15	1.49	1.35	0.76	1.16
2	43	1.01	1.00	0.95	1.15	1.50	1.35	0.79	1.16
2	44	1.01	1.00	0.96	1.15	1.51	1.35	0.81	1.17
2	45	1.02	1.01	0.97	1.16	1.52	1.35	0.84	1.17
2	46	1.02	1.01	0.97	1.16	1.53	1.36	0.86	1.17
2	47	1.03	1.01	0.97	1.16	1.53	1.36	0.88	1.18
2	48	1.03	1.02	0.97	1.16	1.54	1.36	0.90	1.18
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.03	0.02	0.03	0.02	0.02	0.02	0.03	0.02
3	3	0.05	0.04	0.05	0.04	0.05	0.04	0.05	0.04
3	4	0.07	0.05	0.08	0.05	0.07	0.05	0.06	0.06
3	5	0.10	0.06	0.10	0.06	0.10	0.07	0.08	0.07
3	6	0.12	0.07	0.13	0.07	0.12	0.08	0.09	0.08
3	7	0.14	0.08	0.14	0.08	0.14	0.08	0.09	0.08
3	8	0.16	0.09	0.15	0.09	0.16	0.09	0.10	0.09
3	9	0.18	0.09	0.18	0.10	0.15	0.10	0.11	0.10
3	10	0.21	0.10	0.19	0.11	0.18	0.11	0.12	0.10
3	11	0.23	0.11	0.21	0.12	0.21	0.13	0.12	0.11
3	12	0.24	0.12	0.22	0.13	0.30	0.16	0.13	0.12
3	13	0.27	0.13	0.24	0.14	0.41	0.27	0.13	0.14
3	14	0.29	0.14	0.26	0.16	0.51	0.51	0.14	0.15
3	15	0.33	0.15	0.28	0.18	0.65	0.72	0.15	0.17
3	16	0.33	0.17	0.30	0.19	0.76	0.83	0.16	0.20
3	17	0.35	0.19	0.31	0.21	0.88	0.88	0.17	0.23
3	18	0.36	0.21	0.33	0.24	0.97	0.93	0.17	0.28
3	19	0.38	0.23	0.34	0.28	1.02	0.97	0.18	0.33
3	20	0.41	0.26	0.36	0.31	1.05	1.01	0.19	0.39

Table A2-3$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 1 % honeys under aerobic condition

Rep	Time (h)	A1	AC1	B1	BC1	C1	CC1	K1	KC1
3	21	0.41	0.30	0.38	0.36	1.08	1.06	0.21	0.45
3	22	0.45	0.34	0.40	0.40	1.11	1.10	0.22	0.52
3	23	0.47	0.38	0.43	0.45	1.13	1.12	0.23	0.58
3	24	0.51	0.43	0.46	0.50	1.15	1.15	0.25	0.64
3	25	0.54	0.49	0.50	0.54	1.16	1.19	0.27	0.69
3	26	0.58	0.54	0.55	0.60	1.16	1.23	0.29	0.77
3	27	0.61	0.61	0.60	0.66	1.17	1.26	0.31	0.84
3	28	0.71	0.68	0.65	0.72	1.17	1.27	0.33	0.91
3	29	0.72	0.75	0.69	0.78	1.17	1.28	0.36	0.97
3	30	0.76	0.80	0.74	0.84	1.17	1.28	0.38	1.02
3	31	0.80	0.85	0.77	0.88	1.17	1.28	0.41	1.06
3	32	0.83	0.90	0.80	0.92	1.17	1.29	0.43	1.10
3	33	0.86	0.93	0.83	0.94	1.18	1.29	0.46	1.12
3	34	0.89	0.96	0.85	0.97	1.18	1.29	0.49	1.15
3	35	0.92	0.98	0.88	0.99	1.18	1.30	0.52	1.17
3	36	0.94	1.00	0.90	1.00	1.17	1.30	0.55	1.18
3	37	0.97	1.01	0.92	1.02	1.17	1.30	0.59	1.19
3	38	0.98	1.02	0.93	1.03	1.17	1.30	0.63	1.20
3	39	1.00	1.03	0.95	1.04	1.16	1.30	0.66	1.21
3	40	1.01	1.04	0.96	1.05	1.16	1.30	0.69	1.22
3	41	1.02	1.05	0.97	1.05	1.16	1.30	0.73	1.23
3	42	1.02	1.05	0.97	1.06	1.16	1.30	0.76	1.23
3	43	1.02	1.06	0.98	1.06	1.15	1.30	0.78	1.24
3	44	1.03	1.06	0.98	1.07	1.15	1.31	0.81	1.24
3	45	1.04	1.07	0.99	1.07	1.15	1.31	0.84	1.25
3	46	1.05	1.07	0.99	1.08	1.15	1.31	0.87	1.25
3	47	1.05	1.07	0.99	1.08	1.15	1.32	0.89	1.25
3	48	1.06	1.07	0.99	1.08	1.15	1.32	0.91	1.25

Notes: A1=1 % Manuka honey 20+ UMF; AC1=1 % Manuka honey 20+ UMF with catalase;
B1=1 % Manuka honey 15+ UMF; BC1=1 % Manuka honey 15+ UMF with catalase;
C1=1 % Manuka honey 10+ UMF; CC1=1 % Manuka honey 10+ UMF with catalase;
K1=1 % Kanuka honey; KC1=1 % Kanuka honey with catalase

Table A2-4 Growth of *P. acnes* (Abs $_{595nm}$) in 1 % honeys under anaerobic condition

Rep	Time (h)	A1	AC1	B1	BC1	C1	CC1	K1	KC1
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.01	0.03	0.02	0.03	0.02	0.04	0.02	0.04
1	3	0.03	0.06	0.04	0.06	0.04	0.06	0.04	0.07
1	4	0.04	0.08	0.05	0.08	0.05	0.08	0.05	0.09
1	5	0.05	0.10	0.07	0.09	0.05	0.11	0.07	0.11
1	6	0.07	0.12	0.09	0.11	0.05	0.13	0.09	0.14
1	7	0.08	0.13	0.11	0.12	0.06	0.15	0.10	0.16
1	8	0.10	0.14	0.12	0.14	0.06	0.17	0.12	0.19
1	9	0.11	0.16	0.14	0.16	0.06	0.19	0.14	0.21
1	10	0.13	0.18	0.16	0.18	0.06	0.20	0.16	0.24
1	11	0.15	0.19	0.17	0.19	0.07	0.22	0.18	0.26
1	12	0.16	0.20	0.19	0.21	0.08	0.24	0.19	0.29
1	13	0.18	0.22	0.20	0.23	0.09	0.26	0.21	0.31
1	14	0.19	0.24	0.22	0.25	0.08	0.28	0.23	0.34
1	15	0.21	0.26	0.24	0.27	0.08	0.31	0.25	0.36
1	16	0.23	0.28	0.25	0.29	0.08	0.33	0.26	0.39
1	17	0.25	0.30	0.26	0.31	0.09	0.36	0.28	0.42
1	18	0.26	0.32	0.28	0.33	0.09	0.38	0.30	0.44
1	19	0.28	0.34	0.29	0.35	0.09	0.40	0.31	0.47
1	20	0.30	0.36	0.31	0.37	0.10	0.43	0.33	0.50
1	21	0.31	0.38	0.32	0.39	0.12	0.45	0.34	0.52
1	22	0.34	0.40	0.44	0.41	0.16	0.48	0.35	0.55
1	23	0.39	0.42	0.49	0.43	0.02	0.50	0.37	0.58
1	24	0.43	0.44	0.52	0.45	0.05	0.52	0.38	0.61
1	25	0.45	0.46	0.53	0.48	0.07	0.55	0.40	0.64
1	26	0.46	0.48	0.55	0.50	0.09	0.58	0.42	0.67
1	27	0.48	0.51	0.56	0.53	0.10	0.61	0.43	0.71
1	28	0.49	0.53	0.57	0.55	0.12	0.64	0.45	0.74
1	29	0.51	0.56	0.58	0.58	0.13	0.67	0.46	0.77
1	30	0.52	0.58	0.61	0.61	0.14	0.71	0.49	0.81
1	31	0.53	0.62	0.63	0.65	0.16	0.74	0.51	0.84
1	32	0.54	0.65	0.66	0.68	0.19	0.78	0.53	0.88
1	33	0.57	0.68	0.66	0.71	0.21	0.81	0.55	0.91
1	34	0.60	0.71	0.68	0.75	0.23	0.84	0.57	0.94
1	35	0.60	0.74	0.71	0.78	0.25	0.88	0.59	0.97
1	36	0.62	0.78	0.73	0.81	0.27	0.90	0.62	1.00
1	37	0.71	0.81	0.75	0.84	0.29	0.93	0.64	1.03
1	38	0.71	0.84	0.77	0.87	0.31	0.96	0.67	1.05
1	39	0.67	0.87	0.79	0.90	0.33	0.99	0.69	1.08

Table A2-4$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 1 % honeys under anaerobic condition

Rep	Time (h)	A1	AC1	B1	BC1	C1	CC1	K1	KC1
1	40	0.69	0.90	0.81	0.93	0.34	1.02	0.71	1.10
1	41	0.71	0.93	0.83	0.95	0.36	1.04	0.73	1.12
1	42	0.73	0.96	0.85	0.98	0.38	1.06	0.75	1.14
1	43	0.75	0.98	0.87	1.00	0.40	1.09	0.78	1.16
1	44	0.77	1.01	0.89	1.03	0.42	1.11	0.80	1.18
1	45	0.79	1.03	0.91	1.05	0.44	1.13	0.82	1.20
1	46	0.80	1.05	0.93	1.07	0.46	1.15	0.84	1.22
1	47	0.82	1.08	0.95	1.09	0.48	1.17	0.86	1.24
1	48	0.84	1.10	0.97	1.11	0.49	1.19	0.87	1.25
2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.01	0.04	0.02	0.03	0.01	0.04	0.02	0.03
2	3	0.03	0.06	0.03	0.06	0.02	0.06	0.03	0.06
2	4	0.04	0.09	0.04	0.07	0.03	0.08	0.05	0.08
2	5	0.05	0.10	0.06	0.09	0.04	0.11	0.06	0.10
2	6	0.06	0.12	0.07	0.11	0.05	0.13	0.08	0.13
2	7	0.08	0.13	0.09	0.13	0.06	0.15	0.10	0.15
2	8	0.09	0.14	0.10	0.15	0.08	0.16	0.11	0.18
2	9	0.11	0.16	0.12	0.17	0.10	0.18	0.14	0.20
2	10	0.12	0.18	0.14	0.18	0.11	0.20	0.15	0.23
2	11	0.13	0.19	0.15	0.20	0.12	0.22	0.18	0.25
2	12	0.14	0.21	0.16	0.22	0.14	0.24	0.20	0.28
2	13	0.16	0.23	0.18	0.24	0.15	0.26	0.22	0.31
2	14	0.17	0.24	0.20	0.25	0.16	0.28	0.25	0.33
2	15	0.18	0.27	0.21	0.28	0.18	0.30	0.28	0.35
2	16	0.19	0.30	0.22	0.30	0.20	0.33	0.30	0.38
2	17	0.20	0.35	0.24	0.32	0.21	0.35	0.33	0.41
2	18	0.21	0.45	0.25	0.34	0.22	0.37	0.35	0.44
2	19	0.22	0.61	0.26	0.36	0.24	0.39	0.38	0.46
2	20	0.23	0.77	0.27	0.38	0.25	0.41	0.40	0.49
2	21	0.26	0.87	0.31	0.40	0.26	0.44	0.45	0.51
2	22	0.32	0.93	0.39	0.42	0.28	0.46	0.44	0.54
2	23	0.34	0.95	0.40	0.44	0.30	0.48	0.45	0.57
2	24	0.35	0.96	0.44	0.47	0.31	0.51	0.45	0.59
2	25	0.36	0.97	0.47	0.50	0.32	0.54	0.46	0.63
2	26	0.36	0.98	0.50	0.54	0.33	0.56	0.47	0.66
2	27	0.37	0.98	0.56	0.58	0.33	0.59	0.49	0.69
2	28	0.37	0.99	0.54	0.64	0.34	0.62	0.50	0.73
2	29	0.38	0.99	0.54	0.70	0.34	0.66	0.52	0.76
2	30	0.38	0.99	0.55	0.76	0.34	0.69	0.54	0.80

Table A2-4$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 1 % honeys under anaerobic condition

Rep	Time (h)	A1	AC1	B1	BC1	C1	CC1	K1	KC1
2	31	0.39	1.00	0.56	0.82	0.35	0.72	0.57	0.83
2	32	0.40	1.00	0.57	0.87	0.36	0.76	0.60	0.87
2	33	0.40	1.00	0.58	0.91	0.36	0.79	0.61	0.90
2	34	0.41	1.00	0.58	0.94	0.37	0.82	0.64	0.93
2	35	0.42	1.00	0.60	0.97	0.38	0.85	0.66	0.96
2	36	0.43	1.00	0.61	0.99	0.39	0.88	0.69	0.99
2	37	0.44	1.00	0.62	1.00	0.40	0.91	0.71	1.02
2	38	0.44	1.00	0.63	1.01	0.41	0.94	0.74	1.05
2	39	0.45	1.00	0.64	1.02	0.42	0.97	0.76	1.07
2	40	0.46	1.00	0.65	1.03	0.43	0.99	0.79	1.10
2	41	0.47	1.00	0.66	1.03	0.44	1.02	0.81	1.13
2	42	0.48	1.00	0.67	1.04	0.46	1.04	0.84	1.15
2	43	0.49	1.00	0.68	1.04	0.47	1.06	0.86	1.17
2	44	0.50	1.00	0.70	1.04	0.48	1.08	0.89	1.20
2	45	0.51	1.00	0.71	1.05	0.49	1.11	0.91	1.22
2	46	0.52	1.00	0.72	1.05	0.51	1.13	0.93	1.24
2	47	0.54	1.00	0.73	1.05	0.52	1.15	0.95	1.26
2	48	0.55	1.00	0.75	1.05	0.54	1.17	0.97	1.28
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.01	0.04	0.01	0.03	0.01	0.04	0.02	0.04
3	3	0.02	0.06	0.03	0.06	0.03	0.07	0.03	0.07
3	4	0.02	0.08	0.04	0.08	0.05	0.09	0.05	0.10
3	5	0.04	0.09	0.06	0.10	0.06	0.11	0.07	0.12
3	6	0.05	0.12	0.07	0.12	0.08	0.13	0.09	0.15
3	7	0.06	0.14	0.09	0.13	0.10	0.15	0.11	0.17
3	8	0.08	0.15	0.10	0.15	0.11	0.17	0.13	0.20
3	9	0.09	0.17	0.12	0.17	0.13	0.19	0.16	0.22
3	10	0.11	0.18	0.14	0.19	0.15	0.21	0.18	0.25
3	11	0.12	0.19	0.15	0.20	0.16	0.23	0.21	0.28
3	12	0.13	0.21	0.17	0.22	0.18	0.25	0.24	0.30
3	13	0.14	0.23	0.19	0.24	0.20	0.27	0.27	0.33
3	14	0.15	0.24	0.23	0.26	0.22	0.29	0.29	0.35
3	15	0.17	0.26	0.24	0.28	0.24	0.31	0.32	0.38
3	16	0.19	0.28	0.26	0.30	0.26	0.33	0.35	0.41
3	17	0.20	0.30	0.28	0.32	0.27	0.36	0.37	0.43
3	18	0.21	0.32	0.29	0.34	0.29	0.38	0.39	0.46
3	19	0.22	0.34	0.31	0.36	0.31	0.40	0.41	0.49
3	20	0.23	0.36	0.34	0.39	0.33	0.43	0.43	0.51

Table A2-4$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 1 % honeys under anaerobic condition

Rep	Time (h)	A1	AC1	B1	BC1	C1	CC1	K1	KC1
3	21	0.24	0.38	0.36	0.41	0.35	0.45	0.45	0.54
3	22	0.27	0.40	0.40	0.43	0.35	0.47	0.48	0.57
3	23	0.31	0.42	0.41	0.45	0.33	0.50	0.48	0.59
3	24	0.34	0.43	0.43	0.47	0.34	0.53	0.49	0.62
3	25	0.36	0.46	0.44	0.49	0.34	0.56	0.49	0.65
3	26	0.37	0.48	0.45	0.52	0.36	0.60	0.51	0.69
3	27	0.38	0.50	0.47	0.54	0.38	0.64	0.52	0.72
3	28	0.38	0.53	0.55	0.57	0.39	0.70	0.53	0.75
3	29	0.39	0.55	0.55	0.60	0.40	0.75	0.55	0.78
3	30	0.39	0.58	0.57	0.63	0.41	0.81	0.57	0.82
3	31	0.40	0.61	0.57	0.67	0.41	0.86	0.60	0.85
3	32	0.41	0.64	0.58	0.70	0.42	0.91	0.62	0.88
3	33	0.41	0.67	0.59	0.73	0.42	0.95	0.64	0.92
3	34	0.42	0.71	0.60	0.77	0.43	0.98	0.66	0.94
3	35	0.43	0.74	0.61	0.80	0.44	1.01	0.69	0.97
3	36	0.44	0.77	0.62	0.83	0.45	1.03	0.71	1.00
3	37	0.45	0.80	0.63	0.86	0.46	1.04	0.74	1.03
3	38	0.46	0.84	0.65	0.89	0.47	1.06	0.76	1.05
3	39	0.47	0.87	0.66	0.92	0.48	1.07	0.79	1.08
3	40	0.48	0.89	0.67	0.95	0.49	1.08	0.81	1.10
3	41	0.49	0.92	0.69	0.97	0.51	1.09	0.84	1.12
3	42	0.50	0.95	0.70	1.00	0.52	1.10	0.86	1.14
3	43	0.52	0.97	0.71	1.02	0.53	1.10	0.89	1.16
3	44	0.53	1.00	0.73	1.05	0.54	1.11	0.91	1.18
3	45	0.54	1.03	0.74	1.07	0.55	1.11	0.93	1.20
3	46	0.55	1.05	0.75	1.09	0.57	1.11	0.95	1.22
3	47	0.56	1.07	0.77	1.11	0.58	1.12	0.97	1.23
3	48	0.57	1.10	0.79	1.14	0.60	1.12	0.99	1.25

Notes: A1=1 % Manuka honey 20+ UMF; AC1=1 % Manuka honey 20+ UMF with catalase;
B1=1 % Manuka honey 15+ UMF; BC1=1 % Manuka honey 15+ UMF with catalase;
C1=1 % Manuka honey 10+ UMF; CC1=1 % Manuka honey 10+ UMF with catalase;
K1=1 % Kanuka honey; KC1=1 % Kanuka honey with catalase

Table A2-5 Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under aerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01
1	3	0.05	0.03	0.04	0.03	0.04	0.04	0.02	0.03
1	4	0.07	0.04	0.06	0.04	0.06	0.05	0.03	0.04
1	5	0.08	0.05	0.08	0.05	0.07	0.06	0.04	0.05
1	6	0.10	0.06	0.10	0.06	0.08	0.07	0.04	0.06
1	7	0.12	0.07	0.11	0.07	0.10	0.08	0.05	0.07
1	8	0.13	0.08	0.13	0.08	0.12	0.09	0.06	0.08
1	9	0.14	0.09	0.14	0.09	0.13	0.10	0.06	0.09
1	10	0.15	0.10	0.15	0.10	0.17	0.12	0.07	0.10
1	11	0.16	0.11	0.17	0.11	0.30	0.16	0.07	0.11
1	12	0.17	0.13	0.18	0.12	0.54	0.23	0.08	0.13
1	13	0.18	0.14	0.18	0.13	0.66	0.35	0.08	0.14
1	14	0.19	0.15	0.19	0.14	0.74	0.51	0.09	0.16
1	15	0.19	0.16	0.20	0.16	0.80	0.66	0.09	0.18
1	16	0.20	0.17	0.21	0.17	0.85	0.75	0.10	0.21
1	17	0.19	0.18	0.21	0.18	0.88	0.80	0.10	0.25
1	18	0.19	0.19	0.22	0.20	0.91	0.85	0.10	0.29
1	19	0.20	0.21	0.23	0.22	0.93	0.90	0.11	0.33
1	20	0.20	0.23	0.23	0.24	0.96	0.92	0.12	0.37
1	21	0.21	0.25	0.24	0.26	0.98	0.95	0.13	0.42
1	22	0.22	0.27	0.25	0.29	1.01	0.97	0.13	0.46
1	23	0.22	0.29	0.25	0.32	1.02	1.00	0.14	0.51
1	24	0.23	0.32	0.26	0.36	1.05	1.03	0.16	0.57
1	25	0.23	0.34	0.27	0.39	1.07	1.06	0.17	0.62
1	26	0.24	0.37	0.28	0.43	1.09	1.09	0.18	0.67
1	27	0.24	0.40		0.47	1.11	1.11	0.20	0.73
1	28	0.25	0.43	0.29	0.50	1.14	1.14	0.22	0.77
1	29	0.26	0.46	0.30	0.54	1.16	1.16	0.24	0.83
1	30	0.27	0.50	0.31	0.57	1.18	1.19	0.26	0.88
1	31	0.27	0.53	0.32	0.61	1.20	1.21	0.29	0.92
1	32	0.28	0.56	0.33	0.66	1.21	1.24	0.32	0.96
1	33	0.29	0.60	0.34	0.70	1.23	1.26	0.35	1.01
1	34	0.30	0.64	0.35	0.75	1.25	1.28	0.38	1.05
1	35	0.31	0.68	0.36	0.78	1.26	1.30	0.41	1.09
1	36	0.32	0.71	0.38	0.82	1.27	1.32	0.44	1.12
1	37	0.33	0.74	0.40	0.85	1.28	1.33	0.47	1.15
1	38	0.34	0.77	0.42	0.87	1.29	1.35	0.50	1.18
1	39	0.34	0.79	0.44	0.90	1.29	1.37	0.53	1.21

Table A2-5$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under aerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
1	40	0.35	0.82	0.46	0.92	1.30	1.38	0.56	1.24
1	41	0.37	0.84	0.47	0.94	1.31	1.39	0.59	1.26
1	42	0.39	0.85	0.49	0.96	1.31	1.40	0.62	1.28
1	43	0.40	0.87	0.52	0.97	1.32	1.41	0.65	1.30
1	44	0.42	0.88	0.54	0.99	1.33	1.42	0.68	1.32
1	45	0.44	0.90	0.56	1.00	1.33	1.43	0.71	1.33
1	46	0.45	0.91	0.58	1.01	1.34	1.44	0.74	1.35
1	47	0.47	0.92	0.60	1.02	1.34	1.44	0.77	1.36
1	48	0.49	0.93	0.62	1.02	1.35	1.45	0.80	1.37
2	0	0.00	0.00	0.64	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.01	0.02	0.00	0.02	0.02	0.02	0.02	0.02
2	3	0.02	0.04	0.02	0.03	0.04	0.04	0.04	0.03
2	4	0.02	0.05	0.03	0.04	0.06	0.05	0.05	0.05
2	5	0.03	0.07	0.05	0.06	0.08	0.06	0.06	0.07
2	6	0.04	0.08	0.06	0.07	0.10	0.07	0.07	0.08
2	7	0.05	0.09	0.07	0.08	0.12	0.08	0.08	0.09
2	8	0.06	0.10	0.08	0.09	0.13	0.09	0.09	0.10
2	9	0.07	0.11	0.09	0.10	0.16	0.10	0.09	0.11
2	10	0.08	0.12	0.10	0.11	0.23	0.12	0.10	0.13
2	11	0.09	0.13	0.11	0.12	0.40	0.13	0.11	0.14
2	12	0.09	0.14	0.12	0.13	0.61	0.19	0.12	0.16
2	13	0.10	0.15	0.12	0.14	0.72	0.33	0.12	0.18
2	14	0.11	0.16	0.12	0.16	0.79	0.57	0.13	0.20
2	15	0.11	0.18	0.13	0.17	0.85	0.70	0.13	0.22
2	16	0.12	0.19	0.13	0.18	0.90	0.77	0.14	0.26
2	17	0.12	0.20	0.13	0.20	0.92	0.83	0.14	0.30
2	18	0.12	0.21	0.14	0.21	0.95	0.87	0.15	0.35
2	19	0.13	0.23	0.14	0.23	0.98	0.90	0.16	0.40
2	20	0.14	0.25	0.14	0.25	1.00	0.93	0.17	0.46
2	21	0.14	0.28	0.14	0.28	1.03	0.97	0.18	0.51
2	22	0.14	0.30	0.15	0.31	1.05	1.00	0.20	0.57
2	23	0.14	0.33	0.15	0.34	1.07	1.04	0.21	0.62
2	24	0.14	0.36	0.15	0.38	1.10	1.06	0.22	0.68
2	25	0.14	0.39	0.15	0.41	1.12	1.09	0.24	0.74
2	26	0.15	0.42	0.15	0.45	1.14	1.12	0.25	0.79
2	27	0.15	0.45	0.15	0.49	1.17	1.15	0.27	0.83
2	28	0.15	0.49	0.16	0.52	1.19	1.18	0.30	0.89
2	29	0.15	0.52	0.16	0.56	1.21	1.20	0.32	0.95
2	30	0.15	0.55	0.17	0.59	1.23	1.23	0.35	1.00

Table A2-5$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under aerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
2	31	0.15	0.59	0.17	0.63	1.24	1.25	0.38	1.04
2	32	0.16	0.63	0.17	0.68	1.26	1.26	0.41	1.08
2	33	0.16	0.67	0.18	0.72	1.27	1.28	0.44	1.12
2	34	0.16	0.70	0.19	0.75	1.29	1.29	0.47	1.15
2	35	0.17	0.74	0.19	0.79	1.30	1.31	0.50	1.18
2	36	0.17	0.77	0.20	0.82	1.31	1.32	0.53	1.21
2	37	0.18	0.80	0.21	0.85	1.31	1.33	0.56	1.24
2	38	0.18	0.83	0.22	0.87	1.32	1.34	0.59	1.27
2	39	0.19	0.85	0.23	0.89	1.33	1.35	0.62	1.29
2	40	0.19	0.87	0.24	0.91	1.33	1.36	0.66	1.30
2	41	0.20	0.89	0.24	0.93	1.34	1.36	0.69	1.32
2	42	0.21	0.90	0.26	0.94	1.34	1.37	0.72	1.34
2	43	0.21	0.92	0.27	0.96	1.35	1.38	0.75	1.35
2	44	0.23	0.93	0.28	0.97	1.36	1.39	0.77	1.36
2	45	0.23	0.94	0.30	0.98	1.37	1.39	0.79	1.37
2	46	0.24	0.95	0.31	0.99	1.37	1.40	0.82	1.39
2	47	0.25	0.96	0.32	0.99	1.38	1.41	0.85	1.39
2	48	0.26	0.96	0.33	1.00	1.38	1.41	0.89	1.40
3	0	0.00	0.00	0.35	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.02	0.02	0.00	0.02	0.03	0.02	0.02	0.02
3	3	0.04	0.04	0.02	0.04	0.05	0.03	0.04	0.03
3	4	0.06	0.05	0.04	0.06	0.08	0.04	0.05	0.05
3	5	0.08	0.07	0.06	0.07	0.10	0.05	0.06	0.06
3	6	0.10	0.08	0.08	0.08	0.12	0.06	0.07	0.07
3	7	0.11	0.09	0.09	0.09	0.14	0.07	0.08	0.08
3	8	0.12	0.10	0.11	0.10	0.15	0.08	0.09	0.09
3	9	0.14	0.11	0.12	0.11	0.17	0.09	0.09	0.11
3	10	0.15	0.12	0.13	0.12	0.19	0.10	0.10	0.12
3	11	0.15	0.13	0.14	0.14	0.20	0.12	0.10	0.13
3	12	0.16	0.14	0.15	0.15	0.22	0.13	0.11	0.15
3	13	0.17	0.15	0.16	0.16	0.24	0.15	0.11	0.17
3	14	0.18	0.16	0.16	0.18	0.26	0.20	0.12	0.19
3	15	0.18	0.18	0.17	0.19	0.30	0.27	0.12	0.22
3	16	0.19	0.19	0.17	0.21	0.38	0.39	0.13	0.25
3	17	0.20	0.20	0.18	0.22	0.47	0.52	0.13	0.29
3	18	0.20	0.21	0.18	0.24	0.58	0.64	0.13	0.33
3	19	0.21	0.23	0.18	0.26	0.72	0.75	0.14	0.38
3	20	0.22	0.25	0.19	0.29	0.78	0.81	0.15	0.43

Table A2-5$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under aerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
3	21	0.22	0.28	0.19	0.32	0.82	0.87	0.16	0.48
3	22	0.23	0.30	0.20	0.35	0.87	0.90	0.17	0.53
3	23	0.23	0.33	0.20	0.39	0.92	0.92	0.18	0.59
3	24	0.23	0.36	0.20	0.42	0.94	0.94	0.19	0.65
3	25	0.24	0.39	0.20	0.47	0.97	0.95	0.20	0.72
3	26	0.25	0.42	0.20	0.50	1.00	0.96	0.21	0.77
3	27	0.25	0.45	0.21	0.54	1.03	0.97	0.23	0.82
3	28	0.26	0.49	0.22	0.58	1.06	0.98	0.25	0.87
3	29	0.27	0.52	0.22	0.62	1.09	0.99	0.27	0.92
3	30	0.28	0.55	0.23	0.66	1.12	0.99	0.30	0.96
3	31	0.29	0.59	0.23	0.70	1.14	1.00	0.32	1.00
3	32	0.30	0.63	0.24	0.75	1.17	1.00	0.35	1.04
3	33	0.32	0.67	0.25	0.79	1.19	1.01	0.38	1.08
3	34	0.33	0.70	0.26	0.83	1.22	1.01	0.41	1.11
3	35	0.34	0.74	0.27	0.86	1.24	1.02	0.44	1.14
3	36	0.36	0.77	0.28	0.89	1.26	1.04	0.48	1.17
3	37	0.37	0.80	0.29	0.92	1.28	1.06	0.51	1.19
3	38	0.39	0.83	0.30	0.94	1.30	1.07	0.55	1.21
3	39	0.41	0.85	0.31	0.96	1.32	1.08	0.58	1.24
3	40	0.42	0.87	0.33	0.98	1.33	1.09	0.62	1.25
3	41	0.44	0.89	0.34	0.99	1.35	1.11	0.66	1.27
3	42	0.47	0.90	0.35	1.00	1.36	1.12	0.69	1.29
3	43	0.48	0.92	0.37	1.02	1.38	1.13	0.72	1.29
3	44	0.50	0.93	0.39	1.03	1.39	1.14	0.75	1.31
3	45	0.52	0.94	0.40	1.04	1.40	1.15	0.78	1.32
3	46	0.54	0.95	0.41	1.04	1.41	1.16	0.80	1.33
3	47	0.55	0.96	0.43	1.05	1.42	1.16	0.82	1.34
3	48	0.57	0.96	0.44	1.05	1.43	1.17	0.85	1.34
4	0	0.00	0.00	0.45	0.00	0.00	0.00	0.00	0.00
4	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	2	0.02	0.02	0.00	0.01	0.02	0.02	0.02	0.01
4	3	0.04	0.03	0.02	0.02	0.04	0.03	0.04	0.02
4	4	0.07	0.04	0.05	0.04	0.06	0.05	0.05	0.04
4	5	0.09	0.05	0.08	0.05	0.08	0.06	0.06	0.04
4	6	0.11	0.06	0.10	0.05	0.10	0.07	0.07	0.05
4	7	0.13	0.07	0.12	0.06	0.11	0.08	0.08	0.05
4	8	0.14	0.08	0.13	0.06	0.13	0.08	0.09	0.06
4	9	0.16	0.09	0.14	0.07	0.15	0.09	0.09	0.06
4	10	0.17	0.09	0.15	0.07	0.17	0.11	0.10	0.06

Table A2-5$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under aerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
4	11	0.18	0.10	0.16	0.08	0.30	0.17	0.10	0.07
4	12	0.18	0.11	0.16	0.09	0.48	0.26	0.11	0.08
4	13	0.19	0.12	0.17	0.10	0.63	0.37	0.11	0.09
4	14	0.20	0.13	0.18	0.11	0.73	0.49	0.12	0.10
4	15	0.20	0.14	0.18	0.12	0.77	0.61	0.13	0.11
4	16	0.21	0.15	0.19	0.13	0.82	0.72	0.13	0.14
4	17	0.22	0.17	0.19	0.14	0.85	0.81	0.14	0.17
4	18	0.22	0.18	0.20	0.16	0.89	0.83	0.15	0.21
4	19	0.22	0.20	0.20	0.18	0.91	0.87	0.16	0.26
4	20	0.22	0.22	0.21	0.21	0.94	0.91	0.17	0.31
4	21	0.22	0.25	0.22	0.23	0.96	0.93	0.18	0.37
4	22	0.22	0.28	0.23	0.27	0.99	0.94	0.19	0.42
4	23	0.23	0.32	0.24	0.30	1.01	0.96	0.21	0.49
4	24	0.22	0.36	0.25	0.34	1.04	0.97	0.23	0.56
4	25	0.22	0.40	0.26	0.38	1.07	0.98	0.25	0.63
4	26	0.23	0.44	0.27	0.42	1.09	0.99	0.28	0.70
4	27	0.22	0.48	0.29	0.46	1.11	1.00	0.31	0.77
4	28	0.23	0.52	0.30	0.50	1.14	1.01	0.34	0.83
4	29	0.23	0.56	0.31	0.55	1.16	1.02	0.38	0.89
4	30	0.23	0.62	0.33	0.60	1.18	1.04	0.41	0.94
4	31	0.23	0.66	0.34	0.65	1.20	1.04	0.45	0.99
4	32	0.24	0.70	0.35	0.69	1.22	1.05	0.48	1.04
4	33	0.24	0.75	0.37	0.74	1.23	1.05	0.52	1.08
4	34	0.25	0.78	0.40	0.77	1.25	1.06	0.56	1.11
4	35	0.25	0.82	0.42	0.80	1.26	1.08	0.60	1.15
4	36	0.26	0.85	0.44	0.83	1.27	1.09	0.64	1.18
4	37	0.27	0.88	0.47	0.86	1.28	1.10	0.68	1.20
4	38	0.29	0.91	0.49	0.88	1.29	1.11	0.72	1.23
4	39	0.32	0.93	0.52	0.90	1.29	1.13	0.75	1.25
4	40	0.33	0.95	0.54	0.91	1.30	1.14	0.78	1.27
4	41	0.36	0.96	0.57	0.93	1.31	1.15	0.81	1.29
4	42	0.40	0.97	0.59	0.94	1.32	1.17	0.83	1.30
4	43	0.44	0.99	0.62	0.96	1.33	1.18	0.87	1.32
4	44	0.47	1.00	0.65	0.96	1.33	1.20	0.88	1.33
4	45	0.53	1.01	0.68	0.97	1.34	1.21	0.89	1.35
4	46	0.57	1.02	0.70	0.99	1.35	1.22	0.92	1.36
4	47	0.62	1.03	0.73	0.99	1.35	1.23	0.95	1.37
4	48	0.66	1.03	0.75	1.00	1.36	1.24	0.97	1.38

Table A2-5$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under aerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
5	0	0.00	0.00	0.78	0.00	0.00	0.00	0.00	0.00
5	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	2	0.02	0.02	0.00	0.01	0.04	0.02	0.02	0.02
5	3	0.04	0.03	0.02	0.02	0.06	0.04	0.04	0.03
5	4	0.07	0.04	0.04	0.04	0.09	0.05	0.05	0.05
5	5	0.09	0.05	0.07	0.04	0.11	0.06	0.06	0.06
5	6	0.11	0.06	0.09	0.05	0.13	0.07	0.07	0.06
5	7	0.12	0.06	0.10	0.05	0.15	0.08	0.08	0.07
5	8	0.14	0.07	0.12	0.06	0.17	0.09	0.09	0.07
5	9	0.15	0.08	0.13	0.07	0.21	0.10	0.09	0.08
5	10	0.16	0.09	0.15	0.08	0.31	0.11	0.10	0.10
5	11	0.17	0.10	0.16	0.08	0.49	0.12	0.10	0.10
5	12	0.17	0.10	0.17	0.09	0.63	0.13	0.11	0.11
5	13	0.17	0.11	0.18	0.10	0.72	0.14	0.11	0.12
5	14	0.18	0.13	0.19	0.11	0.77	0.17	0.12	0.14
5	15	0.18	0.14	0.19	0.12	0.83	0.27	0.12	0.16
5	16	0.18	0.15	0.20	0.13	0.87	0.46	0.13	0.18
5	17	0.18	0.17	0.21	0.14	0.90	0.66	0.14	0.21
5	18	0.18	0.18	0.22	0.16	0.93	0.80	0.15	0.25
5	19	0.18	0.20	0.22	0.18	0.95	0.89	0.16	0.29
5	20	0.19	0.22	0.23	0.21	0.97	0.93	0.17	0.33
5	21	0.19	0.24	0.24	0.23	0.99	0.99	0.18	0.38
5	22	0.19	0.27	0.25	0.27	1.03	1.05	0.19	0.44
5	23	0.20	0.31	0.26	0.30	1.05	1.10	0.21	0.51
5	24	0.20	0.35	0.28	0.34	1.08	1.14	0.23	0.58
5	25	0.21	0.39	0.29	0.38	1.11	1.18	0.25	0.65
5	26	0.22	0.42	0.31	0.42	1.14	1.21	0.28	0.71
5	27	0.23	0.46	0.33	0.46	1.16	1.24	0.31	0.78
5	28	0.24	0.50	0.35	0.50	1.18	1.26	0.34	0.84
5	29	0.26	0.54	0.38	0.55	1.20	1.28	0.38	0.90
5	30	0.27	0.58	0.41	0.60	1.22	1.30	0.42	0.95
5	31	0.28	0.63	0.43	0.65	1.24	1.32	0.45	1.00
5	32	0.29	0.67	0.46	0.70	1.26	1.34	0.48	1.05
5	33	0.31	0.71	0.48	0.73	1.27	1.35	0.52	1.09
5	34	0.33	0.75	0.51	0.77	1.28	1.37	0.56	1.13
5	35	0.34	0.79	0.54	0.80	1.29	1.39	0.62	1.16
5	36	0.36	0.82	0.56	0.83	1.30	1.40	0.66	1.19
5	37	0.38	0.85	0.59	0.85	1.31	1.42	0.70	1.21
5	38	0.40	0.87	0.61	0.87	1.31	1.43	0.74	1.23
5	39	0.43	0.89	0.64	0.89	1.32	1.44	0.78	1.26

Table A2-5$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under aerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
5	40	0.45	0.91	0.67	0.91	1.33	1.45	0.81	1.27
5	41	0.47	0.93	0.69	0.92	1.34	1.46	0.85	1.30
5	42	0.50	0.94	0.73	0.93	1.35	1.46	0.88	1.31
5	43	0.52	0.96	0.75	0.94	1.36	1.47	0.92	1.33
5	44	0.55	0.97	0.78	0.95	1.36	1.48	0.95	1.33
5	45	0.57	0.98	0.81	0.97	1.37	1.49	0.98	1.35
5	46	0.60	0.99	0.83	0.97	1.38	1.49	1.01	1.36
5	47	0.62	1.00	0.86	0.98	1.39	1.50	1.03	1.37
5	48	0.64	1.01	0.88	0.98	1.40	1.51	1.06	1.38
6	0	0.00	0.00	0.91	0.00	0.00	0.00	0.00	0.00
6	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	2	0.02	0.02	0.00	0.02	0.02	0.01	0.02	0.02
6	3	0.04	0.04	0.02	0.03	0.04	0.02	0.04	0.03
6	4	0.06	0.05	0.04	0.04	0.07	0.03	0.06	0.04
6	5	0.08	0.06	0.06	0.05	0.09	0.04	0.07	0.05
6	6	0.09	0.07	0.08	0.06	0.11	0.05	0.08	0.05
6	7	0.10	0.08	0.10	0.07	0.13	0.06	0.09	0.05
6	8	0.11	0.09	0.11	0.07	0.15	0.07	0.09	0.06
6	9	0.13	0.09	0.12	0.08	0.17	0.07	0.10	0.06
6	10	0.14	0.10	0.14	0.08	0.17	0.08	0.10	0.06
6	11	0.14	0.11	0.15	0.09	0.18	0.09	0.11	0.07
6	12	0.16	0.12	0.16	0.10	0.20	0.10	0.11	0.08
6	13	0.16	0.13	0.17	0.11	0.21	0.11	0.12	0.09
6	14	0.18	0.14	0.18	0.12	0.23	0.13	0.12	0.10
6	15	0.19	0.15	0.19	0.14	0.24	0.14	0.13	0.12
6	16	0.20	0.17	0.19	0.15	0.26	0.16	0.14	0.14
6	17	0.20	0.18	0.20	0.16	0.28	0.18	0.15	0.16
6	18	0.21	0.20	0.21	0.18	0.29	0.20	0.15	0.20
6	19	0.21	0.22	0.21	0.21	0.31	0.23	0.16	0.25
6	20	0.22	0.24	0.22	0.24	0.34	0.26	0.18	0.30
6	21	0.22	0.28	0.23	0.27	0.37	0.30	0.19	0.36
6	22	0.23	0.31	0.24	0.31	0.41	0.35	0.21	0.41
6	23	0.24	0.35	0.26	0.35	0.46	0.40	0.22	0.48
6	24	0.25	0.40	0.27	0.39	0.56	0.45	0.25	0.54
6	25	0.25	0.44	0.29	0.43	0.60	0.50	0.27	0.61
6	26	0.27	0.48	0.31	0.47	0.69	0.56	0.30	0.67
6	27	0.27	0.53	0.32	0.51	0.77	0.63	0.34	0.77
6	28	0.28	0.57	0.35	0.56	0.85	0.69	0.38	0.83
6	29	0.30	0.61	0.37	0.61	0.88	0.74	0.43	0.89
6	30	0.31	0.66	0.40	0.66	0.89	0.79	0.47	0.94

Table A2-5$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under aerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
6	31	0.34	0.71	0.43	0.71	0.91	0.83	0.52	1.00
6	32	0.35	0.75	0.46	0.75	0.92	0.86	0.56	1.04
6	33	0.37	0.80	0.48	0.79	0.92	0.88	0.61	1.08
6	34	0.39	0.84	0.51	0.82	0.93	0.90	0.65	1.11
6	35	0.41	0.87	0.54	0.85	0.93	0.92	0.69	1.14
6	36	0.43	0.90	0.58	0.87	0.94	0.94	0.73	1.17
6	37	0.46	0.92	0.61	0.90	0.94	0.95	0.76	1.20
6	38	0.48	0.95	0.63	0.92	0.94	0.96	0.79	1.22
6	39	0.50	0.97	0.66	0.93	0.95	0.96	0.83	1.24
6	40	0.53	0.98	0.69	0.95	0.96	0.97	0.86	1.25
6	41	0.55	1.00	0.72	0.96	0.96	0.98	0.89	1.27
6	42	0.58	1.01	0.74	0.98	0.96	0.98	0.92	1.28
6	43	0.61	1.02	0.77	0.98	0.96	0.99	0.95	1.29
6	44	0.64	1.03	0.79	0.99	0.96	0.99	0.98	1.30
6	45	0.66	1.04	0.81	1.00	0.96	0.99	1.01	1.31
6	46	0.70	1.05	0.82	1.01	0.95	1.00	1.04	1.32
6	47	0.73	1.06	0.85	1.01	0.96	1.00	1.06	1.33
6	48	0.75	1.06	0.86	1.02	0.96	1.00	1.09	1.34

Notes: A2.5=2.5 % Manuka honey 20+ UMF; AC2.5=2.5 % Manuka honey 20+ UMF with catalase; B2.5=2.5 % Manuka honey 15+ UMF; BC2.5=2.5 % Manuka honey 15+ UMF with catalase; C2.5=2.5 % Manuka honey 10+ UMF; CC2.5=2.5 % Manuka honey 10+ UMF with catalase; K2.5=2.5 % Kanuka honey; KC2.5=2.5 % Kanuka honey with catalase

Table A2-6 Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under anaerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.02	0.03	0.01	0.03	0.01	0.03	0.01	0.02
1	3	0.02	0.05	0.03	0.04	0.02	0.05	0.02	0.05
1	4	0.03	0.06	0.04	0.06	0.04	0.06	0.03	0.07
1	5	0.05	0.07	0.05	0.08	0.05	0.08	0.04	0.09
1	6	0.06	0.08	0.06	0.09	0.07	0.11	0.05	0.11
1	7	0.07	0.09	0.07	0.10	0.09	0.11	0.06	0.13
1	8	0.08	0.10	0.09	0.11	0.11	0.12	0.07	0.16
1	9	0.10	0.11	0.10	0.12	0.13	0.14	0.09	0.18
1	10	0.11	0.13	0.11	0.13	0.15	0.16	0.10	0.21
1	11	0.12	0.14	0.12	0.14	0.16	0.17	0.12	0.24
1	12	0.14	0.15	0.13	0.15	0.18	0.19	0.14	0.26
1	13	0.15	0.16	0.15	0.17	0.20	0.21	0.14	0.29
1	14	0.16	0.17	0.16	0.18	0.22	0.22	0.16	0.33
1	15	0.17	0.19	0.17	0.19	0.24	0.24	0.17	0.38
1	16	0.19	0.20	0.18	0.21	0.26	0.26	0.19	0.47
1	17	0.20	0.21	0.19	0.23	0.28	0.29	0.21	0.61
1	18	0.21	0.23	0.20	0.24	0.29	0.30	0.22	0.77
1	19	0.22	0.24	0.21	0.26	0.31	0.33	0.24	0.87
1	20	0.24	0.25	0.22	0.28	0.32	0.35	0.27	0.93
1	21	0.25	0.27	0.23	0.29	0.34	0.37	0.29	0.96
1	22	0.26	0.28	0.29	0.31	0.35	0.39	0.31	0.97
1	23	0.27	0.29	0.31	0.32	0.36	0.40	0.35	0.98
1	24	0.28	0.31	0.33	0.34	0.37	0.42	0.37	0.99
1	25	0.29	0.32	0.34	0.35	0.37	0.44	0.39	0.99
1	26	0.30	0.34	0.35	0.37	0.37	0.46	0.41	0.99
1	27	0.31	0.35	0.37	0.39	0.37	0.48	0.42	0.99
1	28	0.32	0.37	0.38	0.41	0.37	0.51	0.43	0.99
1	29	0.33	0.39	0.39	0.43	0.37	0.53	0.45	0.99
1	30	0.34	0.40	0.42	0.45	0.37	0.55	0.47	0.99
1	31	0.35	0.42	0.42	0.47	0.37	0.58	0.49	1.00
1	32	0.36	0.44	0.44	0.49	0.37	0.61	0.51	1.00
1	33	0.37	0.46	0.45	0.51	0.37	0.64	0.54	1.00
1	34	0.38	0.48	0.47	0.53	0.38	0.67	0.53	1.00
1	35	0.39	0.50	0.49	0.56	0.39	0.70	0.54	1.00
1	36	0.41	0.52	0.48	0.59	0.39	0.73	0.55	1.00
1	37	0.42	0.54	0.50	0.61	0.40	0.76	0.56	1.00
1	38	0.43	0.56	0.52	0.65	0.41	0.79	0.58	1.00
1	39	0.45	0.58	0.54	0.68	0.42	0.82	0.60	0.99

Table A2-6$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under anaerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
1	40	0.46	0.61	0.55	0.70	0.43	0.85	0.63	0.99
1	41	0.47	0.63	0.57	0.74	0.44	0.88	0.66	0.99
1	42	0.49	0.66	0.59	0.77	0.45	0.91	0.69	0.99
1	43	0.50	0.69	0.60	0.80	0.46	0.93	0.71	0.99
1	44	0.52	0.71	0.62	0.82	0.47	0.96	0.74	0.99
1	45	0.53	0.74	0.64	0.85	0.48	0.98	0.75	0.99
1	46	0.55	0.77	0.65	0.88	0.50	1.01	0.77	0.99
1	47	0.56	0.80	0.67	0.90	0.51	1.03	0.79	1.00
1	48	0.59	0.83	0.69	0.93	0.53	1.05	0.81	1.00
2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.03	0.03	0.00	0.03	0.01	0.03	0.01	0.03
2	3	0.01	0.05	0.01	0.05	0.03	0.05	0.02	0.05
2	4	0.02	0.06	0.01	0.06	0.04	0.06	0.03	0.07
2	5	0.02	0.07	0.02	0.07	0.05	0.08	0.04	0.09
2	6	0.03	0.09	0.03	0.09	0.06	0.10	0.06	0.11
2	7	0.04	0.10	0.04	0.10	0.08	0.11	0.07	0.13
2	8	0.05	0.11	0.05	0.11	0.09	0.12	0.09	0.15
2	9	0.05	0.12	0.06	0.12	0.10	0.14	0.11	0.18
2	10	0.06	0.13	0.08	0.13	0.12	0.16	0.13	0.20
2	11	0.07	0.14	0.09	0.15	0.13	0.17	0.14	0.23
2	12	0.08	0.15	0.10	0.16	0.14	0.19	0.17	0.26
2	13	0.09	0.16	0.11	0.17	0.15	0.20	0.18	0.29
2	14	0.10	0.17	0.12	0.19	0.17	0.22	0.20	0.31
2	15	0.11	0.18	0.13	0.20	0.18	0.24	0.22	0.34
2	16	0.11	0.20	0.14	0.21	0.20	0.26	0.24	0.37
2	17	0.12	0.21	0.15	0.23	0.21	0.28	0.26	0.40
2	18	0.13	0.22	0.16	0.25	0.23	0.30	0.27	0.43
2	19	0.14	0.24	0.18	0.26	0.24	0.32	0.29	0.45
2	20	0.15	0.25	0.19	0.28	0.26	0.34	0.29	0.48
2	21	0.15	0.27	0.20	0.30	0.27	0.36	0.30	0.51
2	22	0.16	0.28	0.22	0.31	0.32	0.38	0.30	0.53
2	23	0.18	0.29	0.24	0.33	0.39	0.39	0.30	0.56
2	24	0.22	0.31	0.27	0.34	0.49	0.41	0.31	0.58
2	25	0.24	0.32	0.27	0.36	0.50	0.43	0.32	0.61
2	26	0.25	0.34	0.28	0.38	0.49	0.45	0.34	0.64
2	27	0.26	0.35	0.29	0.40	0.49	0.48	0.36	0.67
2	28	0.27	0.37	0.30	0.42	0.52	0.50	0.39	0.70
2	29	0.28	0.39	0.30	0.44	0.57	0.52	0.41	0.73
2	30	0.29	0.40	0.34	0.45	0.61	0.54	0.43	0.77

Table A2-6$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under anaerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
2	31	0.30	0.42	0.35	0.48	0.66	0.57	0.45	0.80
2	32	0.32	0.44	0.37	0.50	0.71	0.59	0.46	0.83
2	33	0.34	0.46	0.37	0.52	0.77	0.62	0.48	0.86
2	34	0.36	0.48	0.38	0.54	0.79	0.65	0.50	0.89
2	35	0.38	0.50	0.39	0.57	0.79	0.68	0.52	0.92
2	36	0.38	0.52	0.39	0.60	0.80	0.71	0.54	0.95
2	37	0.38	0.54	0.40	0.63	0.82	0.75	0.56	0.98
2	38	0.39	0.56	0.41	0.66	0.83	0.78	0.58	1.01
2	39	0.40	0.59	0.42	0.69	0.84	0.81	0.60	1.03
2	40	0.41	0.61	0.42	0.72	0.86	0.83	0.62	1.06
2	41	0.42	0.63	0.43	0.74	0.88	0.86	0.64	1.08
2	42	0.43	0.66	0.44	0.78	0.90	0.89	0.66	1.11
2	43	0.45	0.68	0.44	0.80	0.91	0.92	0.68	1.13
2	44	0.46	0.71	0.45	0.83	0.93	0.94	0.70	1.15
2	45	0.47	0.74	0.46	0.86	0.94	0.96	0.73	1.17
2	46	0.48	0.77	0.46	0.88	0.96	0.99	0.75	1.19
2	47	0.50	0.79	0.47	0.91	0.98	1.01	0.77	1.21
2	48	0.51	0.82	0.48	0.93	0.99	1.04	0.79	1.23
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.01	0.03	0.00	0.02	0.01	0.03	0.01	0.03
3	3	0.02	0.04	0.01	0.05	0.03	0.06	0.03	0.06
3	4	0.02	0.06	0.02	0.06	0.04	0.07	0.04	0.07
3	5	0.04	0.07	0.03	0.08	0.05	0.09	0.05	0.10
3	6	0.05	0.08	0.04	0.09	0.07	0.10	0.06	0.12
3	7	0.06	0.09	0.05	0.10	0.08	0.12	0.08	0.14
3	8	0.07	0.11	0.06	0.11	0.10	0.13	0.09	0.17
3	9	0.08	0.12	0.08	0.13	0.11	0.15	0.10	0.19
3	10	0.10	0.13	0.09	0.14	0.13	0.16	0.12	0.22
3	11	0.11	0.14	0.10	0.15	0.14	0.18	0.13	0.25
3	12	0.12	0.15	0.11	0.16	0.15	0.20	0.15	0.28
3	13	0.14	0.16	0.12	0.18	0.17	0.22	0.16	0.31
3	14	0.15	0.18	0.14	0.19	0.18	0.23	0.18	0.33
3	15	0.17	0.19	0.16	0.21	0.19	0.25	0.20	0.36
3	16	0.18	0.20	0.17	0.23	0.21	0.28	0.21	0.39
3	17	0.20	0.22	0.18	0.24	0.22	0.30	0.23	0.42
3	18	0.21	0.23	0.20	0.26	0.23	0.33	0.25	0.45
3	19	0.22	0.25	0.22	0.28	0.24	0.38	0.28	0.48
3	20	0.24	0.26	0.23	0.30	0.25	0.48	0.31	0.50

Table A2-6$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under anaerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
3	21	0.25	0.28	0.26	0.31	0.27	0.58	0.33	0.53
3	22	0.26	0.29	0.27	0.33	0.31	0.66	0.36	0.56
3	23	0.27	0.31	0.27	0.34	0.35	0.74	0.39	0.58
3	24	0.28	0.32	0.30	0.36	0.37	0.80	0.40	0.61
3	25	0.30	0.34	0.28	0.38	0.40	0.85	0.41	0.64
3	26	0.31	0.35	0.30	0.40	0.42	0.87	0.41	0.67
3	27	0.32	0.37	0.30	0.42	0.42	0.90	0.40	0.70
3	28	0.33	0.39	0.30	0.44	0.43	0.90	0.41	0.73
3	29	0.33	0.40	0.30	0.46	0.43	0.91	0.42	0.76
3	30	0.34	0.42	0.31	0.48	0.42	0.91	0.43	0.79
3	31	0.35	0.44	0.31	0.50	0.43	0.91	0.45	0.82
3	32	0.36	0.46	0.32	0.52	0.43	0.91	0.47	0.85
3	33	0.37	0.48	0.32	0.54	0.43	0.91	0.49	0.88
3	34	0.38	0.49	0.33	0.57	0.49	0.91	0.51	0.91
3	35	0.38	0.52	0.33	0.60	0.43	0.91	0.53	0.94
3	36	0.40	0.54	0.34	0.62	0.45	0.91	0.55	0.97
3	37	0.41	0.56	0.35	0.65	0.47	0.90	0.57	1.00
3	38	0.42	0.58	0.35	0.68	0.49	0.90	0.59	1.02
3	39	0.43	0.61	0.36	0.71	0.52	0.90	0.61	1.04
3	40	0.44	0.63	0.37	0.74	0.54	0.90	0.63	1.07
3	41	0.45	0.65	0.38	0.77	0.55	0.90	0.65	1.09
3	42	0.46	0.68	0.38	0.80	0.57	0.90	0.67	1.12
3	43	0.47	0.71	0.39	0.83	0.59	0.90	0.69	1.14
3	44	0.48	0.74	0.40	0.85	0.61	0.90	0.71	1.16
3	45	0.49	0.76	0.41	0.88	0.62	0.90	0.73	1.18
3	46	0.51	0.79	0.42	0.90	0.64	0.90	0.75	1.19
3	47	0.52	0.82	0.43	0.93	0.67	0.90	0.77	1.21
3	48	0.53	0.84	0.44	0.96	0.69	0.90	0.79	1.23
4	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	2	0.01	0.02	0.02	0.03	0.01	0.03	0.02	0.03
4	3	0.03	0.04	0.03	0.04	0.02	0.05	0.04	0.05
4	4	0.05	0.05	0.04	0.06	0.03	0.06	0.05	0.08
4	5	0.06	0.06	0.06	0.07	0.04	0.09	0.06	0.10
4	6	0.08	0.07	0.07	0.08	0.06	0.10	0.08	0.13
4	7	0.10	0.08	0.09	0.09	0.07	0.12	0.11	0.16
4	8	0.11	0.09	0.10	0.10	0.08	0.13	0.14	0.19
4	9	0.13	0.10	0.11	0.12	0.09	0.14	0.17	0.23
4	10	0.14	0.12	0.12	0.13	0.11	0.17	0.19	0.27

Table A2-6$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under anaerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
4	11	0.16	0.13	0.14	0.15	0.12	0.18	0.22	0.31
4	12	0.18	0.14	0.15	0.16	0.13	0.21	0.25	0.34
4	13	0.19	0.16	0.16	0.18	0.15	0.23	0.27	0.37
4	14	0.21	0.17	0.17	0.20	0.16	0.25	0.30	0.40
4	15	0.22	0.18	0.18	0.21	0.18	0.27	0.32	0.44
4	16	0.24	0.19	0.20	0.23	0.21	0.29	0.34	0.47
4	17	0.26	0.21	0.22	0.25	0.22	0.32	0.35	0.50
4	18	0.27	0.22	0.23	0.28	0.23	0.34	0.38	0.53
4	19	0.29	0.24	0.25	0.29	0.24	0.36	0.40	0.56
4	20	0.31	0.25	0.26	0.32	0.24	0.38	0.43	0.59
4	21	0.32	0.26	0.27	0.34	0.25	0.41	0.46	0.61
4	22	0.34	0.28	0.29	0.36	0.25	0.43	0.48	0.64
4	23	0.35	0.29	0.30	0.38	0.26	0.45	0.50	0.68
4	24	0.37	0.30	0.32	0.40	0.26	0.48	0.52	0.71
4	25	0.38	0.32	0.33	0.43	0.26	0.50	0.54	0.74
4	26	0.39	0.33	0.35	0.45	0.26	0.53	0.56	0.77
4	27	0.40	0.34	0.36	0.47	0.26	0.56	0.59	0.81
4	28	0.41	0.36	0.37	0.50	0.27	0.59	0.61	0.84
4	29	0.42	0.37	0.39	0.52	0.27	0.62	0.64	0.88
4	30	0.43	0.39	0.40	0.55	0.27	0.65	0.67	0.91
4	31	0.44	0.41	0.42	0.57	0.27	0.68	0.69	0.94
4	32	0.45	0.42	0.43	0.60	0.27	0.71	0.71	0.97
4	33	0.46	0.44	0.45	0.63	0.28	0.74	0.74	1.00
4	34	0.47	0.46	0.47	0.66	0.28	0.77	0.76	1.03
4	35	0.48	0.48	0.48	0.69	0.29	0.81	0.79	1.05
4	36	0.49	0.50	0.50	0.72	0.29	0.83	0.80	1.08
4	37	0.50	0.52	0.51	0.75	0.30	0.86	0.83	1.10
4	38	0.51	0.54	0.52	0.78	0.31	0.89	0.85	1.13
4	39	0.52	0.56	0.54	0.81	0.31	0.92	0.87	1.15
4	40	0.53	0.58	0.55	0.84	0.32	0.94	0.89	1.17
4	41	0.55	0.61	0.57	0.86	0.33	0.97	0.91	1.19
4	42	0.56	0.63	0.58	0.89	0.34	0.99	0.94	1.21
4	43	0.57	0.66	0.59	0.92	0.35	1.01	0.95	1.22
4	44	0.58	0.68	0.60	0.94	0.37	1.03	0.96	1.23
4	45	0.59	0.71	0.62	0.97	0.39	1.05	0.98	1.25
4	46	0.60	0.75	0.64	1.00	0.41	1.07	1.02	1.26
4	47	0.62	0.77	0.66	1.03	0.43	1.09	1.05	1.28
4	48	0.64	0.81	0.68	1.06	0.45	1.11	1.08	1.29

Table A2-6$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under anaerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
5	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	2	0.01	0.03	0.01	0.02	0.01	0.03	0.02	0.05
5	3	0.02	0.03	0.02	0.04	0.03	0.05	0.04	0.08
5	4	0.03	0.05	0.03	0.06	0.04	0.06	0.06	0.11
5	5	0.04	0.07	0.04	0.07	0.06	0.08	0.08	0.14
5	6	0.05	0.07	0.06	0.08	0.07	0.10	0.10	0.16
5	7	0.06	0.08	0.07	0.09	0.08	0.11	0.13	0.19
5	8	0.07	0.10	0.08	0.11	0.10	0.13	0.14	0.23
5	9	0.09	0.11	0.10	0.12	0.11	0.15	0.17	0.26
5	10	0.11	0.12	0.13	0.13	0.13	0.17	0.19	0.30
5	11	0.13	0.13	0.15	0.15	0.14	0.19	0.22	0.33
5	12	0.16	0.15	0.17	0.16	0.15	0.21	0.25	0.37
5	13	0.17	0.17	0.18	0.18	0.17	0.23	0.27	0.40
5	14	0.19	0.18	0.19	0.20	0.18	0.25	0.29	0.44
5	15	0.20	0.19	0.21	0.21	0.21	0.28	0.31	0.47
5	16	0.20	0.21	0.25	0.23	0.23	0.30	0.32	0.50
5	17	0.24	0.22	0.26	0.25	0.25	0.32	0.34	0.52
5	18	0.25	0.24	0.26	0.27	0.27	0.34	0.37	0.55
5	19	0.26	0.26	0.27	0.29	0.28	0.37	0.39	0.58
5	20	0.26	0.27	0.28	0.31	0.30	0.39	0.43	0.61
5	21	0.27	0.29	0.28	0.34	0.31	0.41	0.46	0.63
5	22	0.27	0.31	0.29	0.36	0.33	0.44	0.49	0.67
5	23	0.28	0.32	0.29	0.38	0.34	0.46	0.53	0.70
5	24	0.29	0.34	0.30	0.41	0.34	0.48	0.56	0.73
5	25	0.29	0.35	0.30	0.43	0.35	0.51	0.58	0.77
5	26	0.29	0.37	0.30	0.45	0.35	0.54	0.61	0.80
5	27	0.29	0.39	0.30	0.47	0.34	0.56	0.63	0.83
5	28	0.30	0.40	0.31	0.50	0.34	0.59	0.66	0.87
5	29	0.30	0.42	0.31	0.52	0.34	0.62	0.68	0.90
5	30	0.31	0.44	0.32	0.55	0.34	0.65	0.70	0.93
5	31	0.31	0.46	0.32	0.57	0.34	0.68	0.73	0.96
5	32	0.31	0.47	0.33	0.60	0.34	0.71	0.75	0.99
5	33	0.32	0.49	0.33	0.63	0.34	0.74	0.77	1.02
5	34	0.32	0.51	0.34	0.66	0.33	0.77	0.80	1.04
5	35	0.33	0.53	0.35	0.69	0.34	0.80	0.82	1.07
5	36	0.33	0.55	0.36	0.72	0.34	0.83	0.85	1.09
5	37	0.34	0.57	0.37	0.75	0.34	0.85	0.86	1.11
5	38	0.34	0.59	0.38	0.77	0.34	0.88	0.89	1.14
5	39	0.35	0.61	0.40	0.81	0.35	0.91	0.91	1.16

Table A2-6$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under anaerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
5	40	0.36	0.63	0.41	0.83	0.36	0.93	0.93	1.18
5	41	0.37	0.66	0.42	0.86	0.36	0.95	0.95	1.20
5	42	0.37	0.68	0.44	0.89	0.37	0.98	0.97	1.22
5	43	0.38	0.70	0.45	0.92	0.38	1.00	0.99	1.23
5	44	0.40	0.73	0.48	0.94	0.38	1.01	1.00	1.25
5	45	0.46	0.76	0.56	0.97	0.39	1.04	1.01	1.26
5	46	0.48	0.79	0.59	1.00	0.40	1.06	1.05	1.27
5	47	0.49	0.82	0.61	1.02	0.42	1.08	1.08	1.27
5	48	0.51	0.85	0.64	1.05	0.44	1.10	1.11	1.28
6	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	2	0.01	0.03	0.02	0.03	0.01	0.03	0.02	0.03
6	3	0.03	0.04	0.03	0.05	0.03	0.05	0.04	0.06
6	4	0.03	0.05	0.04	0.06	0.05	0.07	0.06	0.08
6	5	0.04	0.07	0.05	0.08	0.06	0.08	0.08	0.13
6	6	0.05	0.08	0.07	0.09	0.07	0.10	0.10	0.15
6	7	0.07	0.09	0.08	0.10	0.09	0.12	0.12	0.18
6	8	0.08	0.10	0.09	0.11	0.10	0.14	0.15	0.22
6	9	0.09	0.11	0.11	0.13	0.12	0.16	0.17	0.26
6	10	0.11	0.12	0.13	0.14	0.12	0.18	0.20	0.30
6	11	0.13	0.14	0.15	0.15	0.16	0.20	0.22	0.35
6	12	0.14	0.15	0.17	0.17	0.17	0.22	0.25	0.39
6	13	0.16	0.17	0.19	0.19	0.19	0.25	0.27	0.43
6	14	0.17	0.18	0.21	0.20	0.20	0.27	0.30	0.48
6	15	0.18	0.20	0.23	0.22	0.21	0.29	0.31	0.51
6	16	0.20	0.22	0.26	0.24	0.22	0.32	0.33	0.54
6	17	0.23	0.23	0.27	0.25	0.24	0.34	0.35	0.56
6	18	0.24	0.26	0.28	0.28	0.25	0.36	0.37	0.59
6	19	0.25	0.27	0.29	0.30	0.27	0.38	0.40	0.61
6	20	0.25	0.29	0.31	0.32	0.28	0.41	0.44	0.63
6	21	0.26	0.31	0.31	0.34	0.29	0.43	0.47	0.66
6	22	0.27	0.34	0.32	0.36	0.30	0.46	0.51	0.68
6	23	0.27	0.35	0.33	0.39	0.31	0.49	0.53	0.71
6	24	0.28	0.37	0.34	0.41	0.31	0.52	0.56	0.73
6	25	0.28	0.39	0.35	0.43	0.32	0.56	0.59	0.78
6	26	0.28	0.40	0.36	0.45	0.32	0.60	0.61	0.82
6	27	0.29	0.42	0.36	0.47	0.31	0.65	0.64	0.85
6	28	0.29	0.44	0.37	0.50	0.31	0.70	0.66	0.88
6	29	0.30	0.46	0.38	0.52	0.31	0.75	0.69	0.91
6	30	0.30	0.47	0.39	0.55	0.31	0.79	0.71	0.94

Table A2-6$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 2.5 % honeys under anaerobic condition

Rep	Time (h)	A2.5	AC2.5	B2.5	BC2.5	C2.5	CC2.5	K2.5	KC2.5
6	31	0.30	0.49	0.40	0.57	0.31	0.82	0.74	0.97
6	32	0.31	0.51	0.41	0.60	0.31	0.85	0.76	1.00
6	33	0.31	0.52	0.43	0.63	0.32	0.87	0.78	1.03
6	34	0.31	0.54	0.44	0.65	0.32	0.89	0.80	1.05
6	35	0.32	0.56	0.45	0.68	0.32	0.90	0.83	1.07
6	36	0.32	0.58	0.47	0.71	0.32	0.91	0.85	1.09
6	37	0.33	0.59	0.48	0.74	0.33	0.92	0.88	1.12
6	38	0.33	0.62	0.50	0.77	0.33	0.92	0.90	1.14
6	39	0.34	0.63	0.52	0.80	0.34	0.93	0.92	1.16
6	40	0.35	0.65	0.53	0.83	0.34	0.93	0.94	1.18
6	41	0.35	0.68	0.55	0.85	0.35	0.93	0.96	1.20
6	42	0.36	0.70	0.56	0.88	0.36	0.93	0.98	1.21
6	43	0.37	0.72	0.58	0.91	0.37	0.93	1.00	1.23
6	44	0.38	0.75	0.61	0.93	0.38	0.93	1.00	1.23
6	45	0.41	0.77	0.64	0.96	0.39	0.93	1.02	1.24
6	46	0.43	0.80	0.67	0.99	0.40	0.93	1.06	1.25
6	47	0.46	0.83	0.69	1.02	0.42	0.93	1.09	1.27
6	48	0.48	0.86	0.72	1.05	0.44	0.93	1.12	1.28

Notes: A2.5=2.5 % Manuka honey 20+ UMF; AC2.5=2.5 % Manuka honey 20+ UMF with catalase; B2.5=2.5 % Manuka honey 15+ UMF; BC2.5=2.5 % Manuka honey 15+ UMF with catalase; C2.5=2.5 % Manuka honey 10+ UMF; CC2.5=2.5 % Manuka honey 10+ UMF with catalase; K2.5=2.5 % Kanuka honey; KC2.5=2.5 % Kanuka honey with catalase

Table A2-7 Growth of *P. acnes* (Abs $_{595nm}$) in 5 % honeys under aerobic condition

Rep	Time (h)	A5	AC5	B5	BC5	C5	CC5	K5	KC5
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.01
1	3	0.02	0.03	0.02	0.02	0.02	0.03	0.01	0.02
1	4	0.02	0.04	0.03	0.03	0.03	0.04	0.01	0.03
1	5	0.03	0.05	0.04	0.04	0.04	0.06	0.01	0.04
1	6	0.04	0.06	0.04	0.05	0.05	0.07	0.02	0.05
1	7	0.04	0.07	0.05	0.05	0.06	0.08	0.02	0.06
1	8	0.05	0.07	0.05	0.06	0.07	0.09	0.02	0.07
1	9	0.05	0.08	0.06	0.07	0.08	0.09	0.02	0.08
1	10	0.06	0.09	0.07	0.08	0.09	0.11	0.03	0.09
1	11	0.06	0.10	0.08	0.09	0.14	0.12	0.03	0.10
1	12	0.07	0.12	0.08	0.10	0.24	0.14	0.03	0.11
1	13	0.07	0.12	0.09	0.11	0.38	0.21	0.04	0.13
1	14	0.07	0.13	0.09	0.12	0.53	0.37	0.06	0.15
1	15	0.07	0.14	0.09	0.13	0.61	0.62	0.09	0.17
1	16	0.07	0.15	0.10	0.14	0.62	0.75	0.14	0.20
1	17	0.08	0.16	0.10	0.15	0.64	0.80	0.20	0.23
1	18	0.07	0.18	0.10	0.17	0.67	0.82	0.26	0.27
1	19	0.08	0.19	0.11	0.19	0.69	0.83	0.33	0.32
1	20	0.08	0.20	0.11	0.21	0.71	0.84	0.41	0.36
1	21	0.08	0.22	0.12	0.24	0.73	0.85	0.50	0.41
1	22	0.08	0.24	0.12	0.26	0.74	0.85	0.57	0.47
1	23	0.08	0.26	0.12	0.29	0.76	0.85	0.65	0.51
1	24	0.07	0.28	0.12	0.32	0.77	0.85	0.72	0.56
1	25	0.07	0.30	0.13	0.35	0.79	0.86	0.77	0.61
1	26	0.08	0.33	0.13	0.39	0.80	0.86	0.81	0.66
1	27	0.07	0.36	0.13	0.43	0.82	0.86	0.84	0.72
1	28	0.07	0.39	0.13	0.47	0.83	0.87	0.87	0.77
1	29	0.07	0.42	0.13	0.51	0.85	0.87	0.90	0.82
1	30	0.07	0.45	0.13	0.55	0.86	0.87	0.92	0.86
1	31	0.07	0.49	0.13	0.59	0.87	0.87	0.94	0.90
1	32	0.07	0.52	0.13	0.63	0.89	0.87	0.95	0.94
1	33	0.07	0.55	0.13	0.67	0.90	0.87	0.95	0.97
1	34	0.07	0.58	0.13	0.70	0.92	0.87	0.96	1.00
1	35	0.07	0.61	0.13	0.73	0.93	0.87	0.97	1.03
1	36	0.06	0.65	0.13	0.76	0.94	0.87	0.97	1.07
1	37	0.07	0.68	0.12	0.79	0.96	0.88	0.97	1.09
1	38	0.07	0.71	0.12	0.82	0.97	0.88	0.97	1.12
1	39	0.06	0.74	0.12	0.85	0.98	0.88	0.98	1.15

Table A2-7$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 5 % honeys under aerobic condition

Rep	Time (h)	A5	AC5	B5	BC5	C5	CC5	K5	KC5
1	40	0.06	0.76	0.12	0.88	0.99	0.88	0.98	1.17
1	41	0.06	0.79	0.11	0.91	1.00	0.87	0.98	1.19
1	42	0.06	0.81	0.12	0.94	1.01	0.88	0.99	1.21
1	43	0.06	0.84	0.11	0.96	1.03	0.88	0.99	1.23
1	44	0.06	0.86	0.11	0.99	1.04	0.88	0.99	1.24
1	45	0.06	0.89	0.11	1.01	1.05	0.88	0.99	1.26
1	46	0.06	0.91	0.11	1.03	1.06	0.88	1.00	1.27
1	47	0.06	0.93	0.11	1.05	1.06	0.88	1.00	1.28
1	48	0.06	0.95	0.11	1.07	1.07	0.88	1.01	1.29
2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.00	0.02	0.00	0.02	0.02	0.01	0.01	0.02
2	3	0.01	0.03	0.01	0.03	0.04	0.02	0.02	0.03
2	4	0.01	0.04	0.01	0.04	0.06	0.03	0.02	0.04
2	5	0.01	0.05	0.02	0.05	0.08	0.05	0.03	0.05
2	6	0.01	0.06	0.03	0.06	0.09	0.06	0.03	0.06
2	7	0.01	0.07	0.02	0.07	0.11	0.07	0.04	0.07
2	8	0.01	0.08	0.02	0.08	0.12	0.08	0.04	0.08
2	9	0.01	0.09	0.02	0.09	0.14	0.09	0.04	0.09
2	10	0.01	0.10	0.02	0.10	0.15	0.10	0.05	0.11
2	11	0.00	0.11	0.02	0.11	0.17	0.11	0.05	0.12
2	12	0.00	0.12	0.02	0.12	0.20	0.14	0.05	0.13
2	13	0.00	0.13	0.02	0.13	0.25	0.23	0.06	0.14
2	14	0.00	0.14	0.02	0.14	0.36	0.39	0.06	0.16
2	15	0.00	0.15	0.01	0.15	0.51	0.56	0.06	0.19
2	16	0.00	0.16	0.01	0.17	0.62	0.65	0.06	0.22
2	17	0.00	0.17	0.00	0.18	0.71	0.72	0.06	0.25
2	18	0.00	0.18	0.00	0.19	0.75	0.77	0.06	0.29
2	19	0.00	0.20	0.00	0.22	0.78	0.81	0.07	0.34
2	20	0.00	0.21	0.00	0.24	0.81	0.83	0.08	0.38
2	21	0.00	0.23	0.00	0.27	0.84	0.86	0.08	0.44
2	22	0.00	0.25	-0.01	0.29	0.85	0.89	0.08	0.49
2	23	0.00	0.27	-0.01	0.32	0.88	0.92	0.08	0.55
2	24	0.00	0.30	-0.02	0.35	0.89	0.95	0.07	0.60
2	25	0.00	0.32	-0.02	0.39	0.90	0.99	0.07	0.66
2	26	0.00	0.35	-0.02	0.42	0.92	1.02	0.08	0.72
2	27	0.00	0.38	-0.02	0.46	0.94	1.05	0.08	0.77
2	28	0.00	0.41	-0.02	0.50	0.96	1.08	0.08	0.82
2	29	0.00	0.44	-0.02	0.54	0.98	1.11	0.08	0.87
2	30	0.00	0.48	-0.03	0.58	1.00	1.14	0.08	0.91

Table A2-7$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 5 % honeys under aerobic condition

Rep	Time (h)	A5	AC5	B5	BC5	C5	CC5	K5	KC5
2	31	0.00	0.51	-0.03	0.63	1.02	1.17	0.08	0.95
2	32	0.00	0.55	-0.03	0.65	1.04	1.19	0.08	0.99
2	33	0.00	0.57	-0.03	0.68	1.06	1.21	0.08	1.02
2	34	0.00	0.61	-0.02	0.72	1.08	1.23	0.08	1.06
2	35	0.00	0.64	-0.02	0.75	1.10	1.24	0.08	1.08
2	36	0.00	0.68	-0.03	0.78	1.11	1.25	0.08	1.11
2	37	0.00	0.71	-0.04	0.80	1.13	1.27	0.07	1.14
2	38	0.00	0.74	-0.04	0.83	1.15	1.27	0.07	1.17
2	39	0.00	0.76	-0.03	0.86	1.16	1.29	0.07	1.19
2	40	0.00	0.79	-0.03	0.89	1.18	1.29	0.07	1.21
2	41	0.00	0.81	-0.03	0.92	1.19	1.30	0.07	1.23
2	42	0.00	0.84	-0.03	0.94	1.20	1.31	0.07	1.25
2	43	0.00	0.86	-0.03	0.97	1.22	1.32	0.07	1.27
2	44	0.00	0.89	-0.03	0.99	1.23	1.33	0.07	1.28
2	45	0.00	0.91	-0.03	1.02	1.24	1.34	0.07	1.30
2	46	0.00	0.93	-0.03	1.03	1.25	1.34	0.07	1.31
2	47	0.00	0.95	-0.03	1.06	1.26	1.35	0.07	1.32
2	48	0.00	0.98	-0.03	1.07	1.27	1.35	0.07	1.33
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.01	0.02	0.01	0.02	0.02	0.02	0.01	0.02
3	3	0.02	0.03	0.02	0.03	0.04	0.03	0.02	0.03
3	4	0.03	0.04	0.04	0.04	0.06	0.04	0.03	0.04
3	5	0.05	0.05	0.05	0.05	0.08	0.05	0.03	0.05
3	6	0.06	0.06	0.06	0.06	0.10	0.06	0.03	0.06
3	7	0.07	0.07	0.07	0.07	0.11	0.07	0.04	0.07
3	8	0.08	0.08	0.08	0.08	0.12	0.09	0.04	0.08
3	9	0.08	0.09	0.08	0.09	0.14	0.10	0.04	0.09
3	10	0.09	0.10	0.09	0.10	0.15	0.11	0.05	0.10
3	11	0.10	0.11	0.10	0.11	0.17	0.14	0.05	0.12
3	12	0.10	0.12	0.11	0.12	0.25	0.24	0.05	0.13
3	13	0.11	0.13	0.11	0.13	0.36	0.45	0.05	0.14
3	14	0.11	0.14	0.12	0.14	0.53	0.60	0.06	0.16
3	15	0.12	0.15	0.12	0.16	0.65	0.69	0.06	0.19
3	16	0.12	0.17	0.12	0.17	0.72	0.75	0.06	0.22
3	17	0.13	0.18	0.13	0.18	0.76	0.81	0.06	0.26
3	18	0.13	0.19	0.13	0.20	0.79	0.84	0.06	0.30
3	19	0.14	0.21	0.13	0.22	0.82	0.89	0.07	0.36
3	20	0.14	0.22	0.13	0.24	0.84	0.91	0.07	0.41

Table A2-7$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 5 % honeys under aerobic condition

Rep	Time (h)	A5	AC5	B5	BC5	C5	CC5	K5	KC5
3	21	0.14	0.24	0.14	0.27	0.87	0.94	0.08	0.48
3	22	0.15	0.27	0.14	0.30	0.88	0.96	0.08	0.54
3	23	0.15	0.29	0.14	0.33	0.90	0.99	0.08	0.60
3	24	0.15	0.31	0.13	0.36	0.91	1.02	0.08	0.66
3	25	0.16	0.34	0.13	0.40	0.93	1.06	0.08	0.72
3	26	0.16	0.37	0.14	0.43	0.95	1.08	0.08	0.79
3	27	0.16	0.40	0.13	0.48	0.97	1.11	0.08	0.84
3	28	0.17	0.43	0.13	0.52	0.99	1.14	0.08	0.89
3	29	0.17	0.47	0.13	0.56	1.01	1.17	0.08	0.93
3	30	0.18	0.50	0.13	0.60	1.03	1.20	0.08	0.96
3	31	0.18	0.54	0.13	0.64	1.05	1.23	0.08	1.00
3	32	0.18	0.57	0.13	0.67	1.07	1.24	0.08	1.04
3	33	0.19	0.61	0.13	0.71	1.09	1.26	0.08	1.07
3	34	0.19	0.64	0.13	0.74	1.10	1.27	0.08	1.10
3	35	0.19	0.68	0.12	0.77	1.12	1.28	0.08	1.13
3	36	0.19	0.71	0.12	0.80	1.14	1.29	0.08	1.16
3	37	0.20	0.74	0.12	0.83	1.15	1.30	0.08	1.18
3	38	0.20	0.77	0.12	0.85	1.17	1.31	0.08	1.21
3	39	0.20	0.80	0.12	0.88	1.18	1.32	0.08	1.23
3	40	0.20	0.82	0.11	0.91	1.19	1.33	0.08	1.24
3	41	0.20	0.85	0.11	0.94	1.21	1.34	0.08	1.26
3	42	0.20	0.87	0.11	0.96	1.22	1.34	0.08	1.28
3	43	0.20	0.89	0.11	0.99	1.23	1.35	0.08	1.29
3	44	0.21	0.92	0.11	1.01	1.24	1.36	0.08	1.30
3	45	0.21	0.94	0.11	1.04	1.25	1.36	0.08	1.32
3	46	0.21	0.96	0.11	1.06	1.26	1.37	0.08	1.32
3	47	0.21	0.98	0.10	1.07	1.27	1.37	0.08	1.33
3	48	0.21	1.00	0.10	1.09	1.28	1.38	0.08	1.34

Notes: A5=5 % Manuka honey 20+ UMF; AC5=5 % Manuka honey 20+ UMF with catalase; B5=5 % Manuka honey 15+ UMF; BC5=5 % Manuka honey 15+ UMF with catalase; C5=5 % Manuka honey 10+ UMF; CC5=5 % Manuka honey 10+ UMF with catalase; K5=5 % Kanuka honey; KC5=5 % Kanuka honey with catalase

Table A2-8 Growth of *P. acnes* (Abs $_{595nm}$) in 5 % honeys under anaerobic condition

Rep	Time (h)	A5	AC5	B5	BC5	C5	CC5	K5	KC5
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.01	0.02	0.01	0.01	0.01	0.03	0.02	0.02
1	3	0.01	0.03	0.03	0.02	0.02	0.04	0.03	0.04
1	4	0.02	0.04	0.04	0.03	0.03	0.05	0.04	0.06
1	5	0.03	0.05	0.05	0.04	0.04	0.06	0.05	0.07
1	6	0.04	0.06	0.06	0.05	0.06	0.08	0.07	0.09
1	7	0.05	0.06	0.07	0.05	0.07	0.08	0.09	0.11
1	8	0.05	0.07	0.08	0.06	0.08	0.09	0.12	0.14
1	9	0.05	0.07	0.09	0.06	0.09	0.10	0.14	0.16
1	10	0.06	0.08	0.10	0.07	0.11	0.11	0.16	0.20
1	11	0.06	0.09	0.11	0.08	0.12	0.12	0.18	0.22
1	12	0.07	0.10	0.12	0.09	0.13	0.13	0.20	0.25
1	13	0.07	0.11	0.13	0.09	0.15	0.14	0.23	0.28
1	14	0.08	0.11	0.14	0.10	0.16	0.15	0.25	0.31
1	15	0.08	0.12	0.14	0.11	0.18	0.17	0.26	0.33
1	16	0.09	0.13	0.17	0.12	0.21	0.18	0.28	0.36
1	17	0.10	0.14	0.18	0.13	0.22	0.20	0.30	0.38
1	18	0.10	0.15	0.19	0.14	0.23	0.22	0.32	0.41
1	19	0.11	0.16	0.20	0.15	0.24	0.23	0.34	0.43
1	20	0.12	0.17	0.21	0.17	0.24	0.24	0.37	0.46
1	21	0.13	0.18	0.22	0.18	0.25	0.26	0.40	0.50
1	22	0.14	0.19	0.23	0.19	0.25	0.27	0.42	0.53
1	23	0.15	0.20	0.25	0.20	0.26	0.28	0.46	0.58
1	24	0.15	0.21	0.26	0.22	0.26	0.29	0.49	0.61
1	25	0.16	0.22	0.27	0.23	0.26	0.30	0.51	0.64
1	26	0.17	0.23	0.28	0.24	0.26	0.31	0.53	0.68
1	27	0.18	0.24	0.29	0.25	0.26	0.33	0.56	0.71
1	28	0.19	0.25	0.30	0.26	0.27	0.34	0.59	0.74
1	29	0.20	0.26	0.31	0.27	0.27	0.35	0.60	0.77
1	30	0.21	0.27	0.32	0.29	0.27	0.37	0.61	0.80
1	31	0.21	0.28	0.33	0.30	0.27	0.38	0.64	0.84
1	32	0.22	0.28	0.34	0.31	0.27	0.39	0.66	0.87
1	33	0.23	0.29	0.35	0.33	0.28	0.40	0.69	0.90
1	34	0.24	0.30	0.36	0.34	0.28	0.42	0.71	0.92
1	35	0.24	0.31	0.37	0.36	0.29	0.44	0.73	0.95
1	36	0.25	0.32	0.38	0.38	0.29	0.46	0.75	0.98
1	37	0.26	0.32	0.38	0.40	0.30	0.48	0.77	1.00
1	38	0.27	0.33	0.39	0.41	0.31	0.50	0.79	1.03
1	39	0.27	0.33	0.40	0.44	0.31	0.52	0.82	1.05

Table A2-8$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 5 % honeys under anaerobic condition

Rep	Time (h)	A5	AC5	B5	BC5	C5	CC5	K5	KC5
1	40	0.28	0.34	0.41	0.46	0.32	0.54	0.84	1.08
1	41	0.29	0.35	0.42	0.48	0.33	0.57	0.87	1.10
1	42	0.30	0.35	0.43	0.51	0.34	0.59	0.89	1.12
1	43	0.30	0.36	0.43	0.53	0.35	0.61	0.91	1.14
1	44	0.31	0.37	0.45	0.54	0.37	0.64	0.94	1.16
1	45	0.32	0.38	0.46	0.55	0.39	0.67	0.93	1.18
1	46	0.33	0.40	0.47	0.58	0.41	0.70	0.96	1.20
1	47	0.34	0.41	0.48	0.61	0.43	0.73	1.01	1.22
1	48	0.35	0.43	0.49	0.64	0.45	0.76	1.03	1.23
2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.00	0.02	0.00	0.02	0.01	0.02	0.02	0.02
2	3	0.01	0.03	0.02	0.03	0.03	0.03	0.03	0.04
2	4	0.01	0.04	0.02	0.04	0.04	0.04	0.05	0.06
2	5	0.02	0.05	0.03	0.05	0.06	0.05	0.06	0.08
2	6	0.02	0.06	0.04	0.05	0.07	0.06	0.08	0.10
2	7	0.02	0.07	0.05	0.06	0.08	0.07	0.10	0.12
2	8	0.03	0.06	0.06	0.07	0.10	0.08	0.12	0.14
2	9	0.04	0.07	0.07	0.07	0.11	0.09	0.14	0.17
2	10	0.04	0.08	0.08	0.08	0.13	0.10	0.16	0.20
2	11	0.05	0.09	0.08	0.08	0.14	0.11	0.18	0.22
2	12	0.05	0.10	0.09	0.09	0.15	0.12	0.20	0.25
2	13	0.05	0.10	0.11	0.10	0.17	0.13	0.22	0.28
2	14	0.07	0.11	0.12	0.11	0.18	0.15	0.23	0.31
2	15	0.08	0.12	0.12	0.11	0.21	0.16	0.24	0.34
2	16	0.08	0.12	0.13	0.12	0.23	0.17	0.26	0.37
2	17	0.09	0.13	0.13	0.13	0.25	0.19	0.28	0.42
2	18	0.09	0.14	0.14	0.15	0.27	0.21	0.31	0.47
2	19	0.10	0.15	0.15	0.15	0.28	0.22	0.33	0.53
2	20	0.10	0.17	0.15	0.16	0.30	0.23	0.37	0.59
2	21	0.11	0.17	0.16	0.18	0.31	0.25	0.41	0.64
2	22	0.12	0.19	0.16	0.19	0.33	0.26	0.44	0.69
2	23	0.12	0.20	0.17	0.20	0.34	0.27	0.48	0.74
2	24	0.13	0.21	0.17	0.21	0.34	0.28	0.50	0.78
2	25	0.14	0.21	0.18	0.22	0.35	0.29	0.52	0.81
2	26	0.14	0.22	0.18	0.23	0.35	0.30	0.54	0.84
2	27	0.15	0.23	0.18	0.24	0.34	0.31	0.56	0.86
2	28	0.16	0.24	0.18	0.25	0.34	0.32	0.58	0.87
2	29	0.16	0.25	0.18	0.26	0.34	0.33	0.60	0.89
2	30	0.18	0.26	0.19	0.27	0.34	0.34	0.61	0.90

Table A2-8$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 5 % honeys under anaerobic condition

Rep	Time (h)	A5	AC5	B5	BC5	C5	CC5	K5	KC5
2	31	0.18	0.27	0.19	0.29	0.34	0.35	0.63	0.91
2	32	0.19	0.27	0.19	0.30	0.34	0.36	0.65	0.92
2	33	0.20	0.28	0.19	0.31	0.34	0.38	0.68	0.92
2	34	0.21	0.29	0.19	0.32	0.33	0.39	0.68	0.92
2	35	0.22	0.30	0.20	0.34	0.34	0.41	0.71	0.93
2	36	0.22	0.31	0.20	0.36	0.34	0.42	0.73	0.93
2	37	0.23	0.31	0.20	0.38	0.34	0.44	0.75	0.93
2	38	0.24	0.32	0.20	0.40	0.34	0.46	0.77	0.93
2	39	0.25	0.33	0.21	0.42	0.35	0.49	0.79	0.93
2	40	0.25	0.34	0.21	0.44	0.36	0.51	0.81	0.94
2	41	0.26	0.35	0.22	0.47	0.36	0.53	0.81	0.94
2	42	0.27	0.35	0.22	0.49	0.37	0.56	0.85	0.93
2	43	0.27	0.37	0.23	0.51	0.38	0.58	0.88	0.93
2	44	0.28	0.37	0.24	0.53	0.38	0.60	0.88	0.93
2	45	0.28	0.38	0.27	0.53	0.39	0.63	0.91	0.93
2	46	0.28	0.39	0.29	0.56	0.40	0.66	0.93	0.93
2	47	0.28	0.40	0.30	0.60	0.42	0.70	0.98	0.93
2	48	0.29	0.42	0.31	0.62	0.44	0.72	1.00	0.93
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.01	0.02	0.01	0.03	0.01	0.03	0.02	0.02
3	3	0.02	0.03	0.02	0.04	0.03	0.04	0.03	0.04
3	4	0.03	0.04	0.04	0.05	0.05	0.05	0.04	0.06
3	5	0.04	0.05	0.05	0.05	0.06	0.06	0.06	0.08
3	6	0.05	0.06	0.06	0.06	0.07	0.07	0.08	0.09
3	7	0.05	0.07	0.07	0.07	0.09	0.08	0.10	0.12
3	8	0.06	0.07	0.08	0.08	0.10	0.09	0.12	0.15
3	9	0.07	0.07	0.09	0.08	0.12	0.10	0.14	0.17
3	10	0.08	0.08	0.11	0.09	0.12	0.12	0.17	0.21
3	11	0.08	0.09	0.12	0.10	0.16	0.13	0.19	0.24
3	12	0.09	0.09	0.13	0.11	0.17	0.14	0.21	0.28
3	13	0.10	0.10	0.13	0.11	0.19	0.15	0.24	0.31
3	14	0.11	0.11	0.16	0.12	0.20	0.17	0.26	0.35
3	15	0.12	0.12	0.16	0.13	0.21	0.18	0.28	0.38
3	16	0.14	0.13	0.17	0.14	0.22	0.20	0.31	0.42
3	17	0.15	0.14	0.18	0.15	0.24	0.21	0.35	0.46
3	18	0.16	0.15	0.19	0.16	0.25	0.23	0.40	0.49
3	19	0.17	0.17	0.20	0.17	0.27	0.24	0.43	0.51
3	20	0.18	0.19	0.20	0.18	0.28	0.26	0.45	0.54

Table A2-8$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 5 % honeys under anaerobic condition

Rep	Time (h)	A5	AC5	B5	BC5	C5	CC5	K5	KC5
3	21	0.18	0.21	0.21	0.19	0.29	0.27	0.48	0.57
3	22	0.19	0.24	0.22	0.21	0.30	0.28	0.51	0.60
3	23	0.20	0.27	0.23	0.22	0.31	0.30	0.50	0.64
3	24	0.21	0.31	0.23	0.24	0.31	0.31	0.49	0.66
3	25	0.21	0.37	0.24	0.24	0.32	0.32	0.49	0.69
3	26	0.22	0.44	0.24	0.26	0.32	0.33	0.50	0.73
3	27	0.22	0.49	0.25	0.27	0.31	0.34	0.53	0.76
3	28	0.23	0.52	0.25	0.28	0.31	0.35	0.53	0.78
3	29	0.24	0.55	0.25	0.29	0.31	0.36	0.58	0.80
3	30	0.24	0.57	0.26	0.31	0.31	0.38	0.62	0.83
3	31	0.24	0.58	0.26	0.32	0.31	0.39	0.61	0.86
3	32	0.24	0.59	0.27	0.33	0.31	0.40	0.65	0.89
3	33	0.24	0.60	0.27	0.34	0.32	0.41	0.68	0.91
3	34	0.25	0.60	0.28	0.36	0.32	0.42	0.71	0.94
3	35	0.25	0.61	0.29	0.37	0.32	0.44	0.73	0.96
3	36	0.25	0.61	0.29	0.38	0.32	0.46	0.75	0.99
3	37	0.25	0.62	0.30	0.40	0.33	0.47	0.77	1.01
3	38	0.25	0.62	0.31	0.41	0.33	0.50	0.79	1.03
3	39	0.25	0.62	0.32	0.43	0.34	0.52	0.82	1.06
3	40	0.25	0.62	0.32	0.45	0.34	0.55	0.85	1.08
3	41	0.25	0.63	0.33	0.47	0.35	0.57	0.86	1.10
3	42	0.25	0.63	0.34	0.49	0.36	0.59	0.88	1.12
3	43	0.25	0.63	0.35	0.51	0.37	0.61	0.91	1.14
3	44	0.25	0.63	0.37	0.52	0.38	0.63	0.91	1.15
3	45	0.27	0.63	0.41	0.53	0.39	0.65	0.94	1.17
3	46	0.29	0.63	0.44	0.55	0.40	0.68	0.95	1.19
3	47	0.31	0.63	0.46	0.58	0.42	0.71	1.01	1.21
3	48	0.32	0.63	0.48	0.61	0.44	0.74	1.04	1.22

Notes: A5=5 % Manuka honey 20+ UMF; AC5=5 % Manuka honey 20+ UMF with catalase;
B5=5 % Manuka honey 15+ UMF; BC5=5 % Manuka honey 15+ UMF with catalase;
C5=5 % Manuka honey 10+ UMF; CC5=5 % Manuka honey 10+ UMF with catalase;
K5=5 % Kanuka honey; KC5=5 % Kanuka honey with catalase

Table A2-9 Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under aerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.01	0.02	0.01	0.01	0.01	0.02	0.00	0.01
1	3	0.02	0.04	0.02	0.02	0.02	0.02	0.00	0.02
1	4	0.03	0.04	0.03	0.02	0.02	0.03	0.00	0.03
1	5	0.03	0.05	0.04	0.03	0.03	0.04	0.00	0.03
1	6	0.04	0.06	0.05	0.03	0.04	0.06	0.00	0.04
1	7	0.05	0.07	0.06	0.04	0.04	0.07	0.00	0.05
1	8	0.05	0.08	0.07	0.04	0.04	0.09	0.00	0.06
1	9	0.05	0.09	0.07	0.05	0.05	0.10	0.01	0.07
1	10	0.06	0.10	0.08	0.06	0.05	0.11	0.01	0.08
1	11	0.06	0.11	0.08	0.06	0.06	0.13	0.01	0.09
1	12	0.06	0.12	0.08	0.09	0.07	0.14	0.01	0.10
1	13	0.06	0.12	0.08	0.10	0.08	0.15	0.01	0.12
1	14	0.06	0.14	0.08	0.11	0.12	0.18	0.00	0.13
1	15	0.06	0.15	0.08	0.12	0.21	0.24	0.00	0.15
1	16	0.06	0.16	0.08	0.13	0.30	0.37	0.00	0.18
1	17	0.06	0.17	0.08	0.14	0.43	0.51	0.00	0.21
1	18	0.07	0.18	0.07	0.16	0.51	0.61	0.00	0.24
1	19	0.07	0.20	0.07	0.17	0.60	0.68	0.00	0.28
1	20	0.07	0.22	0.07	0.19	0.65	0.73	0.00	0.31
1	21	0.07	0.24	0.07	0.21	0.68	0.79	0.00	0.36
1	22	0.07	0.26	0.07	0.23	0.70	0.84	0.00	0.40
1	23	0.07	0.28	0.07	0.26	0.73	0.88	0.00	0.43
1	24	0.07	0.31	0.07	0.28	0.74	0.90	0.00	0.48
1	25	0.08	0.33	0.07	0.31	0.75	0.93	0.00	0.52
1	26	0.08	0.36	0.07	0.34	0.77	0.97	0.00	0.57
1	27	0.08	0.39	0.07	0.36	0.79	1.00	0.00	0.62
1	28	0.08	0.42	0.07	0.39	0.81	1.03	0.00	0.66
1	29	0.08	0.45	0.07	0.42	0.82	1.06	0.00	0.70
1	30	0.08	0.48	0.07	0.44	0.84	1.08	0.00	0.75
1	31	0.08	0.50	0.07	0.47	0.85	1.11	0.00	0.78
1	32	0.08	0.52	0.07	0.49	0.87	1.14	0.00	0.82
1	33	0.08	0.55	0.07	0.52	0.88	1.17	0.00	0.85
1	34	0.08	0.57	0.07	0.54	0.89	1.20	0.00	0.87
1	35	0.08	0.59	0.07	0.57	0.90	1.22	0.00	0.90
1	36	0.08	0.61	0.07	0.59	0.91	1.24	0.00	0.92
1	37	0.08	0.64	0.07	0.62	0.93	1.26	0.00	0.94
1	38	0.08	0.67	0.07	0.64	0.94	1.28	0.00	0.96
1	39	0.08	0.69	0.06	0.66	0.95	1.29	0.00	0.98

Table A2-9$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under aerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
1	40	0.08	0.71	0.06	0.68	0.97	1.30	0.00	1.00
1	41	0.08	0.72	0.06	0.70	0.98	1.31	0.00	1.02
1	42	0.09	0.74	0.06	0.72	0.99	1.32	0.00	1.03
1	43	0.09	0.76	0.06	0.74	1.00	1.33	0.00	1.05
1	44	0.09	0.78	0.06	0.76	1.01	1.34	0.00	1.06
1	45	0.09	0.80	0.06	0.78	1.03	1.35	0.00	1.07
1	46	0.09	0.81	0.06	0.80	1.04	1.36	0.00	1.09
1	47	0.09	0.83	0.05	0.82	1.05	1.37	0.00	1.10
1	48	0.09	0.84	0.05	0.84	1.06	1.37	0.00	1.11
2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.01	0.02	0.00	0.01	0.02	0.02	0.01	0.01
2	3	0.01	0.04	0.00	0.02	0.04	0.03	0.01	0.02
2	4	0.01	0.04	0.00	0.03	0.05	0.04	0.01	0.03
2	5	0.02	0.05	0.01	0.03	0.06	0.06	0.01	0.04
2	6	0.02	0.07	0.02	0.04	0.07	0.08	0.01	0.05
2	7	0.02	0.08	0.02	0.05	0.08	0.09	0.01	0.06
2	8	0.03	0.09	0.03	0.06	0.09	0.10	0.01	0.07
2	9	0.03	0.10	0.03	0.06	0.09	0.11	0.02	0.08
2	10	0.04	0.11	0.04	0.07	0.10	0.13	0.02	0.09
2	11	0.04	0.12	0.04	0.08	0.11	0.13	0.02	0.11
2	12	0.05	0.13	0.04	0.09	0.11	0.15	0.02	0.12
2	13	0.05	0.14	0.04	0.10	0.12	0.20	0.02	0.13
2	14	0.05	0.15	0.05	0.11	0.12	0.32	0.04	0.15
2	15	0.05	0.16	0.05	0.12	0.13	0.47	0.03	0.18
2	16	0.05	0.18	0.05	0.13	0.17	0.59	0.03	0.23
2	17	0.05	0.19	0.05	0.14	0.27	0.67	0.03	0.30
2	18	0.05	0.20	0.05	0.16	0.37	0.71	0.02	0.38
2	19	0.05	0.22	0.05	0.17	0.51	0.77	0.02	0.46
2	20	0.04	0.24	0.06	0.19	0.61	0.83	0.02	0.53
2	21	0.04	0.26	0.06	0.21	0.65	0.88	0.03	0.59
2	22	0.04	0.28	0.05	0.23	0.67	0.91	0.02	0.65
2	23	0.04	0.31	0.04	0.26	0.72	0.94	0.02	0.71
2	24	0.04	0.33	0.04	0.28	0.76	0.98	0.03	0.76
2	25	0.04	0.36	0.04	0.31	0.78	1.01	0.02	0.80
2	26	0.04	0.39	0.05	0.34	0.80	1.04	0.03	0.86
2	27	0.04	0.42	0.05	0.36	0.81	1.06	0.02	0.90
2	28	0.05	0.45	0.05	0.39	0.83	1.08	0.02	0.94
2	29	0.05	0.48	0.05	0.42	0.85	1.11	0.02	0.98
2	30	0.05	0.51	0.05	0.44	0.85	1.14	0.02	1.03

Table A2-9$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under aerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
2	31	0.06	0.53	0.05	0.47	0.87	1.16	0.02	1.06
2	32	0.06	0.56	0.05	0.49	0.89	1.18	0.02	1.10
2	33	0.06	0.58	0.06	0.52	0.91	1.21	0.02	1.13
2	34	0.07	0.60	0.06	0.54	0.93	1.23	0.02	1.14
2	35	0.07	0.62	0.06	0.57	0.95	1.25	0.02	1.17
2	36	0.07	0.64	0.06	0.59	0.97	1.27	0.02	1.18
2	37	0.07	0.67	0.06	0.62	0.99	1.28	0.02	1.20
2	38	0.07	0.69	0.06	0.64	1.00	1.30	0.02	1.22
2	39	0.07	0.72	0.06	0.66	1.02	1.31	0.02	1.23
2	40	0.07	0.74	0.06	0.68	1.04	1.32	0.02	1.25
2	41	0.07	0.76	0.06	0.70	1.06	1.33	0.02	1.26
2	42	0.07	0.78	0.06	0.72	1.08	1.33	0.03	1.27
2	43	0.08	0.79	0.06	0.74	1.09	1.34	0.02	1.28
2	44	0.08	0.81	0.07	0.76	1.11	1.35	0.03	1.29
2	45	0.08	0.83	0.07	0.78	1.13	1.36	0.03	1.30
2	46	0.08	0.84	0.07	0.80	1.14	1.37	0.03	1.31
2	47	0.08	0.86	0.06	0.82	1.16	1.38	0.03	1.31
2	48	0.08	0.87	0.06	0.84	1.17	1.38	0.03	1.31
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.01	0.02	0.01	0.01	0.02	0.01	0.01	0.01
3	3	0.02	0.04	0.02	0.02	0.04	0.03	0.01	0.03
3	4	0.02	0.05	0.03	0.03	0.05	0.04	0.01	0.04
3	5	0.03	0.06	0.04	0.04	0.06	0.06	0.01	0.05
3	6	0.03	0.07	0.05	0.04	0.07	0.07	0.01	0.06
3	7	0.04	0.08	0.05	0.05	0.08	0.08	0.01	0.07
3	8	0.04	0.09	0.06	0.05	0.09	0.09	0.02	0.08
3	9	0.05	0.10	0.07	0.06	0.09	0.10	0.02	0.09
3	10	0.05	0.11	0.08	0.07	0.10	0.11	0.02	0.10
3	11	0.05	0.12	0.09	0.08	0.11	0.12	0.02	0.11
3	12	0.05	0.13	0.09	0.09	0.11	0.14	0.02	0.12
3	13	0.05	0.15	0.10	0.10	0.12	0.15	0.02	0.14
3	14	0.05	0.16	0.10	0.11	0.13	0.20	0.03	0.16
3	15	0.05	0.17	0.10	0.12	0.13	0.36	0.03	0.19
3	16	0.05	0.19	0.11	0.13	0.14	0.47	0.03	0.22
3	17	0.05	0.24	0.11	0.14	0.14	0.57	0.03	0.25
3	18	0.05	0.33	0.11	0.16	0.15	0.65	0.03	0.29
3	19	0.05	0.45	0.11	0.18	0.17	0.71	0.03	0.34
3	20	0.05	0.55	0.11	0.19	0.23	0.78	0.02	0.40

Table A2-9$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under aerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
3	21	0.05	0.62	0.12	0.21	0.32	0.85	0.03	0.47
3	22	0.05	0.67	0.11	0.24	0.42	0.89	0.02	0.53
3	23	0.05	0.69	0.11	0.26	0.53	0.93	0.02	0.60
3	24	0.04	0.73	0.11	0.28	0.61	0.96	0.02	0.66
3	25	0.04	0.75	0.11	0.31	0.63	0.99	0.02	0.72
3	26	0.03	0.77	0.11	0.34	0.66	1.01	0.02	0.78
3	27	0.03	0.77	0.11	0.37	0.69	1.04	0.03	0.81
3	28	0.02	0.78	0.11	0.40	0.73	1.07	0.03	0.86
3	29	0.02	0.78	0.11	0.42	0.75	1.09	0.03	0.90
3	30	0.01	0.79	0.11	0.45	0.76	1.11	0.03	0.94
3	31	0.01	0.79	0.11	0.47	0.78	1.13	0.03	0.98
3	32	0.00	0.78	0.11	0.49	0.78	1.16	0.04	1.01
3	33	0.00	0.79	0.11	0.51	0.79	1.18	0.03	1.04
3	34	0.00	0.79	0.11	0.54	0.82	1.21	0.03	1.07
3	35	0.00	0.79	0.11	0.56	0.83	1.23	0.03	1.09
3	36	0.00	0.78	0.11	0.59	0.85	1.25	0.04	1.11
3	37	0.00	0.79	0.11	0.61	0.86	1.27	0.03	1.13
3	38	0.00	0.79	0.11	0.63	0.88	1.28	0.04	1.15
3	39	0.00	0.79	0.11	0.65	0.90	1.30	0.03	1.17
3	40	0.00	0.79	0.11	0.67	0.92	1.31	0.03	1.18
3	41	0.00	0.79	0.11	0.69	0.94	1.31	0.04	1.20
3	42	0.00	0.80	0.11	0.72	0.95	1.32	0.03	1.20
3	43	0.00	0.80	0.11	0.74	0.97	1.33	0.04	1.21
3	44	0.00	0.80	0.11	0.76	0.99	1.34	0.03	1.22
3	45	0.00	0.80	0.11	0.78	1.01	1.35	0.03	1.22
3	46	0.00	0.80	0.11	0.79	1.02	1.36	0.03	1.23
3	47	0.00	0.80	0.11	0.81	1.04	1.36	0.04	1.23
3	48	0.00	0.80	0.10	0.83	1.06	1.37	0.03	1.24
4	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	2	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01
4	3	0.00	0.02	0.01	0.02	0.01	0.02	0.00	0.03
4	4	0.00	0.02	0.01	0.03	0.02	0.03	0.00	0.04
4	5	0.00	0.03	0.02	0.03	0.03	0.04	0.00	0.06
4	6	0.00	0.04	0.03	0.04	0.03	0.06	0.00	0.07
4	7	0.00	0.04	0.03	0.05	0.04	0.07	0.00	0.09
4	8	0.00	0.05	0.04	0.05	0.05	0.08	0.00	0.10
4	9	0.00	0.05	0.04	0.05	0.05	0.10	0.00	0.12
4	10	0.00	0.06	0.04	0.06	0.05	0.11	0.00	0.14

Table A2-9$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under aerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
4	11	0.00	0.06	0.04	0.06	0.05	0.13	0.00	0.16
4	12	0.00	0.07	0.04	0.07	0.06	0.17	0.00	0.18
4	13	0.00	0.07	0.04	0.07	0.05	0.26	0.01	0.20
4	14	0.00	0.08	0.04	0.08	0.06	0.41	0.01	0.22
4	15	0.00	0.08	0.04	0.08	0.06	0.58	0.01	0.25
4	16	0.00	0.09	0.04	0.09	0.13	0.68	0.00	0.28
4	17	0.00	0.09	0.04	0.10	0.24	0.73	0.00	0.33
4	18	0.00	0.10	0.04	0.12	0.35	0.78	0.01	0.39
4	19	0.00	0.10	0.04	0.17	0.50	0.82	0.00	0.44
4	20	0.00	0.11	0.04	0.28	0.57	0.86	0.00	0.50
4	21	0.00	0.12	0.05	0.39	0.59	0.90	0.00	0.56
4	22	0.00	0.12	0.04	0.49	0.63	0.93	0.00	0.61
4	23	0.00	0.13	0.05	0.59	0.67	0.96	0.00	0.66
4	24	0.00	0.14	0.04	0.66	0.68	0.98	0.00	0.71
4	25	0.00	0.15	0.04	0.73	0.70	1.02	-0.01	0.76
4	26	0.00	0.16	0.05	0.78	0.71	1.04	0.00	0.81
4	27	0.00	0.17	0.05	0.81	0.72	1.07	0.00	0.86
4	28	0.00	0.18	0.04	0.84	0.71	1.10	0.00	0.91
4	29	0.00	0.18	0.04	0.87	0.72	1.13	-0.01	0.95
4	30	0.00	0.19	0.04	0.88	0.73	1.16	-0.01	0.99
4	31	0.00	0.20	0.04	0.90	0.74	1.19	-0.01	1.01
4	32	0.00	0.20	0.04	0.91	0.75	1.22	-0.02	1.03
4	33	0.00	0.20	0.04	0.92	0.76	1.24	-0.02	1.05
4	34	0.00	0.21	0.04	0.93	0.77	1.26	-0.02	1.08
4	35	0.00	0.23	0.04	0.93	0.78	1.28	-0.02	1.10
4	36	0.00	0.24	0.04	0.94	0.79	1.30	-0.01	1.12
4	37	0.00	0.24	0.04	0.95	0.80	1.31	-0.02	1.14
4	38	0.00	0.24	0.04	0.95	0.81	1.33	-0.01	1.16
4	39	0.00	0.25	0.04	0.96	0.83	1.34	-0.02	1.17
4	40	0.00	0.25	0.04	0.96	0.84	1.35	-0.02	1.18
4	41	0.00	0.26	0.04	0.96	0.85	1.36	-0.02	1.19
4	42	0.00	0.26	0.04	0.97	0.86	1.37	-0.02	1.20
4	43	0.00	0.26	0.04	0.97	0.87	1.37	-0.02	1.21
4	44	0.00	0.26	0.03	0.97	0.88	1.38	-0.02	1.22
4	45	0.00	0.27	0.03	0.97	0.90	1.38	-0.02	1.22
4	46	0.00	0.27	0.03	0.98	0.91	1.39	-0.02	1.23
4	47	0.00	0.27	0.03	0.98	0.92	1.40	-0.03	1.23
4	48	0.00	0.28	0.03	0.98	0.93	1.40	-0.02	1.24
5	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table A2-9$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under aerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
5	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	2	0.00	0.02	0.00	0.01	0.00	0.01	0.00	0.01
5	3	0.00	0.02	0.01	0.01	0.01	0.03	0.01	0.02
5	4	0.01	0.04	0.01	0.02	0.01	0.04	0.01	0.04
5	5	0.02	0.04	0.02	0.03	0.02	0.05	0.01	0.05
5	6	0.02	0.05	0.02	0.03	0.02	0.06	0.01	0.07
5	7	0.03	0.06	0.03	0.04	0.03	0.08	0.01	0.09
5	8	0.03	0.06	0.04	0.05	0.03	0.09	0.01	0.10
5	9	0.04	0.07	0.04	0.05	0.03	0.10	0.01	0.12
5	10	0.04	0.07	0.05	0.05	0.04	0.12	0.01	0.14
5	11	0.04	0.08	0.05	0.06	0.04	0.13	0.01	0.15
5	12	0.04	0.09	0.05	0.06	0.04	0.15	0.01	0.17
5	13	0.04	0.10	0.06	0.07	0.05	0.16	0.01	0.20
5	14	0.05	0.10	0.06	0.07	0.13	0.19	0.01	0.23
5	15	0.05	0.11	0.06	0.08	0.24	0.23	0.02	0.28
5	16	0.05	0.11	0.06	0.09	0.37	0.36	0.02	0.35
5	17	0.05	0.12	0.06	0.10	0.50	0.49	0.01	0.43
5	18	0.06	0.12	0.06	0.12	0.56	0.63	0.01	0.50
5	19	0.06	0.13	0.06	0.16	0.57	0.72	0.01	0.57
5	20	0.07	0.13	0.06	0.24	0.64	0.76	0.01	0.62
5	21	0.07	0.14	0.06	0.38	0.67	0.80	0.01	0.68
5	22	0.08	0.15	0.06	0.49	0.69	0.88	0.01	0.73
5	23	0.08	0.15	0.06	0.59	0.70	0.92	0.01	0.79
5	24	0.09	0.16	0.06	0.68	0.70	0.96	0.01	0.81
5	25	0.09	0.17	0.06	0.76	0.71	1.00	0.01	0.81
5	26	0.10	0.18	0.07	0.82	0.72	1.06	0.01	0.83
5	27	0.11	0.18	0.07	0.86	0.73	1.11	0.01	0.87
5	28	0.11	0.19	0.06	0.90	0.75	1.15	0.01	0.89
5	29	0.11	0.20	0.06	0.92	0.76	1.19	0.00	0.91
5	30	0.11	0.20	0.06	0.94	0.77	1.23	0.00	0.94
5	31	0.11	0.21	0.06	0.96	0.79	1.27	0.00	0.96
5	32	0.10	0.22	0.06	0.97	0.81	1.30	0.00	0.97
5	33	0.10	0.23	0.06	0.98	0.83	1.33	0.00	0.99
5	34	0.10	0.24	0.06	0.99	0.86	1.35	0.00	1.00
5	35	0.10	0.25	0.07	1.00	0.89	1.37	0.01	1.02
5	36	0.09	0.25	0.06	1.00	0.92	1.38	0.00	1.03
5	37	0.09	0.26	0.07	1.01	0.95	1.39	0.00	1.05
5	38	0.09	0.26	0.07	1.02	0.98	1.40	0.01	1.07
5	39	0.08	0.28	0.07	1.02	1.01	1.41	0.01	1.08
5	40	0.08	0.30	0.07	1.03	1.04	1.41	0.01	1.10

Table A2-9$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under aerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
5	41	0.08	0.31	0.07	1.03	1.05	1.42	0.00	1.12
5	42	0.08	0.32	0.07	1.03	1.06	1.43	0.00	1.12
5	43	0.08	0.32	0.07	1.03	1.06	1.43	0.00	1.12
5	44	0.07	0.33	0.07	1.03	1.07	1.44	0.00	1.14
5	45	0.07	0.34	0.07	1.04	1.07	1.44	0.00	1.15
5	46	0.07	0.34	0.07	1.04	1.07	1.45	0.00	1.15
5	47	0.07	0.35	0.07	1.04	1.07	1.45	0.00	1.15
5	48	0.07	0.36	0.07	1.04	1.08	1.46	0.00	1.17
6	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	2	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.02
6	3	0.01	0.02	0.01	0.02	0.01	0.03	0.01	0.03
6	4	0.02	0.03	0.01	0.02	0.02	0.04	0.01	0.04
6	5	0.02	0.04	0.02	0.03	0.03	0.05	0.01	0.06
6	6	0.02	0.05	0.02	0.04	0.03	0.06	0.01	0.08
6	7	0.03	0.05	0.03	0.04	0.04	0.07	0.01	0.10
6	8	0.03	0.06	0.04	0.05	0.04	0.08	0.02	0.12
6	9	0.03	0.07	0.04	0.05	0.05	0.09	0.02	0.14
6	10	0.03	0.07	0.05	0.06	0.05	0.10	0.02	0.16
6	11	0.04	0.07	0.05	0.07	0.05	0.12	0.03	0.18
6	12	0.04	0.08	0.06	0.07	0.06	0.13	0.03	0.21
6	13	0.04	0.09	0.06	0.08	0.06	0.14	0.02	0.23
6	14	0.04	0.09	0.06	0.08	0.06	0.16	0.02	0.27
6	15	0.04	0.10	0.07	0.08	0.06	0.22	0.02	0.34
6	16	0.04	0.11	0.07	0.09	0.07	0.36	0.02	0.45
6	17	0.04	0.11	0.07	0.10	0.13	0.49	0.02	0.58
6	18	0.04	0.12	0.07	0.12	0.25	0.62	0.02	0.66
6	19	0.04	0.12	0.08	0.18	0.38	0.69	0.03	0.68
6	20	0.04	0.13	0.08	0.29	0.50	0.76	0.07	0.73
6	21	0.05	0.14	0.08	0.44	0.57	0.81	0.18	0.76
6	22	0.05	0.17	0.08	0.53	0.58	0.87	0.33	0.80
6	23	0.05	0.21	0.08	0.58	0.61	0.92	0.55	0.83
6	24	0.05	0.29	0.08	0.62	0.65	0.94	0.73	0.86
6	25	0.05	0.37	0.08	0.65	0.68	0.98	0.84	0.89
6	26	0.06	0.42	0.08	0.67	0.70	1.01	0.89	0.92
6	27	0.06	0.47	0.09	0.69	0.71	1.05	0.93	0.96
6	28	0.06	0.55	0.08	0.69	0.72	1.07	0.95	1.00
6	29	0.06	0.59	0.08	0.70	0.72	1.10	0.97	1.02
6	30	0.06	0.63	0.09	0.70	0.73	1.13	0.99	1.04

Table A2-9$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under aerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
6	31	0.07	0.66	0.09	0.70	0.73	1.15	1.01	1.06
6	32	0.07	0.69	0.09	0.70	0.74	1.18	1.02	1.08
6	33	0.08	0.72	0.09	0.70	0.76	1.20	1.03	1.09
6	34	0.08	0.74	0.09	0.71	0.77	1.23	1.05	1.11
6	35	0.09	0.76	0.09	0.70	0.78	1.26	1.06	1.12
6	36	0.09	0.77	0.09	0.71	0.79	1.28	1.06	1.14
6	37	0.09	0.78	0.09	0.71	0.81	1.29	1.07	1.14
6	38	0.09	0.80	0.10	0.71	0.82	1.31	1.08	1.15
6	39	0.10	0.81	0.10	0.71	0.83	1.33	1.08	1.15
6	40	0.10	0.82	0.10	0.72	0.85	1.34	1.09	1.16
6	41	0.10	0.83	0.10	0.72	0.86	1.35	1.09	1.17
6	42	0.10	0.84	0.10	0.72	0.87	1.36	1.10	1.17
6	43	0.10	0.85	0.10	0.71	0.89	1.37	1.10	1.18
6	44	0.10	0.86	0.10	0.72	0.90	1.38	1.11	1.18
6	45	0.10	0.86	0.10	0.72	0.91	1.39	1.11	1.19
6	46	0.11	0.87	0.10	0.71	0.93	1.39	1.12	1.19
6	47	0.11	0.88	0.10	0.72	0.94	1.40	1.13	1.20
6	48	0.10	0.88	0.10	0.72	0.96	1.41	1.13	1.20

Notes: A7.5=7.5 % Manuka honey 20+ UMF; AC7.5=7.5 % Manuka honey 20+ UMF with catalase; B7.5=7.5 % Manuka honey 15+ UMF; BC7.5=7.5 % Manuka honey 15+ UMF with catalase; C7.5=7.5 % Manuka honey 10+ UMF; CC7.5=7.5 % Manuka honey 10+ UMF with catalase; K7.5=7.5 % Kanuka honey; KC7.5=7.5 % Kanuka honey with catalase

Table A2-10 Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under anaerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.01	0.02	0.01	0.02	0.01	0.01	0.00	0.01
1	3	0.02	0.02	0.02	0.03	0.02	0.03	0.01	0.02
1	4	0.03	0.03	0.03	0.04	0.03	0.03	0.01	0.04
1	5	0.04	0.04	0.04	0.05	0.04	0.05	0.01	0.05
1	6	0.04	0.04	0.05	0.05	0.05	0.05	0.00	0.06
1	7	0.05	0.05	0.05	0.06	0.05	0.06	0.01	0.06
1	8	0.06	0.06	0.06	0.07	0.06	0.06	0.01	0.08
1	9	0.07	0.06	0.06	0.07	0.06	0.09	0.00	0.09
1	10	0.08	0.07	0.07	0.07	0.07	0.08	0.01	0.10
1	11	0.08	0.07	0.07	0.08	0.08	0.09	0.01	0.12
1	12	0.09	0.08	0.07	0.08	0.09	0.10	0.01	0.14
1	13	0.09	0.08	0.07	0.08	0.10	0.11	0.01	0.16
1	14	0.10	0.09	0.07	0.08	0.11	0.11	0.01	0.18
1	15	0.10	0.09	0.07	0.08	0.12	0.12	0.01	0.21
1	16	0.11	0.09	0.07	0.08	0.13	0.13	0.00	0.23
1	17	0.12	0.10	0.07	0.09	0.13	0.14	0.01	0.26
1	18	0.12	0.10	0.07	0.09	0.14	0.16	0.00	0.29
1	19	0.13	0.11	0.07	0.09	0.15	0.17	0.00	0.31
1	20	0.13	0.11	0.07	0.09	0.16	0.18	0.00	0.34
1	21	0.14	0.12	0.07	0.09	0.17	0.20	0.00	0.36
1	22	0.15	0.12	0.07	0.10	0.18	0.21	0.00	0.40
1	23	0.16	0.13	0.08	0.10	0.19	0.22	0.00	0.43
1	24	0.17	0.13	0.08	0.10	0.20	0.24	0.00	0.47
1	25	0.17	0.13	0.08	0.11	0.20	0.25	0.01	0.50
1	26	0.18	0.14	0.08	0.11	0.21	0.26	0.00	0.54
1	27	0.18	0.14	0.09	0.11	0.22	0.27	0.00	0.58
1	28	0.19	0.14	0.08	0.11	0.24	0.28	0.00	0.62
1	29	0.19	0.15	0.08	0.11	0.25	0.30	0.00	0.64
1	30	0.19	0.15	0.08	0.12	0.26	0.31	0.00	0.67
1	31	0.20	0.16	0.08	0.12	0.28	0.32	0.00	0.71
1	32	0.20	0.16	0.08	0.12	0.28	0.33	0.00	0.74
1	33	0.21	0.16	0.08	0.12	0.30	0.34	0.00	0.78
1	34	0.21	0.17	0.08	0.12	0.31	0.35	0.00	0.79
1	35	0.21	0.17	0.08	0.13	0.32	0.37	0.00	0.83
1	36	0.21	0.18	0.07	0.13	0.33	0.37	0.00	0.86
1	37	0.22	0.19	0.07	0.13	0.33	0.38	0.00	0.88
1	38	0.22	0.19	0.07	0.14	0.34	0.39	0.00	0.91
1	39	0.22	0.20	0.07	0.14	0.35	0.40	0.00	0.94

Table A2-10 Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under anaerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
1	40	0.23	0.20	0.07	0.15	0.34	0.40	0.00	0.96
1	41	0.23	0.21	0.07	0.15	0.35	0.41	0.00	0.99
1	42	0.24	0.21	0.07	0.15	0.35	0.41	0.00	1.01
1	43	0.24	0.22	0.07	0.16	0.34	0.42	0.00	1.03
1	44	0.24	0.23	0.07	0.16	0.35	0.43	0.00	1.04
1	45	0.24	0.23	0.07	0.17	0.35	0.44	0.00	1.06
1	46	0.24	0.24	0.07	0.17	0.35	0.46	0.00	1.08
1	47	0.25	0.25	0.07	0.18	0.36	0.48	0.00	1.09
1	48	0.26	0.26	0.07	0.19	0.37	0.50	0.00	1.11
2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.01	0.01	0.00	0.02	0.01	0.02	0.00	0.01
2	3	0.02	0.02	0.01	0.03	0.02	0.03	0.00	0.03
2	4	0.02	0.03	0.02	0.05	0.03	0.04	0.01	0.04
2	5	0.02	0.04	0.02	0.05	0.04	0.05	0.01	0.05
2	6	0.03	0.05	0.02	0.06	0.05	0.05	0.01	0.06
2	7	0.04	0.05	0.03	0.07	0.05	0.06	0.01	0.06
2	8	0.04	0.06	0.04	0.07	0.06	0.08	0.01	0.07
2	9	0.05	0.06	0.04	0.08	0.06	0.07	0.01	0.09
2	10	0.05	0.07	0.04	0.08	0.07	0.08	0.01	0.10
2	11	0.06	0.08	0.05	0.09	0.11	0.09	0.01	0.12
2	12	0.06	0.08	0.05	0.09	0.09	0.10	0.01	0.13
2	13	0.07	0.08	0.06	0.09	0.10	0.11	0.02	0.15
2	14	0.07	0.09	0.06	0.09	0.11	0.12	0.02	0.17
2	15	0.08	0.09	0.06	0.09	0.12	0.13	0.02	0.19
2	16	0.09	0.10	0.06	0.09	0.13	0.14	0.01	0.22
2	17	0.10	0.10	0.07	0.10	0.13	0.15	0.00	0.25
2	18	0.10	0.11	0.07	0.10	0.14	0.16	0.00	0.28
2	19	0.11	0.11	0.07	0.10	0.15	0.17	0.00	0.31
2	20	0.11	0.12	0.07	0.10	0.16	0.19	0.00	0.34
2	21	0.11	0.12	0.06	0.10	0.17	0.20	0.00	0.36
2	22	0.12	0.13	0.07	0.11	0.19	0.21	0.00	0.39
2	23	0.12	0.13	0.07	0.11	0.20	0.23	0.00	0.42
2	24	0.12	0.14	0.07	0.11	0.21	0.24	0.00	0.44
2	25	0.12	0.14	0.07	0.12	0.23	0.25	0.02	0.46
2	26	0.12	0.14	0.07	0.12	0.24	0.26	0.02	0.49
2	27	0.12	0.15	0.07	0.12	0.25	0.27	0.02	0.53
2	28	0.12	0.15	0.08	0.12	0.27	0.28	0.01	0.55
2	29	0.11	0.16	0.08	0.13	0.28	0.29	0.01	0.58
2	30	0.11	0.16	0.08	0.13	0.30	0.31	0.00	0.61

Table A2-10_{cont}Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under anaerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
2	31	0.11	0.17	0.08	0.13	0.31	0.32	0.00	0.65
2	32	0.11	0.17	0.08	0.14	0.32	0.34	0.01	0.68
2	33	0.11	0.17	0.08	0.14	0.32	0.35	0.01	0.71
2	34	0.11	0.18	0.08	0.14	0.33	0.36	0.01	0.73
2	35	0.11	0.18	0.08	0.14	0.33	0.37	0.01	0.77
2	36	0.11	0.18	0.08	0.15	0.33	0.38	0.01	0.80
2	37	0.11	0.19	0.08	0.15	0.33	0.39	0.01	0.84
2	38	0.12	0.20	0.08	0.15	0.33	0.40	0.01	0.87
2	39	0.12	0.20	0.08	0.16	0.33	0.41	0.00	0.90
2	40	0.12	0.20	0.08	0.16	0.32	0.42	0.00	0.92
2	41	0.12	0.21	0.08	0.17	0.32	0.43	0.00	0.94
2	42	0.12	0.21	0.08	0.17	0.32	0.44	0.00	0.95
2	43	0.12	0.22	0.08	0.18	0.32	0.45	0.00	0.96
2	44	0.12	0.23	0.08	0.18	0.32	0.47	0.00	0.97
2	45	0.11	0.23	0.08	0.19	0.32	0.48	0.00	0.98
2	46	0.12	0.23	0.08	0.19	0.32	0.49	0.00	0.99
2	47	0.12	0.24	0.08	0.20	0.33	0.51	0.00	1.00
2	48	0.12	0.25	0.08	0.21	0.34	0.55	0.01	1.01
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.00	0.01	0.00	0.02	0.00	0.02	0.00	0.00
3	3	0.01	0.02	0.01	0.03	0.02	0.04	0.00	0.02
3	4	0.01	0.03	0.02	0.05	0.03	0.04	0.00	0.02
3	5	0.02	0.04	0.02	0.05	0.05	0.05	0.01	0.03
3	6	0.03	0.04	0.03	0.06	0.05	0.06	0.01	0.04
3	7	0.04	0.05	0.03	0.06	0.06	0.07	0.01	0.05
3	8	0.04	0.05	0.04	0.07	0.08	0.08	0.01	0.05
3	9	0.05	0.08	0.04	0.07	0.08	0.09	0.01	0.07
3	10	0.06	0.07	0.05	0.08	0.09	0.10	0.01	0.08
3	11	0.07	0.07	0.05	0.08	0.10	0.11	0.01	0.10
3	12	0.07	0.07	0.05	0.09	0.11	0.12	0.02	0.11
3	13	0.08	0.08	0.06	0.09	0.13	0.13	0.01	0.13
3	14	0.08	0.08	0.06	0.09	0.14	0.14	0.01	0.15
3	15	0.09	0.08	0.06	0.09	0.15	0.15	0.01	0.17
3	16	0.10	0.09	0.06	0.09	0.16	0.17	0.00	0.20
3	17	0.10	0.09	0.07	0.10	0.17	0.18	0.00	0.23
3	18	0.11	0.10	0.07	0.10	0.18	0.19	0.00	0.26
3	19	0.11	0.10	0.07	0.10	0.19	0.21	0.00	0.30
3	20	0.12	0.11	0.08	0.10	0.21	0.22	0.00	0.34

Table A2-10$_{cont}$Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under anaerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
3	21	0.13	0.11	0.08	0.10	0.22	0.23	0.00	0.37
3	22	0.13	0.12	0.08	0.11	0.23	0.25	0.00	0.41
3	23	0.14	0.12	0.08	0.11	0.25	0.27	0.00	0.45
3	24	0.15	0.12	0.09	0.11	0.26	0.28	0.00	0.46
3	25	0.15	0.13	0.09	0.12	0.28	0.29	0.00	0.50
3	26	0.16	0.13	0.09	0.12	0.28	0.29	0.00	0.53
3	27	0.17	0.13	0.09	0.12	0.30	0.30	0.00	0.56
3	28	0.18	0.14	0.09	0.12	0.32	0.30	0.00	0.58
3	29	0.19	0.14	0.09	0.13	0.32	0.31	0.00	0.60
3	30	0.19	0.14	0.09	0.12	0.33	0.33	0.00	0.62
3	31	0.20	0.15	0.09	0.13	0.34	0.33	0.00	0.65
3	32	0.21	0.15	0.09	0.13	0.34	0.34	0.01	0.67
3	33	0.22	0.15	0.09	0.13	0.34	0.35	0.01	0.69
3	34	0.22	0.16	0.09	0.14	0.34	0.37	0.01	0.71
3	35	0.22	0.16	0.09	0.14	0.34	0.38	0.01	0.74
3	36	0.23	0.17	0.09	0.15	0.34	0.39	0.00	0.76
3	37	0.23	0.17	0.09	0.15	0.34	0.40	0.00	0.78
3	38	0.23	0.17	0.09	0.15	0.34	0.41	0.00	0.79
3	39	0.24	0.18	0.09	0.15	0.34	0.42	0.00	0.81
3	40	0.24	0.18	0.08	0.16	0.34	0.43	0.00	0.83
3	41	0.24	0.19	0.08	0.16	0.34	0.44	0.00	0.84
3	42	0.24	0.19	0.08	0.17	0.34	0.46	0.00	0.86
3	43	0.24	0.20	0.08	0.17	0.34	0.46	0.00	0.87
3	44	0.24	0.20	0.08	0.18	0.34	0.46	0.00	0.88
3	45	0.23	0.21	0.08	0.18	0.33	0.48	0.00	0.89
3	46	0.23	0.22	0.08	0.19	0.34	0.49	0.00	0.90
3	47	0.23	0.23	0.08	0.19	0.35	0.52	0.00	0.91
3	48	0.24	0.23	0.08	0.20	0.36	0.55	0.00	0.92
4	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	2	0.01	0.02	0.01	0.02	0.02	0.01	0.01	0.02
4	3	0.03	0.03	0.02	0.03	0.03	0.03	0.02	0.03
4	4	0.03	0.04	0.03	0.03	0.04	0.04	0.03	0.05
4	5	0.04	0.05	0.04	0.04	0.05	0.05	0.04	0.06
4	6	0.05	0.05	0.05	0.04	0.06	0.06	0.05	0.07
4	7	0.05	0.06	0.06	0.05	0.07	0.06	0.06	0.09
4	8	0.06	0.06	0.06	0.06	0.08	0.07	0.08	0.11
4	9	0.06	0.07	0.07	0.06	0.08	0.07	0.10	0.13
4	10	0.06	0.07	0.08	0.07	0.08	0.08	0.11	0.15

Table A2-10$_{cont}$Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under anaerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
4	11	0.06	0.07	0.08	0.07	0.08	0.08	0.13	0.18
4	12	0.06	0.08	0.09	0.07	0.08	0.08	0.15	0.20
4	13	0.06	0.08	0.09	0.08	0.09	0.09	0.17	0.22
4	14	0.06	0.08	0.10	0.08	0.09	0.09	0.19	0.25
4	15	0.07	0.09	0.10	0.09	0.09	0.10	0.19	0.27
4	16	0.07	0.09	0.10	0.09	0.10	0.11	0.20	0.30
4	17	0.06	0.09	0.11	0.10	0.10	0.11	0.22	0.33
4	18	0.06	0.09	0.11	0.11	0.10	0.12	0.23	0.35
4	19	0.06	0.09	0.12	0.12	0.10	0.12	0.26	0.37
4	20	0.06	0.09	0.13	0.12	0.10	0.14	0.26	0.38
4	21	0.06	0.09	0.13	0.13	0.10	0.15	0.26	0.41
4	22	0.06	0.10	0.14	0.14	0.10	0.16	0.26	0.44
4	23	0.06	0.10	0.15	0.15	0.11	0.17	0.27	0.47
4	24	0.06	0.10	0.15	0.16	0.11	0.19	0.29	0.51
4	25	0.07	0.10	0.15	0.16	0.12	0.21	0.30	0.54
4	26	0.07	0.11	0.16	0.17	0.12	0.23	0.31	0.60
4	27	0.07	0.11	0.16	0.18	0.12	0.25	0.32	0.64
4	28	0.07	0.11	0.17	0.19	0.12	0.27	0.35	0.68
4	29	0.07	0.11	0.17	0.20	0.13	0.29	0.38	0.71
4	30	0.07	0.11	0.18	0.21	0.12	0.32	0.40	0.74
4	31	0.07	0.11	0.18	0.22	0.13	0.35	0.42	0.76
4	32	0.07	0.11	0.18	0.22	0.13	0.37	0.45	0.80
4	33	0.07	0.11	0.19	0.23	0.13	0.40	0.48	0.82
4	34	0.08	0.11	0.19	0.24	0.12	0.42	0.50	0.85
4	35	0.08	0.12	0.19	0.25	0.13	0.44	0.53	0.88
4	36	0.08	0.11	0.20	0.25	0.12	0.46	0.56	0.90
4	37	0.08	0.12	0.20	0.26	0.13	0.48	0.59	0.92
4	38	0.08	0.12	0.20	0.27	0.12	0.51	0.61	0.94
4	39	0.08	0.12	0.20	0.28	0.12	0.53	0.63	0.96
4	40	0.08	0.12	0.21	0.29	0.12	0.56	0.65	0.98
4	41	0.08	0.12	0.21	0.29	0.12	0.58	0.68	0.99
4	42	0.09	0.12	0.21	0.30	0.13	0.60	0.70	1.01
4	43	0.09	0.12	0.22	0.31	0.13	0.62	0.72	1.03
4	44	0.09	0.12	0.22	0.32	0.13	0.65	0.75	1.05
4	45	0.08	0.12	0.22	0.33	0.13	0.67	0.79	1.07
4	46	0.08	0.12	0.23	0.34	0.13	0.69	0.81	1.09
4	47	0.09	0.13	0.23	0.37	0.13	0.71	0.82	1.12
4	48	0.09	0.13	0.24	0.39	0.13	0.73	0.84	1.14

Table A2-10$_{cont}$Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under anaerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
5	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	2	0.01	0.02	0.00	0.01	0.01	0.01	0.01	0.02
5	3	0.02	0.03	0.00	0.03	0.02	0.03	0.02	0.03
5	4	0.03	0.04	0.01	0.03	0.03	0.04	0.03	0.05
5	5	0.04	0.05	0.01	0.04	0.03	0.05	0.04	0.07
5	6	0.05	0.05	0.02	0.05	0.04	0.06	0.05	0.08
5	7	0.05	0.06	0.02	0.05	0.04	0.06	0.07	0.10
5	8	0.06	0.07	0.03	0.06	0.05	0.07	0.08	0.11
5	9	0.06	0.07	0.03	0.06	0.05	0.07	0.10	0.14
5	10	0.07	0.08	0.04	0.07	0.05	0.08	0.12	0.16
5	11	0.07	0.08	0.05	0.07	0.05	0.09	0.14	0.18
5	12	0.08	0.08	0.06	0.07	0.05	0.09	0.16	0.20
5	13	0.08	0.09	0.06	0.08	0.05	0.10	0.18	0.23
5	14	0.08	0.09	0.06	0.08	0.04	0.10	0.21	0.25
5	15	0.08	0.09	0.06	0.08	0.04	0.11	0.22	0.27
5	16	0.08	0.09	0.07	0.09	0.04	0.11	0.24	0.29
5	17	0.08	0.09	0.07	0.09	0.04	0.12	0.25	0.31
5	18	0.08	0.10	0.07	0.10	0.03	0.14	0.26	0.33
5	19	0.07	0.10	0.08	0.11	0.03	0.14	0.29	0.35
5	20	0.08	0.10	0.08	0.12	0.03	0.16	0.32	0.38
5	21	0.08	0.10	0.08	0.12	0.02	0.17	0.34	0.40
5	22	0.08	0.10	0.08	0.13	0.02	0.19	0.35	0.43
5	23	0.07	0.11	0.09	0.14	0.02	0.21	0.33	0.46
5	24	0.07	0.11	0.09	0.14	0.01	0.22	0.34	0.49
5	25	0.08	0.11	0.09	0.15	0.02	0.25	0.33	0.52
5	26	0.08	0.12	0.09	0.16	0.02	0.27	0.34	0.56
5	27	0.08	0.12	0.09	0.16	0.02	0.30	0.33	0.59
5	28	0.08	0.12	0.09	0.17	0.02	0.32	0.36	0.63
5	29	0.08	0.12	0.09	0.18	0.01	0.35	0.37	0.66
5	30	0.07	0.12	0.09	0.19	0.01	0.38	0.38	0.69
5	31	0.08	0.12	0.09	0.20	0.01	0.40	0.40	0.73
5	32	0.08	0.12	0.09	0.21	0.02	0.42	0.43	0.76
5	33	0.08	0.12	0.09	0.22	0.02	0.45	0.46	0.79
5	34	0.07	0.12	0.09	0.23	0.01	0.46	0.48	0.82
5	35	0.07	0.12	0.09	0.24	0.02	0.48	0.50	0.85
5	36	0.07	0.12	0.09	0.25	0.02	0.51	0.54	0.87
5	37	0.07	0.13	0.09	0.25	0.02	0.53	0.55	0.89
5	38	0.07	0.13	0.09	0.26	0.02	0.55	0.58	0.91
5	39	0.07	0.13	0.09	0.27	0.03	0.57	0.60	0.93

Table A2-10$_{cont}$Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under anaerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
5	40	0.07	0.13	0.09	0.28	0.03	0.59	0.64	0.95
5	41	0.07	0.13	0.09	0.29	0.03	0.62	0.65	0.97
5	42	0.07	0.13	0.09	0.30	0.03	0.64	0.67	0.98
5	43	0.07	0.13	0.09	0.31	0.03	0.66	0.69	1.00
5	44	0.07	0.13	0.09	0.32	0.04	0.68	0.71	1.01
5	45	0.07	0.13	0.09	0.32	0.04	0.71	0.75	1.04
5	46	0.07	0.13	0.10	0.33	0.04	0.73	0.77	1.06
5	47	0.08	0.14	0.10	0.36	0.05	0.75	0.79	1.08
5	48	0.08	0.14	0.10	0.38	0.05	0.77	0.81	1.09
6	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	2	0.01	0.02	0.00	0.02	0.02	0.02	0.02	0.02
6	3	0.01	0.03	0.02	0.03	0.03	0.03	0.04	0.04
6	4	0.02	0.04	0.02	0.04	0.04	0.05	0.04	0.05
6	5	0.02	0.05	0.03	0.04	0.06	0.06	0.06	0.07
6	6	0.02	0.05	0.04	0.05	0.06	0.06	0.07	0.08
6	7	0.03	0.06	0.05	0.06	0.08	0.07	0.08	0.10
6	8	0.03	0.07	0.05	0.06	0.08	0.08	0.09	0.12
6	9	0.04	0.07	0.06	0.07	0.09	0.08	0.11	0.14
6	10	0.04	0.08	0.07	0.07	0.10	0.08	0.12	0.17
6	11	0.04	0.08	0.08	0.07	0.10	0.09	0.14	0.19
6	12	0.04	0.08	0.09	0.08	0.11	0.09	0.16	0.22
6	13	0.04	0.09	0.11	0.08	0.11	0.10	0.18	0.24
6	14	0.04	0.09	0.11	0.09	0.11	0.10	0.20	0.26
6	15	0.04	0.10	0.12	0.09	0.12	0.11	0.22	0.28
6	16	0.05	0.10	0.12	0.10	0.12	0.12	0.23	0.30
6	17	0.05	0.10	0.12	0.10	0.13	0.12	0.25	0.33
6	18	0.05	0.10	0.13	0.11	0.13	0.13	0.26	0.35
6	19	0.05	0.10	0.13	0.12	0.14	0.13	0.28	0.37
6	20	0.05	0.11	0.14	0.12	0.14	0.15	0.32	0.40
6	21	0.05	0.11	0.14	0.13	0.15	0.16	0.34	0.42
6	22	0.05	0.11	0.15	0.14	0.15	0.17	0.35	0.45
6	23	0.05	0.11	0.15	0.15	0.15	0.18	0.36	0.48
6	24	0.05	0.11	0.16	0.16	0.15	0.19	0.39	0.51
6	25	0.05	0.12	0.16	0.16	0.16	0.22	0.38	0.54
6	26	0.05	0.12	0.16	0.17	0.16	0.24	0.40	0.57
6	27	0.05	0.13	0.17	0.18	0.16	0.26	0.40	0.60
6	28	0.05	0.13	0.17	0.19	0.16	0.28	0.41	0.63
6	29	0.04	0.13	0.17	0.19	0.16	0.30	0.41	0.66
6	30	0.04	0.13	0.18	0.20	0.15	0.31	0.43	0.70

Table A2-10$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 7.5 % honeys under anaerobic condition

Rep	Time (h)	A7.5	AC7.5	B7.5	BC7.5	C7.5	CC7.5	K7.5	KC7.5
6	31	0.04	0.13	0.18	0.21	0.15	0.34	0.45	0.72
6	32	0.04	0.13	0.19	0.22	0.15	0.36	0.46	0.76
6	33	0.04	0.14	0.19	0.23	0.15	0.39	0.48	0.79
6	34	0.04	0.13	0.19	0.24	0.14	0.40	0.51	0.82
6	35	0.04	0.14	0.20	0.25	0.14	0.43	0.53	0.84
6	36	0.04	0.14	0.20	0.26	0.14	0.45	0.56	0.87
6	37	0.04	0.14	0.20	0.27	0.14	0.46	0.57	0.91
6	38	0.03	0.14	0.21	0.27	0.13	0.48	0.59	0.93
6	39	0.03	0.14	0.21	0.28	0.13	0.51	0.62	0.95
6	40	0.03	0.14	0.21	0.29	0.13	0.53	0.64	0.96
6	41	0.03	0.14	0.22	0.30	0.13	0.55	0.67	0.98
6	42	0.03	0.14	0.22	0.31	0.12	0.57	0.69	0.99
6	43	0.03	0.14	0.23	0.32	0.12	0.59	0.71	1.01
6	44	0.03	0.14	0.23	0.33	0.12	0.61	0.74	1.02
6	45	0.03	0.15	0.24	0.33	0.12	0.63	0.77	1.04
6	46	0.03	0.15	0.25	0.34	0.12	0.64	0.81	1.06
6	47	0.03	0.15	0.27	0.36	0.12	0.66	0.83	1.08
6	48	0.03	0.15	0.28	0.38	0.12	0.67	0.86	1.10

Notes: A7.5=7.5 % Manuka honey 20+ UMF; AC7.5=7.5 % Manuka honey 20+ UMF with catalase; B7.5=7.5 % Manuka honey 15+ UMF; BC7.5=7.5 % Manuka honey 15+ UMF with catalase; C7.5=7.5 % Manuka honey 10+ UMF; CC7.5=7.5 % Manuka honey 10+ UMF with catalase; K7.5=7.5 % Kanuka honey; KC7.5=7.5 % Kanuka honey with catalase

Table A2-11 Growth of *P. acnes* (Abs $_{595nm}$) in 10 % honeys under aerobic condition

Rep	Time (h)	A10	AC10	B10	BC10	C10	CC10	K10	KC10
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.01
1	3	0.00	0.01	0.00	0.02	0.01	0.01	0.00	0.02
1	4	0.00	0.01	0.00	0.02	0.00	0.01	0.00	0.02
1	5	0.00	0.02	0.00	0.02	0.01	0.02	0.00	0.02
1	6	0.00	0.02	0.00	0.03	0.01	0.02	0.00	0.03
1	7	0.00	0.02	0.00	0.03	0.01	0.02	0.00	0.03
1	8	0.00	0.03	0.00	0.03	0.02	0.03	0.00	0.04
1	9	0.00	0.03	0.00	0.03	0.02	0.03	0.00	0.05
1	10	0.00	0.03	0.00	0.03	0.03	0.03	0.00	0.05
1	11	0.00	0.03	0.00	0.04	0.03	0.03	0.00	0.06
1	12	0.00	0.03	0.00	0.04	0.03	0.03	0.00	0.07
1	13	0.00	0.03	0.00	0.04	0.04	0.04	0.00	0.08
1	14	0.00	0.04	0.00	0.04	0.04	0.04	0.00	0.10
1	15	0.00	0.04	0.00	0.05	0.04	0.04	0.00	0.11
1	16	0.00	0.04	0.00	0.05	0.05	0.04	0.00	0.13
1	17	0.00	0.05	0.00	0.05	0.05	0.04	0.00	0.15
1	18	0.00	0.05	0.00	0.06	0.05	0.04	0.00	0.18
1	19	0.00	0.06	0.00	0.07	0.05	0.05	0.00	0.21
1	20	0.00	0.06	0.00	0.07	0.05	0.05	0.00	0.24
1	21	0.00	0.07	0.00	0.08	0.06	0.05	0.00	0.28
1	22	0.00	0.08	0.00	0.09	0.06	0.05	0.00	0.32
1	23	0.00	0.09	0.00	0.09	0.06	0.06	0.00	0.36
1	24	0.00	0.10	0.01	0.10	0.07	0.06	0.00	0.40
1	25	0.00	0.11	0.01	0.11	0.07	0.06	0.00	0.44
1	26	0.00	0.12	0.01	0.12	0.08	0.07	0.00	0.48
1	27	0.00	0.13	0.01	0.12	0.08	0.07	0.00	0.52
1	28	0.00	0.14	0.01	0.12	0.08	0.08	0.00	0.56
1	29	0.00	0.15	0.01	0.12	0.08	0.07	0.00	0.60
1	30	0.00	0.15	0.01	0.14	0.09	0.08	0.00	0.65
1	31	0.00	0.15	0.01	0.15	0.09	0.08	0.00	0.69
1	32	0.00	0.15	0.00	0.15	0.10	0.08	0.00	0.74
1	33	0.00	0.15	0.00	0.15	0.11	0.09	0.00	0.78
1	34	0.00	0.15	0.00	0.16	0.13	0.13	0.00	0.82
1	35	0.00	0.15	0.00	0.16	0.15	0.17	0.00	0.86
1	36	0.00	0.15	0.00	0.16	0.19	0.25	0.00	0.90
1	37	0.00	0.15	0.00	0.16	0.25	0.34	0.00	0.93
1	38	0.00	0.15	0.00	0.17	0.30	0.40	0.00	0.96
1	39	0.00	0.15	0.00	0.17	0.36	0.47	0.00	0.99

Table A2-11$_{cont}$Growth of *P. acnes* (Abs $_{595nm}$) in 10% honeys under aerobic condition

Rep	Time (h)	A10	AC10	B10	BC10	C10	CC10	K10	KC10
1	40	0.00	0.15	0.00	0.17	0.42	0.52	0.00	1.01
1	41	0.00	0.15	0.00	0.17	0.46	0.58	0.00	1.03
1	42	0.00	0.16	0.00	0.17	0.49	0.61	0.00	1.05
1	43	0.00	0.16	0.00	0.17	0.51	0.64	0.00	1.06
1	44	0.00	0.16	0.00	0.18	0.53	0.66	0.00	1.07
1	45	0.00	0.16	0.00	0.18	0.55	0.67	0.00	1.08
1	46	0.00	0.16	0.00	0.18	0.57	0.68	0.00	1.08
1	47	0.00	0.16	0.00	0.18	0.58	0.69	0.00	1.09
1	48	0.00	0.17	0.00	0.18	0.60	0.69	0.00	1.09
2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.01	0.00	0.00	0.00	0.01	0.02	0.00	0.01
2	3	0.02	0.01	0.00	0.01	0.01	0.03	0.00	0.02
2	4	0.02	0.01	0.01	0.01	0.01	0.03	0.00	0.02
2	5	0.03	0.02	0.01	0.02	0.02	0.03	0.00	0.04
2	6	0.03	0.02	0.01	0.02	0.02	0.04	0.01	0.04
2	7	0.03	0.02	0.01	0.03	0.03	0.04	0.01	0.05
2	8	0.03	0.03	0.01	0.03	0.04	0.05	0.00	0.06
2	9	0.04	0.03	0.01	0.03	0.04	0.04	0.01	0.06
2	10	0.03	0.03	0.01	0.03	0.04	0.05	0.00	0.07
2	11	0.04	0.03	0.01	0.03	0.05	0.05	0.00	0.07
2	12	0.04	0.03	0.00	0.03	0.05	0.05	0.00	0.08
2	13	0.04	0.03	0.00	0.04	0.05	0.05	0.00	0.10
2	14	0.04	0.04	0.00	0.04	0.06	0.05	0.01	0.11
2	15	0.04	0.04	0.00	0.04	0.06	0.05	0.01	0.12
2	16	0.04	0.04	0.00	0.04	0.07	0.06	0.01	0.16
2	17	0.04	0.05	0.00	0.05	0.07	0.06	0.00	0.23
2	18	0.04	0.05	0.00	0.05	0.07	0.06	0.00	0.38
2	19	0.04	0.06	0.00	0.06	0.07	0.06	0.00	0.53
2	20	0.04	0.06	0.00	0.06	0.07	0.06	0.00	0.65
2	21	0.04	0.07	0.00	0.07	0.07	0.06	0.00	0.69
2	22	0.04	0.08	0.00	0.07	0.07	0.06	0.00	0.71
2	23	0.04	0.09	0.00	0.08	0.08	0.07	0.00	0.71
2	24	0.04	0.09	0.00	0.09	0.08	0.07	0.00	0.71
2	25	0.04	0.11	0.00	0.10	0.08	0.10	0.00	0.72
2	26	0.04	0.11	0.00	0.11	0.09	0.17	0.00	0.73
2	27	0.05	0.12	0.00	0.11	0.09	0.28	0.00	0.73
2	28	0.05	0.12	0.00	0.12	0.09	0.41	0.00	0.73
2	29	0.05	0.13	0.00	0.12	0.09	0.50	0.00	0.74
2	30	0.04	0.13	0.00	0.12	0.09	0.55	0.00	0.74

Table A2-11$_{cont}$Growth of *P. acnes* (Abs $_{595nm}$) in 10 % honeys under aerobic condition

Rep	Time (h)	A10	AC10	B10	BC10	C10	CC10	K10	KC10
2	31	0.04	0.14	0.00	0.11	0.09	0.57	0.00	0.74
2	32	0.04	0.15	0.00	0.11	0.09	0.61	0.00	0.74
2	33	0.04	0.16	0.00	0.12	0.09	0.63	0.00	0.74
2	34	0.04	0.16	0.00	0.12	0.09	0.65	0.00	0.75
2	35	0.04	0.17	0.00	0.12	0.09	0.65	0.00	0.75
2	36	0.04	0.17	0.00	0.12	0.09	0.66	0.00	0.75
2	37	0.04	0.18	0.00	0.12	0.09	0.66	0.00	0.75
2	38	0.04	0.18	0.00	0.12	0.09	0.67	0.00	0.76
2	39	0.05	0.18	0.00	0.13	0.10	0.68	0.00	0.76
2	40	0.05	0.18	0.00	0.13	0.12	0.70	0.00	0.76
2	41	0.05	0.18	0.00	0.13	0.15	0.72	0.00	0.76
2	42	0.05	0.19	0.00	0.13	0.17	0.74	0.00	0.76
2	43	0.05	0.19	0.00	0.13	0.22	0.76	0.00	0.76
2	44	0.05	0.19	0.00	0.13	0.27	0.78	0.00	0.76
2	45	0.05	0.19	0.00	0.13	0.32	0.80	0.00	0.76
2	46	0.05	0.19	0.00	0.13	0.35	0.82	0.00	0.76
2	47	0.06	0.19	0.00	0.14	0.37	0.84	0.00	0.76
2	48	0.06	0.19	0.00	0.14	0.39	0.85	0.00	0.76
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.00	0.01	0.00	0.02	0.00	0.02	0.00	0.01
3	3	0.00	0.02	0.00	0.02	0.00	0.02	0.00	0.02
3	4	0.00	0.02	0.00	0.02	0.00	0.03	0.00	0.03
3	5	0.00	0.03	0.01	0.03	0.00	0.04	0.00	0.04
3	6	0.00	0.03	0.01	0.03	0.00	0.04	0.00	0.05
3	7	0.00	0.04	0.01	0.04	0.00	0.05	0.00	0.05
3	8	0.00	0.03	0.01	0.04	0.00	0.05	0.00	0.06
3	9	0.00	0.04	0.01	0.04	0.00	0.06	0.00	0.07
3	10	0.00	0.04	0.01	0.04	0.00	0.06	0.00	0.08
3	11	0.00	0.04	0.01	0.04	0.00	0.06	0.00	0.09
3	12	0.00	0.04	0.01	0.04	0.00	0.06	0.00	0.09
3	13	0.00	0.04	0.01	0.05	0.01	0.07	0.00	0.11
3	14	0.00	0.05	0.02	0.05	0.01	0.07	0.00	0.12
3	15	0.00	0.05	0.02	0.05	0.01	0.07	0.00	0.13
3	16	0.00	0.05	0.02	0.06	0.01	0.08	0.00	0.14
3	17	0.00	0.05	0.02	0.06	0.01	0.12	0.00	0.15
3	18	0.00	0.06	0.02	0.06	0.01	0.21	0.00	0.17
3	19	0.00	0.07	0.02	0.07	0.01	0.40	0.00	0.18
3	20	0.00	0.07	0.02	0.07	0.01	0.55	0.00	0.20

Table A2-11$_{cont}$Growth of *P. acnes* (Abs $_{595nm}$) in 10 % honeys under aerobic condition

Rep	Time (h)	A10	AC10	B10	BC10	C10	CC10	K10	KC10
3	21	0.00	0.08	0.02	0.08	0.02	0.64	0.00	0.22
3	22	0.00	0.09	0.02	0.09	0.02	0.69	0.00	0.25
3	23	0.00	0.10	0.02	0.10	0.02	0.75	0.00	0.28
3	24	0.00	0.11	0.02	0.10	0.02	0.78	0.00	0.33
3	25	0.00	0.12	0.02	0.11	0.02	0.80	0.00	0.38
3	26	0.00	0.13	0.03	0.12	0.03	0.81	0.00	0.42
3	27	0.01	0.14	0.03	0.13	0.03	0.81	0.00	0.48
3	28	0.01	0.14	0.03	0.13	0.03	0.82	0.00	0.54
3	29	0.00	0.14	0.03	0.13	0.02	0.82	0.00	0.60
3	30	0.00	0.14	0.03	0.13	0.02	0.83	0.00	0.66
3	31	0.00	0.14	0.03	0.13	0.02	0.83	0.00	0.72
3	32	0.00	0.14	0.03	0.12	0.02	0.83	0.00	0.77
3	33	0.00	0.14	0.03	0.13	0.01	0.83	0.00	0.82
3	34	0.00	0.15	0.03	0.13	0.01	0.83	0.00	0.86
3	35	0.00	0.15	0.03	0.13	0.01	0.83	0.00	0.90
3	36	0.00	0.15	0.03	0.13	0.01	0.83	0.00	0.93
3	37	0.00	0.15	0.03	0.13	0.01	0.83	0.00	0.96
3	38	0.00	0.15	0.03	0.13	0.03	0.84	0.00	0.98
3	39	0.00	0.15	0.03	0.13	0.06	0.84	0.00	1.01
3	40	0.00	0.15	0.03	0.13	0.09	0.84	0.00	1.03
3	41	0.00	0.15	0.03	0.14	0.14	0.84	0.00	1.05
3	42	0.00	0.15	0.03	0.14	0.19	0.84	0.00	1.07
3	43	0.00	0.16	0.03	0.14	0.24	0.84	0.00	1.09
3	44	0.00	0.16	0.03	0.14	0.28	0.84	0.00	1.10
3	45	0.00	0.16	0.03	0.14	0.32	0.84	0.00	1.11
3	46	0.00	0.16	0.03	0.14	0.34	0.83	0.00	1.12
3	47	0.00	0.16	0.03	0.15	0.36	0.84	0.00	1.12
3	48	0.00	0.16	0.03	0.15	0.38	0.83	0.00	1.13
4	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	2	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.01
4	3	0.00	0.01	0.00	0.01	0.02	0.02	0.00	0.02
4	4	0.00	0.02	0.01	0.02	0.03	0.03	0.00	0.03
4	5	0.00	0.02	0.01	0.03	0.03	0.04	0.00	0.04
4	6	0.00	0.03	0.02	0.03	0.04	0.05	0.00	0.06
4	7	0.00	0.03	0.02	0.03	0.04	0.06	0.00	0.07
4	8	0.00	0.04	0.03	0.04	0.05	0.07	0.00	0.09
4	9	0.00	0.04	0.03	0.04	0.06	0.08	0.00	0.11
4	10	0.00	0.04	0.03	0.05	0.06	0.09	0.00	0.13

Table A2-11$_{cont}$Growth of *P. acnes* (Abs $_{595nm}$) in 10 % honeys under aerobic condition

Rep	Time (h)	A10	AC10	B10	BC10	C10	CC10	K10	KC10
4	11	0.00	0.04	0.03	0.05	0.07	0.10	0.00	0.14
4	12	0.00	0.04	0.03	0.06	0.07	0.11	0.00	0.17
4	13	0.00	0.05	0.03	0.06	0.08	0.12	0.00	0.19
4	14	0.00	0.05	0.03	0.06	0.09	0.13	0.00	0.22
4	15	0.00	0.05	0.03	0.07	0.13	0.14	0.00	0.24
4	16	0.00	0.06	0.03	0.08	0.24	0.15	0.00	0.28
4	17	0.00	0.06	0.03	0.08	0.38	0.16	0.00	0.31
4	18	0.00	0.07	0.03	0.09	0.49	0.17	0.00	0.35
4	19	0.00	0.08	0.03	0.09	0.57	0.19	0.00	0.40
4	20	0.00	0.09	0.03	0.10	0.58	0.23	0.00	0.44
4	21	0.00	0.09	0.03	0.11	0.61	0.27	0.00	0.49
4	22	0.00	0.10	0.03	0.12	0.63	0.36	0.00	0.53
4	23	0.00	0.11	0.03	0.13	0.66	0.42	0.00	0.58
4	24	0.00	0.12	0.03	0.14	0.67	0.49	0.00	0.63
4	25	0.00	0.13	0.04	0.15	0.68	0.55	0.00	0.66
4	26	0.00	0.14	0.04	0.15	0.69	0.61	0.00	0.71
4	27	0.00	0.15	0.04	0.16	0.69	0.64	0.00	0.77
4	28	0.00	0.15	0.04	0.17	0.70	0.66	0.00	0.81
4	29	0.00	0.15	0.03	0.17	0.71	0.69	0.00	0.86
4	30	0.00	0.15	0.03	0.17	0.72	0.71	0.00	0.90
4	31	0.00	0.15	0.03	0.17	0.73	0.72	0.00	0.94
4	32	0.00	0.15	0.03	0.17	0.75	0.73	0.00	0.98
4	33	0.00	0.16	0.03	0.17	0.76	0.76	0.00	1.01
4	34	0.00	0.16	0.03	0.18	0.77	0.78	0.00	1.04
4	35	0.00	0.16	0.03	0.18	0.78	0.80	0.00	1.06
4	36	0.00	0.16	0.03	0.18	0.79	0.80	0.00	1.08
4	37	0.00	0.17	0.03	0.18	0.80	0.82	0.00	1.10
4	38	0.00	0.17	0.03	0.19	0.82	0.84	0.00	1.13
4	39	0.00	0.17	0.03	0.19	0.83	0.86	0.00	1.14
4	40	0.00	0.18	0.03	0.19	0.85	0.88	0.00	1.16
4	41	0.00	0.18	0.03	0.20	0.86	0.90	0.00	1.17
4	42	0.00	0.18	0.03	0.20	0.87	0.93	0.00	1.18
4	43	0.00	0.18	0.03	0.20	0.88	0.95	0.00	1.19
4	44	0.00	0.18	0.03	0.20	0.90	0.97	0.00	1.19
4	45	0.00	0.18	0.03	0.20	0.91	0.99	0.00	1.20
4	46	0.00	0.18	0.03	0.21	0.92	1.01	0.00	1.20
4	47	0.00	0.18	0.03	0.21	0.94	1.03	0.00	1.21
4	48	0.00	0.19	0.03	0.21	0.95	1.05	0.00	1.21

Table A2-11$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 10 % honeys under aerobic condition

Rep	Time (h)	A10	AC10	B10	BC10	C10	CC10	K10	KC10
5	0	0.00		0.00	0.00	0.00	0.00	0.00	0.00
5	1	0.00		0.00	0.00	0.00	0.00	0.00	0.00
5	2	0.00		0.00	0.01	0.00	0.01	0.00	0.01
5	3	0.00		0.00	0.02	0.00	0.02	0.00	0.02
5	4	0.01		0.00	0.02	0.01	0.03	0.00	0.03
5	5	0.01		0.01	0.03	0.01	0.04	0.00	0.04
5	6	0.01		0.01	0.03	0.01	0.05	0.00	0.06
5	7	0.01		0.01	0.03	0.02	0.05	0.00	0.07
5	8	0.02		0.01	0.04	0.02	0.06	0.01	0.09
5	9	0.02		0.01	0.04	0.02	0.07	0.02	0.10
5	10	0.02		0.01	0.04	0.02	0.08	0.01	0.12
5	11	0.02		0.01	0.05	0.02	0.09	0.00	0.14
5	12	0.02		0.00	0.05	0.02	0.10	0.00	0.15
5	13	0.02		0.00	0.06	0.02	0.11	0.00	0.17
5	14	0.01		0.00	0.06	0.02	0.12	0.00	0.19
5	15	0.01		0.00	0.06	0.01	0.14	0.00	0.22
5	16	0.02		0.00	0.07	0.02	0.19	0.00	0.25
5	17	0.01		0.00	0.07	0.03	0.27	0.00	0.29
5	18	0.01		0.00	0.08	0.11	0.37	0.00	0.36
5	19	0.01		0.00	0.09	0.20	0.47	0.00	0.45
5	20	0.01		0.00	0.09	0.32	0.58	0.00	0.57
5	21	0.02		0.00	0.10	0.44	0.65	0.00	0.65
5	22	0.02		0.00	0.11	0.47	0.71	0.00	0.70
5	23	0.01		0.00	0.12	0.48	0.74	0.00	0.71
5	24	0.01		0.00	0.13	0.53	0.77	0.00	0.75
5	25	0.01		0.00	0.14	0.58	0.83	0.00	0.79
5	26	0.02		0.00	0.15	0.61	0.88	0.00	0.82
5	27	0.02		0.00	0.16	0.62	0.93	0.00	0.85
5	28	0.02		0.00	0.16	0.62	0.95	0.00	0.88
5	29	0.01		0.00	0.16	0.61	0.98	0.00	0.91
5	30	0.01		0.00	0.17	0.62	1.02	0.00	0.93
5	31	0.01		0.00	0.16	0.62	1.05	0.00	0.95
5	32	0.01		0.00	0.16	0.63	1.08	0.00	0.97
5	33	0.01		0.00	0.17	0.64	1.11	0.00	0.99
5	34	0.01		0.00	0.18	0.65	1.14	0.00	1.00
5	35	0.01		0.00	0.18	0.66	1.18	0.00	1.01
5	36	0.01		0.00	0.18	0.67	1.21	0.00	1.02
5	37	0.01		0.00	0.18	0.68	1.24	0.00	1.03
5	38	0.01		0.00	0.19	0.69	1.26	0.00	1.05
5	39	0.02		0.00	0.19	0.71	1.29	0.00	1.06

Table A2-11$_{cont}$Growth of *P. acnes* (Abs $_{595nm}$) in 10 % honeys under aerobic condition

Rep	Time (h)	A10	AC10	B10	BC10	C10	CC10	K10	KC10
5	40	0.02		0.00	0.19	0.72	1.31	0.00	1.06
5	41	0.01		0.00	0.20	0.73	1.33	0.00	1.07
5	42	0.01		0.00	0.20	0.74	1.35	0.00	1.08
5	43	0.01		0.00	0.20	0.75	1.37	0.00	1.09
5	44	0.01		0.00	0.21	0.76	1.38	0.00	1.10
5	45	0.01		0.00	0.21	0.78	1.39	0.00	1.10
5	46	0.01		0.00	0.21	0.79	1.41	0.00	1.11
5	47	0.01		0.00	0.21	0.80	1.42	0.00	1.12
5	48	0.01		0.00	0.21	0.81	1.42	0.00	1.12
6	0	0.00		0.00	0.00	0.00	0.00	0.00	0.00
6	1	0.00		0.00	0.00	0.00	0.00	0.00	0.00
6	2	0.00		0.00	0.01	0.01	0.01	0.00	0.00
6	3	0.00		0.00	0.02	0.01	0.02	0.00	0.02
6	4	0.01		0.00	0.03	0.02	0.03	0.00	0.03
6	5	0.01		0.01	0.03	0.02	0.04	0.00	0.04
6	6	0.01		0.01	0.04	0.03	0.05	0.00	0.06
6	7	0.01		0.01	0.04	0.03	0.06	0.01	0.07
6	8	0.02		0.01	0.05	0.04	0.07	0.01	0.08
6	9	0.02		0.01	0.05	0.04	0.08	0.01	0.10
6	10	0.03		0.01	0.05	0.05	0.09	0.00	0.12
6	11	0.03		0.01	0.06	0.05	0.10	0.00	0.13
6	12	0.04		0.01	0.06	0.05	0.12	0.00	0.15
6	13	0.04		0.01	0.07	0.06	0.13	0.00	0.17
6	14	0.04		0.00	0.07	0.06	0.15	0.00	0.18
6	15	0.04		0.00	0.07	0.06	0.17	0.00	0.21
6	16	0.04		0.00	0.08	0.06	0.25	0.00	0.24
6	17	0.05		0.00	0.08	0.06	0.37	0.00	0.28
6	18	0.05		0.00	0.09	0.07	0.51	0.00	0.35
6	19	0.05		0.00	0.10	0.13	0.61	0.00	0.43
6	20	0.05		0.00	0.11	0.21	0.67	0.00	0.53
6	21	0.06		0.00	0.11	0.33	0.71	0.00	0.61
6	22	0.06		0.00	0.12	0.46	0.75	0.00	0.64
6	23	0.06		0.00	0.13	0.57	0.80	0.00	0.66
6	24	0.06		0.00	0.14	0.58	0.85	0.00	0.68
6	25	0.06		0.00	0.15	0.60	0.88	0.00	0.70
6	26	0.07		0.00	0.16	0.64	0.91	0.00	0.72
6	27	0.07		0.00	0.17	0.65	0.93	0.00	0.74
6	28	0.06		0.00	0.17	0.66	0.95	0.00	0.77
6	29	0.07		0.00	0.18	0.67	0.98	0.00	0.79
6	30	0.07		0.00	0.18	0.67	0.99	0.00	0.81

Table A2-11$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 10 % honeys under aerobic condition

Rep	Time (h)	A10	AC10	B10	BC10	C10	CC10	K10	KC10
6	31	0.07		0.00	0.18	0.67	1.01	0.00	0.83
6	32	0.08		0.00	0.18	0.67	1.04	0.00	0.86
6	33	0.08		0.00	0.18	0.67	1.07	0.00	0.87
6	34	0.09		0.00	0.19	0.68	1.10	0.00	0.89
6	35	0.09		0.00	0.19	0.69	1.13	0.00	0.90
6	36	0.10		0.00	0.20	0.69	1.15	0.00	0.92
6	37	0.10		0.00	0.20	0.70	1.17	0.00	0.93
6	38	0.10		0.00	0.21	0.71	1.19	0.00	0.94
6	39	0.10		0.00	0.22	0.72	1.21	0.00	0.96
6	40	0.10		0.00	0.22	0.73	1.22	0.00	0.97
6	41	0.10		0.00	0.22	0.74	1.23	0.00	0.98
6	42	0.11		0.00	0.23	0.75	1.24	0.00	0.99
6	43	0.11		0.00	0.23	0.76	1.24	0.00	1.00
6	44	0.10		0.00	0.24	0.77	1.25	0.00	1.01
6	45	0.10		0.00	0.25	0.78	1.25	0.00	1.02
6	46	0.11		0.00	0.26	0.79	1.26	0.00	1.02
6	47	0.11		0.00	0.28	0.80	1.26	0.00	1.02
6	48	0.10		0.00	0.30	0.81	1.26	0.00	1.03

Notes: A10=10 % Manuka honey 20+ UMF; AC10=10 % Manuka honey 20+ UMF with catalase; B10=10 % Manuka honey 15+ UMF; BC10=10 % Manuka honey 15+ UMF with catalase;C10=10 % Manuka honey 10+ UMF; CC10=10 % Manuka honey 10+ UMF with catalase; K10=10 % Kanuka honey; KC10=10 % Kanuka honey with catalase

Table A2-12 Growth of *P. acnes* (Abs $_{595nm}$) in 10 % honeys under anaerobic condition

Rep	Time (h)	A10	AC10	B10	BC10	C10	CC10	K10	KC10
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.01	0.01	0.01	0.01	0.01	0.02	0.00	0.01
1	3	0.01	0.02	0.03	0.02	0.02	0.03	0.01	0.02
1	4	0.02	0.03	0.03	0.02	0.03	0.04	0.01	0.04
1	5	0.03	0.03	0.04	0.03	0.04	0.05	0.01	0.05
1	6	0.03	0.03	0.05	0.04	0.04	0.06	0.01	0.06
1	7	0.04	0.04	0.06	0.04	0.05	0.07	0.01	0.07
1	8	0.04	0.05	0.07	0.04	0.05	0.08	0.01	0.08
1	9	0.04	0.05	0.07	0.05	0.05	0.08	0.01	0.10
1	10	0.05	0.05	0.08	0.05	0.06	0.08	0.01	0.11
1	11	0.05	0.06	0.08	0.05	0.06	0.09	0.01	0.13
1	12	0.05	0.06	0.08	0.06	0.07	0.09	0.01	0.15
1	13	0.05	0.06	0.08	0.06	0.07	0.09	0.01	0.18
1	14	0.05	0.06	0.08	0.06	0.07	0.09	0.01	0.20
1	15	0.05	0.06	0.08	0.06	0.07	0.09	0.01	0.23
1	16	0.04	0.06	0.08	0.06	0.08	0.09	0.01	0.26
1	17	0.04	0.06	0.08	0.07	0.08	0.10	0.00	0.30
1	18	0.04	0.07	0.08	0.07	0.08	0.10	0.00	0.34
1	19	0.04	0.06	0.08	0.07	0.09	0.10	0.00	0.36
1	20	0.04	0.07	0.08	0.07	0.09	0.10	0.00	0.39
1	21	0.04	0.07	0.08	0.07	0.09	0.11	0.00	0.42
1	22	0.04	0.07	0.08	0.07	0.09	0.11	0.00	0.45
1	23	0.04	0.07	0.08	0.07	0.09	0.11	0.00	0.49
1	24	0.04	0.07	0.08	0.08	0.09	0.12	0.00	0.52
1	25	0.04	0.08	0.09	0.08	0.10	0.13	0.01	0.56
1	26	0.04	0.08	0.09	0.08	0.10	0.13	0.01	0.60
1	27	0.04	0.08	0.09	0.09	0.10	0.14	0.00	0.64
1	28	0.04	0.08	0.09	0.08	0.09	0.14	0.00	0.68
1	29	0.03	0.09	0.09	0.09	0.09	0.14	0.00	0.73
1	30	0.03	0.08	0.08	0.09	0.09	0.15	0.00	0.76
1	31	0.03	0.09	0.08	0.09	0.09	0.15	0.00	0.81
1	32	0.02	0.09	0.08	0.09	0.09	0.16	0.00	0.84
1	33	0.02	0.09	0.08	0.09	0.09	0.17	0.00	0.88
1	34	0.02	0.09	0.08	0.09	0.09	0.17	0.00	0.90
1	35	0.02	0.09	0.07	0.09	0.08	0.18	0.00	0.94
1	36	0.02	0.10	0.07	0.09	0.08	0.19	0.01	0.96
1	37	0.02	0.09	0.07	0.10	0.08	0.20	0.01	0.99
1	38	0.02	0.10	0.07	0.10	0.08	0.21	0.00	1.01
1	39	0.01	0.09	0.07	0.10	0.07	0.22	0.01	1.03

Table A2-12$_{cont}$Growth of *P. acnes* (Abs $_{595nm}$) in 10 % honeys under anaerobic condition

Rep	Time (h)	A10	AC10	B10	BC10	C10	CC10	K10	KC10
1	40	0.01	0.10	0.07	0.10	0.07	0.23	0.00	1.05
1	41	0.01	0.10	0.07	0.10	0.07	0.25	0.00	1.07
1	42	0.01	0.10	0.07	0.10	0.07	0.26	0.00	1.08
1	43	0.01	0.10	0.07	0.10	0.06	0.27	0.00	1.09
1	44	0.01	0.10	0.07	0.10	0.07	0.29	0.00	1.11
1	45	0.01	0.10	0.07	0.11	0.07	0.31	0.00	1.11
1	46	0.01	0.10	0.07	0.11	0.06	0.32	0.00	1.13
1	47	0.01	0.10	0.07	0.11	0.06	0.34	0.00	1.14
1	48	0.01	0.11	0.07	0.11	0.07	0.36	0.00	1.15
2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.01	0.01	0.00	0.02	0.00	0.01	0.00	0.02
2	3	0.01	0.02	0.01	0.03	0.01	0.02	0.00	0.03
2	4	0.02	0.03	0.01	0.03	0.02	0.04	0.00	0.04
2	5	0.02	0.04	0.01	0.04	0.02	0.05	0.00	0.05
2	6	0.02	0.04	0.01	0.05	0.02	0.05	0.00	0.06
2	7	0.03	0.04	0.02	0.05	0.02	0.06	0.00	0.07
2	8	0.03	0.05	0.02	0.06	0.02	0.06	0.00	0.09
2	9	0.03	0.05	0.02	0.06	0.02	0.06	0.00	0.10
2	10	0.03	0.05	0.02	0.06	0.02	0.07	0.00	0.12
2	11	0.04	0.05	0.03	0.07	0.02	0.07	0.00	0.14
2	12	0.04	0.06	0.03	0.07	0.02	0.07	0.00	0.16
2	13	0.04	0.05	0.03	0.07	0.02	0.07	0.00	0.19
2	14	0.04	0.06	0.03	0.07	0.02	0.07	0.00	0.21
2	15	0.04	0.06	0.03	0.08	0.02	0.08	0.00	0.25
2	16	0.04	0.06	0.03	0.08	0.02	0.08	0.00	0.28
2	17	0.04	0.06	0.03	0.08	0.02	0.08	0.01	0.31
2	18	0.04	0.06	0.03	0.08	0.02	0.09	0.01	0.35
2	19	0.03	0.06	0.03	0.08	0.02	0.09	0.00	0.38
2	20	0.03	0.06	0.03	0.08	0.02	0.09	0.01	0.41
2	21	0.03	0.06	0.03	0.08	0.02	0.10	0.00	0.44
2	22	0.03	0.07	0.03	0.08	0.02	0.10	0.00	0.46
2	23	0.03	0.07	0.03	0.08	0.01	0.11	0.00	0.49
2	24	0.03	0.07	0.03	0.09	0.01	0.11	0.01	0.52
2	25	0.03	0.07	0.03	0.09	0.02	0.13	0.02	0.55
2	26	0.03	0.08	0.03	0.09	0.02	0.13	0.01	0.59
2	27	0.03	0.08	0.03	0.10	0.01	0.14	0.01	0.62
2	28	0.03	0.08	0.03	0.10	0.01	0.14	0.01	0.65
2	29	0.03	0.08	0.03	0.10	0.01	0.15	0.01	0.68
2	30	0.03	0.08	0.03	0.10	0.01	0.16	0.00	0.71

Table A2-12 $_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 10 % honeys under anaerobic condition

Rep	Time (h)	A10	AC10	B10	BC10	C10	CC10	K10	KC10
2	31	0.03	0.08	0.03	0.10	0.01	0.17	0.00	0.75
2	32	0.02	0.08	0.03	0.10	0.00	0.19	0.00	0.78
2	33	0.03	0.09	0.03	0.10	0.01	0.20	0.00	0.81
2	34	0.02	0.09	0.03	0.10	0.00	0.21	0.00	0.83
2	35	0.02	0.09	0.03	0.10	0.00	0.22	0.01	0.86
2	36	0.02	0.09	0.02	0.10	0.00	0.24	0.00	0.89
2	37	0.02	0.09	0.02	0.11	0.00	0.26	0.00	0.91
2	38	0.02	0.09	0.02	0.11	0.00	0.27	0.00	0.93
2	39	0.02	0.09	0.02	0.11	-0.01	0.29	0.00	0.96
2	40	0.02	0.09	0.02	0.11	-0.01	0.31	0.00	0.97
2	41	0.01	0.09	0.02	0.11	-0.01	0.33	0.00	0.99
2	42	0.01	0.09	0.02	0.11	-0.01	0.35	0.00	1.00
2	43	0.01	0.09	0.02	0.11	-0.01	0.37	0.00	1.01
2	44	0.01	0.10	0.01	0.12	-0.01	0.39	0.00	1.02
2	45	0.01	0.10	0.01	0.12	-0.01	0.40	0.00	1.03
2	46	0.01	0.10	0.01	0.12	-0.01	0.42	0.00	1.04
2	47	0.01	0.10	0.01	0.12	-0.02	0.44	0.01	1.05
2	48	0.01	0.10	0.02	0.13	-0.01	0.46	0.01	1.05
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.00	0.02	0.00	0.02	0.01	0.01	0.01	0.02
3	3	0.00	0.03	0.01	0.03	0.03	0.02	0.01	0.04
3	4	0.00	0.03	0.01	0.04	0.03	0.04	0.01	0.05
3	5	0.01	0.04	0.01	0.04	0.04	0.05	0.01	0.06
3	6	0.00	0.04	0.02	0.05	0.05	0.05	0.01	0.07
3	7	0.01	0.05	0.02	0.05	0.06	0.06	0.02	0.08
3	8	0.01	0.05	0.02	0.06	0.06	0.07	0.02	0.10
3	9	0.00	0.05	0.03	0.06	0.06	0.07	0.02	0.12
3	10	0.01	0.06	0.03	0.07	0.07	0.08	0.02	0.14
3	11	0.01	0.06	0.04	0.07	0.07	0.08	0.02	0.16
3	12	0.00	0.06	0.04	0.07	0.07	0.08	0.02	0.18
3	13	0.00	0.06	0.05	0.07	0.07	0.09	0.02	0.21
3	14	0.00	0.07	0.05	0.07	0.07	0.08	0.02	0.23
3	15	0.00	0.07	0.05	0.08	0.07	0.08	0.02	0.26
3	16	0.00	0.07	0.06	0.08	0.07	0.09	0.01	0.30
3	17	0.00	0.07	0.06	0.08	0.06	0.09	0.01	0.33
3	18	0.00	0.07	0.06	0.08	0.07	0.09	0.01	0.36
3	19	0.00	0.07	0.06	0.07	0.06	0.09	0.00	0.39
3	20	0.00	0.07	0.07	0.08	0.06	0.10	0.00	0.43

Table A2-12 cont Growth of *P. acnes* (Abs $_{595nm}$) in 10 % honeys under anaerobic condition

Rep	Time (h)	A10	AC10	B10	BC10	C10	CC10	K10	KC10
3	21	0.00	0.08	0.07	0.08	0.06	0.10	0.00	0.47
3	22	0.00	0.08	0.07	0.08	0.06	0.10	0.00	0.51
3	23	0.00	0.08	0.08	0.09	0.06	0.11	0.00	0.57
3	24	0.01	0.08	0.08	0.08	0.06	0.11	0.00	0.63
3	25	0.01	0.09	0.08	0.09	0.06	0.12	0.01	0.68
3	26	0.00	0.09	0.08	0.09	0.06	0.13	0.01	0.74
3	27	0.00	0.09	0.08	0.10	0.06	0.13	0.01	0.77
3	28	0.00	0.09	0.08	0.10	0.06	0.14	0.01	0.80
3	29	0.00	0.09	0.08	0.10	0.06	0.14	0.01	0.83
3	30	0.00	0.09	0.08	0.10	0.05	0.15	0.00	0.86
3	31	0.00	0.10	0.08	0.10	0.06	0.15	0.00	0.89
3	32	0.00	0.09	0.08	0.10	0.05	0.16	0.01	0.92
3	33	0.00	0.10	0.08	0.10	0.05	0.17	0.00	0.94
3	34	0.00	0.10	0.08	0.10	0.05	0.18	0.00	0.96
3	35	0.00	0.10	0.08	0.10	0.05	0.19	0.00	0.98
3	36	0.00	0.10	0.08	0.11	0.04	0.20	0.00	1.00
3	37	0.00	0.10	0.08	0.11	0.04	0.21	0.01	1.01
3	38	0.00	0.10	0.08	0.11	0.04	0.22	0.00	1.03
3	39	0.00	0.10	0.08	0.11	0.04	0.23	0.00	1.05
3	40	0.00	0.11	0.08	0.11	0.04	0.25	0.01	1.06
3	41	0.00	0.11	0.08	0.12	0.03	0.26	0.00	1.07
3	42	0.01	0.11	0.08	0.12	0.03	0.27	0.00	1.08
3	43	0.01	0.11	0.08	0.12	0.03	0.29	0.00	1.09
3	44	0.02	0.11	0.08	0.12	0.03	0.30	0.00	1.11
3	45	0.02	0.11	0.08	0.12	0.03	0.31	0.00	1.11
3	46	0.02	0.11	0.08	0.13	0.03	0.33	0.00	1.12
3	47	0.02	0.11	0.08	0.13	0.03	0.34	0.01	1.12
3	48	0.02	0.12	0.08	0.13	0.03	0.36	0.01	1.13

Notes: A10=10 % Manuka honey 20+ UMF; AC10=10 % Manuka honey 20+ UMF with catalase; B10=10 % Manuka honey 15+ UMF; BC10=10 % Manuka honey 15+ UMF with catalase;C10=10 % Manuka honey 10+ UMF; CC10=10 % Manuka honey 10+ UMF with catalase; K10=10 % Kanuka honey; KC10=10 % Kanuka honey with catalase

Table A2-13 Growth of *P. acnes* (Abs $_{595nm}$) in 12.5 % honeys under aerobic condition

Rep	Time (h)	A12.5	AC12.5	B12.5	BC12.5	C12.5	CC12.5	K12.5	KC12.5
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01
1	3	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.01
1	4	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.02
1	5	0.00	0.02	0.00	0.02	0.00	0.02	0.00	0.03
1	6	0.01	0.02	0.00	0.02	0.01	0.02	0.00	0.04
1	7	0.01	0.02	0.00	0.02	0.01	0.02	0.00	0.04
1	8	0.01	0.02	0.00	0.02	0.01	0.02	0.00	0.05
1	9	0.00	0.02	0.00	0.02	0.01	0.02	0.00	0.06
1	10	0.00	0.02	0.00	0.02	0.02	0.02	0.00	0.06
1	11	0.00	0.03	0.00	0.03	0.02	0.02	0.00	0.07
1	12	0.00	0.02	0.00	0.03	0.02	0.02	0.00	0.08
1	13	0.00	0.03	0.00	0.03	0.03	0.02	0.00	0.08
1	14	0.00	0.03	0.00	0.03	0.03	0.03	0.00	0.10
1	15	0.00	0.03	0.00	0.04	0.03	0.03	0.00	0.11
1	16	0.00	0.04	0.00	0.04	0.04	0.03	0.00	0.13
1	17	0.00	0.04	0.00	0.05	0.04	0.03	0.00	0.14
1	18	0.00	0.04	0.00	0.05	0.04	0.03	0.00	0.17
1	19	0.00	0.05	0.00	0.06	0.04	0.03	0.00	0.19
1	20	0.00	0.05	0.00	0.07	0.04	0.03	0.00	0.22
1	21	0.00	0.06	0.00	0.07	0.05	0.04	0.00	0.24
1	22	0.00	0.07	0.00	0.08	0.05	0.04	0.00	0.28
1	23	0.00	0.07	0.00	0.09	0.05	0.04	0.00	0.31
1	24	0.00	0.08	0.00	0.10	0.05	0.04	0.00	0.35
1	25	0.00	0.09	0.00	0.10	0.05	0.05	0.00	0.38
1	26	0.00	0.10	0.00	0.11	0.06	0.05	0.00	0.42
1	27	0.00	0.11	0.00	0.11	0.06	0.07	0.00	0.47
1	28	0.00	0.12	0.00	0.11	0.06	0.12	0.00	0.51
1	29	0.00	0.12	0.00	0.11	0.06	0.18	0.00	0.54
1	30	0.00	0.12	0.00	0.10	0.06	0.25	0.00	0.59
1	31	0.00	0.12	0.00	0.09	0.06	0.30	0.00	0.63
1	32	0.00	0.12	0.00	0.09	0.06	0.34	0.00	0.67
1	33	0.00	0.12	0.00	0.09	0.06	0.39	0.00	0.72
1	34	0.00	0.12	0.00	0.09	0.06	0.43	0.00	0.76
1	35	0.00	0.13	0.00	0.09	0.06	0.46	0.00	0.80
1	36	0.00	0.14	0.00	0.09	0.06	0.48	0.00	0.84
1	37	0.00	0.15	0.00	0.09	0.06	0.48	0.00	0.88
1	38	0.00	0.16	0.00	0.09	0.06	0.49	0.00	0.91
1	39	0.00	0.17	0.00	0.09	0.06	0.52	0.00	0.95

Table A2-13 $_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 12.5 % honeys under aerobic condition

Rep	Time (h)	A12.5	AC12.5	B12.5	BC12.5	C12.5	CC12.5	K12.5	KC12.5
1	40	0.00	0.17	0.00	0.09	0.06	0.54	0.00	0.98
1	41	0.00	0.17	0.00	0.09	0.06	0.55	0.00	1.00
1	42	0.00	0.17	0.00	0.09	0.06	0.57	0.00	1.03
1	43	0.00	0.17	0.00	0.09	0.06	0.58	0.00	1.05
1	44	0.00	0.17	0.00	0.09	0.06	0.60	0.00	1.07
1	45	0.00	0.17	0.00	0.09	0.07	0.61	0.00	1.09
1	46	0.00	0.17	0.00	0.09	0.07	0.62	0.00	1.10
1	47	0.00	0.17	0.00	0.09	0.07	0.63	0.00	1.11
1	48	0.00	0.17	0.00	0.10	0.07	0.63	0.00	1.12
2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.01
2	3	0.00	0.01	0.00	0.01	0.01	0.02	0.00	0.02
2	4	0.00	0.01	0.00	0.01	0.01	0.02	0.01	0.03
2	5	0.01	0.01	0.01	0.02	0.02	0.03	0.01	0.04
2	6	0.01	0.02	0.01	0.02	0.02	0.03	0.01	0.04
2	7	0.01	0.02	0.01	0.03	0.02	0.03	0.01	0.05
2	8	0.01	0.02	0.01	0.03	0.03	0.04	0.01	0.06
2	9	0.01	0.02	0.01	0.03	0.03	0.04	0.01	0.06
2	10	0.01	0.02	0.01	0.03	0.03	0.04	0.00	0.07
2	11	0.01	0.02	0.01	0.03	0.04	0.04	0.00	0.07
2	12	0.01	0.02	0.01	0.03	0.04	0.04	0.00	0.08
2	13	0.01	0.02	0.01	0.04	0.05	0.04	0.01	0.09
2	14	0.01	0.03	0.01	0.04	0.05	0.04	0.01	0.10
2	15	0.01	0.03	0.00	0.04	0.05	0.04	0.00	0.11
2	16	0.01	0.03	0.00	0.05	0.05	0.04	0.00	0.12
2	17	0.01	0.03	0.00	0.05	0.06	0.04	0.00	0.13
2	18	0.01	0.04	0.00	0.06	0.06	0.04	0.00	0.15
2	19	0.01	0.04	0.00	0.06	0.06	0.04	0.00	0.17
2	20	0.01	0.04	0.00	0.07	0.06	0.04	0.00	0.19
2	21	0.01	0.05	0.00	0.07	0.07	0.04	0.00	0.22
2	22	0.01	0.06	0.00	0.08	0.07	0.05	0.00	0.24
2	23	0.01	0.07	0.00	0.09	0.07	0.05	0.00	0.27
2	24	0.02	0.07	0.01	0.10	0.08	0.05	0.00	0.30
2	25	0.02	0.08	0.01	0.11	0.08	0.06	0.00	0.34
2	26	0.02	0.09	0.01	0.11	0.09	0.06	0.00	0.38
2	27	0.02	0.10	0.01	0.12	0.09	0.07	0.00	0.41
2	28	0.02	0.11	0.01	0.12	0.09	0.09	0.00	0.44
2	29	0.02	0.11	0.01	0.12	0.09	0.15	0.00	0.49
2	30	0.02	0.12	0.01	0.12	0.10	0.21	0.00	0.52

Table A2-13$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 12.5 % honeys under aerobic condition

Rep	Time (h)	A12.5	AC12.5	B12.5	BC12.5	C12.5	CC12.5	K12.5	KC12.5
2	31	0.02	0.12	0.01	0.11	0.10	0.26	0.00	0.56
2	32	0.01	0.12	0.01	0.11	0.10	0.30	0.00	0.60
2	33	0.01	0.12	0.01	0.12	0.10	0.34	0.00	0.65
2	34	0.01	0.12	0.01	0.12	0.10	0.38	0.00	0.71
2	35	0.01	0.12	0.01	0.12	0.10	0.43	0.00	0.76
2	36	0.01	0.12	0.01	0.12	0.10	0.46	0.00	0.81
2	37	0.01	0.12	0.01	0.12	0.10	0.50	0.00	0.87
2	38	0.01	0.12	0.01	0.13	0.10	0.52	0.00	0.91
2	39	0.01	0.12	0.01	0.13	0.10	0.53	0.00	0.95
2	40	0.01	0.12	0.01	0.13	0.10	0.53	0.00	0.98
2	41	0.01	0.12	0.01	0.13	0.10	0.54	0.00	1.01
2	42	0.02	0.12	0.02	0.13	0.10	0.55	0.00	1.04
2	43	0.02	0.12	0.02	0.13	0.10	0.57	0.00	1.07
2	44	0.01	0.12	0.02	0.13	0.10	0.58	0.00	1.09
2	45	0.02	0.12	0.02	0.13	0.10	0.59	0.00	1.10
2	46	0.02	0.12	0.02	0.14	0.10	0.60	0.00	1.11
2	47	0.02	0.12	0.02	0.14	0.10	0.61	0.00	1.12
2	48	0.02	0.12	0.02	0.14	0.10	0.62	0.00	1.14
3	0	0.00	0.00	0.00	0.00	0.00		0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00		0.00	0.00
3	2	0.00	0.01	0.00	0.01	0.00		0.00	0.02
3	3	0.00	0.01	0.00	0.01	0.00		0.00	0.03
3	4	0.00	0.01	0.00	0.01	0.01		0.00	0.03
3	5	0.00	0.02	0.01	0.02	0.01		0.00	0.05
3	6	0.00	0.02	0.01	0.02	0.01		0.00	0.05
3	7	0.00	0.02	0.02	0.02	0.02		0.00	0.06
3	8	0.00	0.02	0.02	0.03	0.02		0.00	0.07
3	9	0.00	0.02	0.02	0.03	0.02		0.00	0.07
3	10	0.00	0.02	0.02	0.03	0.02		0.00	0.08
3	11	0.00	0.03	0.03	0.03	0.03		0.00	0.08
3	12	0.00	0.03	0.03	0.03	0.03		0.00	0.09
3	13	0.00	0.03	0.03	0.03	0.04		0.00	0.11
3	14	0.00	0.03	0.03	0.03	0.04		0.00	0.11
3	15	0.00	0.03	0.03	0.04	0.04		0.00	0.13
3	16	0.00	0.03	0.03	0.04	0.05		0.00	0.14
3	17	0.00	0.04	0.03	0.04	0.05		0.00	0.15
3	18	0.00	0.04	0.03	0.05	0.05		0.00	0.16
3	19	0.00	0.05	0.04	0.06	0.06		0.00	0.18
3	20	0.00	0.05	0.04	0.06	0.06		0.00	0.20

Table A2-13$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 12.5 % honeys under aerobic condition

Rep	Time (h)	A12.5	AC12.5	B12.5	BC12.5	C12.5	CC12.5	K12.5	KC12.5
3	21	0.00	0.06	0.04	0.07	0.07		0.00	0.22
3	22	0.00	0.07	0.04	0.08	0.07		0.00	0.24
3	23	0.00	0.08	0.04	0.08	0.07		0.00	0.27
3	24	0.00	0.08	0.05	0.09	0.08		0.00	0.31
3	25	0.00	0.10	0.05	0.10	0.09		0.00	0.34
3	26	0.00	0.10	0.05	0.10	0.09		0.00	0.37
3	27	0.01	0.11	0.06	0.11	0.09		0.00	0.41
3	28	0.01	0.12	0.06	0.11	0.10		0.00	0.44
3	29	0.01	0.12	0.06	0.11	0.10		0.00	0.48
3	30	0.01	0.13	0.06	0.12	0.10		0.00	0.51
3	31	0.01	0.13	0.06	0.13	0.10		0.00	0.55
3	32	0.01	0.13	0.06	0.12	0.10		0.00	0.59
3	33	0.01	0.13	0.06	0.12	0.10		0.00	0.63
3	34	0.01	0.13	0.06	0.12	0.10		0.00	0.67
3	35	0.00	0.13	0.06	0.13	0.10		0.00	0.71
3	36	0.00	0.14	0.06	0.12	0.10		0.00	0.74
3	37	0.00	0.14	0.06	0.12	0.10		0.00	0.77
3	38	0.00	0.14	0.06	0.12	0.11		0.00	0.81
3	39	0.00	0.14	0.06	0.12	0.11		0.00	0.84
3	40	0.00	0.14	0.06	0.12	0.11		0.00	0.87
3	41	0.00	0.14	0.07	0.12	0.11		0.00	0.90
3	42	0.00	0.14	0.06	0.12	0.11		0.00	0.93
3	43	0.00	0.15	0.07	0.12	0.11		0.00	0.95
3	44	0.00	0.15	0.07	0.12	0.12		0.00	0.97
3	45	0.00	0.15	0.07	0.13	0.12		0.00	0.99
3	46	0.00	0.15	0.07	0.13	0.12		0.00	1.00
3	47	0.00	0.15	0.07	0.13	0.12		0.00	1.01
3	48	0.00	0.15	0.07	0.13	0.12		0.00	1.02
4	0	0.00	0.00	0.00	0.00		0.00	0.00	0.00
4	1	0.00	0.00	0.00	0.00		0.00	0.00	0.00
4	2	0.00	0.00	0.00	0.00		0.00	0.00	0.01
4	3	0.00	0.01	0.00	0.01		0.01	0.00	0.02
4	4	0.00	0.01	0.01	0.01		0.01	0.00	0.02
4	5	0.00	0.02	0.01	0.02		0.02	0.00	0.03
4	6	0.00	0.02	0.01	0.02		0.02	0.00	0.04
4	7	0.00	0.02	0.02	0.02		0.02	0.00	0.06
4	8	0.00	0.02	0.02	0.03		0.03	0.00	0.07
4	9	0.00	0.03	0.02	0.03		0.03	0.00	0.08
4	10	0.00	0.03	0.02	0.03		0.03	0.00	0.10

Table A2-13$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 12.5 % honeys under aerobic condition

Rep	Time (h)	A12.5	AC12.5	B12.5	BC12.5	C12.5	CC12.5	K12.5	KC12.5
4	11	0.00	0.03	0.02	0.04		0.03	0.00	0.11
4	12	0.00	0.04	0.02	0.04		0.03	0.00	0.12
4	13	0.00	0.04	0.02	0.05		0.04	0.00	0.14
4	14	0.00	0.04	0.02	0.05		0.04	0.00	0.18
4	15	0.00	0.04	0.02	0.06		0.04	0.00	0.19
4	16	0.00	0.05	0.02	0.07		0.05	0.00	0.21
4	17	0.00	0.05	0.03	0.08		0.05	0.00	0.24
4	18	0.00	0.06	0.03	0.09		0.05	0.00	0.27
4	19	0.00	0.07	0.03	0.10		0.06	0.00	0.30
4	20	0.00	0.07	0.03	0.10		0.06	0.00	0.34
4	21	0.00	0.08	0.03	0.11		0.06	0.00	0.37
4	22	0.00	0.09	0.03	0.12		0.08	0.00	0.40
4	23	0.00	0.09	0.03	0.13		0.10	0.00	0.43
4	24	0.00	0.10	0.03	0.14		0.12	0.00	0.47
4	25	0.00	0.11	0.03	0.14		0.17	0.00	0.50
4	26	0.00	0.12	0.03	0.15		0.22	0.00	0.54
4	27	0.00	0.12	0.03	0.15		0.28	0.00	0.57
4	28	0.00	0.13	0.03	0.15		0.33	0.00	0.60
4	29	0.00	0.13	0.03	0.15		0.39	0.00	0.64
4	30	0.00	0.13	0.03	0.15		0.42	0.00	0.67
4	31	0.00	0.14	0.03	0.15		0.45	0.00	0.70
4	32	0.00	0.14	0.02	0.15		0.48	0.00	0.73
4	33	0.00	0.14	0.02	0.15		0.50	0.00	0.76
4	34	0.00	0.14	0.02	0.15		0.52	0.00	0.79
4	35	0.00	0.14	0.03	0.15		0.53	0.00	0.81
4	36	0.00	0.14	0.02	0.15		0.54	0.00	0.84
4	37	0.00	0.14	0.02	0.15		0.55	0.00	0.86
4	38	0.00	0.14	0.03	0.16		0.56	0.00	0.89
4	39	0.00	0.14	0.03	0.16		0.57	0.00	0.91
4	40	0.00	0.14	0.03	0.16		0.58	0.00	0.93
4	41	0.00	0.14	0.02	0.16		0.60	0.00	0.94
4	42	0.00	0.14	0.02	0.16		0.62	0.00	0.96
4	43	0.00	0.14	0.03	0.17		0.63	0.00	0.97
4	44	0.00	0.14	0.02	0.16		0.64	0.00	0.98
4	45	0.00	0.14	0.02	0.16		0.66	0.00	1.00
4	46	0.00	0.14	0.02	0.16		0.67	0.00	1.00
4	47	0.00	0.14	0.02	0.16		0.68	0.00	1.01
4	48	0.00	0.14	0.02	0.16		0.70	0.00	1.02

Table A2-13$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 12.5 % honeys under aerobic condition

Rep	Time (h)	A12.5	AC12.5	B12.5	BC12.5	C12.5	CC12.5	K12.5	KC12.5
5	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	2	0.00	0.00	-0.01	0.01	0.00	0.01	0.00	0.00
5	3	0.00	0.01	0.00	0.02	0.00	0.01	0.00	0.01
5	4	0.00	0.01	0.00	0.02	0.00	0.02	0.00	0.02
5	5	0.00	0.02	0.00	0.02	0.00	0.02	0.00	0.03
5	6	0.00	0.02	0.00	0.03	0.00	0.03	0.00	0.04
5	7	0.00	0.02	0.00	0.03	0.00	0.03	0.00	0.05
5	8	0.00	0.02	0.00	0.03	0.01	0.03	0.00	0.06
5	9	0.00	0.03	0.00	0.04	0.01	0.04	0.00	0.07
5	10	0.00	0.03	0.00	0.04	0.01	0.04	0.01	0.09
5	11	0.00	0.03	0.00	0.04	0.01	0.04	0.00	0.10
5	12	0.00	0.04	0.00	0.05	0.01	0.05	0.00	0.12
5	13	0.00	0.04	0.00	0.05	0.01	0.05	0.00	0.13
5	14	0.00	0.04	0.00	0.06	0.01	0.05	0.00	0.16
5	15	0.00	0.04	0.00	0.06	0.01	0.05	0.00	0.19
5	16	0.00	0.05	0.00	0.07	0.02	0.06	0.00	0.22
5	17	0.00	0.05	0.00	0.08	0.02	0.06	0.00	0.25
5	18	0.00	0.06	0.00	0.08	0.02	0.07	0.00	0.27
5	19	0.00	0.06	0.00	0.09	0.02	0.07	0.00	0.30
5	20	0.00	0.07	0.00	0.10	0.02	0.07	0.00	0.33
5	21	0.00	0.07	0.00	0.11	0.02	0.08	0.00	0.37
5	22	0.00	0.08	0.00	0.12	0.03	0.09	0.00	0.40
5	23	0.00	0.09	0.00	0.13	0.04	0.10	0.00	0.43
5	24	0.00	0.09	0.00	0.13	0.07	0.12	0.00	0.46
5	25	0.00	0.10	0.00	0.14	0.11	0.14	0.00	0.50
5	26	0.00	0.11	0.00	0.15	0.15	0.16	0.00	0.54
5	27	0.00	0.11	0.00	0.15	0.19	0.20	0.00	0.57
5	28	0.00	0.12	-0.01	0.16	0.24	0.24	0.00	0.61
5	29	0.00	0.12	-0.01	0.16	0.29	0.29	0.00	0.64
5	30	0.00	0.12	-0.01	0.17	0.33	0.33	0.00	0.68
5	31	0.00	0.13	-0.01	0.17	0.36	0.36	0.00	0.71
5	32	0.00	0.13	-0.01	0.17	0.39	0.39	0.00	0.74
5	33	0.00	0.14	-0.01	0.17	0.41	0.42	0.00	0.77
5	34	0.00	0.14	-0.01	0.18	0.44	0.45	0.00	0.80
5	35	0.00	0.14	-0.01	0.18	0.46	0.47	0.00	0.82
5	36	0.00	0.15	-0.01	0.18	0.48	0.49	0.00	0.84
5	37	0.00	0.15	-0.01	0.18	0.50	0.51	0.00	0.86
5	38	0.00	0.15	-0.01	0.18	0.53	0.53	0.00	0.89
5	39	0.00	0.15	-0.01	0.19	0.54	0.55	0.00	0.90

Table A2-13$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 12.5 % honeys under aerobic condition

Rep	Time (h)	A12.5	AC12.5	B12.5	BC12.5	C12.5	CC12.5	K12.5	KC12.5
5	40	0.00	0.16	-0.01	0.19	0.56	0.56	0.00	0.92
5	41	0.00	0.16	-0.01	0.19	0.57	0.58	0.00	0.93
5	42	0.00	0.16	-0.01	0.19	0.59	0.60	0.00	0.94
5	43	0.00	0.16	-0.01	0.19	0.60	0.62	0.00	0.94
5	44	0.00	0.16	-0.01	0.19	0.60	0.64	0.00	0.95
5	45	0.00	0.17	-0.01	0.19	0.61	0.66	0.00	0.96
5	46	0.00	0.17	-0.01	0.19	0.61	0.68	0.00	0.96
5	47	0.00	0.17	-0.01	0.19	0.62	0.71	0.00	0.96
5	48	0.00	0.17	-0.01	0.19	0.62	0.73	0.00	0.97
6	0	0.00		0.00	0.00			0.00	0.00
6	1	0.00		0.00	0.00			0.00	0.00
6	2	0.00		-0.01	0.01			0.00	0.00
6	3	0.00		0.00	0.01			0.00	0.01
6	4	0.00		0.00	0.02			0.00	0.02
6	5	0.00		0.00	0.02			0.00	0.04
6	6	0.00		0.00	0.02			0.00	0.05
6	7	0.01		0.00	0.03			0.00	0.06
6	8	0.01		0.00	0.03			0.00	0.08
6	9	0.01		0.00	0.03			0.00	0.09
6	10	0.01		0.01	0.03			0.00	0.10
6	11	0.02		0.01	0.04			0.00	0.12
6	12	0.02		0.01	0.04			0.02	0.13
6	13	0.02		0.01	0.04			0.01	0.15
6	14	0.02		0.01	0.05			0.00	0.17
6	15	0.02		0.01	0.05			0.00	0.19
6	16	0.02		0.01	0.06			0.00	0.22
6	17	0.02		0.01	0.07			0.00	0.24
6	18	0.03		0.01	0.07			0.00	0.27
6	19	0.03		0.02	0.08			0.00	0.30
6	20	0.03		0.02	0.09			0.00	0.33
6	21	0.03		0.02	0.10			0.00	0.37
6	22	0.04		0.02	0.11			0.00	0.40
6	23	0.04		0.02	0.12			0.00	0.43
6	24	0.04		0.02	0.12			0.00	0.47
6	25	0.04		0.02	0.13			0.00	0.51
6	26	0.05		0.02	0.14			0.00	0.55
6	27	0.05		0.02	0.15			0.00	0.59
6	28	0.05		0.02	0.15			0.00	0.62
6	29	0.05		0.02	0.15			0.00	0.65
6	30	0.05		0.02	0.15			0.00	0.67

Table A2-13$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 12.5 % honeys under aerobic condition

Rep	Time (h)	A12.5	AC12.5	B12.5	BC12.5	C12.5	CC12.5	K12.5	KC12.5
6	31	0.04		0.02	0.15			0.00	0.71
6	32	0.04		0.02	0.15			0.00	0.73
6	33	0.04		0.02	0.14			0.00	0.75
6	34	0.04		0.02	0.14			0.00	0.78
6	35	0.04		0.02	0.14			0.00	0.81
6	36	0.04		0.02	0.14			0.00	0.85
6	37	0.04		0.02	0.14			0.00	0.87
6	38	0.04		0.02	0.15			0.00	0.87
6	39	0.04		0.02	0.15			0.00	0.88
6	40	0.04		0.02	0.15			0.00	0.89
6	41	0.04		0.02	0.15			0.00	0.91
6	42	0.04		0.02	0.15			0.00	0.92
6	43	0.04		0.02	0.15			0.00	0.94
6	44	0.04		0.02	0.15			0.00	0.96
6	45	0.05		0.02	0.15			0.00	0.98
6	46	0.05		0.02	0.15			0.00	0.99
6	47	0.05		0.02	0.15			0.00	1.00
6	48	0.05		0.02	0.15			0.00	1.01

Notes: A12.5=12.5 % Manuka honey 20+ UMF; AC12.5=12.5 % Manuka honey 20+ UMF with catalase; B12.5=12.5 % Manuka honey 15+ UMF; BC12.5=12.5 % Manuka honey 15+ UMF with catalase; C12.5=12.5 % Manuka honey 10+ UMF; CC12.5=12.5 % Manuka honey 10+ UMF with catalase; K12.5=12.5 % Kanuka honey; KC12.5=12.5 % Kanuka honey with catalase

Table A2-14 Growth of *P. acnes* (Abs $_{595nm}$) in 12.5 % honeys under anaerobic condition

Rep	Time (h)	A12.5	AC12.5	B12.5	BC12.5	C12.5	CC12.5	K12.5	KC12.5
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.01	0.01	0.01	0.01	0.02	0.02	0.00	0.01
1	3	0.02	0.02	0.02	0.02	0.02	0.03	0.01	0.02
1	4	0.02	0.02	0.03	0.02	0.03	0.05	0.01	0.04
1	5	0.03	0.03	0.04	0.03	0.04	0.05	0.01	0.05
1	6	0.03	0.03	0.05	0.03	0.04	0.06	0.00	0.06
1	7	0.04	0.04	0.05	0.04	0.05	0.07	0.01	0.06
1	8	0.04	0.04	0.06	0.04	0.06	0.07	0.01	0.08
1	9	0.04	0.04	0.06	0.04	0.06	0.07	0.00	0.09
1	10	0.05	0.05	0.07	0.04	0.07	0.08	0.01	0.10
1	11	0.06	0.05	0.07	0.05	0.07	0.08	0.01	0.12
1	12	0.06	0.05	0.08	0.05	0.08	0.08	0.01	0.14
1	13	0.06	0.05	0.08	0.05	0.08	0.09	0.01	0.16
1	14	0.06	0.05	0.08	0.05	0.08	0.09	0.01	0.18
1	15	0.06	0.05	0.08	0.05	0.08	0.09	0.01	0.21
1	16	0.06	0.05	0.08	0.05	0.08	0.09	0.00	0.23
1	17	0.06	0.05	0.08	0.05	0.09	0.09	0.01	0.26
1	18	0.06	0.05	0.08	0.06	0.09	0.09	0.00	0.29
1	19	0.06	0.06	0.08	0.06	0.09	0.09	0.00	0.31
1	20	0.06	0.06	0.08	0.06	0.09	0.09	0.00	0.34
1	21	0.06	0.06	0.08	0.06	0.10	0.09	0.00	0.36
1	22	0.06	0.06	0.08	0.06	0.10	0.09	0.00	0.40
1	23	0.06	0.06	0.08	0.06	0.10	0.10	0.00	0.43
1	24	0.06	0.06	0.08	0.06	0.10	0.09	0.00	0.47
1	25	0.06	0.07	0.08	0.07	0.10	0.10	0.01	0.50
1	26	0.06	0.07	0.08	0.07	0.11	0.10	0.00	0.54
1	27	0.06	0.07	0.08	0.07	0.11	0.10	0.00	0.58
1	28	0.06	0.07	0.07	0.07	0.11	0.11	0.00	0.62
1	29	0.06	0.07	0.07	0.07	0.11	0.11	0.00	0.64
1	30	0.05	0.07	0.06	0.07	0.11	0.10	0.00	0.67
1	31	0.05	0.07	0.06	0.07	0.11	0.11	0.00	0.71
1	32	0.05	0.08	0.06	0.08	0.11	0.11	0.00	0.74
1	33	0.05	0.08	0.06	0.08	0.11	0.11	0.00	0.78
1	34	0.05	0.08	0.06	0.08	0.11	0.11	0.00	0.79
1	35	0.05	0.08	0.05	0.08	0.11	0.11	0.00	0.83
1	36	0.05	0.08	0.05	0.08	0.11	0.11	0.00	0.86
1	37	0.05	0.08	0.05	0.08	0.11	0.11	0.00	0.88
1	38	0.04	0.08	0.05	0.08	0.11	0.11	0.00	0.91
1	39	0.04	0.08	0.05	0.08	0.10	0.11	0.00	0.94

Table A2-14cont   Growth of *P. acnes* (Abs $_{595nm}$) in 12.5 % honeys under anaerobic condition

Rep	Time (h)	A12.5	AC12.5	B12.5	BC12.5	C12.5	CC12.5	K12.5	KC12.5
1	40	0.04	0.08	0.04	0.08	0.10	0.11	0.00	0.96
1	41	0.04	0.08	0.04	0.09	0.10	0.11	0.00	0.99
1	42	0.04	0.08	0.04	0.09	0.10	0.12	0.00	1.01
1	43	0.04	0.08	0.04	0.09	0.10	0.11	0.00	1.03
1	44	0.04	0.08	0.04	0.09	0.10	0.12	0.00	1.04
1	45	0.04	0.08	0.04	0.09	0.09	0.12	0.00	1.06
1	46	0.04	0.09	0.04	0.09	0.09	0.12	0.00	1.08
1	47	0.04	0.09	0.04	0.10	0.09	0.12	0.00	1.09
1	48	0.04	0.09	0.04	0.10	0.09	0.12	0.00	1.11
2	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.00	0.02	0.00	0.02	0.00	0.01	0.00	0.01
2	3	0.01	0.02	0.00	0.03	0.01	0.02	0.00	0.03
2	4	0.01	0.03	0.01	0.04	0.01	0.03	0.01	0.04
2	5	0.01	0.04	0.01	0.04	0.02	0.04	0.01	0.05
2	6	0.01	0.04	0.02	0.04	0.01	0.04	0.01	0.06
2	7	0.01	0.04	0.02	0.05	0.02	0.05	0.01	0.06
2	8	0.02	0.05	0.03	0.05	0.03	0.05	0.01	0.07
2	9	0.02	0.05	0.03	0.05	0.03	0.05	0.01	0.09
2	10	0.02	0.05	0.04	0.06	0.03	0.06	0.01	0.10
2	11	0.03	0.06	0.04	0.06	0.03	0.06	0.01	0.12
2	12	0.03	0.06	0.05	0.06	0.04	0.06	0.01	0.13
2	13	0.03	0.06	0.05	0.06	0.04	0.06	0.02	0.15
2	14	0.03	0.06	0.05	0.06	0.04	0.06	0.02	0.17
2	15	0.04	0.06	0.05	0.07	0.05	0.06	0.02	0.19
2	16	0.04	0.06	0.05	0.07	0.05	0.06	0.01	0.22
2	17	0.04	0.06	0.06	0.07	0.05	0.06	0.00	0.25
2	18	0.04	0.06	0.06	0.07	0.05	0.07	0.00	0.28
2	19	0.04	0.06	0.06	0.07	0.05	0.06	0.00	0.31
2	20	0.04	0.07	0.06	0.07	0.06	0.07	0.00	0.34
2	21	0.04	0.07	0.06	0.07	0.06	0.07	0.00	0.36
2	22	0.05	0.07	0.06	0.07	0.06	0.07	0.00	0.39
2	23	0.05	0.07	0.06	0.08	0.06	0.07	0.00	0.42
2	24	0.05	0.07	0.06	0.08	0.06	0.07	0.00	0.44
2	25	0.05	0.07	0.06	0.08	0.07	0.08	0.02	0.46
2	26	0.05	0.08	0.06	0.09	0.07	0.08	0.02	0.49
2	27	0.05	0.08	0.06	0.09	0.08	0.08	0.02	0.53
2	28	0.06	0.08	0.06	0.09	0.07	0.08	0.01	0.55
2	29	0.05	0.08	0.06	0.09	0.08	0.08	0.01	0.58
2	30	0.05	0.08	0.06	0.09	0.07	0.08	0.00	0.61

Table A2-14$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 12.5 % honeys under anaerobic condition

Rep	Time (h)	A12.5	AC12.5	B12.5	BC12.5	C12.5	CC12.5	K12.5	KC12.5
2	31	0.05	0.08	0.06	0.09	0.07	0.09	0.00	0.65
2	32	0.05	0.08	0.06	0.09	0.08	0.09	0.01	0.68
2	33	0.06	0.08	0.06	0.09	0.08	0.09	0.01	0.71
2	34	0.05	0.08	0.06	0.09	0.07	0.09	0.01	0.73
2	35	0.06	0.08	0.05	0.10	0.08	0.09	0.01	0.77
2	36	0.06	0.08	0.05	0.10	0.08	0.09	0.01	0.80
2	37	0.06	0.08	0.05	0.10	0.08	0.09	0.01	0.84
2	38	0.06	0.08	0.05	0.10	0.08	0.09	0.01	0.87
2	39	0.06	0.08	0.05	0.10	0.08	0.09	0.00	0.90
2	40	0.06	0.08	0.05	0.10	0.07	0.10	0.00	0.92
2	41	0.06	0.08	0.05	0.10	0.08	0.10	0.00	0.94
2	42	0.06	0.08	0.05	0.10	0.08	0.10	0.00	0.95
2	43	0.06	0.09	0.05	0.10	0.08	0.10	0.00	0.96
2	44	0.06	0.09	0.05	0.11	0.08	0.10	0.00	0.97
2	45	0.06	0.09	0.05	0.11	0.08	0.10	0.00	0.98
2	46	0.06	0.09	0.05	0.11	0.08	0.11	0.00	0.99
2	47	0.06	0.09	0.05	0.11	0.08	0.11	0.00	1.00
2	48	0.06	0.09	0.05	0.11	0.08	0.11	0.01	1.01
3	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.00	0.01	0.00	0.01	0.01	0.02	0.00	0.00
3	3	0.00	0.02	0.01	0.03	0.02	0.03	0.00	0.02
3	4	0.01	0.03	0.01	0.03	0.03	0.04	0.00	0.02
3	5	0.01	0.03	0.01	0.04	0.04	0.05	0.01	0.03
3	6	0.00	0.04	0.01	0.04	0.04	0.06	0.01	0.04
3	7	0.01	0.04	0.02	0.05	0.05	0.06	0.01	0.05
3	8	0.01	0.04	0.03	0.05	0.05	0.07	0.01	0.05
3	9	0.01	0.05	0.03	0.05	0.06	0.07	0.01	0.07
3	10	0.01	0.05	0.03	0.05	0.06	0.08	0.01	0.08
3	11	0.02	0.05	0.04	0.05	0.07	0.09	0.01	0.10
3	12	0.02	0.05	0.04	0.05	0.07	0.09	0.02	0.11
3	13	0.01	0.05	0.04	0.06	0.08	0.09	0.01	0.13
3	14	0.02	0.05	0.05	0.06	0.08	0.09	0.01	0.15
3	15	0.01	0.05	0.05	0.06	0.08	0.09	0.01	0.17
3	16	0.01	0.05	0.05	0.06	0.08	0.10	0.00	0.20
3	17	0.01	0.05	0.06	0.06	0.08	0.09	0.00	0.23
3	18	0.01	0.06	0.06	0.06	0.08	0.10	0.00	0.26
3	19	0.01	0.06	0.06	0.06	0.08	0.10	0.00	0.30
3	20	0.01	0.06	0.07	0.06	0.08	0.10	0.00	0.34

Table A2-14$_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in 12.5 % honeys under anaerobic condition

Rep	Time (h)	A12.5	AC12.5	B12.5	BC12.5	C12.5	CC12.5	K12.5	KC12.5
3	21	0.01	0.06	0.07	0.06	0.09	0.10	0.00	0.37
3	22	0.01	0.06	0.07	0.07	0.09	0.10	0.00	0.41
3	23	0.01	0.06	0.07	0.07	0.09	0.11	0.00	0.45
3	24	0.01	0.06	0.07	0.07	0.09	0.10	0.00	0.46
3	25	0.02	0.07	0.08	0.08	0.10	0.11	0.00	0.50
3	26	0.02	0.07	0.08	0.08	0.10	0.11	0.00	0.53
3	27	0.02	0.07	0.08	0.08	0.11	0.11	0.00	0.56
3	28	0.02	0.07	0.08	0.08	0.11	0.11	0.00	0.58
3	29	0.02	0.07	0.08	0.08	0.11	0.11	0.00	0.60
3	30	0.01	0.07	0.08	0.08	0.11	0.11	0.00	0.62
3	31	0.02	0.08	0.08	0.08	0.11	0.11	0.00	0.65
3	32	0.02	0.08	0.08	0.08	0.12	0.11	0.01	0.67
3	33	0.02	0.08	0.08	0.09	0.12	0.11	0.01	0.69
3	34	0.02	0.08	0.08	0.09	0.12	0.11	0.01	0.71
3	35	0.02	0.08	0.08	0.09	0.12	0.11	0.01	0.74
3	36	0.02	0.08	0.08	0.09	0.12	0.11	0.00	0.76
3	37	0.02	0.08	0.08	0.09	0.12	0.11	0.00	0.78
3	38	0.02	0.08	0.08	0.09	0.12	0.11	0.00	0.79
3	39	0.01	0.08	0.08	0.09	0.12	0.11	0.00	0.81
3	40	0.01	0.08	0.08	0.09	0.12	0.11	0.00	0.83
3	41	0.01	0.08	0.08	0.10	0.12	0.11	0.00	0.84
3	42	0.01	0.08	0.08	0.10	0.12	0.11	0.00	0.86
3	43	0.00	0.08	0.07	0.10	0.12	0.11	0.00	0.87
3	44	0.00	0.08	0.07	0.10	0.12	0.11	0.00	0.88
3	45	0.00	0.08	0.07	0.10	0.12	0.11	0.00	0.89
3	46	0.00	0.09	0.07	0.10	0.12	0.12	0.00	0.90
3	47	0.00	0.09	0.07	0.11	0.12	0.12	0.00	0.91
3	48	0.00	0.09	0.07	0.11	0.13	0.12	0.00	0.92

Notes: A12.5=12.5 % Manuka honey 20+ UMF; AC12.5=12.5 % Manuka honey 20+ UMF with catalase; B12.5=12.5 % Manuka honey 15+ UMF; BC12.5=12.5 % Manuka honey 15+ UMF with catalase; C12.5=12.5 % Manuka honey 10+ UMF; CC12.5=12.5 % Manuka honey 10+ UMF with catalase;K12.5=12.5 % Kanuka honey; KC12.5=12.5 % Kanuka honey with catalase

Table A2-15 Growth of *P. acnes* (Abs $_{595nm}$) in artificial honeys under aerobic condition

Rep	Time (h)	7.5 ar	7.5ar+c	10ar	10ar+c	12ar	12ar+c
1	0	0.00		0.00	0.00	0.00	
1	1	0.00		0.00	0.00	0.00	
1	2	0.02		0.02	0.02	0.01	
1	3	0.03		0.04	0.03	0.02	
1	4	0.05		0.06	0.03	0.04	
1	5	0.07		0.08	0.05	0.05	
1	6	0.09		0.10	0.06	0.07	
1	7	0.11		0.12	0.08	0.08	
1	8	0.13		0.15	0.09	0.10	
1	9	0.15		0.16	0.11	0.12	
1	10	0.17		0.18	0.12	0.14	
1	11	0.19		0.21	0.14	0.16	
1	12	0.21		0.23	0.16	0.17	
1	13	0.23		0.25	0.18	0.19	
1	14	0.25		0.27	0.20	0.21	
1	15	0.27		0.29	0.22	0.23	
1	16	0.29		0.32	0.23	0.25	
1	17	0.31		0.34	0.25	0.27	
1	18	0.32		0.34	0.27	0.28	
1	19	0.34		0.37	0.29	0.30	
1	20	0.35		0.39	0.31	0.31	
1	21	0.37		0.40	0.34	0.32	
1	22	0.38		0.44	0.37	0.33	
1	23	0.40		0.46	0.39	0.35	
1	24	0.42		0.48	0.43	0.36	
1	25	0.45		0.50	0.46	0.37	
1	26	0.49		0.53	0.50	0.37	
1	27	0.51		0.56	0.54	0.41	
1	28	0.52		0.58	0.57	0.40	
1	29	0.54		0.60	0.61	0.43	
1	30	0.56		0.62	0.64	0.44	
1	31	0.58		0.65	0.67	0.44	
1	32	0.60		0.67	0.70	0.47	
1	33	0.63		0.70	0.75	0.47	
1	34	0.65		0.71	0.79	0.48	
1	35	0.67		0.73	0.82	0.49	
1	36	0.69		0.75	0.86	0.50	
1	37	0.71		0.76	0.89	0.51	
1	38	0.74		0.78	0.92	0.52	
1	39	0.76		0.79	0.95	0.53	

Table A2-15 $_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in artificial honeys under aerobic condition

Rep	Time (h)	7.5 ar	7.5ar+c	10ar	10ar+c	12ar	12ar+c
1	40	0.78		0.81	0.98	0.54	
1	41	0.81		0.83	1.00	0.55	
1	42	0.83		0.86	1.03	0.56	
1	43	0.85		0.89	1.05	0.57	
1	44	0.87		0.91	1.07	0.59	
1	45	0.90		0.93	1.09	0.61	
1	46	0.92		0.94	1.11	0.62	
1	47	0.95		0.96	1.13	0.64	
1	48	0.97		0.98	1.14	0.66	
2	0	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.01	0.01	0.02	0.02	0.01	0.01
2	3	0.03	0.02	0.04	0.03	0.03	0.02
2	4	0.05	0.02	0.06	0.04	0.04	0.02
2	5	0.07	0.03	0.08	0.06	0.06	0.03
2	6	0.09	0.04	0.11	0.07	0.07	0.04
2	7	0.11	0.05	0.13	0.08	0.09	0.05
2	8	0.13	0.06	0.16	0.10	0.11	0.06
2	9	0.16	0.07	0.18	0.12	0.13	0.07
2	10	0.18	0.08	0.21	0.13	0.14	0.08
2	11	0.20	0.09	0.23	0.15	0.17	0.09
2	12	0.22	0.10	0.25	0.16	0.19	0.10
2	13	0.25	0.11	0.28	0.19	0.21	0.11
2	14	0.27	0.13	0.30	0.21	0.23	0.13
2	15	0.30	0.14	0.32	0.22	0.25	0.14
2	16	0.32	0.15	0.33	0.24	0.27	0.15
2	17	0.34	0.16	0.35	0.26	0.29	0.16
2	18	0.36	0.18	0.37	0.28	0.31	0.18
2	19	0.37	0.19	0.38	0.30	0.33	0.19
2	20	0.38	0.21	0.40	0.32	0.33	0.21
2	21	0.40	0.22	0.42	0.35	0.35	0.22
2	22	0.42	0.24	0.43	0.38	0.36	0.24
2	23	0.42	0.26	0.45	0.41	0.37	0.26
2	24	0.43	0.28	0.47	0.44	0.38	0.28
2	25	0.45	0.30	0.50	0.47	0.39	0.30
2	26	0.47	0.33	0.53	0.51	0.40	0.33
2	27	0.49	0.35	0.53	0.55	0.42	0.35
2	28	0.52	0.38	0.55	0.59	0.43	0.38
2	29	0.55	0.41	0.59	0.62	0.45	0.41
2	30	0.58	0.44	0.58	0.66	0.46	0.44

Table A2-15 $_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in artificial honeys under aerobic condition

Rep	Time (h)	7.5 ar	7.5ar+c	10ar	10ar+c	12ar	12ar+c
2	31	0.58	0.47	0.60	0.69	0.47	0.47
2	32	0.60	0.50	0.62	0.72	0.49	0.50
2	33	0.62	0.54	0.64	0.76	0.50	0.54
2	34	0.64	0.58	0.66	0.79	0.51	0.58
2	35	0.67	0.62	0.67	0.83	0.52	0.62
2	36	0.69	0.66	0.69	0.86	0.53	0.66
2	37	0.71	0.70	0.71	0.89	0.54	0.70
2	38	0.74	0.74	0.73	0.91	0.55	0.74
2	39	0.76	0.77	0.75	0.94	0.56	0.77
2	40	0.79	0.81	0.77	0.96	0.57	0.81
2	41	0.81	0.85	0.79	0.98	0.58	0.85
2	42	0.84	0.88	0.81	1.00	0.59	0.88
2	43	0.86	0.91	0.83	1.03	0.61	0.91
2	44	0.89	0.94	0.85	1.04	0.62	0.94
2	45	0.91	0.97	0.87	1.06	0.64	0.97
2	46	0.93	1.00	0.90	1.08	0.65	1.00
2	47	0.95	1.02	0.91	1.09	0.67	1.02
2	48	0.97	1.04	0.93	1.10	0.69	1.04
3	0	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.01	0.01	0.02	0.02	0.01	0.01
3	3	0.03	0.03	0.03	0.03	0.03	0.03
3	4	0.04	0.04	0.05	0.04	0.04	0.04
3	5	0.06	0.05	0.07	0.05	0.05	0.05
3	6	0.08	0.06	0.10	0.07	0.07	0.06
3	7	0.10	0.07	0.12	0.08	0.09	0.07
3	8	0.12	0.08	0.14	0.09	0.11	0.08
3	9	0.14	0.09	0.17	0.11	0.13	0.09
3	10	0.16	0.10	0.19	0.13	0.14	0.10
3	11	0.19	0.11	0.21	0.15	0.16	0.11
3	12	0.21	0.13	0.24	0.16	0.18	0.13
3	13	0.23	0.14	0.26	0.18	0.20	0.14
3	14	0.26	0.16	0.28	0.20	0.22	0.16
3	15	0.28	0.18	0.30	0.22	0.24	0.18
3	16	0.30	0.19	0.32	0.24	0.26	0.19
3	17	0.32	0.21	0.34	0.26	0.27	0.21
3	18	0.35	0.22	0.35	0.28	0.29	0.22
3	19	0.37	0.24	0.37	0.30	0.30	0.24
3	20	0.35	0.26	0.38	0.32	0.32	0.26

Table A2-15 $_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in artificial honeys under aerobic condition

Rep	Time (h)	7.5 ar	7.5ar+c	10ar	10ar+c	12ar	12ar+c
3	21	0.36	0.28	0.39	0.34	0.34	0.28
3	22	0.39	0.30	0.41	0.37	0.35	0.30
3	23	0.40	0.32	0.43	0.40	0.35	0.32
3	24	0.42	0.35	0.46	0.43	0.36	0.35
3	25	0.40	0.37	0.46	0.46	0.37	0.37
3	26	0.44	0.41	0.48	0.50	0.39	0.41
3	27	0.46	0.44	0.51	0.54	0.40	0.44
3	28	0.47	0.47	0.53	0.57	0.41	0.47
3	29	0.48	0.51	0.55	0.61	0.43	0.51
3	30	0.50	0.54	0.57	0.64	0.44	0.54
3	31	0.54	0.56	0.60	0.68	0.45	0.56
3	32	0.54	0.59	0.62	0.71	0.46	0.59
3	33	0.57	0.62	0.63	0.74	0.47	0.62
3	34	0.59	0.66	0.64	0.78	0.49	0.66
3	35	0.61	0.69	0.65	0.81	0.50	0.69
3	36	0.63	0.74	0.67	0.86	0.51	0.74
3	37	0.64	0.77	0.68	0.89	0.52	0.77
3	38	0.66	0.81	0.69	0.91	0.53	0.81
3	39	0.68	0.85	0.71	0.94	0.54	0.85
3	40	0.70	0.88	0.72	0.97	0.55	0.88
3	41	0.72	0.91	0.74	0.99	0.56	0.91
3	42	0.74	0.95	0.76	1.01	0.58	0.95
3	43	0.75	0.98	0.79	1.03	0.59	0.98
3	44	0.77	1.01	0.81	1.05	0.61	1.01
3	45	0.78	1.03	0.83	1.07	0.63	1.03
3	46	0.81	1.05	0.85	1.08	0.64	1.05
3	47	0.82	1.07	0.87	1.10	0.66	1.07
3	48	0.84	1.10	0.89	1.11	0.68	1.10
4	0	0.00	0.00	0.00	0.00	0.00	0.00
4	1	0.00	0.00	0.00	0.00	0.00	0.00
4	2	0.02	0.02	0.02	0.02	0.01	0.02
4	3	0.04	0.04	0.04	0.04	0.03	0.04
4	4	0.06	0.06	0.07	0.07	0.05	0.06
4	5	0.08	0.07	0.09	0.08	0.06	0.07
4	6	0.11	0.09	0.11	0.11	0.08	0.09
4	7	0.13	0.11	0.13	0.13	0.10	0.11
4	8	0.16	0.13	0.15	0.16	0.13	0.13
4	9	0.18	0.15	0.17	0.19	0.15	0.15
4	10	0.21	0.17	0.19	0.21	0.17	0.17

Table A2-15 cont Growth of *P. acnes* (Abs $_{595nm}$) in artificial honeys under aerobic condition

Rep	Time (h)	7.5 ar	7.5ar+c	10ar	10ar+c	12ar	12ar+c
4	11	0.23	0.19	0.21	0.24	0.19	0.19
4	12	0.26	0.21	0.23	0.26	0.21	0.21
4	13	0.28	0.23	0.25	0.28	0.23	0.23
4	14	0.30	0.24	0.28	0.31	0.25	0.24
4	15	0.32	0.26	0.30	0.33	0.27	0.26
4	16	0.34	0.28	0.32	0.36	0.28	0.28
4	17	0.35	0.31	0.35	0.39	0.31	0.31
4	18	0.37	0.33	0.36	0.42	0.31	0.33
4	19	0.39	0.35	0.39	0.45	0.32	0.35
4	20	0.39	0.38	0.41	0.49	0.33	0.38
4	21	0.41	0.40	0.45	0.52	0.33	0.40
4	22	0.43	0.43	0.45	0.55	0.34	0.43
4	23	0.44	0.45	0.46	0.59	0.35	0.45
4	24	0.46	0.48	0.50	0.62	0.36	0.48
4	25	0.48	0.52	0.53	0.66	0.37	0.52
4	26	0.49	0.55	0.56	0.70	0.38	0.55
4	27	0.54	0.59	0.58	0.74	0.39	0.59
4	28	0.53	0.62	0.60	0.78	0.40	0.62
4	29	0.56	0.67	0.62	0.82	0.41	0.67
4	30	0.57	0.71	0.63	0.86	0.42	0.71
4	31	0.59	0.75	0.65	0.91	0.43	0.75
4	32	0.62	0.79	0.67	0.95	0.44	0.79
4	33	0.63	0.83	0.69	0.98	0.45	0.83
4	34	0.64	0.87	0.70	1.01	0.46	0.87
4	35	0.66	0.91	0.72	1.04	0.47	0.91
4	36	0.67	0.94	0.73	1.07	0.48	0.94
4	37	0.68	0.97	0.75	1.09	0.50	0.97
4	38	0.70	1.01	0.77	1.11	0.51	1.01
4	39	0.72	1.03	0.79	1.13	0.52	1.03
4	40	0.74	1.06	0.81	1.14	0.54	1.06
4	41	0.76	1.08	0.84	1.16	0.55	1.08
4	42	0.78	1.10	0.86	1.17	0.57	1.10
4	43	0.80	1.13	0.89	1.18	0.59	1.13
4	44	0.82	1.15	0.92	1.19	0.61	1.15
4	45	0.83	1.16	0.95	1.20	0.63	1.16
4	46	0.85	1.18	0.97	1.21	0.65	1.18
4	47	0.87	1.20	0.99	1.22	0.67	1.20
4	48	0.89	1.21	1.02	1.22	0.70	1.21

Table A2-15 $_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in artificial honeys under aerobic condition

Rep	Time (h)	7.5 ar	7.5ar+c	10ar	10ar+c	12ar	12ar+c
5	0	0.00	0.00	0.00	0.00	0.00	0.00
5	1	0.00	0.00	0.00	0.00	0.00	0.00
5	2	0.02	0.01	0.02	0.03	0.01	0.01
5	3	0.04	0.03	0.04	0.04	0.03	0.03
5	4	0.06	0.04	0.07	0.07	0.05	0.04
5	5	0.08	0.06	0.09	0.10	0.07	0.06
5	6	0.10	0.07	0.11	0.12	0.09	0.07
5	7	0.12	0.09	0.13	0.15	0.11	0.09
5	8	0.14	0.11	0.16	0.17	0.13	0.11
5	9	0.16	0.13	0.18	0.19	0.15	0.13
5	10	0.19	0.15	0.21	0.22	0.17	0.15
5	11	0.21	0.17	0.24	0.25	0.19	0.17
5	12	0.23	0.19	0.26	0.27	0.22	0.19
5	13	0.25	0.20	0.28	0.29	0.24	0.20
5	14	0.27	0.22	0.31	0.31	0.26	0.22
5	15	0.31	0.23	0.33	0.33	0.28	0.23
5	16	0.32	0.26	0.35	0.36	0.29	0.26
5	17	0.34	0.28	0.37	0.39	0.31	0.28
5	18	0.34	0.30	0.39	0.42	0.31	0.30
5	19	0.37	0.32	0.39	0.45	0.33	0.32
5	20	0.36	0.35	0.41	0.49	0.33	0.35
5	21	0.37	0.37	0.45	0.53	0.34	0.37
5	22	0.41	0.40	0.44	0.57	0.35	0.40
5	23	0.41	0.43	0.47	0.62	0.36	0.43
5	24	0.44	0.46	0.49	0.67	0.37	0.46
5	25	0.43	0.50	0.53	0.73	0.38	0.50
5	26	0.46	0.53	0.53	0.79	0.39	0.53
5	27	0.48	0.57	0.57	0.86	0.40	0.57
5	28	0.50	0.60	0.58	0.91	0.41	0.60
5	29	0.52	0.64	0.62	0.95	0.42	0.64
5	30	0.54	0.68	0.64	0.98	0.43	0.68
5	31	0.56	0.72	0.63	1.01	0.44	0.72
5	32	0.58	0.76	0.65	1.03	0.44	0.76
5	33	0.60	0.80	0.66	1.05	0.46	0.80
5	34	0.61	0.84	0.67	1.06	0.47	0.84
5	35	0.63	0.88	0.69	1.07	0.48	0.88
5	36	0.66	0.91	0.70	1.08	0.49	0.91
5	37	0.67	0.95	0.71	1.08	0.50	0.95
5	38	0.69	0.98	0.73	1.09	0.51	0.98
5	39	0.71	1.01	0.75	1.09	0.53	1.01

Table A2-15 cont Growth of *P. acnes* (Abs $_{595nm}$) in artificial honeys under aerobic condition

Rep	Time (h)	7.5 ar	7.5ar+c	10ar	10ar+c	12ar	12ar+c
5	40	0.73	1.04	0.77	1.09	0.54	1.04
5	41	0.75	1.06	0.79	1.09	0.56	1.06
5	42	0.76	1.08	0.80	1.09	0.58	1.08
5	43	0.78	1.11	0.82	1.09	0.59	1.11
5	44	0.80	1.13	0.84	1.09	0.61	1.13
5	45	0.82	1.15	0.86	1.09	0.63	1.15
5	46	0.84	1.16	0.88	1.09	0.66	1.16
5	47	0.85	1.18	0.90	1.09	0.68	1.18
5	48	0.87	1.19	0.91	1.09	0.70	1.19
6	0	0.00	0.00	0.00	0.00	0.00	0.00
6	1	0.00	0.00	0.00	0.00	0.00	0.00
6	2	0.02	0.03	0.02	0.02	0.02	0.03
6	3	0.04	0.04	0.04	0.04	0.03	0.04
6	4	0.06	0.06	0.06	0.07	0.05	0.06
6	5	0.08	0.09	0.08	0.09	0.07	0.09
6	6	0.10	0.11	0.10	0.12	0.09	0.11
6	7	0.13	0.13	0.12	0.14	0.11	0.13
6	8	0.15	0.15	0.15	0.17	0.13	0.15
6	9	0.17	0.17	0.17	0.19	0.15	0.17
6	10	0.20	0.20	0.19	0.22	0.17	0.20
6	11	0.22	0.22	0.21	0.24	0.20	0.22
6	12	0.25	0.24	0.24	0.26	0.22	0.24
6	13	0.27	0.26	0.26	0.28	0.25	0.26
6	14	0.29	0.27	0.28	0.30	0.26	0.27
6	15	0.30	0.30	0.30	0.33	0.27	0.30
6	16	0.32	0.32	0.31	0.35	0.29	0.32
6	17	0.35	0.34	0.33	0.38	0.31	0.34
6	18	0.35	0.37	0.36	0.41	0.32	0.37
6	19	0.35	0.40	0.36	0.45	0.32	0.40
6	20	0.37	0.43	0.38	0.48	0.33	0.43
6	21	0.37	0.45	0.39	0.51	0.33	0.45
6	22	0.38	0.48	0.42	0.55	0.35	0.48
6	23	0.40	0.51	0.45	0.58	0.35	0.51
6	24	0.41	0.55	0.44	0.62	0.36	0.55
6	25	0.42	0.59	0.46	0.66	0.37	0.59
6	26	0.44	0.62	0.48	0.70	0.38	0.62
6	27	0.45	0.65	0.50	0.74	0.39	0.65
6	28	0.46	0.68	0.53	0.78	0.40	0.68
6	29	0.47	0.72	0.55	0.82	0.41	0.72

Table A2-15 $_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in artificial honeys under aerobic condition

Rep	Time (h)	7.5 ar	7.5ar+c	10ar	10ar+c	12ar	12ar+c
6	30	0.48	0.76	0.58	0.87	0.42	0.76
6	31	0.50	0.80	0.59	0.90	0.43	0.80
6	32	0.51	0.84	0.62	0.94	0.43	0.84
6	33	0.52	0.88	0.62	0.98	0.44	0.88
6	34	0.54	0.92	0.64	1.01	0.45	0.92
6	35	0.55	0.95	0.65	1.04	0.46	0.95
6	36	0.58	0.98	0.65	1.06	0.48	0.98
6	37	0.60	1.01	0.68	1.08	0.49	1.01
6	38	0.63	1.04	0.70	1.10	0.50	1.04
6	39	0.66	1.06	0.71	1.12	0.52	1.06
6	40	0.69	1.08	0.73	1.13	0.54	1.08
6	41	0.71	1.10	0.75	1.15	0.55	1.10
6	42	0.73	1.12	0.77	1.16	0.57	1.12
6	43	0.76	1.14	0.78	1.17	0.60	1.14
6	44	0.78	1.15	0.80	1.18	0.61	1.15
6	45	0.82	1.16	0.82	1.19	0.64	1.16
6	46	0.85	1.17	0.84	1.20	0.67	1.17
6	47	0.87	1.19	0.86	1.20	0.69	1.19
6	48	0.89	1.20	0.88	1.21	0.72	1.20

Notes: 7.5 ar=7.5 % artificial honey; 7.5 ar+c =7.5 % artificial honey with catalase; 10 ar= 10 % artificial honey;10 ar+c =10 % artificial honey with catalase; 12.5 ar= 12.5 % artificial honey;12.5 ar+c =12.5 % artificial honey with catalase

Table A2-16 Growth of *P. acnes* (Abs $_{595nm}$) in artificial honeys under anaerobic condition

Rep	Time (h)	7.5 ar	7.5ar+c	10ar	10ar+c	12ar	12ar+c
1	0	0.00	0.00	0.00	0.00	0.00	0.00
1	1	0.00	0.00	0.00	0.00	0.00	0.00
1	2	0.02	0.03	0.02	0.02	0.02	0.02
1	3	0.04	0.06	0.04	0.04	0.03	0.04
1	4	0.05	0.08	0.07	0.07	0.05	0.06
1	5	0.07	0.12	0.08	0.08	0.07	0.07
1	6	0.09	0.15	0.11	0.11	0.09	0.09
1	7	0.11	0.18	0.14	0.14	0.11	0.11
1	8	0.14	0.22	0.17	0.17	0.14	0.13
1	9	0.16	0.25	0.21	0.21	0.16	0.16
1	10	0.19	0.29	0.24	0.24	0.18	0.17
1	11	0.22	0.32	0.27	0.27	0.20	0.20
1	12	0.26	0.35	0.29	0.29	0.22	0.22
1	13	0.30	0.38	0.33	0.33	0.24	0.24
1	14	0.32	0.41	0.36	0.36	0.25	0.26
1	15	0.32	0.44	0.39	0.39	0.27	0.29
1	16	0.34	0.47	0.41	0.41	0.29	0.31
1	17	0.37	0.50	0.44	0.44	0.30	0.33
1	18	0.40	0.53	0.47	0.47	0.32	0.35
1	19	0.43	0.56	0.49	0.49	0.33	0.38
1	20	0.45	0.59	0.53	0.53	0.35	0.40
1	21	0.46	0.62	0.57	0.57	0.36	0.42
1	22	0.48	0.65	0.61	0.61	0.38	0.45
1	23	0.50	0.69	0.65	0.65	0.39	0.48
1	24	0.52	0.73	0.69	0.69	0.41	0.51
1	25	0.54	0.76	0.71	0.71	0.42	0.54
1	26	0.56	0.80	0.74	0.74	0.42	0.57
1	27	0.58	0.84	0.76	0.76	0.43	0.59
1	28	0.60	0.87	0.78	0.78	0.44	0.61
1	29	0.62	0.91	0.80	0.80	0.45	0.63
1	30	0.65	0.94	0.81	0.81	0.45	0.65
1	31	0.67	0.97	0.83	0.83	0.46	0.67
1	32	0.70	1.00	0.84	0.84	0.47	0.68
1	33	0.72	1.03	0.86	0.86	0.47	0.70
1	34	0.74	1.05	0.87	0.87	0.48	0.72
1	35	0.77	1.08	0.89	0.89	0.49	0.73
1	36	0.79	1.10	0.90	0.90	0.50	0.75
1	37	0.81	1.11	0.92	0.92	0.52	0.77
1	38	0.84	1.13	0.93	0.93	0.54	0.78
1	39	0.86	1.15	0.94	0.94	0.56	0.80

Table A2-16 $_{cont}$ Growth of *P. acnes* (Abs $_{595nm}$) in artificial honeys under anaerobic condition

Rep	Time (h)	7.5 ar	7.5ar+c	10ar	10ar+c	12ar	12ar+c
1	40	0.88	1.17	0.95	0.95	0.58	0.82
1	41	0.90	1.18	0.97	0.97	0.60	0.84
1	42	0.92	1.20	0.98	0.98	0.62	0.85
1	43	0.94	1.21	0.98	0.98	0.65	0.87
1	44	0.95	1.22	0.99	0.99	0.67	0.88
1	45	0.97	1.23	1.01	1.01	0.69	0.90
1	46	0.98	1.24	1.02	1.02	0.71	0.92
1	47	1.01	1.25	1.03	1.03	0.73	0.93
1	48	1.03	1.26	1.04	1.04	0.75	0.95
2	0	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.00	0.00	0.00	0.00	0.00	0.00
2	2	0.01	0.03	0.03	0.03	0.02	0.03
2	3	0.03	0.06	0.05	0.05	0.04	0.04
2	4	0.05	0.10	0.07	0.07	0.05	0.06
2	5	0.06	0.14	0.09	0.09	0.07	0.08
2	6	0.08	0.17	0.11	0.11	0.09	0.09
2	7	0.10	0.20	0.14	0.14	0.12	0.12
2	8	0.11	0.24	0.17	0.17	0.14	0.14
2	9	0.13	0.27	0.20	0.20	0.16	0.16
2	10	0.15	0.30	0.23	0.23	0.18	0.19
2	11	0.18	0.34	0.26	0.26	0.20	0.22
2	12	0.21	0.37	0.29	0.29	0.22	0.24
2	13	0.24	0.40	0.31	0.31	0.23	0.26
2	14	0.26	0.45	0.34	0.34	0.25	0.29
2	15	0.28	0.54	0.37	0.37	0.26	0.31
2	16	0.31	0.64	0.40	0.40	0.28	0.33
2	17	0.34	0.74	0.42	0.42	0.30	0.36
2	18	0.35	0.83	0.45	0.45	0.31	0.38
2	19	0.40	0.90	0.47	0.47	0.33	0.40
2	20	0.41	0.94	0.50	0.50	0.34	0.43
2	21	0.42	0.97	0.55	0.55	0.35	0.46
2	22	0.43	0.98	0.58	0.58	0.37	0.49
2	23	0.44	0.99	0.62	0.62	0.38	0.52
2	24	0.45	1.00	0.65	0.65	0.40	0.56
2	25	0.46	1.00	0.68	0.68	0.41	0.59
2	26	0.47	1.01	0.70	0.70	0.41	0.62
2	27	0.48	1.01	0.72	0.72	0.42	0.64
2	28	0.49	1.01	0.74	0.74	0.43	0.66
2	29	0.51	1.01	0.76	0.76	0.44	0.68
2	30	0.52	1.01	0.77	0.77	0.44	0.69

Table A2-16 cont Growth of *P. acnes* (Abs $_{595nm}$) in artificial honeys under anaerobic condition

Rep	Time (h)	7.5 ar	7.5ar+c	10ar	10ar+c	12ar	12ar+c
2	31	0.54	1.01	0.79	0.79	0.45	0.71
2	32	0.56	1.01	0.80	0.80	0.45	0.72
2	33	0.57	1.01	0.82	0.82	0.46	0.74
2	34	0.59	1.01	0.83	0.83	0.47	0.75
2	35	0.61	1.01	0.85	0.85	0.48	0.77
2	36	0.63	1.01	0.86	0.86	0.49	0.78
2	37	0.64	1.01	0.87	0.87	0.51	0.80
2	38	0.66	1.01	0.89	0.89	0.53	0.81
2	39	0.68	1.01	0.90	0.90	0.55	0.83
2	40	0.70	1.01	0.91	0.91	0.56	0.84
2	41	0.71	1.01	0.92	0.92	0.59	0.86
2	42	0.73	1.01	0.93	0.93	0.61	0.87
2	43	0.74	1.00	0.94	0.94	0.63	0.88
2	44	0.76	1.00	0.95	0.95	0.66	0.90
2	45	0.78	1.00	0.96	0.96	0.68	0.91
2	46	0.81	1.00	0.97	0.97	0.70	0.92
2	47	0.83	0.99	0.98	0.98	0.72	0.93
2	48	0.86	0.99	0.98	0.98	0.75	0.95
3	0	0.00	0.00	0.00	0.00	0.00	0.00
3	1	0.00	0.00	0.00	0.00	0.00	0.00
3	2	0.02	0.03	0.02	0.02	0.02	0.02
3	3	0.03	0.06	0.04	0.04	0.03	0.04
3	4	0.05	0.08	0.06	0.06	0.05	0.05
3	5	0.07	0.11	0.08	0.08	0.07	0.07
3	6	0.08	0.14	0.11	0.11	0.09	0.09
3	7	0.10	0.17	0.14	0.14	0.11	0.11
3	8	0.12	0.20	0.17	0.17	0.13	0.14
3	9	0.14	0.24	0.20	0.20	0.15	0.16
3	10	0.16	0.27	0.24	0.24	0.18	0.19
3	11	0.19	0.31	0.26	0.26	0.20	0.22
3	12	0.22	0.34	0.29	0.29	0.22	0.24
3	13	0.25	0.37	0.32	0.32	0.23	0.27
3	14	0.27	0.40	0.35	0.35	0.25	0.30
3	15	0.30	0.43	0.38	0.38	0.27	0.32
3	16	0.33	0.45	0.41	0.41	0.28	0.35
3	17	0.37	0.48	0.43	0.43	0.30	0.37
3	18	0.38	0.51	0.46	0.46	0.32	0.40
3	19	0.41	0.54	0.48	0.48	0.33	0.41
3	20	0.43	0.57	0.51	0.51	0.35	0.44

Table A2-16 cont Growth of *P. acnes* (Abs $_{595nm}$) in artificial honeys under anaerobic condition

Rep	Time (h)	7.5 ar	7.5ar+c	10ar	10ar+c	12ar	12ar+c
3	21	0.50	0.61	0.55	0.55	0.36	0.47
3	22	0.51	0.64	0.59	0.59	0.38	0.50
3	23	0.51	0.68	0.63	0.63	0.39	0.53
3	24	0.52	0.71	0.67	0.67	0.40	0.56
3	25	0.52	0.75	0.70	0.70	0.41	0.59
3	26	0.53	0.79	0.73	0.73	0.42	0.61
3	27	0.54	0.82	0.75	0.75	0.43	0.64
3	28	0.56	0.85	0.77	0.77	0.44	0.66
3	29	0.57	0.89	0.79	0.79	0.45	0.67
3	30	0.59	0.92	0.80	0.80	0.45	0.68
3	31	0.60	0.95	0.81	0.81	0.46	0.70
3	32	0.63	0.98	0.83	0.83	0.46	0.72
3	33	0.65	1.01	0.85	0.85	0.47	0.73
3	34	0.67	1.03	0.86	0.86	0.48	0.75
3	35	0.69	1.05	0.87	0.87	0.49	0.77
3	36	0.71	1.07	0.89	0.89	0.51	0.79
3	37	0.73	1.09	0.90	0.90	0.52	0.80
3	38	0.75	1.11	0.91	0.91	0.54	0.82
3	39	0.78	1.13	0.93	0.93	0.56	0.83
3	40	0.80	1.14	0.94	0.94	0.58	0.84
3	41	0.82	1.16	0.95	0.95	0.61	0.86
3	42	0.84	1.17	0.96	0.96	0.63	0.88
3	43	0.86	1.18	0.97	0.97	0.65	0.89
3	44	0.88	1.19	0.99	0.99	0.67	0.90
3	45	0.90	1.20	0.99	0.99	0.69	0.92
3	46	0.92	1.21	1.00	1.00	0.72	0.93
3	47	0.93	1.23	1.01	1.01	0.74	0.94
3	48	0.95	1.24	1.03	1.03	0.76	0.96

Notes: 7.5 ar=7.5 % artificial honey; 7.5 ar+c =7.5 % artificial honey with catalase; 10 ar= 10 % artificial honey;10 ar+c =10 % artificial honey with catalase; 12.5 ar= 12.5 % artificial honey;12.5 ar+c =12.5 % artificial honey with catalase

A.3 The growth of *P. acnes* in Bioactives (Abs $_{595nm}$)

Table A3-1 Growth of *P. acnes* (Abs $_{595nm}$) in propolis solution

Rep	Propolis (mg/mL)				
	0.08	0.15	0.30	0.60	1.20
1	0.73	0.53	0.36	0.39	0.33
2	0.75	0.65	0.41	0.37	0.29
3	0.60	0.58	0.40	0.32	0.27
4	0.68	0.56	0.34	0.39	0.25
5	0.78	0.46	0.40	0.32	0.17
6	0.71	0.46	0.31	0.38	0.25
Average	0.71	0.54	0.37	0.36	0.26
SEM	0.06	0.07	0.03	0.03	0.05

Table A3-2 Growth of *P. acnes* (Abs $_{595nm}$) in control ofpropolis solution

Rep	Control of propolis (mg/mL)				
	0.63	1.25	2.50	5.00	10.00
1	0.63	0.68	0.63	0.48	0.34
2	0.63	0.68	0.63	0.48	0.34
3	0.62	0.66	0.66	0.53	0.12
4	0.62	0.66	0.66	0.53	0.12
Average	0.63	0.67	0.64	0.50	0.23
SEM	0.00	0.01	0.02	0.02	0.11

Table A3-3 Growth of *P. acnes* (Abs $_{595nm}$) in olive leave extracts solution

Rep	Olive leaves extracts (mg/mL)				
	15	30	60	120	240
1	0.42	0.25	0.26	0.16	-0.08
2	0.43	0.25	0.26	0.18	-0.09
3	0.38	0.36	0.17	0.09	-0.04
4	0.36	0.31	0.17	0.09	-0.03
Average	0.40	0.29	0.21	0.13	-0.06
SEM	0.03	0.04	0.04	0.04	0.03

Table A3-4 Growth of *P. acnes* (Abs $_{595nm}$) in Manuka tree oil solution

Rep	Manuka tree oil (mg/mL)				
	0.625	1.25	2.5	5	10
1	0.07	-0.04	-0.11	-0.19	-0.47
2	0.06	-0.03	-0.09	-0.16	-0.41
3	0.21	0.12	0.07	0.12	0.09
4	0.29	0.09	0.07	0.12	0.11
Average	0.16	0.03	-0.01	-0.03	-0.17
SEM	0.11	0.08	0.10	0.17	0.31

Table A3-5 Growth of *P. acnes* (Abs $_{595nm}$) in DMSO solution

Rep	DMSO (ug/mL)				
	0.03125	0.625	1.25	2.5	5
1	0.51	0.53	0.53	0.47	0.32
2	0.55	0.48	0.48	0.46	0.41
3	0.50	0.52	0.53	0.47	0.32
4	0.44	0.52	0.48	0.46	0.41
Average	0.50	0.51	0.51	0.46	0.36
SEM	0.04	0.02	0.02	0.01	0.04

Table A3-6 Growth of *P. acnes* (Abs $_{595nm}$) in Lavender oil solution

Rep	Lavender oil (mg/mL)				
	2.75	5.5	11	22.13	44.25
1	0.45	0.38	0.30	0.09	0.02
2	0.43	0.40	0.32	0.14	0.01
3	0.45	0.42	0.28	0.29	0.16
4	0.50	0.38	0.29	0.14	0.16
Average	0.46	0.39	0.30	0.17	0.09
SEM	0.03	0.02	0.01	0.07	0.07

A.4 The zone of inhibition (Diameter=mm)

Table A4 Zone of inhibitions measured in mm in diameter

Samples	Replications							
	1	2	3	4	5	6	7	8
MTO100	26.8	22.7	16	19.4				
MTO50	11	8.5	13.8	16.6				
MTO25	ND	ND	ND	ND				
GTE100	19.4	16.6	17.7	16.6	18	16.4	17.6	17.7
GTE50	15.3	14	11.4	10.8	11	11.7	15	14.3
GTE25	10	8.05	9	10	9			
p100	ND	ND	ND	ND	ND	ND	ND	ND
p50	ND	ND	ND	ND	ND	ND	ND	ND
p25	ND	ND	ND	ND	ND	ND	ND	ND
LO100	ND	ND	ND	ND	ND	ND	ND	ND
LO50	ND	ND	ND	ND	ND	ND	ND	ND
LO25	ND	ND	ND	ND	ND	ND	ND	ND
OLE100	ND	ND	ND	ND	ND	ND	ND	ND
OLE50	ND	ND	ND	ND	ND	ND	ND	ND
OLE25	ND	ND	ND	ND	ND	ND	ND	ND

Note: ND mean not defined

A.5 Viable cell counts in different bioactive solution (CFU/mL) throughout the incubation period

Table A5 Viable cell counts in different bioactive solution (CFU/mL) at different times

Incubation (hr)	Replication	Viable cell counts in the testing samples (CFU/mL)					
		Honey	GTE	MTO	H+GTE	H+MTO	P.acnes
t=0	1	<100	<100	<100	<100	<100	$5X10^{8}$
	2	<100	<100	<100	<100	<100	$5X10^{8}$
	3	<100	<100	<100	<100	<100	
	Average						$5X10^{8}$
	SEM						$2X10^{7}$
t=6	1	$1 X10^{8}$	$8 X10^{5}$	$8 X10^{7}$	$1 X10^{5}$	$3 X10^{7}$	$5 X10^{8}$
	2	$1 X10^{8}$	$7 X10^{5}$	$6 X10^{7}$			$5 X10^{8}$
	3						
	average	$1 X10^{8}$	$7 X10^{5}$	$7 X10^{7}$	$1 X10^{5}$	$3 X10^{7}$	$5 X10^{8}$
	SEM	$3 X10^{6}$	$7 X10^{4}$	$1 X10^{7}$	<100	<100	$2 X10^{7}$
t=24	1	$6 X10^{8}$	<100	$2 X10^{6}$	$5 X10^{2}$	$6 X10^{3}$	$1 X10^{9}$
	2	$6 X10^{8}$	<100	$1 X10^{6}$	$7 X10^{2}$	$5 X10^{3}$	$9 X10^{9}$
	3	$5 X10^{8}$		$1 X10^{6}$			$2 X10^{9}$
	4	$2 X10^{8}$					
	average	$5 X10^{8}$	<100	$1 X10^{6}$	$6 X10^{2}$	$6 X10^{3}$	$4 X10^{9}$
	SEM	$2 X10^{8}$	<100	$5 X10^{5}$	$1 X10^{2}$	$5 X10^{2}$	$4 X10^{9}$
t=48	1	$7 X10^{9}$	<100	$3 X10^{3}$	<100	<100	$1 X10^{11}$
	2	$8 X10^{9}$	<100	$3 X10^{3}$	<100	<100	$1 X10^{11}$
	3		0				$2 X10^{10}$
	4		0				
	average	$7 X10^{9}$	0	$3 X10^{3}$	0	0	$8 X10^{10}$
	SEM	$2 X10^{8}$	0	$3 X10^{2}$	0	0	$4 X10^{10}$

A.6 Viable cell counts of P. acnes in four different honey treatments signally and in combination with GTE and MTO

Table A6 Viable cell counts of P. acnes in four different honey treatments signally and in combination with GTE and MTO

Samples	Replication						average	SEM
	1	2	3	4	5	6		
P. acnes initial	1 x 10^{8}	1 x 10^{8}	9 x 10^{7}	9 x 10^{7}	3 x 10^{7}	3 x 10^{7}	7. x 10^{7}	32359525
P. acnes end	1 x 10^{8}	9 x 10^{8}	4 x 10^{8}	3 x 10^{8}	1 x 10^{8}	1 x 10^{8}	3 x 10^{8}	272723150
Emulsion control	1 x 10^{8}	2 x 10^{8}	2 x 10^{8}	2 x 10^{8}	2 x 10^{8}	2 x 10^{8}	2x 10^{8}	40928461
Honey 10%	1 x 10^{8}	1 x 10^{8}	1 x 10^{8}	1 x 10^{8}	5 x 10^{7}	5 x 10^{7}	1 x 10^{8}	49825529
derma cream	2 x 10^{7}		6 x 10^{7}	5 x 10^{7}	1 x 10^{7}	1 x 10^{7}	3 x 10^{7}	20043952
moisturiser	<100	<100	<100	<100	<100	<100	<100	160.4
H+0.5GTE	6 x 10^{3}	3 x 10^{3}	5 x 10^{3}	6 x 10^{3}	3 x 10^{3}	3 x 10^{3}	4 x 10^{3}	1404.3583
H+1%GTE	2 x 10^{2}		2 x 10^{2}	2 x 10^{2}	4 x 10^{2}	4 x 10^{2}	3 x 10^{2}	97.97959
1% GTE	3 x 10^{3}	2 x 10^{3}	8 x 10^{2}	8 x 10^{2}	3 x 10^{3}	4 x 10^{3}	2 x 10^{3}	1172.8408
H+0.125%MTO	6 x 10^{5}	8 x 10^{5}	6 x 10^{5}	1 x 10^{6}	6 x 10^{5}	7 x 10^{5}	7 x 10^{5}	185779.14
H+0.25%MTO	4 x 10^{5}	3 x 10^{5}	3 x 10^{5}	3 x 10^{5}	3 x 10^{5}	3 x 10^{5}	3 x 10^{5}	31841.622
0.125% MTO	8 x 10^{5}	6 x 10^{5}	7 x 10^{6}	7 x 10^{6}	3 x 10^{6}	7 x 10^{6}	6 x 10^{6}	1380116.7
0.25% MTO			1 x 10^{5}	1 x 10^{5}	2 x 10^{5}	2 x 10^{5}	1 x 10^{5}	25495.098
Honey 10% + GTE 1%+ MTO 0.125%	<100	<100	<100	<100	<100	<100	<100	0
honey 30%	<100	<100	<100	<100	<100	<100	<100	0

Appendix B: Statistic analysis

B.1 Minitab output for *Propionibacterium acnes*

One-way ANOVA: P-aerobic versus replications

```
Source        DF       SS      MS     F      P
replications   7   1.2377  0.1768  1.87  0.074
Error        384  36.3859  0.0948
Total        391  37.6235
S = 0.3078   R-Sq = 3.29%   R-Sq(adj) = 1.53%
                                 Individual 95% CIs For Mean
Based on
                                 Pooled StDev
Level   N    Mean   StDev  ------+---------+---------+---------+---
1      49  0.4631  0.2872      (-------*--------)
2      49  0.4275  0.2542  (--------*-------)
3      49  0.4727  0.2774       (-------*--------)
4      49  0.5339  0.3050             (-------*--------)
5      49  0.5525  0.3016               (-------*--------)
6      49  0.6161  0.3544                     (--------*-------)
7      49  0.5483  0.3427              (--------*-------)
8      49  0.5096  0.3269          (--------*--------)
                           ------+---------+---------+---------+---
                               0.40      0.50      0.60      0.70
```

One-way ANOVA: P-anaerobic versus replications

```
Source        DF       SS      MS     F      P
replications   7   0.9846  0.1407  1.51  0.163
Error        384  35.8054  0.0932
Total        391  36.7900
S = 0.3054   R-Sq = 2.68%   R-Sq(adj) = 0.90%
                     Individual 95% CIs For Mean Based on
                            Pooled StDev
Level   N    Mean   StDev  ---+---------+---------+---------+------
1      49  0.5521  0.2983               (-------*--------)
2      49  0.5919  0.3216                   (-------*--------)
3      49  0.6296  0.3567                      (--------*--------)
4      49  0.4997  0.2991         (--------*--------)
5      49  0.4538  0.2916     (-------*--------)
6      49  0.5353  0.3253             (--------*-------)
7      49  0.5573  0.2691               (--------*-------)
8      49  0.5549  0.2712               (-------*--------)

---+---------+---------+---------+------
                            0.40      0.50      0.60      0.70
```

Two-Sample T-Test and CI: P-anaerobic, P-aerobic

```
Two-sample T for P-anaerobic vs P-aerobic
               N   Mean  StDev  SE Mean
P-anaerobic  392  0.547  0.307    0.015
P-aerobic    392  0.515  0.310    0.016
```

```
Difference = mu (P-anaerobic) - mu (P-aerobic)
Estimate for difference:  0.0314
95% CI for difference:  (-0.0119, 0.0746)
T-Test of difference = 0 (vs not =): T-Value = 1.42
P-Value = 0.155  DF = 781
```

B.2 The growth of *P. acnes* in honeys ranged from 0.5-12.5 %, compared with One-way ANOVA

One-way ANOVA: Manuka honey 20+ UMF ranged from 0.5 %-12.5% at aerobic condition

```
Source  DF      SS      MS      F      P
Factor      7   97.8334  13.9762  348.25  0.000
Error    1903   76.3730   0.0401
Total    1910  174.2064
S = 0.2003   R-Sq = 56.16%   R-Sq(adj) = 56.00%
                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level            N    Mean   StDev  -+---------+---------+---------+--------
A0.5-aerobic   147  0.5971  0.3959                                (-*)
A1-aerobic     147  0.5299  0.3539                             (*-)
A2.5-aerobic   294  0.2400  0.1559               (*)
A5-aerobic     147  0.0671  0.0681    (*-)
A7.5-aerobic   294  0.0436  0.0342    (*)
A10-aerobic    294  0.0186  0.0274   (*)
A12.5-aerobic  294  0.0073  0.0130  (*-)
P-aerobic      294  0.5110  0.3024                            (-*)
                                    -+---------+---------+---------+--------
                                    0.00      0.20      0.40      0.60
Pooled StDev = 0.2003
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
A0.5-aerobic subtracted from:
                 Lower   Center    Upper
A1-aerobic     -0.1381  -0.0672   0.0037
A2.5-aerobic   -0.4185  -0.3572  -0.2958
A5-aerobic     -0.6010  -0.5301  -0.4592
A7.5-aerobic   -0.6149  -0.5535  -0.4921
A10-aerobic    -0.6399  -0.5785  -0.5171
A12.5-aerobic  -0.6512  -0.5899  -0.5285
P-aerobic      -0.1476  -0.0862  -0.0248
            --------+--------+--------+--------
A1-aerobic                  (-*-)
A2.5-aerobic               (-*-)
A5-aerobic                (-*-)
A7.5-aerobic             (-*-)
A10-aerobic               (*-)
A12.5-aerobic            (-*-)
P-aerobic                            (-*)
              ---------+---------+---------+---------+
                    -0.35      0.00      0.35      0.70


              ---------+---------+---------+---------+
A5-aerobic                  (-*-)
A7.5-aerobic                 (*-)
A10-aerobic                 (-*)
A12.5-aerobic               (*-)
P-aerobic                                 (-*)
              ---------+---------+---------+---------+
                    -0.35      0.00      0.35      0.70
A5-aerobic subtracted from:
                 Lower   Center   Upper
A7.5-aerobic   -0.0848  -0.0235  0.0379
A10-aerobic    -0.1098  -0.0484  0.0130
A12.5-aerobic  -0.1212  -0.0598  0.0016
P-aerobic       0.3825   0.4439  0.5053
              ---------+---------+---------+---------+
A7.5-aerobic                     (*-)
A10-aerobic                     (-*)
A12.5-aerobic                   (*-)
P-aerobic                                    (-*)
              ---------+---------+---------+---------+
                    -0.35      0.00      0.35      0.70


A7.5-aerobic subtracted from:
              ---------+---------+---------+---------+
                    -0.35      0.00      0.35      0.70
A1-aerobic subtracted from:
                 Lower   Center    Upper
A2.5-aerobic   -0.3513  -0.2899  -0.2285
A5-aerobic     -0.5337  -0.4628  -0.3920
A7.5-aerobic   -0.5477  -0.4863  -0.4249
A10-aerobic    -0.5727  -0.5113  -0.4499
A12.5-aerobic  -0.5840  -0.5226  -0.4612
P-aerobic      -0.0803  -0.0190   0.0424
              ---------+---------+---------+---------+
A2.5-aerobic                (-*)
A5-aerobic                 (-*-)
A7.5-aerobic              (-*-)
A10-aerobic               (*-)
A12.5-aerobic            (-*-)
P-aerobic                            (*-)
              ---------+---------+---------+---------+
                    -0.35      0.00      0.35      0.70
A2.5-aerobic subtracted from:
                 Lower   Center    Upper
A5-aerobic     -0.2343  -0.1729  -0.1115
A7.5-aerobic   -0.2465  -0.1964  -0.1463
A10-aerobic    -0.2715  -0.2213  -0.1712
A12.5-aerobic  -0.2828  -0.2327  -0.1826
P-aerobic       0.2208   0.2710   0.3211


                 Lower   Center   Upper
A10-aerobic    -0.0751  -0.0250  0.0252
A12.5-aerobic  -0.0864  -0.0363  0.0138
P-aerobic       0.4172   0.4673  0.5175

              ---------+---------+---------+---------+
A10-aerobic                          (*-)
A12.5-aerobic                        (*)
P-aerobic                                        (*-)
              ---------+---------+---------+---------+
                    -0.35      0.00      0.35      0.70
A10-aerobic subtracted from:
                 Lower   Center   Upper
A12.5-aerobic  -0.0615  -0.0114  0.0388
P-aerobic       0.4422   0.4923  0.5424
              ---------+---------+---------+---------+
A12.5-aerobic                        (-*)
P-aerobic                                         (*)
              ---------+---------+---------+---------+
                    -0.35      0.00      0.35      0.70
A12.5-aerobic subtracted from:
           Lower  Center   Upper  ---------+---------+---------+---------+
P-aerobic  0.4535  0.5037  0.5538                                   (*-)
                                  ---------+---------+---------+---------+
                                        -0.35      0.00      0.35      0.70
```

One-way ANOVA: Manuka honey 20+ UMF with Catalase ranged from 0.5 %-12.5% at aerobic condition

```
Source    DF        SS      MS      F      P
Factor     7   47.6227  6.8032  80.72  0.000
Error   1756  147.9945  0.0843
Total   1763  195.6172
S = 0.2903   R-Sq = 24.34%   R-Sq(adj) = 24.04%
                                     Individual 95% CIs For Mean Based on
                                     Pooled StDev
Level             N    Mean   StDev  -------+---------+---------+---------+--
AC0.5-aerobic   147  0.5071  0.3910                              (--*--)
AC1-AERO        147  0.5037  0.3969                             (---*--)
AC2.5-aerobic   294  0.4453  0.3492                          (--*-)
AC5-aerobic     147  0.3941  0.3153                      (--*--)
AC7.5-aerobic   294  0.3295  0.2871                   (-*-)
AC10-aerobic    196  0.0979  0.0619   (--*-)
AC12.5-aerobic  245  0.0835  0.0553  (--*-)
P-aerobic       294  0.5110  0.3024                               (-*-)
                                     -------+---------+---------+---------+--
                                          0.15      0.30      0.45      0.60
Pooled StDev = 0.2903
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
AC0.5-aerobic subtracted from:
                  Lower   Center    Upper
AC1-AERO        -0.1062  -0.0034   0.0993
AC2.5-aerobic   -0.1507  -0.0618   0.0272
AC5-aerobic     -0.2158  -0.1131  -0.0103
AC7.5-aerobic   -0.2665  -0.1776  -0.0886
AC10-aerobic    -0.5053  -0.4092  -0.3131
AC12.5-aerobic  -0.5155  -0.4236  -0.3317
P-aerobic       -0.0851   0.0038   0.0928
                -------+---------+---------+---------+--
AC1-AERO                     (---*--)
AC2.5-aerobic               (--*--)
AC5-aerobic               (--*---)
AC7.5-aerobic           (--*--)
AC10-aerobic    (--*---)
AC12.5-aerobic  (--*--)
P-aerobic                     (--*--)
                -------+---------+---------+---------+--
                    -0.30      0.00      0.30      0.60
AC1-AERO subtracted from:
                  Lower   Center    Upper
AC2.5-aerobic   -0.1473  -0.0583   0.0306
AC5-aerobic     -0.2123  -0.1096  -0.0069
AC7.5-aerobic   -0.2631  -0.1741  -0.0852
AC10-aerobic    -0.5019  -0.4058  -0.3097
AC12.5-aerobic  -0.5120  -0.4201  -0.3283
P-aerobic       -0.0817   0.0073   0.0962
                -------+---------+---------+---------+--
AC2.5-aerobic               (--*--)
AC5-aerobic               (--*---)
AC7.5-aerobic           (--*--)
AC10-aerobic    (--*---)
AC12.5-aerobic  (--*--)
P-aerobic                     (--*--)
                -------+---------+---------+---------+--
                    -0.30      0.00      0.30      0.60
AC2.5-aerobic subtracted from:
                  Lower   Center    Upper
AC5-aerobic     -0.1402  -0.0513   0.0377
AC7.5-aerobic   -0.1884  -0.1158  -0.0432
AC10-aerobic    -0.4286  -0.3474  -0.2662
AC12.5-aerobic  -0.4380  -0.3618  -0.2856
P-aerobic       -0.0070   0.0656   0.1383
                -------+---------+---------+---------+--
AC5-aerobic                 (--*--)
AC7.5-aerobic              (-*--)
AC10-aerobic       (-*--)
AC12.5-aerobic    (--*-)
P-aerobic                        (-*--)
                -------+---------+---------+---------+--
                    -0.30      0.00      0.30      0.60
AC5-aerobic subtracted from:
                  Lower   Center    Upper
AC7.5-aerobic   -0.1535  -0.0645   0.0244
AC10-aerobic    -0.3922  -0.2961  -0.2001
AC12.5-aerobic  -0.4024  -0.3105  -0.2186
P-aerobic        0.0279   0.1169   0.2059
                -------+---------+---------+---------+--
AC7.5-aerobic               (--*--)
AC10-aerobic        (--*--)
AC12.5-aerobic      (--*--)
P-aerobic                         (--*--)
                -------+---------+---------+---------+--
                    -0.30      0.00      0.30      0.60
AC7.5-aerobic subtracted from:
                  Lower   Center    Upper
AC10-aerobic    -0.3128  -0.2316  -0.1504
AC12.5-aerobic  -0.3222  -0.2460  -0.1698
P-aerobic        0.1088   0.1814   0.2541
                -------+---------+---------+---------+--
AC10-aerobic           (-*--)
AC12.5-aerobic        (--*-)
P-aerobic                            (-*-)
                -------+---------+---------+---------+--
                    -0.30      0.00      0.30      0.60
AC10-aerobic subtracted from:
                  Lower   Center   Upper
AC12.5-aerobic  -0.0988  -0.0144  0.0700
P-aerobic        0.3318   0.4130  0.4943
                -------+---------+---------+---------+--
AC12.5-aerobic                (--*-)
P-aerobic                                   (--*-)
                -------+---------+---------+---------+--
                   -0.30      0.00      0.30      0.60
AC12.5-aerobic subtracted from:
            Lower  Center   Upper  -------+---------+---------+---------+--
P-aerobic  0.3512  0.4274  0.5036                               (-*--)
                                   -------+---------+---------+---------+--
                                       -0.30     0.00      0.30      0.60
```

One-way ANOVA: Manuka honey 15+ UMF ranged from 0.5 %-12.5% at aerobic condition

```
Source    DF       SS      MS       F      P
Factor     7   89.7758  12.8251  317.32  0.000
Error   1902   76.8741   0.0404
Total   1909  166.6499
S = 0.2010   R-Sq = 53.87%   R-Sq(adj) = 53.70%
                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level            N    Mean   StDev  -+---------+---------+---------+--------
B0.5-aerobic   147  0.5310  0.3649                                  (-*--)
B1-AERO        147  0.5122  0.3386                                 (-*-)
B2.5-aerobic   293  0.2889  0.2105                     (*-)
B5-aerobic     147  0.0642  0.0616      (-*-)
B7.5-aerobic   294  0.0597  0.0299      (-*-)
B10-aerobic    294  0.0091  0.0121   (-*)
B12.5-aerobic  294  0.0137  0.0188   (-*)
P-aerobic      294  0.5110  0.3024                                  (*-)
                                    -+---------+---------+---------+--------
                                    0.00      0.15      0.30      0.45
Pooled StDev = 0.2010
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
B0.5-aerobic subtracted from:
                 Lower   Center    Upper
B1-AERO        -0.0899  -0.0188   0.0523
B2.5-aerobic   -0.3038  -0.2421  -0.1805
B5-aerobic     -0.5380  -0.4669  -0.3958
B7.5-aerobic   -0.5330  -0.4714  -0.4098
B10-aerobic    -0.5836  -0.5219  -0.4603
B12.5-aerobic  -0.5790  -0.5174  -0.4558
P-aerobic      -0.0817  -0.0201   0.0415
               ---------+---------+---------+---------+
B1-AERO                          (-*--)
B2.5-aerobic             (-*-)
B5-aerobic       (-*--)
B7.5-aerobic     (-*-)
B10-aerobic     (-*-)
B12.5-aerobic   (-*-)
P-aerobic                        (-*-)
               ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
B1-AERO subtracted from:

                 Lower   Center    Upper
B2.5-aerobic   -0.2850  -0.2233  -0.1617
B5-aerobic     -0.5192  -0.4481  -0.3769
B7.5-aerobic   -0.5142  -0.4526  -0.3910
B10-aerobic    -0.5647  -0.5031  -0.4415
B12.5-aerobic  -0.5602  -0.4986  -0.4370
P-aerobic      -0.0629  -0.0013   0.0603
               ---------+---------+---------+---------+
B2.5-aerobic              (-*-)
B5-aerobic        (-*-)
B7.5-aerobic      (-*-)
B10-aerobic     (-*-)
B12.5-aerobic   (-*-)
P-aerobic                         (-*-)
               ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
```

```
B2.5-aerobic subtracted from:
                 Lower   Center    Upper
B5-aerobic     -0.2864  -0.2247  -0.1631
B7.5-aerobic   -0.2796  -0.2292  -0.1789
B10-aerobic    -0.3301  -0.2798  -0.2295
B12.5-aerobic  -0.3256  -0.2752  -0.2249
P-aerobic       0.1717   0.2221   0.2724
               ---------+---------+---------+---------+
B5-aerobic           (--*-)
B7.5-aerobic          (*-)
B10-aerobic          (-*)
B12.5-aerobic        (-*-)
P-aerobic                             (*-)
               ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
B5-aerobic subtracted from:
                 Lower   Center   Upper
B7.5-aerobic   -0.0661  -0.0045  0.0571
B10-aerobic    -0.1167  -0.0551  0.0065
B12.5-aerobic  -0.1121  -0.0505  0.0111
P-aerobic       0.3852   0.4468  0.5084
               ---------+---------+---------+---------+
B7.5-aerobic                    (-*-)
B10-aerobic                   (-*-)
B12.5-aerobic                 (-*-)
P-aerobic                                     (-*-)
               ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
B7.5-aerobic subtracted from:
                 Lower   Center    Upper
B10-aerobic    -0.1009  -0.0506  -0.0003
B12.5-aerobic  -0.0963  -0.0460   0.0043
P-aerobic       0.4010   0.4513   0.5016
               ---------+---------+---------+---------+
B10-aerobic                    (*-)
B12.5-aerobic                  (*-)
P-aerobic                                     (-*-)
               ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60

B10-aerobic subtracted from:

                 Lower  Center   Upper
B12.5-aerobic  -0.0457  0.0046  0.0549
P-aerobic       0.4516  0.5019  0.5522

               ---------+---------+---------+---------+
B12.5-aerobic                   (-*-)
P-aerobic                                      (-*)
               ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60


B12.5-aerobic subtracted from:

             Lower  Center   Upper  ---------+---------+---------+---------+
P-aerobic   0.4470  0.4973  0.5476                                    (-*)
                                    ---------+---------+---------+---------+
                                          -0.30      0.00      0.30      0.60
```

One-way ANOVA: Manuka honey 15+ UMF with catalase ranged from 0.5 %-12.5% at aerobic condition

```
Source    DF        SS      MS       F      P
Factor     7   64.9835  9.2834  100.24  0.000
Error   1903  176.2459  0.0926
Total   1910  241.2294
S = 0.3043   R-Sq = 26.94%   R-Sq(adj) = 26.67%
                                     Individual 95% CIs For Mean Based on
                                     Pooled StDev
Level              N    Mean   StDev  ------+---------+---------+---------+---
BC0.5-aerobic    147  0.5653  0.4229                              (---*--)
BC1-AERO         147  0.5768  0.4342                               (--*---)
BC2.5-aerobic    294  0.4588  0.3642                        (--*-)
BC5-aerobic      147  0.4442  0.3606                       (---*--)
BC7.5-aerobic    294  0.4199  0.3494                       (-*-)
BC10-aerobic     294  0.1067  0.0665   (-*-)
BC12.5-aerobic   294  0.0901  0.0555  (-*-)
P-aerobic        294  0.5110  0.3024                             (-*-)
                                      ------+---------+---------+---------+---
                                          0.15      0.30      0.45      0.60
Pooled StDev = 0.3043
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
BC0.5-aerobic subtracted from:
                  Lower   Center    Upper
BC1-AERO        -0.0962   0.0114   0.1191
BC2.5-aerobic   -0.1998  -0.1066  -0.0133
BC5-aerobic     -0.2288  -0.1211  -0.0134
BC7.5-aerobic   -0.2387  -0.1454  -0.0522
BC10-aerobic    -0.5519  -0.4586  -0.3654
BC12.5-aerobic  -0.5685  -0.4752  -0.3820
P-aerobic       -0.1476  -0.0544   0.0389
                ---------+---------+---------+---------+
BC1-AERO                        (--*---)
BC2.5-aerobic               (--*---)
BC5-aerobic                (---*---)
BC7.5-aerobic              (--*--)
BC10-aerobic     (--*--)
BC12.5-aerobic  (--*--)
P-aerobic                     (--*--)
               ---------+---------+---------+---------+
                    -0.30      0.00      0.30      0.60
BC1-AERO subtracted from:
                  Lower   Center    Upper
BC2.5-aerobic   -0.2112  -0.1180  -0.0247
BC5-aerobic     -0.2402  -0.1325  -0.0249
BC7.5-aerobic   -0.2501  -0.1569  -0.0636
BC10-aerobic    -0.5633  -0.4701  -0.3768
BC12.5-aerobic  -0.5799  -0.4867  -0.3934
P-aerobic       -0.1591  -0.0658   0.0274
                ---------+---------+---------+---------+
BC2.5-aerobic               (--*--)
BC5-aerobic                (---*--)
BC7.5-aerobic              (--*--)
BC10-aerobic    (--*--)
BC12.5-aerobic  (--*--)
P-aerobic                     (--*--)
                ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
BC2.5-aerobic subtracted from:
                  Lower   Center    Upper
BC5-aerobic     -0.1078  -0.0146   0.0787
BC7.5-aerobic   -0.1150  -0.0389   0.0373
BC10-aerobic    -0.4282  -0.3521  -0.2760
BC12.5-aerobic  -0.4448  -0.3687  -0.2925
P-aerobic       -0.0240   0.0522   0.1283
                ---------+---------+---------+---------+
BC5-aerobic                    (---*--)
BC7.5-aerobic                  (--*-)
BC10-aerobic         (-*--)
BC12.5-aerobic      (--*-)
P-aerobic                         (--*-)
                ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
BC5-aerobic subtracted from:
                  Lower   Center    Upper
BC7.5-aerobic   -0.1176  -0.0243   0.0689
BC10-aerobic    -0.4308  -0.3375  -0.2443
BC12.5-aerobic  -0.4474  -0.3541  -0.2609
P-aerobic       -0.0265   0.0667   0.1600
                ---------+---------+---------+---------+
BC7.5-aerobic                  (--*--)
BC10-aerobic         (--*--)
BC12.5-aerobic      (--*--)
P-aerobic                         (--*--)
                ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
BC7.5-aerobic subtracted from:
                  Lower   Center    Upper
BC10-aerobic    -0.3894  -0.3132  -0.2371
BC12.5-aerobic  -0.4060  -0.3298  -0.2537
P-aerobic        0.0149   0.0910   0.1672
                ---------+---------+---------+---------+
BC10-aerobic          (--*-)
BC12.5-aerobic       (--*--)
P-aerobic                          (--*--)
                ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
BC10-aerobic subtracted from:
                  Lower   Center   Upper
BC12.5-aerobic  -0.0927  -0.0166  0.0595
P-aerobic        0.3281   0.4043  0.4804
                ---------+---------+---------+---------+
BC12.5-aerobic                  (-*--)
P-aerobic                                     (-*--)
                ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
BC12.5-aerobic subtracted from:
            Lower  Center   Upper  ---------+---------+---------+---------+
P-aerobic  0.3447  0.4209  0.4970                              (--*--)
                                   ---------+---------+---------+---------+
                                        -0.30      0.00      0.30      0.60
```

One-way ANOVA: Manuka honey 10+ UMF ranged from 0.5 %-12.5% at aerobic condition

```
Source    DF       SS      MS       F      P
Factor     7  119.366  17.052  112.00  0.000
Error   1805  274.810   0.152
Total   1812  394.176
S = 0.3902   R-Sq = 30.28%   R-Sq(adj) = 30.01%
                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level            N    Mean   StDev  --------+---------+---------+---------+-
C0.5-aerobic   147  0.9178  0.5020                                  (--*-)
C1-AERO        147  0.8433  0.5251                               (--*-)
C2.5-aerobic   294  0.8119  0.4845                               (*-)
C5-aerobic     147  0.7122  0.4262                          (-*--)
C7.5-aerobic   294  0.5381  0.3887                    (-*)
C10-aerobic    294  0.2708  0.3050         (-*-)
C12.5-aerobic  196  0.1011  0.1459  (-*-)
P-aerobic      294  0.5110  0.3024                   (*-)
                                    --------+---------+---------+---------+-
                                    0.25      0.50      0.75      1.00
Pooled StDev = 0.3902
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
C0.5-aerobic subtracted from:
                 Lower   Center    Upper
C1-AERO        -0.2125  -0.0745   0.0636
C2.5-aerobic   -0.2255  -0.1059   0.0136
C5-aerobic     -0.3437  -0.2056  -0.0676
C7.5-aerobic   -0.4993  -0.3797  -0.2602
C10-aerobic    -0.7666  -0.6470  -0.5275
C12.5-aerobic  -0.9458  -0.8166  -0.6875
P-aerobic      -0.5264  -0.4068  -0.2873
               ---------+---------+---------+---------+
C1-AERO                       (--*-)
C2.5-aerobic                 (--*-)
C5-aerobic                 (--*--)
C7.5-aerobic            (-*--)
C10-aerobic        (-*-)
C12.5-aerobic  (--*-)
P-aerobic              (--*-)
               ---------+---------+---------+---------+
                     -0.50      0.00      0.50      1.00
C1-AERO subtracted from:
                 Lower   Center    Upper
C2.5-aerobic   -0.1510  -0.0314   0.0881
C5-aerobic     -0.2692  -0.1311   0.0069
C7.5-aerobic   -0.4248  -0.3052  -0.1857
C10-aerobic    -0.6921  -0.5725  -0.4530
C12.5-aerobic  -0.8713  -0.7422  -0.6130
P-aerobic      -0.4519  -0.3324  -0.2128
               ---------+---------+---------+---------+
C2.5-aerobic                   (-*--)
C5-aerobic                   (-*--)
C7.5-aerobic              (-*-)
C10-aerobic         (--*-)
C12.5-aerobic    (-*--)
P-aerobic                 (-*--)
               ---------+---------+---------+---------+
                     -0.50      0.00      0.50      1.00
C2.5-aerobic subtracted from:
                 Lower   Center    Upper
C5-aerobic     -0.2193  -0.0997   0.0199
C7.5-aerobic   -0.3714  -0.2738  -0.1762
C10-aerobic    -0.6387  -0.5411  -0.4435
C12.5-aerobic  -0.8199  -0.7107  -0.6016
P-aerobic      -0.3985  -0.3009  -0.2033
               ---------+---------+---------+---------+
C5-aerobic                    (-*-)
C7.5-aerobic                (-*)
C10-aerobic          (-*-)
C12.5-aerobic     (-*-)
P-aerobic                  (-*-)
               ---------+---------+---------+---------+
                     -0.50      0.00      0.50      1.00
C5-aerobic subtracted from:
                 Lower   Center    Upper
C7.5-aerobic   -0.2937  -0.1741  -0.0545
C10-aerobic    -0.5610  -0.4414  -0.3218
C12.5-aerobic  -0.7402  -0.6110  -0.4819
P-aerobic      -0.3208  -0.2012  -0.0816
               ---------+---------+---------+---------+
C7.5-aerobic                (--*-)
C10-aerobic            (-*--)
C12.5-aerobic      (--*-)
P-aerobic                   (-*-)
               ---------+---------+---------+---------+
                     -0.50      0.00      0.50      1.00
C7.5-aerobic subtracted from:
                 Lower   Center    Upper
C10-aerobic    -0.3649  -0.2673  -0.1697
C12.5-aerobic  -0.5461  -0.4369  -0.3278
P-aerobic      -0.1247  -0.0271   0.0705
               ---------+---------+---------+---------+
C10-aerobic                 (-*-)
C12.5-aerobic           (-*-)
P-aerobic                        (*-)
               ---------+---------+---------+---------+
                     -0.50      0.00      0.50      1.00
C10-aerobic subtracted from:
                 Lower   Center    Upper
C12.5-aerobic  -0.2788  -0.1696  -0.0605
P-aerobic       0.1426   0.2402   0.3378
               ---------+---------+---------+---------+
C12.5-aerobic               (--*-)
P-aerobic                            (-*-)
               ---------+---------+---------+---------+
                     -0.50      0.00      0.50      1.00
C12.5-aerobic subtracted from:
            Lower  Center   Upper  ---------+---------+---------+---------+
P-aerobic  0.3007  0.4098  0.5190                           (-*-)
                                   ---------+---------+---------+---------+
                                         -0.50      0.00      0.50      1.00
```

One-way ANOVA: Manuka honey 10+ catalase UMF ranged from 0.5 %-12.5% at aerobic condition

```
Source    DF       SS     MS      F      P
Factor     7   67.950  9.707  48.33  0.000
Error   1805  362.573  0.201
Total   1812  430.523
S = 0.4482   R-Sq = 15.78%   R-Sq(adj) = 15.46%
                                     Individual 95% CIs For Mean Based on
                                     Pooled StDev
Level             N    Mean   StDev  -+---------+---------+---------+--------
CC0.5-aerobic   147  0.8654  0.5365                               (--*---)
CC1-AERO        147  0.8136  0.5032                            (---*--)
CC2.5-aerobic   294  0.7538  0.5068                          (--*-)
CC5-aerobic     147  0.7514  0.4728                         (---*--)
CC7.5-aerobic   294  0.7863  0.5272                            (-*--)
CC10-aerobic    294  0.4788  0.4366             (--*--)
CC12.5-aerobic  196  0.2380  0.2439  (--*--)
P-aerobic       294  0.5110  0.3024               (--*-)
                                     -+---------+---------+---------+--------
                                     0.20      0.40      0.60      0.80
Pooled StDev = 0.4482
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
CC0.5-aerobic subtracted from:
                 Lower   Center    Upper
CC1-AERO       -0.2104  -0.0518   0.1068
CC2.5-aerobic  -0.2490  -0.1116   0.0257
CC5-aerobic    -0.2726  -0.1140   0.0446
CC7.5-aerobic  -0.2165  -0.0791   0.0582
CC10-aerobic   -0.5240  -0.3866  -0.2493
CC12.5-aerobic -0.7758  -0.6274  -0.4791
P-aerobic      -0.4918  -0.3545  -0.2171
                ---------+---------+---------+---------+
CC1-AERO                     (---*---)
CC2.5-aerobic                 (--*---)
CC5-aerobic                  (---*---)
CC7.5-aerobic                  (--*--)
CC10-aerobic           (--*---)
CC12.5-aerobic  (--*---)
P-aerobic               (--*---)
                ---------+---------+---------+---------+
                      -0.40      0.00      0.40      0.80
CC1-AERO subtracted from:
                 Lower   Center    Upper
CC2.5-aerobic  -0.1972  -0.0598   0.0775
CC5-aerobic    -0.2208  -0.0622   0.0964
CC7.5-aerobic  -0.1647  -0.0273   0.1100
CC10-aerobic   -0.4722  -0.3348  -0.1975
CC12.5-aerobic -0.7240  -0.5756  -0.4273
P-aerobic      -0.4400  -0.3027  -0.1653
                ---------+---------+---------+---------+
CC2.5-aerobic                  (---*--)
CC5-aerobic                   (---*---)
CC7.5-aerobic                   (--*---)
CC10-aerobic            (---*--)
CC12.5-aerobic   (---*--)
P-aerobic                (--*---)
                ---------+---------+---------+---------+
                      -0.40      0.00      0.40      0.80
CC2.5-aerobic subtracted from:
                 Lower   Center    Upper
CC5-aerobic    -0.1397  -0.0024   0.1350
CC7.5-aerobic  -0.0796   0.0325   0.1446
CC10-aerobic   -0.3872  -0.2750  -0.1629
CC12.5-aerobic -0.6412  -0.5158  -0.3904
P-aerobic      -0.3550  -0.2428  -0.1307
               ---------+---------+---------+---------+
CC5-aerobic                   (--*--)
CC7.5-aerobic                  (--*--)
CC10-aerobic            (--*--)
CC12.5-aerobic     (--*--)
P-aerobic                (--*--)
                ---------+---------+---------+---------+
                      -0.40      0.00      0.40      0.80
CC5-aerobic subtracted from:
                 Lower   Center    Upper
CC7.5-aerobic  -0.1025   0.0349   0.1722
CC10-aerobic   -0.4100  -0.2727  -0.1353
CC12.5-aerobic -0.6618  -0.5134  -0.3651
P-aerobic      -0.3778  -0.2405  -0.1031
                ---------+---------+---------+---------+
CC7.5-aerobic                 (---*--)
CC10-aerobic            (--*---)
CC12.5-aerobic    (---*---)
P-aerobic                (--*--)
                ---------+---------+---------+---------+
                      -0.40      0.00      0.40      0.80
CC7.5-aerobic subtracted from:
                 Lower   Center    Upper
CC10-aerobic   -0.4196  -0.3075  -0.1954
CC12.5-aerobic -0.6737  -0.5483  -0.4229
P-aerobic      -0.3875  -0.2753  -0.1632
                ---------+---------+---------+---------+
CC10-aerobic            (-*--)
CC12.5-aerobic    (--*--)
P-aerobic               (--*--)
                ---------+---------+---------+---------+
                      -0.40      0.00      0.40      0.80
CC10-aerobic subtracted from:
                 Lower   Center    Upper
CC12.5-aerobic -0.3662  -0.2408  -0.1154
P-aerobic      -0.0800   0.0322   0.1443
                ---------+---------+---------+---------+
CC12.5-aerobic           (--*--)
P-aerobic                       (--*--)
                ---------+---------+---------+---------+
                      -0.40      0.00      0.40      0.80
CC12.5-aerobic subtracted from:
             Lower  Center   Upper  ---------+---------+---------+---------+
P-aerobic   0.1476  0.2730  0.3983                         (--*--)
                                    ---------+---------+---------+---------+
                                          -0.40      0.00      0.40      0.80
```

One-way ANOVA: Kanuka honey ranged from 0.5 %-12.5% at aerobic condition

```
Source    DF        SS       MS       F      P
Factor     7   75.5611  10.7944  163.44  0.000
Error   1903  125.6856   0.0660
Total   1910  201.2467
S = 0.2570   R-Sq = 37.55%   R-Sq(adj) = 37.32%
                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level            N    Mean   StDev  --+---------+---------+---------+-------
K0.5-aerobic   147  0.4778  0.3599                               (--*--)
K1-AERO        147  0.3909  0.2956                         (--*--)
K2.5-aerobic   294  0.3413  0.3002                       (-*-)
K5-aerobic     147  0.2244  0.3364              (--*--)
K7.5-aerobic   294  0.1005  0.2879       (-*-)
K10-aerobic    294  0.0005  0.0019  (-*-)
K12.5-aerobic  294  0.0007  0.0020  (-*-)
P-aerobic      294  0.5110  0.3024                                  (-*-)
                                    --+---------+---------+---------+-------
                                    0.00      0.15      0.30      0.45
Pooled StDev = 0.2570
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
K0.5-aerobic subtracted from:
                 Lower   Center    Upper
K1-AERO        -0.1778  -0.0869   0.0040
K2.5-aerobic   -0.2152  -0.1365  -0.0577
K5-aerobic     -0.3443  -0.2534  -0.1625
K7.5-aerobic   -0.4560  -0.3772  -0.2985
K10-aerobic    -0.5560  -0.4773  -0.3985
K12.5-aerobic  -0.5559  -0.4771  -0.3984
P-aerobic      -0.0456   0.0332   0.1119
               ---------+---------+---------+---------+
K1-AERO                     (--*--)
K2.5-aerobic               (-*--)
K5-aerobic             (--*--)
K7.5-aerobic       (-*--)
K10-aerobic    (--*--)
K12.5-aerobic  (--*--)
P-aerobic                       (--*--)
               ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
K1-AERO subtracted from:

                 Lower   Center    Upper
K2.5-aerobic   -0.1283  -0.0496   0.0292
K5-aerobic     -0.2574  -0.1665  -0.0756
K7.5-aerobic   -0.3691  -0.2904  -0.2116
K10-aerobic    -0.4691  -0.3904  -0.3116
K12.5-aerobic  -0.4690  -0.3902  -0.3115
P-aerobic       0.0413   0.1201   0.1988


K10-aerobic subtracted from:

                 Lower  Center   Upper
K12.5-aerobic  -0.0641  0.0002  0.0645
P-aerobic       0.4462  0.5105  0.5748

               ---------+---------+---------+---------+
K12.5-aerobic                   (-*-)
P-aerobic                                        (-*-)
```

```
               ---------+---------+---------+---------+
K2.5-aerobic                  (-*--)
K5-aerobic               (--*--)
K7.5-aerobic          (-*--)
K10-aerobic       (--*--)
K12.5-aerobic     (--*--)
P-aerobic                          (--*--)
               ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
K2.5-aerobic subtracted from:
                 Lower   Center    Upper
K5-aerobic     -0.1957  -0.1169  -0.0382
K7.5-aerobic   -0.3051  -0.2408  -0.1765
K10-aerobic    -0.4051  -0.3408  -0.2765
K12.5-aerobic  -0.4050  -0.3407  -0.2764
P-aerobic       0.1053   0.1696   0.2339
               ---------+---------+---------+---------+
K5-aerobic                 (--*--)
K7.5-aerobic            (-*-)
K10-aerobic         (--*-)
K12.5-aerobic        (-*-)
P-aerobic                             (-*-)
               ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
K5-aerobic subtracted from:
                 Lower   Center    Upper
K7.5-aerobic   -0.2026  -0.1239  -0.0451
K10-aerobic    -0.3026  -0.2239  -0.1451
K12.5-aerobic  -0.3025  -0.2237  -0.1450
P-aerobic       0.2078   0.2866   0.3653
               ---------+---------+---------+---------+
K7.5-aerobic               (--*-)
K10-aerobic             (--*-)
K12.5-aerobic           (--*-)
P-aerobic                                (--*-)
               ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
K7.5-aerobic subtracted from:

                 Lower   Center    Upper
K10-aerobic    -0.1643  -0.1000  -0.0357
K12.5-aerobic  -0.1642  -0.0999  -0.0356
P-aerobic       0.3461   0.4104   0.4747

               ---------+---------+---------+---------+
K10-aerobic                   (-*-)
K12.5-aerobic                 (-*-)
P-aerobic                                      (-*-)
               ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60
               ---------+---------+---------+---------+
                     -0.30      0.00      0.30      0.60


K12.5-aerobic subtracted from:

             Lower  Center   Upper  ---------+---------+---------+---------+
P-aerobic   0.4460  0.5103  0.5746                                    (-*-)
                                    ---------+---------+---------+---------+
                                          -0.30      0.00      0.30      0.60
```

One-way ANOVA: Kanuka honey ranged from 0.5 %-12.5% at aerobic condition

```
Source    DF       SS      MS     F      P
Factor     7    9.620  1.374  7.44  0.000
Error   1903  351.589  0.185
Total   1910  361.209
S = 0.4298   R-Sq = 2.66%   R-Sq(adj) = 2.31%
Level             N    Mean   StDev
KC0.5-aerobic   147  0.6605  0.4729
KC1-AERO        147  0.6034  0.4747
KC2.5-aerobic   294  0.6441  0.5089
KC5-aerobic     147  0.6271  0.4839
KC7.5-aerobic   294  0.6394  0.4553
KC10-aerobic    294  0.5403  0.4062
KC12.5-aerobic  294  0.4607  0.3698
P-aerobic       294  0.5110  0.3024
                Individual 95% CIs For Mean Based on Pooled StDev
Level           ---------+---------+---------+---------+
KC0.5-aerobic                          (--------*-------)
KC1-AERO                        (-------*--------)
KC2.5-aerobic                          (------*-----)
KC5-aerobic                        (-------*--------)
KC7.5-aerobic                          (-----*-----)
KC10-aerobic                   (------*-----)
KC12.5-aerobic  (------*-----)
P-aerobic              (-----*-----)
                ---------+---------+---------+---------+
                       0.480     0.560     0.640     0.720
Pooled StDev = 0.4298
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
KC0.5-aerobic subtracted from:
                  Lower   Center   Upper
KC1-AERO        -0.2092  -0.0571  0.0950
KC2.5-aerobic   -0.1481  -0.0164  0.1153
KC5-aerobic     -0.1854  -0.0334  0.1187
KC7.5-aerobic   -0.1528  -0.0211  0.1106
KC10-aerobic    -0.2519  -0.1202  0.0115
KC12.5-aerobic  -0.3315  -0.1998 -0.0681
P-aerobic       -0.2812  -0.1495 -0.0178
                  -+---------+---------+---------+--------
KC1-AERO                 (--------*---------)
KC2.5-aerobic                (-------*-------)
KC5-aerobic                (---------*--------)
KC7.5-aerobic                  (--------*-------)
KC10-aerobic          (-------*--------)
KC12.5-aerobic    (--------*-------)
P-aerobic           (--------*-------)
                  -+---------+---------+---------+--------
                -0.32     -0.16      0.00      0.16
KC1-AERO subtracted from:
                  Lower   Center   Upper
KC2.5-aerobic   -0.0910   0.0407  0.1724
KC5-aerobic     -0.1283   0.0237  0.1758
KC7.5-aerobic   -0.0957   0.0360  0.1677
KC10-aerobic    -0.1948  -0.0631  0.0686
KC12.5-aerobic  -0.2744  -0.1427 -0.0110
P-aerobic       -0.2241  -0.0924  0.0393
                  -+---------+---------+---------+--------
KC2.5-aerobic                   (--------*-------)
KC5-aerobic                   (--------*---------)
KC7.5-aerobic                   (-------*-------)
KC10-aerobic               (-------*-------)
KC12.5-aerobic       (------*------)
P-aerobic                (------*------)
                  -+---------+---------+---------+--------
                -0.32     -0.16      0.00      0.16
KC2.5-aerobic subtracted from:
                  Lower   Center   Upper
KC5-aerobic     -0.1487  -0.0170  0.1147
KC7.5-aerobic   -0.1122  -0.0047  0.1029
KC10-aerobic    -0.2114  -0.1038  0.0037
KC12.5-aerobic  -0.2910  -0.1834 -0.0759
P-aerobic       -0.2407  -0.1332 -0.0256
                  -+---------+---------+---------+--------
KC5-aerobic                  (-------*-------)
KC7.5-aerobic                  (------*-----)
KC10-aerobic               (-----*-----)
KC12.5-aerobic        (-----*-----)
P-aerobic                (-----*-----)
                  -+---------+---------+---------+--------
                -0.32     -0.16      0.00      0.16
KC5-aerobic subtracted from:
                  Lower   Center   Upper
KC7.5-aerobic   -0.1194   0.0123  0.1440
KC10-aerobic    -0.2186  -0.0868  0.0449
KC12.5-aerobic  -0.2982  -0.1665 -0.0348
P-aerobic       -0.2479  -0.1162  0.0155
                  -+---------+---------+---------+--------
KC7.5-aerobic                  (-------*-------)
KC10-aerobic              (--------*-------)
KC12.5-aerobic       (--------*-------)
P-aerobic                (-------*-------)
                  -+---------+---------+---------+--------
                -0.32     -0.16      0.00      0.16
KC7.5-aerobic subtracted from:
                  Lower   Center   Upper
KC10-aerobic    -0.2067  -0.0991  0.0084
KC12.5-aerobic  -0.2863  -0.1788 -0.0712
P-aerobic       -0.2360  -0.1285 -0.0209
                  -+---------+---------+---------+--------
KC10-aerobic               (------*------)
KC12.5-aerobic        (------*------)
P-aerobic                (------*------)
                  -+---------+---------+---------+--------
                -0.32     -0.16      0.00      0.16
KC10-aerobic subtracted from:
                  Lower   Center   Upper
KC12.5-aerobic  -0.1872  -0.0796  0.0279
P-aerobic       -0.1369  -0.0293  0.0782
                  -+---------+---------+---------+--------
KC12.5-aerobic              (------*------)
P-aerobic                      (------*------)
                  -+---------+---------+---------+--------
                -0.32     -0.16      0.00      0.16
KC12.5-aerobic subtracted from:
             Lower  Center  Upper     -+---------+---------+---------+--------
P-aerobic  -0.0572  0.0503  0.1578                   (------*------)
                                      -+---------+---------+---------+--------
                                    -0.32     -0.16      0.00      0.16
```

One-way ANOVA: Manuka honey 20+ UMF ranged from 0.5 %-12.5% under anaerobic condition

```
Source      DF        SS      MS       F      P
Factor       7   56.0672  8.0096  213.71  0.000
Error     1609   60.3021  0.0375
Total     1616  116.3693
S = 0.1936   R-Sq = 48.18%   R-Sq(adj) = 47.95%
                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level           N    Mean   StDev  -+---------+---------+---------+--------
A0.5-AN       147  0.4555  0.3018                              (-*-)
A1-AN         147  0.3278  0.2158                      (-*-)
A2.5-AN       294  0.2653  0.1621                  (-*)
A5-AN         147  0.1575  0.0977          (-*--)
A7.5-AN       294  0.0906  0.0650       (*-)
A10-AN        147  0.0185  0.0153   (-*-)
A12.5-AN      147  0.0325  0.0210    (-*-)
P-anaerobic   294  0.5437  0.3188                                     (*-)
                                   -+---------+---------+---------+--------
                                  0.00      0.15      0.30      0.45
Pooled StDev = 0.1936
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
A0.5-AN subtracted from:
               Lower   Center    Upper
A1-AN        -0.1962  -0.1277  -0.0592
A2.5-AN      -0.2495  -0.1901  -0.1308
A5-AN        -0.3665  -0.2980  -0.2295
A7.5-AN      -0.4241  -0.3648  -0.3055
A10-AN       -0.5055  -0.4370  -0.3685
A12.5-AN     -0.4915  -0.4230  -0.3545
P-anaerobic   0.0290   0.0883   0.1476
             ---------+---------+---------+---------+
A1-AN                    (--*-)
A2.5-AN                 (-*-)
A5-AN                (-*-)
A7.5-AN             (-*-)
A10-AN            (-*--)
A12.5-AN           (-*-)
P-anaerobic                    (-*-)
             ---------+---------+---------+---------+
                   -0.30      0.00      0.30      0.60
A1-AN subtracted from:
               Lower   Center    Upper
A2.5-AN      -0.1218  -0.0624  -0.0031
A5-AN        -0.2388  -0.1703  -0.1018
A7.5-AN      -0.2965  -0.2371  -0.1778
A10-AN       -0.3778  -0.3093  -0.2408
A12.5-AN     -0.3638  -0.2953  -0.2268
P-anaerobic   0.1567   0.2160   0.2753
             ---------+---------+---------+---------+
A2.5-AN                     (-*-)
A5-AN                    (-*--)
A7.5-AN                 (-*-)
A10-AN                (--*-)
A12.5-AN              (-*-)
P-anaerobic                       (-*-)
             ---------+---------+---------+---------+
                   -0.30      0.00      0.30      0.60
A2.5-AN subtracted from:
               Lower   Center    Upper
A5-AN        -0.1672  -0.1078  -0.0485
A7.5-AN      -0.2231  -0.1747  -0.1262
A10-AN       -0.3062  -0.2468  -0.1875
A12.5-AN     -0.2921  -0.2328  -0.1735
P-anaerobic   0.2300   0.2784   0.3269
             ---------+---------+---------+---------+
A5-AN                      (-*-)
A7.5-AN                   (*-)
A10-AN                  (-*-)
A12.5-AN                (-*-)
P-anaerobic                         (*-)
             ---------+---------+---------+---------+
                   -0.30      0.00      0.30      0.60
A5-AN subtracted from:
               Lower   Center    Upper
A7.5-AN      -0.1262  -0.0669  -0.0075
A10-AN       -0.2075  -0.1390  -0.0705
A12.5-AN     -0.1935  -0.1250  -0.0565
P-anaerobic   0.3269   0.3863   0.4456
             ---------+---------+---------+---------+
A7.5-AN                      (-*-)
A10-AN                     (-*--)
A12.5-AN                    (-*-)
P-anaerobic                            (-*-)
             ---------+---------+---------+---------+
                   -0.30      0.00      0.30      0.60
A7.5-AN subtracted from:
               Lower   Center    Upper
A10-AN       -0.1315  -0.0722  -0.0128
A12.5-AN     -0.1175  -0.0581   0.0012
P-anaerobic   0.4047   0.4531   0.5015
             ---------+---------+---------+---------+
A10-AN                       (-*-)
A12.5-AN                     (-*-)
P-anaerobic                              (-*-)
             ---------+---------+---------+---------+
                   -0.30      0.00      0.30      0.60
A10-AN subtracted from:
               Lower  Center   Upper  ---------+---------+---------+---------+
A12.5-AN     -0.0545  0.0140  0.0825                 (-*--)
P-anaerobic   0.4659  0.5253  0.5846                                 (-*)
                                      ---------+---------+---------+---------+
                                            -0.30      0.00      0.30      0.60
A12.5-AN subtracted from:
              Lower  Center   Upper  ---------+---------+---------+---------+
P-anaerobic  0.4519  0.5112  0.5706                                (-*-)
---------+---------+---------+---------+
                                           -0.30      0.00      0.30      0.60
```

One-way ANOVA: Manuka honey 20+ UMF with catalsae ranged from 0.5 %-12.5% under anaerobic condition

```
Source    DF       SS      MS       F      P
Factor     7   68.0407  9.7201  163.77  0.000
Error   1609   95.4976  0.0594
Total   1616  163.5383
S = 0.2436   R-Sq = 41.61%   R-Sq(adj) = 41.35%
                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level          N    Mean   StDev  ---------+---------+---------+---------+
AC0.5-AN     147  0.6194  0.4010                                    (--*-)
AC1-AN       147  0.5475  0.3643                                (-*--)
AC2.5-AN     294  0.3530  0.2385                    (-*-)
AC5-AN       147  0.2514  0.1869             (--*-)
AC7.5-AN     294  0.1122  0.0556     (-*-)
AC10-AN      147  0.0715  0.0278  (-*--)
AC12.5-AN    147  0.0618  0.0229 (--*-)
P-anaerobic  294  0.5437  0.3188                                (-*-)
                                  ---------+---------+---------+---------+
                                        0.16      0.32      0.48      0.64
Pooled StDev = 0.2436
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
AC0.5-AN subtracted from:
               Lower   Center    Upper
AC1-AN       -0.1581  -0.0719   0.0143
AC2.5-AN     -0.3410  -0.2664  -0.1917
AC5-AN       -0.4542  -0.3680  -0.2818
AC7.5-AN     -0.5818  -0.5071  -0.4325
AC10-AN      -0.6340  -0.5478  -0.4616
AC12.5-AN    -0.6437  -0.5575  -0.4713
P-anaerobic  -0.1503  -0.0756  -0.0010
             --------+---------+---------+---------+-
AC1-AN                    (--*-)
AC2.5-AN             (-*--)
AC5-AN             (-*--)
AC7.5-AN        (--*-)
AC10-AN       (-*--)
AC12.5-AN     (-*--)
P-anaerobic               (-*-)
             --------+---------+---------+---------+-
                  -0.35      0.00      0.35      0.70
AC1-AN subtracted from:
               Lower   Center    Upper
AC2.5-AN     -0.2692  -0.1945  -0.1199
AC5-AN       -0.3823  -0.2961  -0.2099
AC7.5-AN     -0.5099  -0.4353  -0.3606
AC10-AN      -0.5622  -0.4760  -0.3898
AC12.5-AN    -0.5719  -0.4857  -0.3995
P-anaerobic  -0.0784  -0.0038   0.0709
             --------+---------+---------+---------+-
AC2.5-AN                (-*--)
AC5-AN                (--*-)
AC7.5-AN        (--*-)
AC10-AN        (-*--)
AC12.5-AN      (-*--)
P-anaerobic                 (-*-)
             --------+---------+---------+---------+-
                  -0.35      0.00      0.35      0.70
AC2.5-AN subtracted from:
               Lower   Center    Upper
AC5-AN       -0.1763  -0.1016  -0.0269
AC7.5-AN     -0.3017  -0.2407  -0.1798
AC10-AN      -0.3561  -0.2814  -0.2068
AC12.5-AN    -0.3658  -0.2911  -0.2165
P-anaerobic   0.1298   0.1908   0.2517
             --------+---------+---------+---------+-
AC5-AN                    (-*-)
AC7.5-AN               (-*-)
AC10-AN               (-*-)
AC12.5-AN             (-*-)
P-anaerobic                      (*-)
             --------+---------+---------+---------+-
                  -0.35      0.00      0.35      0.70
AC5-AN subtracted from:
               Lower   Center    Upper
AC7.5-AN     -0.2138  -0.1391  -0.0645
AC10-AN      -0.2660  -0.1798  -0.0936
AC12.5-AN    -0.2757  -0.1895  -0.1033
P-anaerobic   0.2177   0.2924   0.3670
             --------+---------+---------+---------+-
AC7.5-AN                 (-*-)
AC10-AN                (--*-)
AC12.5-AN              (--*-)
P-anaerobic                        (-*-)
             --------+---------+---------+---------+-
                  -0.35      0.00      0.35      0.70
AC7.5-AN subtracted from:
               Lower   Center   Upper  --------+---------+---------+---------+-
AC10-AN      -0.1153  -0.0407  0.0340                 (-*-)
AC12.5-AN    -0.1250  -0.0504  0.0243                (--*-)
P-anaerobic   0.3706   0.4315  0.4925                              (*-)
                                       --------+---------+---------+---------+-
                                            -0.35      0.00      0.35      0.70
AC10-AN subtracted from:
               Lower   Center   Upper  --------+---------+---------+---------+-
AC12.5-AN    -0.0959  -0.0097  0.0765                 (--*-)
P-anaerobic   0.3975   0.4722  0.5469                              (-*--)
                                       --------+---------+---------+---------+-
                                           -0.35      0.00      0.35      0.70
AC12.5-AN subtracted from:
              Lower  Center   Upper  --------+---------+---------+---------+-
P-anaerobic  0.4072  0.4819  0.5566                              (-*-)
--------+---------+---------+---------+-
                                          -0.35      0.00      0.35      0.70
```

One-way ANOVA: Manuka honey 15+ UMF ranged from 0.5 %-12.5% under anaerobic condition

```
Source    DF        SS      MS       F      P
Factor     7   52.9442  7.5635  187.12  0.000
Error   1609   65.0369  0.0404
Total   1616  117.9811
S = 0.2010   R-Sq = 44.88%   R-Sq(adj) = 44.64%
                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level          N    Mean   StDev  ---------+---------+---------+---------+
B0.5-AN      147  0.4229  0.2776                           (-*-)
B1-AN        147  0.4200  0.2703                           (-*-)
B2.5-AN      294  0.2875  0.1832                   (*-)
B5-AN        147  0.2053  0.1259             (-*-)
B7.5-AN      294  0.0901  0.0582     (-*-)
B10-AN       147  0.0507  0.0287  (-*--)
B12.5-AN     147  0.0536  0.0232  (--*-)
P-anaerobic  294  0.5437  0.3188                                    (*-)
                                  ---------+---------+---------+---------+
                                        0.15      0.30      0.45      0.60
Pooled StDev = 0.2010
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
B0.5-AN subtracted from:
               Lower   Center    Upper
B1-AN        -0.0741  -0.0029   0.0682
B2.5-AN      -0.1970  -0.1354  -0.0738
B5-AN        -0.2888  -0.2176  -0.1465
B7.5-AN      -0.3944  -0.3328  -0.2712
B10-AN       -0.4434  -0.3722  -0.3011
B12.5-AN     -0.4405  -0.3693  -0.2982
P-anaerobic   0.0592   0.1208   0.1824
             --------+---------+---------+---------+-
B1-AN                         (-*-)
B2.5-AN                   (-*--)
B5-AN                  (--*-)
B7.5-AN             (-*-)
B10-AN            (--*-)
B12.5-AN          (--*-)
P-anaerobic                       (-*-)
             --------+---------+---------+---------+-
                 -0.30      0.00      0.30      0.60
B1-AN subtracted from:
               Lower   Center    Upper
B2.5-AN      -0.1941  -0.1324  -0.0708
B5-AN        -0.2858  -0.2147  -0.1435
B7.5-AN      -0.3915  -0.3299  -0.2683
B10-AN       -0.4404  -0.3693  -0.2982
B12.5-AN     -0.4375  -0.3664  -0.2952
P-anaerobic   0.0622   0.1238   0.1854
             --------+---------+---------+---------+-
B2.5-AN                    (-*-)
B5-AN                  (--*-)
B7.5-AN              (-*-)
B10-AN             (--*-)
B12.5-AN           (--*-)
P-anaerobic                       (-*-)
             --------+---------+---------+---------+-
                 -0.30      0.00      0.30      0.60
B2.5-AN subtracted from:
               Lower   Center    Upper
B5-AN        -0.1438  -0.0822  -0.0206
B7.5-AN      -0.2477  -0.1974  -0.1471
B10-AN       -0.2985  -0.2369  -0.1753
B12.5-AN     -0.2955  -0.2339  -0.1723
P-anaerobic   0.2059   0.2562   0.3065
             --------+---------+---------+---------+-
B5-AN                      (-*-)
B7.5-AN                 (*-)
B10-AN                (-*-)
B12.5-AN              (-*-)
P-anaerobic                           (-*)
             --------+---------+---------+---------+-
                 -0.30      0.00      0.30      0.60
B5-AN subtracted from:
               Lower   Center    Upper
B7.5-AN      -0.1768  -0.1152  -0.0536
B10-AN       -0.2258  -0.1546  -0.0835
B12.5-AN     -0.2228  -0.1517  -0.0806
P-anaerobic   0.2768   0.3385   0.4001
             --------+---------+---------+---------+-
B7.5-AN                   (-*-)
B10-AN                  (--*-)
B12.5-AN                 (-*-)
P-anaerobic                             (-*-)
             --------+---------+---------+---------+-
                 -0.30      0.00      0.30      0.60
B7.5-AN subtracted from:
               Lower   Center   Upper  --------+---------+---------+---------+-
B10-AN       -0.1010  -0.0394  0.0222                (-*-)
B12.5-AN     -0.0981  -0.0365  0.0251                (-*-)
P-anaerobic   0.4033   0.4537  0.5040                               (-*-)
                                       --------+---------+---------+---------+-
                                           -0.30      0.00      0.30      0.60
B10-AN subtracted from:
               Lower  Center   Upper  --------+---------+---------+---------+-
B12.5-AN     -0.0682  0.0029  0.0741                (-*-)
P-anaerobic   0.4315  0.4931  0.5547                               (-*-)
                                      --------+---------+---------+---------+-
                                          -0.30      0.00      0.30      0.60
B12.5-AN subtracted from:
              Lower  Center   Upper  --------+---------+---------+---------+-
P-anaerobic  0.4285  0.4902  0.5518                               (-*-)
--------+---------+---------+---------+-
                                         -0.30      0.00      0.30      0.60
```

One-way ANOVA: Manuka honey 15+ UMF with catalase ranged from 0.5 %-12.5% under anaerobic condition

```
Source    DF        SS      MS       F      P
Factor     7   57.1652  8.1665  135.34  0.000
Error   1609   97.0882  0.0603
Total   1616  154.2534
S = 0.2456   R-Sq = 37.06%   R-Sq(adj) = 36.79%
                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level          N    Mean   StDev  --------+---------+---------+---------+-
BC0.5-AN     147  0.4972  0.3257                              (--*--)
BC1-AN       147  0.5287  0.3565                                 (-*--)
BC2.5-AN     294  0.4228  0.2993                          (-*-)
BC5-AN       147  0.2472  0.1770              (-*--)
BC7.5-AN     294  0.1370  0.0867       (-*-)
BC10-AN      147  0.0790  0.0309   (-*--)
BC12.5-AN    147  0.0682  0.0269  (--*-)
P-anaerobic  294  0.5437  0.3188                                  (-*-)
                                  --------+---------+---------+---------+-
                                        0.15      0.30      0.45      0.60
Pooled StDev = 0.2456
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
BC0.5-AN subtracted from:
               Lower  Center     Upper
BC1-AN       -0.0554   0.0315   0.1184
BC2.5-AN     -0.1496  -0.0744   0.0009
BC5-AN       -0.3369  -0.2499  -0.1630
BC7.5-AN     -0.4354  -0.3601  -0.2849
BC10-AN      -0.5051  -0.4182  -0.3313
BC12.5-AN    -0.5159  -0.4289  -0.3420
P-anaerobic  -0.0287   0.0466   0.1218
             --------+---------+---------+---------+-
BC1-AN                       (--*--)
BC2.5-AN                  (--*-)
BC5-AN              (--*--)
BC7.5-AN        (--*--)
BC10-AN       (--*--)
BC12.5-AN     (--*--)
P-anaerobic                   (--*-)
             --------+---------+---------+---------+-
                  -0.30      0.00      0.30      0.60
BC1-AN subtracted from:
               Lower  Center     Upper
BC2.5-AN     -0.1811  -0.1059  -0.0306
BC5-AN       -0.3684  -0.2814  -0.1945
BC7.5-AN     -0.4669  -0.3917  -0.3164
BC10-AN      -0.5366  -0.4497  -0.3628
BC12.5-AN    -0.5474  -0.4604  -0.3735
P-anaerobic  -0.0602   0.0151   0.0903
             --------+---------+---------+---------+-
BC2.5-AN                 (-*--)
BC5-AN             (--*--)
BC7.5-AN       (--*-)
BC10-AN      (--*--)
BC12.5-AN    (--*--)
P-anaerobic                 (--*-)
             --------+---------+---------+---------+-
                  -0.30      0.00      0.30      0.60
BC2.5-AN subtracted from:
               Lower  Center     Upper
BC5-AN       -0.2509  -0.1756  -0.1003
BC7.5-AN     -0.3473  -0.2858  -0.2243
BC10-AN      -0.4191  -0.3438  -0.2686
BC12.5-AN    -0.4299  -0.3546  -0.2793
P-anaerobic   0.0595   0.1209   0.1824
             --------+---------+---------+---------+-
BC5-AN                 (-*--)
BC7.5-AN           (-*--)
BC10-AN          (--*-)
BC12.5-AN        (-*--)
P-anaerobic                         (-*-)
             --------+---------+---------+---------+-
                  -0.30      0.00      0.30      0.60
BC5-AN subtracted from:
               Lower  Center     Upper
BC7.5-AN     -0.1855  -0.1102  -0.0349
BC10-AN      -0.2552  -0.1682  -0.0813
BC12.5-AN    -0.2659  -0.1790  -0.0921
P-anaerobic   0.2212   0.2965   0.3718
             --------+---------+---------+---------+-
BC7.5-AN                 (-*--)
BC10-AN               (--*--)
BC12.5-AN             (--*--)
P-anaerobic                              (--*-)
             --------+---------+---------+---------+-
                  -0.30      0.00      0.30      0.60
BC7.5-AN subtracted from:
               Lower   Center   Upper  --------+---------+---------+---------+-
BC10-AN      -0.1333  -0.0580  0.0172                 (-*--)
BC12.5-AN    -0.1441  -0.0688  0.0065               (--*-)
P-anaerobic   0.3453   0.4067  0.4682                                (-*-)
                                       --------+---------+---------+---------+-
                                            -0.30      0.00      0.30      0.60
BC10-AN subtracted from:
               Lower   Center   Upper  --------+---------+---------+---------+-
BC12.5-AN    -0.0977  -0.0108  0.0762                 (--*--)
P-anaerobic   0.3895   0.4647  0.5400                                 (-*--)
                                       --------+---------+---------+---------+-
                                            -0.30      0.00      0.30      0.60
BC12.5-AN subtracted from:
              Lower  Center   Upper  --------+---------+---------+---------+-
P-anaerobic  0.4002  0.4755  0.5508                                 (--*-)
--------+---------+---------+---------+-
                                          -0.30      0.00      0.30      0.60
```

One-way ANOVA: Manuka honey 10+ UMF ranged from 0.5 %-12.5% under anaerobic condition

```
Source     DF        SS      MS       F      P
Factor      7   41.0645  5.8664  157.68  0.000
Error    1609   59.8599  0.0372
Total    1616  100.9243
S = 0.1929   R-Sq = 40.69%   R-Sq(adj) = 40.43%
                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level           N    Mean   StDev  ---------+---------+---------+---------+
C0.5-AN       147  0.3411  0.2201                      (-*-)
C1-AN         147  0.2537  0.1703                 (-*-)
C2.5-AN       294  0.3029  0.2074                     (*-)
C5-AN         147  0.2448  0.1225                (-*-)
C7.5-AN       294  0.1442  0.1118           (-*)
C10-AN        147  0.0420  0.0318  (-*-)
C12.5-AN      147  0.0755  0.0337    (-*-)
P-anaerobic   294  0.5437  0.3188                                   (*-)
                                   ---------+---------+---------+---------+
                                         0.15      0.30      0.45      0.60
Pooled StDev = 0.1929
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
C0.5-AN subtracted from:
              Lower   Center    Upper
C1-AN       -0.1556  -0.0874  -0.0191
C2.5-AN     -0.0972  -0.0381   0.0210
C5-AN       -0.1645  -0.0962  -0.0280
C7.5-AN     -0.2559  -0.1968  -0.1377
C10-AN      -0.3673  -0.2991  -0.2309
C12.5-AN    -0.3338  -0.2655  -0.1973
P-anaerobic  0.1436   0.2027   0.2618
            ---------+---------+---------+---------+
C1-AN                     (-*-)
C2.5-AN                     (-*-)
C5-AN                     (-*-)
C7.5-AN               (-*-)
C10-AN             (-*-)
C12.5-AN            (-*-)
P-anaerobic                           (-*-)
             ---------+---------+---------+---------+
                    -0.30      0.00      0.30      0.60
C1-AN subtracted from:
              Lower   Center    Upper
C2.5-AN     -0.0098   0.0493   0.1084
C5-AN       -0.0771  -0.0089   0.0594
C7.5-AN     -0.1685  -0.1094  -0.0503
C10-AN      -0.2800  -0.2117  -0.1435
C12.5-AN    -0.2464  -0.1781  -0.1099
P-anaerobic  0.2310   0.2901   0.3492
             ---------+---------+---------+---------+
C2.5-AN                          (-*-)
C5-AN                         (--*-)
C7.5-AN                    (-*-)
C10-AN                  (-*-)
C12.5-AN                  (-*-)
P-anaerobic                               (-*-)
             ---------+---------+---------+---------+
                    -0.30      0.00      0.30      0.60
C2.5-AN subtracted from:
              Lower   Center    Upper
C5-AN       -0.1172  -0.0581   0.0010
C7.5-AN     -0.2070  -0.1587  -0.1104
C10-AN      -0.3201  -0.2610  -0.2019
C12.5-AN    -0.2865  -0.2274  -0.1683
P-anaerobic  0.1925   0.2408   0.2891
             ---------+---------+---------+---------+
C5-AN                       (-*-)
C7.5-AN                   (-*)
C10-AN                (-*-)
C12.5-AN               (-*-)
P-anaerobic                             (-*-)
             ---------+---------+---------+---------+
                    -0.30      0.00      0.30      0.60
C5-AN subtracted from:
              Lower   Center    Upper
C7.5-AN     -0.1597  -0.1006  -0.0415
C10-AN      -0.2711  -0.2029  -0.1346
C12.5-AN    -0.2375  -0.1693  -0.1010
P-anaerobic  0.2398   0.2989   0.3580
             ---------+---------+---------+---------+
C7.5-AN                      (-*-)
C10-AN                   (-*--)
C12.5-AN                  (-*--)
P-anaerobic                               (-*-)
             ---------+---------+---------+---------+
                    -0.30      0.00      0.30      0.60
C7.5-AN subtracted from:
              Lower   Center    Upper
C10-AN      -0.1614  -0.1023  -0.0432
C12.5-AN    -0.1278  -0.0687  -0.0096
P-anaerobic  0.3512   0.3995   0.4478
             ---------+---------+---------+---------+
C10-AN                       (-*-)
C12.5-AN                      (-*-)
P-anaerobic                                   (*-)
             ---------+---------+---------+---------+
                    -0.30      0.00      0.30      0.60
C10-AN subtracted from:
              Lower  Center   Upper  ---------+---------+---------+---------+
C12.5-AN    -0.0347  0.0336  0.1018                    (-*-)
P-anaerobic  0.4427  0.5018  0.5609                                   (-*-)
                                     ---------+---------+---------+---------+
                                            -0.30      0.00      0.30      0.60
C12.5-AN subtracted from:
             Lower  Center   Upper  ---------+---------+---------+---------+
P-anaerobic 0.4091  0.4682  0.5273                                   (-*-)
---------+---------+---------+---------+
                                           -0.30      0.00      0.30      0.60
```

One-way ANOVA: Manuka honey 10+ UMF + catalase ranged from 0.5 %-12.5% under anaerobic condition

```
Source    DF        SS      MS      F      P
Factor     7   48.2794  6.8971  91.33  0.000
Error   1609  121.5063  0.0755
Total   1616  169.7857
S = 0.2748   R-Sq = 28.44%   R-Sq(adj) = 28.12%
                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level          N    Mean   StDev  -------+---------+---------+---------+--
CC0.5-AN     147  0.5570  0.3578                               (--*--)
CC1-AN       147  0.5734  0.3742                                (--*--)
CC2.5-AN     294  0.5119  0.3390                             (-*-)
CC5-AN       147  0.3075  0.2051              (--*-)
CC7.5-AN     294  0.2642  0.1943            (-*-)
CC10-AN      147  0.1490  0.1042    (--*--)
CC12.5-AN    147  0.0843  0.0301  (--*--)
P-anaerobic  294  0.5437  0.3188                               (-*-)
                                  -------+---------+---------+---------+--
                                      0.15      0.30      0.45      0.60
Pooled StDev = 0.2748
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
CC0.5-AN subtracted from:
               Lower   Center    Upper
CC1-AN       -0.0808   0.0164   0.1137
CC2.5-AN     -0.1292  -0.0450   0.0392
CC5-AN       -0.3467  -0.2494  -0.1522
CC7.5-AN     -0.3770  -0.2928  -0.2085
CC10-AN      -0.5052  -0.4079  -0.3107
CC12.5-AN    -0.5699  -0.4726  -0.3754
P-anaerobic  -0.0974  -0.0132   0.0710
                ---------+---------+---------+---------
CC1-AN                           (---*--)
CC2.5-AN                        (-*--)
CC5-AN                  (---*--)
CC7.5-AN               (--*--)
CC10-AN            (--*---)
CC12.5-AN        (--*--)
P-anaerobic                      (--*-)
                ---------+---------+---------+---------
             -0.60     -0.30      0.00      0.30
CC1-AN subtracted from:
               Lower   Center    Upper
CC2.5-AN     -0.1457  -0.0615   0.0227
CC5-AN       -0.3631  -0.2659  -0.1686
CC7.5-AN     -0.3934  -0.3092  -0.2250
CC10-AN      -0.5216  -0.4244  -0.3271
CC12.5-AN    -0.5863  -0.4891  -0.3918
P-anaerobic  -0.1139  -0.0297   0.0546
                ---------+---------+---------+---------
CC2.5-AN                       (--*--)
CC5-AN                  (--*--)
CC7.5-AN               (--*--)
CC10-AN            (--*--)
CC12.5-AN       (---*--)
P-anaerobic                     (--*--)
                ---------+---------+---------+---------
             -0.60     -0.30      0.00      0.30
CC2.5-AN subtracted from:
               Lower   Center    Upper
CC5-AN       -0.2886  -0.2044  -0.1202
CC7.5-AN     -0.3165  -0.2477  -0.1790
CC10-AN      -0.4471  -0.3629  -0.2787
CC12.5-AN    -0.5118  -0.4276  -0.3434
P-anaerobic  -0.0369   0.0318   0.1006
                ---------+---------+---------+---------
CC5-AN                    (--*--)
CC7.5-AN                 (--*-)
CC10-AN              (--*--)
CC12.5-AN          (--*--)
P-anaerobic                          (-*-)
                ---------+---------+---------+---------
             -0.60     -0.30      0.00      0.30
CC5-AN subtracted from:
               Lower   Center    Upper
CC7.5-AN     -0.1275  -0.0433   0.0409
CC10-AN      -0.2558  -0.1585  -0.0613
CC12.5-AN    -0.3204  -0.2232  -0.1260
P-anaerobic   0.1520   0.2362   0.3204
                ---------+---------+---------+---------
CC7.5-AN                        (--*-)
CC10-AN                     (---*--)
CC12.5-AN                 (---*--)
P-anaerobic                                (--*--)
                ---------+---------+---------+---------
             -0.60     -0.30      0.00      0.30
CC7.5-AN subtracted from:
               Lower   Center    Upper
CC10-AN      -0.1994  -0.1152  -0.0310
CC12.5-AN    -0.2641  -0.1799  -0.0957
P-anaerobic   0.2108   0.2795   0.3483
                ---------+---------+---------+---------
CC10-AN                       (--*--)
CC12.5-AN                   (--*--)
P-anaerobic                                  (-*--)
                ---------+---------+---------+---------
             -0.60     -0.30      0.00      0.30
CC10-AN subtracted from:
               Lower   Center   Upper
CC12.5-AN    -0.1619  -0.0647  0.0325
P-anaerobic   0.3105   0.3947  0.4789
                ---------+---------+---------+---------
CC12.5-AN                        (--*--)
P-anaerobic                                     (--*--)
                ---------+---------+---------+---------
             -0.60     -0.30      0.00      0.30
CC12.5-AN subtracted from:
              Lower  Center   Upper
P-anaerobic  0.3752  0.4594  0.5436
                ---------+---------+---------+---------
P-anaerobic                                        (-*--)
                ---------+---------+---------+---------
             -0.60     -0.30      0.00      0.30
```

One-way ANOVA: Kanuka honey ranged from 0.5 %-12.5% under anaerobic condition

```
Source    DF        SS      MS       F      P
Factor     7   63.4071  9.0582  129.54  0.000
Error   1609  112.5093  0.0699
Total   1616  175.9164
S = 0.2644   R-Sq = 36.04%   R-Sq(adj) = 35.77%
                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level          N    Mean   StDev  --+---------+---------+---------+-------
K0.5-AN      147  0.4778  0.2789                             (--*--)
K1-AN        147  0.4478  0.2841                           (--*--)
K2.5-AN      294  0.4474  0.3061                            (-*-)
K5-AN        147  0.4802  0.3117                             (--*--)
K7.5-AN      294  0.1812  0.2475              (-*-)
K10-AN       147  0.0052  0.0055  (-*--)
K12.5-AN     147  0.0046  0.0054  (-*--)
P-anaerobic  294  0.5437  0.3188                                  (-*-)
                                  --+---------+---------+---------+-------
                                  0.00      0.16      0.32      0.48
Pooled StDev = 0.2644
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
K0.5-AN subtracted from:
               Lower   Center    Upper
K1-AN        -0.1236  -0.0301   0.0635
K2.5-AN      -0.1114  -0.0304   0.0506
K5-AN        -0.0912   0.0023   0.0959
K7.5-AN      -0.3777  -0.2966  -0.2156
K10-AN       -0.5662  -0.4726  -0.3791
K12.5-AN     -0.5668  -0.4732  -0.3796
P-anaerobic  -0.0151   0.0659   0.1470
             --------+---------+---------+---------+-
K1-AN                      (--*--)
K2.5-AN                     (-*-)
K5-AN                       (--*--)
K7.5-AN               (--*-)
K10-AN          (-*--)
K12.5-AN        (-*--)
P-anaerobic                    (-*-)
             --------+---------+---------+---------+-
                 -0.35      0.00      0.35      0.70
K1-AN subtracted from:
               Lower   Center    Upper
K2.5-AN      -0.0814  -0.0003   0.0807
K5-AN        -0.0612   0.0324   0.1260
K7.5-AN      -0.3476  -0.2666  -0.1855
K10-AN       -0.5361  -0.4426  -0.3490
K12.5-AN     -0.5367  -0.4431  -0.3496
P-anaerobic   0.0150   0.0960   0.1770
             --------+---------+---------+---------+-
K2.5-AN                      (-*-)
K5-AN                        (--*--)
K7.5-AN                (-*--)
K10-AN           (-*--)
K12.5-AN        (-*--)
P-anaerobic                   (--*-)
             --------+---------+---------+---------+-
                 -0.35      0.00      0.35      0.70
K2.5-AN subtracted from:
               Lower   Center    Upper
K5-AN        -0.0483   0.0327   0.1138
K7.5-AN      -0.3324  -0.2662  -0.2001
K10-AN       -0.5233  -0.4422  -0.3612
K12.5-AN     -0.5238  -0.4428  -0.3618
P-anaerobic   0.0302   0.0963   0.1625
             --------+---------+---------+---------+-
K5-AN                        (-*-)
K7.5-AN                 (*-)
K10-AN          (-*--)
K12.5-AN        (-*--)
P-anaerobic                    (-*-)
             --------+---------+---------+---------+-
                 -0.35      0.00      0.35      0.70
K5-AN subtracted from:
               Lower   Center    Upper
K7.5-AN      -0.3800  -0.2990  -0.2179
K10-AN       -0.5685  -0.4750  -0.3814
K12.5-AN     -0.5691  -0.4755  -0.3820
P-anaerobic  -0.0174   0.0636   0.1446
             --------+---------+---------+---------+-
K7.5-AN               (-*--)
K10-AN          (-*--)
K12.5-AN        (-*--)
P-anaerobic                   (-*-)
--------+---------+---------+---------+-
                 -0.35      0.00      0.35      0.70
K7.5-AN subtracted from:
               Lower   Center    Upper
K10-AN       -0.2570  -0.1760  -0.0950
K12.5-AN     -0.2576  -0.1766  -0.0955
P-anaerobic   0.2964   0.3626   0.4287
             --------+---------+---------+---------+-
K10-AN                    (-*-)
K12.5-AN                  (-*-)
P-anaerobic                          (-*-)
             --------+---------+---------+---------+-
                 -0.35      0.00      0.35      0.70
K10-AN subtracted from:
               Lower   Center   Upper  --------+---------+---------+---------+-
K12.5-AN     -0.0941  -0.0006  0.0930              (--*--)
P-anaerobic   0.4575   0.5386  0.6196                             (-*--)
                                       --------+---------+---------+---------+-
                                           -0.35      0.00      0.35      0.70
K12.5-AN subtracted from:
              Lower  Center   Upper  --------+---------+---------+---------+-
P-anaerobic  0.4581  0.5391  0.6201                               (-*--)
--------+---------+---------+---------+-
                                         -0.35      0.00      0.35      0.70
```

One-way ANOVA: Kanuka honey + catalase ranged from 0.5 %-12.5% under anaerobic condition

```
Source    DF       SS     MS     F      P
Factor     7    6.777  0.968  7.11  0.000
Error   1609  218.953  0.136
Total   1616  225.730
S = 0.3689   R-Sq = 3.00%   R-Sq(adj) = 2.58%
                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level          N    Mean   StDev  --------+---------+---------+---------+-
KC0.5-AN     147  0.5964  0.3655                (-------*------)
KC1-AN       147  0.6324  0.3959                     (------*-------)
KC2.5-AN     294  0.6719  0.4021                            (----*----)
KC5-AN       147  0.6145  0.3837                  (-------*------)
KC7.5-AN     294  0.5046  0.3571       (----*----)
KC10-AN      147  0.5589  0.3935           (-------*------)
KC12.5-AN    147  0.4755  0.3506  (------*-------)
P-anaerobic  294  0.5437  0.3188            (----*----)
                                  --------+---------+---------+---------+-
                                        0.480     0.560     0.640     0.720
Pooled StDev = 0.3689
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.75%
KC0.5-AN subtracted from:
               Lower   Center   Upper
KC1-AN       -0.0944   0.0361  0.1666
KC2.5-AN     -0.0375   0.0755  0.1885
KC5-AN       -0.1124   0.0181  0.1486
KC7.5-AN     -0.2048  -0.0917  0.0213
KC10-AN      -0.1680  -0.0375  0.0930
KC12.5-AN    -0.2514  -0.1209  0.0097
P-anaerobic  -0.1657  -0.0526  0.0604

---------+---------+---------+---------+
KC1-AN                    (-------*-------)
KC2.5-AN                      (------*------)
KC5-AN                   (-------*-------)
KC7.5-AN            (------*------)
KC10-AN               (--------*-------)
KC12.5-AN        (-------*--------)
P-anaerobic             (------*------)
             ---------+---------+---------+---------+
KC7.5-AN           (------*------)
KC10-AN              (--------*-------)
KC12.5-AN       (-------*-------)
P-anaerobic          (------*------)
             ---------+---------+---------+---------+
                    -0.16      0.00      0.16      0.32
KC7.5-AN subtracted from:
               Lower   Center   Upper
KC10-AN      -0.0588   0.0542  0.1673
KC12.5-AN    -0.1422  -0.0291  0.0839
P-anaerobic  -0.0532   0.0391  0.1314
             ---------+---------+---------+---------+
KC10-AN                     (------*------)
KC12.5-AN              (------*------)
P-anaerobic                   (----*-----)
             ---------+---------+---------+---------+
                    -0.16      0.00      0.16      0.32

                    -0.16      0.00      0.16      0.32

KC1-AN subtracted from:
               Lower   Center    Upper
KC2.5-AN     -0.0736   0.0394   0.1525
KC5-AN       -0.1485  -0.0180   0.1126
KC7.5-AN     -0.2409  -0.1278  -0.0148
KC10-AN      -0.2041  -0.0736   0.0569
KC12.5-AN    -0.2875  -0.1569  -0.0264
P-anaerobic  -0.2017  -0.0887   0.0243
             ---------+---------+---------+---------+
KC2.5-AN                   (------*-------)
KC5-AN                 (-------*-------)
KC7.5-AN          (------*------)
KC10-AN             (-------*--------)
KC12.5-AN      (-------*-------)
P-anaerobic         (------*-------)
             ---------+---------+---------+---------+
                    -0.16      0.00      0.16      0.32
KC2.5-AN subtracted from:
               Lower   Center    Upper
KC5-AN       -0.1704  -0.0574   0.0556
KC7.5-AN     -0.2595  -0.1673  -0.0750
KC10-AN      -0.2261  -0.1130   0.0000
KC12.5-AN    -0.3094  -0.1964  -0.0833
P-anaerobic  -0.2204  -0.1281  -0.0358
             ---------+---------+---------+---------+
KC5-AN                (------*------)
KC7.5-AN         (-----*----)
KC10-AN            (------*------)
KC12.5-AN      (------*------)
P-anaerobic        (-----*-----)
             ---------+---------+---------+---------+
                    -0.16      0.00      0.16      0.32
KC5-AN subtracted from:
               Lower   Center    Upper
KC7.5-AN     -0.2229  -0.1099   0.0032
KC10-AN      -0.1862  -0.0556   0.0749
KC12.5-AN    -0.2695  -0.1390  -0.0085
P-anaerobic  -0.1838  -0.0707   0.0423
             ---------+---------+---------+---------+
KC10-AN subtracted from:
               Lower   Center   Upper
KC12.5-AN    -0.2139  -0.0834  0.0472
P-anaerobic  -0.1281  -0.0151  0.0979
             ---------+---------+---------+---------+
KC12.5-AN           (-------*-------)
P-anaerobic              (------*------)
---------+---------+---------+---------+
                    -0.16      0.00      0.16      0.32
KC12.5-AN subtracted from:
               Lower  Center   Upper  ---------+---------+---------+---------+
P-anaerobic  -0.0448  0.0682  0.1813                (------*------)
                                      ---------+---------+---------+---------+
                                             -0.16      0.00      0.16      0.32
```

One-way ANOVA: artificial honey with and without catalase ranged from 0.5 %-12.5% under aerobic condition

```
Source    DF       SS     MS      F      P
Factor     6    9.054  1.509  14.40  0.000
Error   2051  214.935  0.105
Total   2057  223.989
S = 0.3237   R-Sq = 4.04%   R-Sq(adj) = 3.76%
                                     Individual 95% CIs For Mean Based on
                                     Pooled StDev
Level              N    Mean   StDev  -+---------+---------+---------+--------
P-aerobic        294  0.5110  0.3024                     (----*---)
7.5ar_aero       294  0.4395  0.2668            (----*----)
7.5ar+cat_aer    294  0.5145  0.3843                      (---*----)
10ar_aero        294  0.4694  0.2797                (----*---)
10ar+cat_aero    294  0.5717  0.3988                             (---*----)
12.5ar_aerobic   294  0.3484  0.1956   (----*---)
12.5ar+cat_aero  294  0.5145  0.3843                      (---*----)
                                      -+---------+---------+---------+--------
                                     0.320     0.400     0.480     0.560
Pooled StDev = 0.3237
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.68%
P-aerobic subtracted from:
                   Lower   Center    Upper
7.5ar_aero       -0.1502  -0.0714   0.0073
7.5ar+cat_aer    -0.0751   0.0036   0.0823
10ar_aero        -0.1203  -0.0416   0.0372
10ar+cat_aero    -0.0180   0.0607   0.1394
12.5ar_aerobic   -0.2412  -0.1625  -0.0838
12.5ar+cat_aero  -0.0751   0.0036   0.0823
                 ---------+---------+---------+---------+
7.5ar_aero                 (----*---)
7.5ar+cat_aer                  (----*----)
10ar_aero                   (----*----)
10ar+cat_aero                     (----*----)
12.5ar_aerobic        (----*----)
12.5ar+cat_aero                (----*----)
                 ---------+---------+---------+---------+
                      -0.16      0.00      0.16      0.32
7.5ar_aero subtracted from:
                   Lower   Center    Upper
7.5ar+cat_aer    -0.0037   0.0750   0.1538
10ar_aero        -0.0489   0.0299   0.1086
10ar+cat_aero     0.0534   0.1321   0.2109
12.5ar_aerobic   -0.1698  -0.0911  -0.0124
12.5ar+cat_aero  -0.0037   0.0750   0.1538
                 ---------+---------+---------+---------+
7.5ar+cat_aer                       (----*----)
10ar_aero                        (----*----)
10ar+cat_aero                          (----*----)
12.5ar_aerobic              (----*----)
12.5ar+cat_aero                     (----*----)
                 ---------+---------+---------+---------+
                      -0.16      0.00      0.16      0.32
7.5ar+cat_aer subtracted from:
                   Lower   Center    Upper
10ar_aero        -0.1239  -0.0452   0.0336
10ar+cat_aero    -0.0216   0.0571   0.1358
12.5ar_aerobic   -0.2448  -0.1661  -0.0874
12.5ar+cat_aero  -0.0787   0.0000   0.0787
                 ---------+---------+---------+---------+
10ar_aero                   (----*----)
10ar+cat_aero                      (----*---)
12.5ar_aerobic        (----*----)
12.5ar+cat_aero                (----*----)
                 ---------+---------+---------+---------+
                      -0.16      0.00      0.16      0.32
10ar_aero subtracted from:
                   Lower   Center    Upper
10ar+cat_aero     0.0235   0.1023   0.1810
12.5ar_aerobic   -0.1997  -0.1209  -0.0422
12.5ar+cat_aero  -0.0336   0.0452   0.1239

                 ---------+---------+---------+---------+
10ar+cat_aero                        (----*----)
12.5ar_aerobic           (---*----)
12.5ar+cat_aero                   (----*----)
                 ---------+---------+---------+---------+
                      -0.16      0.00      0.16      0.32
10ar+cat_aero subtracted from:
                   Lower   Center    Upper
12.5ar_aerobic   -0.3020  -0.2232  -0.1445
12.5ar+cat_aero  -0.1358  -0.0571   0.0216
                 ---------+---------+---------+---------+
12.5ar_aerobic   (----*----)
12.5ar+cat_aero             (---*----)
                 ---------+---------+---------+---------+
                      -0.16      0.00      0.16      0.32
12.5ar_aerobic subtracted from:
                  Lower  Center   Upper
12.5ar+cat_aero  0.0874  0.1661  0.2448

---------+---------+---------+---------+
12.5ar+cat_aero                         (----*----)
                 ---------+---------+---------+---------+
                      -0.16      0.00      0.16      0.32
```

One-way ANOVA: artificial honey with and without catalase ranged from 0.5 %-12.5% under anaerobic condition

```
Source    DF       SS     MS      F      P
Factor     5    6.140  1.228  11.83  0.000
Error   1170  121.433  0.104
Total   1175  127.574

S = 0.3222   R-Sq = 4.81%   R-Sq(adj) = 4.41%

                                     Individual 95% CIs For Mean Based on
                                     Pooled StDev
Level           N    Mean   StDev   --------+---------+---------+---------+-
P-anaerobic   294  0.5437  0.3188                     (--*---)
7.5ar+cat_an  294  0.6038  0.3666                           (--*---)
10ar_an       147  0.5822  0.3340                       (----*----)
10ar+cat_an   147  0.5822  0.3340                       (----*----)
12.5ar_am     147  0.3706  0.2070   (----*----)
12.5ar+cat_an 147  0.5032  0.3037                (----*-----)
                                    --------+---------+---------+---------+-
                                          0.40      0.50      0.60      0.70
```

Pooled StDev = 0.3222

Tukey 95% Simultaneous Confidence Intervals

All Pairwise Comparisons

Individual confidence level = 99.55%

```
P-anaerobic subtracted from:

                 Lower   Center    Upper
7.5ar+cat_an   -0.0157   0.0600   0.1358
10ar_an        -0.0543   0.0384   0.1312
10ar+cat_an    -0.0543   0.0384   0.1312
12.5ar_am      -0.2659  -0.1732  -0.0804
12.5ar+cat_an  -0.1333  -0.0406   0.0521

               ------+---------+---------+---------+---
7.5ar+cat_an                  (---*---)
10ar_an                     (----*----)
10ar+cat_an                 (----*----)
12.5ar_am         (---*----)
12.5ar+cat_an              (----*----)
               ------+---------+---------+---------+---
                  -0.20      0.00      0.20      0.40

7.5ar+cat_an subtracted from:

                 Lower   Center    Upper
10ar_an        -0.1143  -0.0216   0.0711
10ar+cat_an    -0.1143  -0.0216   0.0711
12.5ar_am      -0.3260  -0.2332  -0.1405
12.5ar+cat_an  -0.1934  -0.1006  -0.0079

               ------+---------+---------+---------+---
10ar_an                   (----*----)
10ar+cat_an               (----*----)
12.5ar_am      (---*----)
12.5ar+cat_an         (----*----)
               ------+---------+---------+---------+---
                  -0.20      0.00      0.20      0.40

10ar_an subtracted from:

                 Lower   Center    Upper
10ar+cat_an    -0.1071   0.0000   0.1071
12.5ar_am      -0.3187  -0.2116  -0.1045
12.5ar+cat_an  -0.1861  -0.0790   0.0281

               ------+---------+---------+---------+---
10ar+cat_an                (----*----)
12.5ar_am      (----*-----)
12.5ar+cat_an         (----*----)
               ------+---------+---------+---------+---
                  -0.20      0.00      0.20      0.40

10ar+cat_an subtracted from:

                 Lower   Center    Upper
12.5ar_am      -0.3187  -0.2116  -0.1045
12.5ar+cat_an  -0.1861  -0.0790   0.0281

               ------+---------+---------+---------+---
12.5ar_am      (----*-----)
12.5ar+cat_an         (----*----)
               ------+---------+---------+---------+---
                  -0.20      0.00      0.20      0.40

12.5ar_am subtracted from:

Lower  Center   Upper  ------+---------+---------+---------+---
12.5ar+cat_an  0.0255  0.1326  0.2397        (-----*----)
                       ------+---------+---------+---------+---
                          -0.20      0.00      0.20      0.40
```

B.3 Percentage of inhibition of *P. acnes* in the honeys and determination of MIC of honeys

B3-1 Manuka honey 20+ UMF under aerobic condition

Table B3-1 Percentage of inhibition of *P. acnes* in the Comvita Manuka honey 20+ UMF honey solutions ranged from 0.5% to 12.5% under aerobic condition.

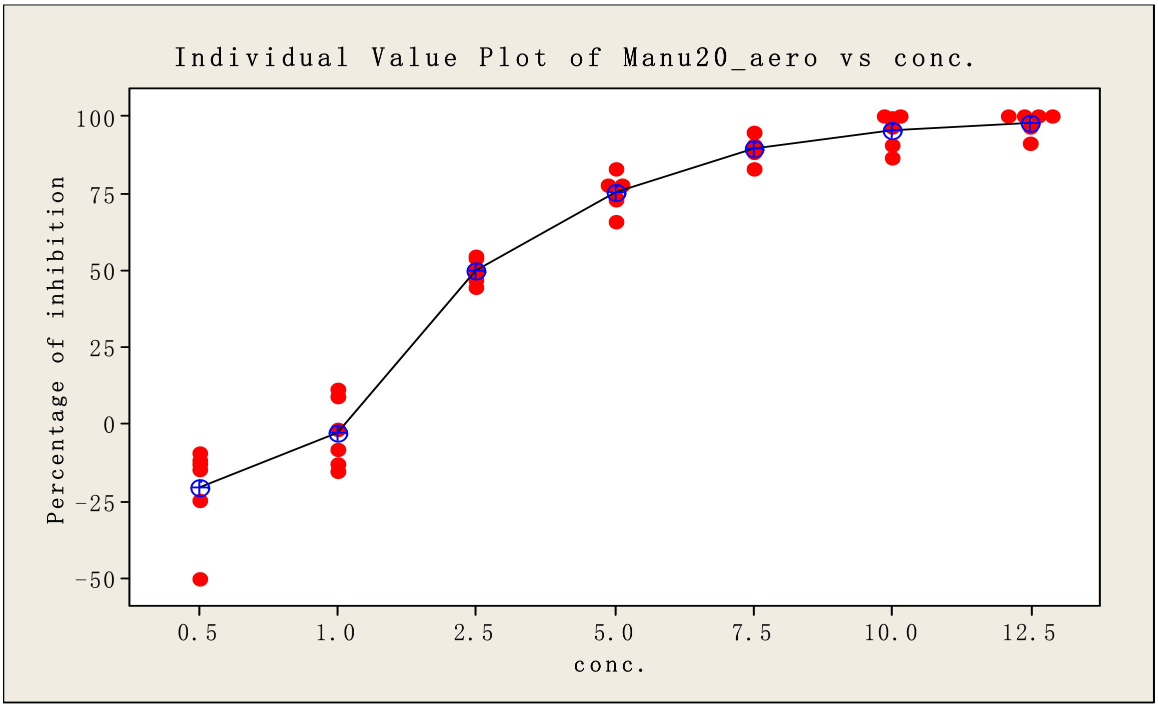
Samples	Concentration		Replications						Average	SEM
	%	mg/ml	1	2	3	4	5	6		
A0.5-aerobic	0.5	5	-14.93	-50.27	-9.75	-24.89	-13.35	-11.99	-20.86	13.99
A1-aerobic	1	10	11.04	-8.53	-15.54	8.78	-1.81	-13.12	-3.20	10.23
A2.5-aerobic	2.5	25	50.11	54.10	44.37	48.87	54.52	46.99	49.83	3.63
A5-aerobic	5	50	83.08	77.38	66.02	72.85	73.64	77.38	75.06	5.22
A7.5-aerobic	7.5	75	83.08	90.79	90.34	89.82	88.21	94.75	89.50	3.49
A10-aerobic	10	100	100.00	96.81	86.65	100.00	90.52	99.64	95.60	5.20
A12.5-aerobic	12.5	125	100.00	100.00	91.29	100.00	96.54	100.00	97.97	3.24

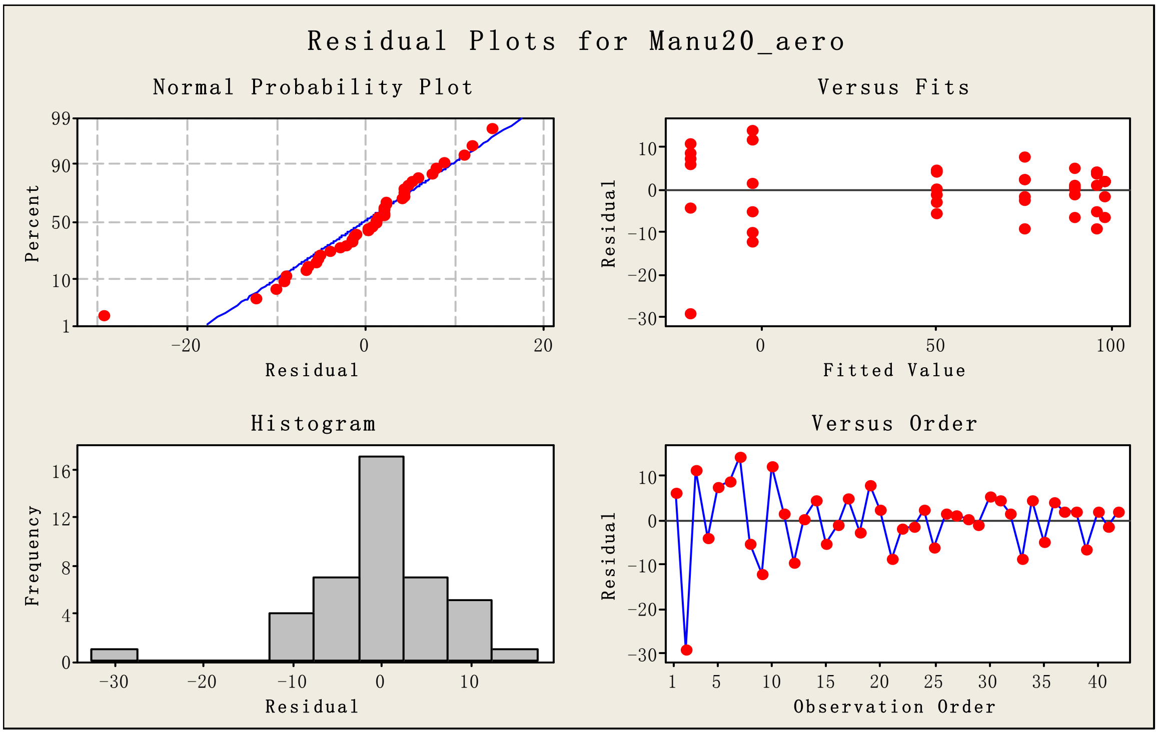
One-way ANOVA: Manu20_aero versus conc.

```
Source  DF       SS       MS       F      P
conc.    6  85538.4  14256.4  212.91  0.000
Error   35   2343.6     67.0
Total   41  87882.0
S = 8.183   R-Sq = 97.33%   R-Sq(adj) = 96.88%
                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 0.5   6  -20.86  15.33  (-*-)
 1.0   6   -3.20  11.20       (-*-)
 2.5   6   49.83   3.98                      (-*-)
 5.0   6   75.06   5.72                              (*-)
 7.5   6   89.50   3.82                                  (-*-)
10.0   6   95.60   5.70                                   (-*-)
12.5   6   97.97   3.55                                    (-*-)
                         --------+---------+---------+---------+-
                                 0        35        70       105
```

Pooled StDev = 8.18
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of conc.
Individual confidence level = 99.64%

```
conc. =  0.5 subtracted from:
conc.    Lower  Center   Upper  -------+---------+---------+---------+--
 1.0      2.90   17.67   32.43                  (--*-)
 2.5     55.92   70.69   85.46                          (-*-)
 5.0     81.15   95.92  110.69                              (-*-)
 7.5     95.60  110.36  125.13                                (-*-)
10.0    101.70  116.47  131.23                                 (-*-)
12.5    104.07  118.83  133.60                                 (-*-)
                                -------+---------+---------+---------+--
                                     -70         0        70       140

conc. =  1.0 subtracted from:
conc.  Lower  Center   Upper  -------+---------+---------+---------+--
 2.5   38.26   53.02   67.79                      (--*-)
 5.0   63.49   78.25   93.02                              (-*-)
 7.5   77.93   92.70  107.46                                (-*-)
10.0   84.04   98.80  113.57                                 (-*-)
12.5   86.40  101.17  115.93                                 (-*--)
                              -------+---------+---------+---------+--
                                   -70         0        70       140

conc. =  2.5 subtracted from:
conc.  Lower  Center  Upper  -------+---------+---------+---------+--
 5.0   10.46   25.23  40.00                (--*-)
 7.5   24.91   39.67  54.44                   (-*-)
10.0   31.01   45.78  60.54                   (--*-)
12.5   33.38   48.14  62.91                    (-*-)
                             -------+---------+---------+---------+--
                                  -70         0        70       140

conc. =  5.0 subtracted from:
conc.  Lower  Center  Upper  -------+---------+---------+---------+--
 7.5   -0.32   14.44  29.21              (-*-)
10.0    5.78   20.55  35.31               (-*-)
12.5    8.15   22.91  37.68               (-*-)
                             -------+---------+---------+---------+--
                                  -70         0        70       140

conc. =  7.5 subtracted from:
conc.  Lower  Center  Upper  -------+---------+---------+---------+--
10.0   -8.66    6.10  20.87             (-*-)
12.5   -6.29    8.47  23.24             (-*-)
                             -------+---------+---------+---------+--
                                -70         0        70       140

conc. = 10.0 subtracted from:
conc.   Lower  Center  Upper  -------+---------+---------+---------+--
12.5   -12.40    2.37  17.13             (-*-)
-------+---------+---------+---------+--
                                   -70         0        70       140
```


Individual Value Plot of Manu20_aero vs conc.
Percentage of inhibition
100
75
50
25
0
-25
-50
0.5
1.0
2.5
5.0
7.5
10.0
12.5
conc.


Residual Plots for Manu20_aero
Normal Probability Plot
Percent
99
90
50
10
1
-20
0
20
Residual
Versus Fits
Residual
10
0
-10
-20
-30
0
50
100
Fitted Value
Histogram
Frequency
16
12
8
4
0
-30
-20
-10
0
10
Residual
Versus Order
Residual
10
0
-10
-20
-30
1
5
10
15
20
25
30
35
40
Observation Order

Polynomial Regression Analysis: Manu20_aero versus conc.

The regression equation is

Manu20_aero = - 40.70 + 44.42 conc. - 4.963 conc.**2 + 0.1845 conc.**3

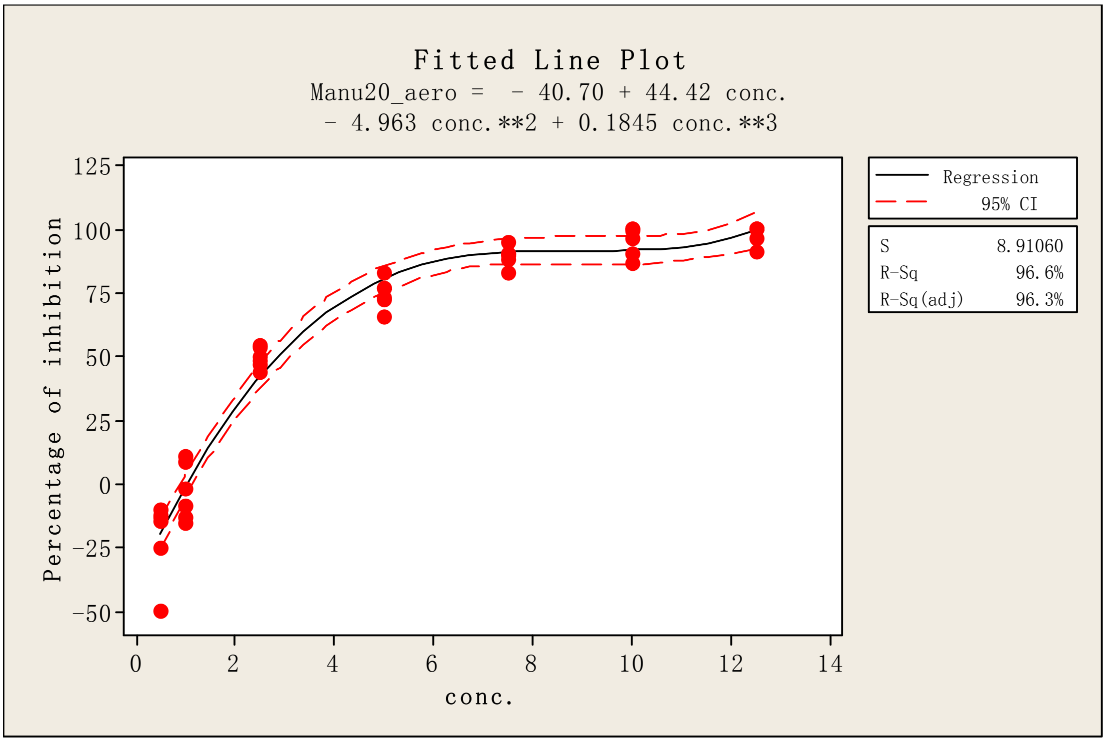
S = 8.91060 R-Sq = 96.6% R-Sq(adj) = 96.3%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	84864.8	28288.3	356.28	0.000
Error	38	3017.2	79.4		
Total	41	87882.0			

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	67570.9	133.07	0.000
Quadratic	1	14822.1	105.31	0.000
Cubic	1	2471.9	31.13	0.000



Base on the regression equation, the MIC therefore should be

MIC	%	Mg/ml
MIC_{50}	2.857	28.56
MIC_{90}	6.849	68.49
MIC_{100}	12.581	125.81

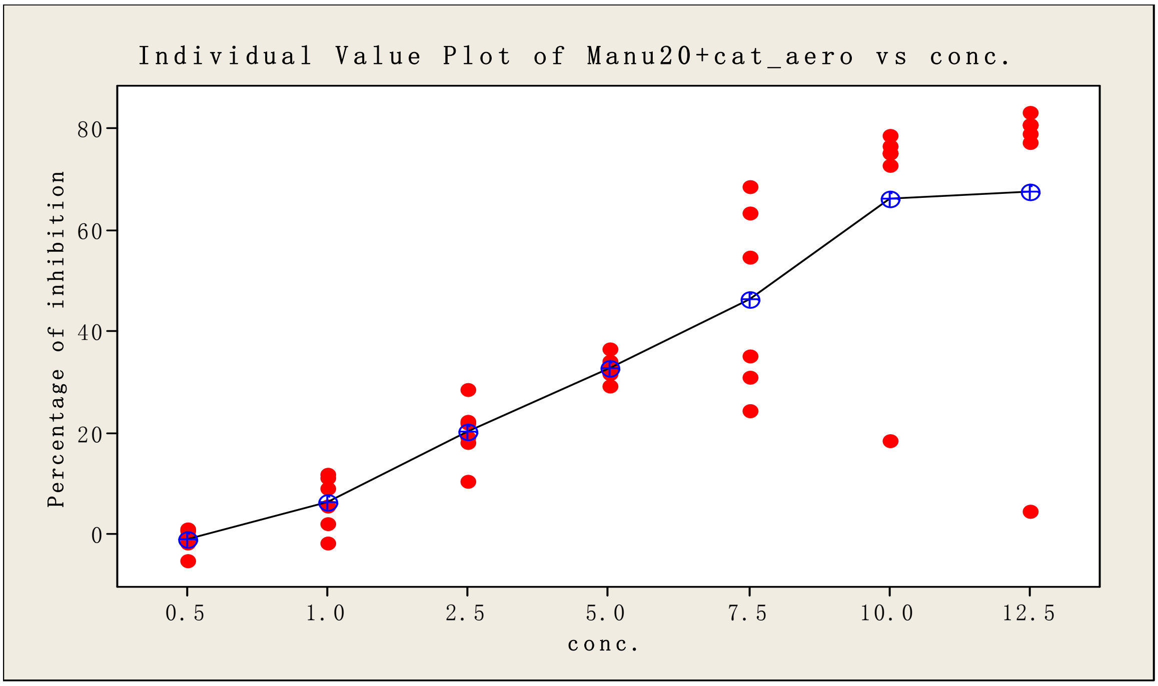
B3-2 Manuka honey 20+ UMF+catalase under aerobic condition

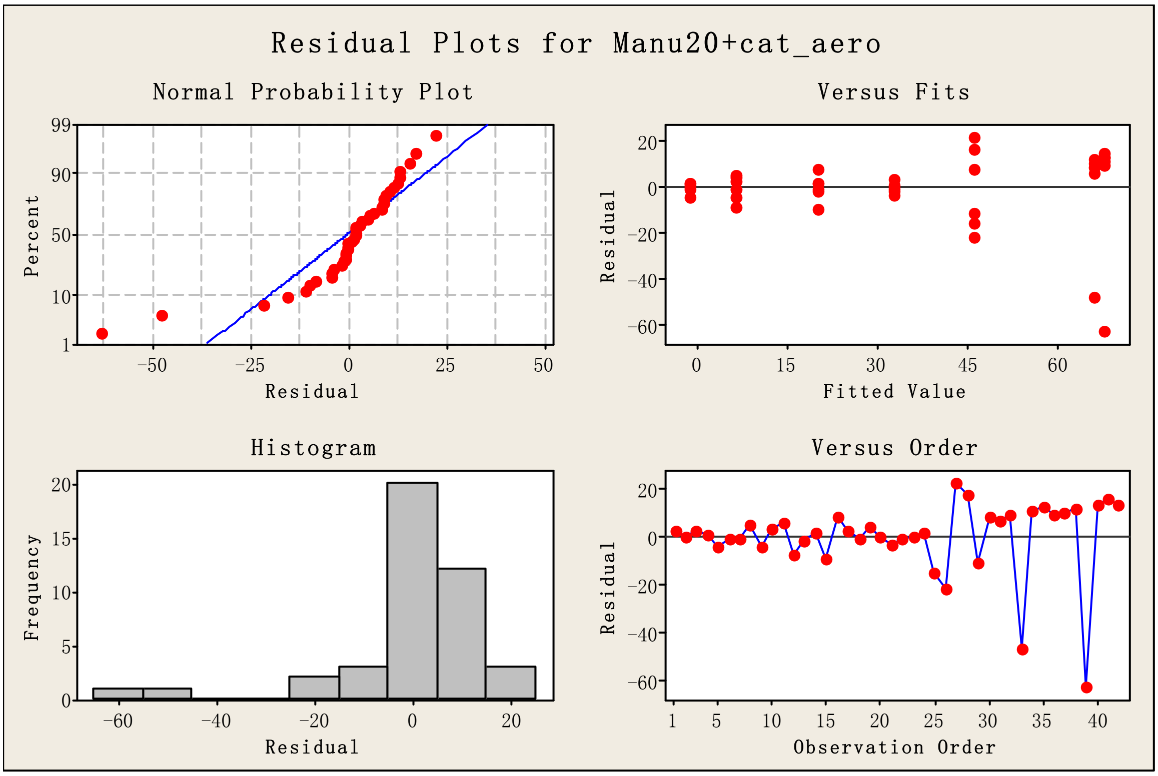
TableB3-2: Percentage of inhibition of *P. acnes* in the Comvita Manuka honey 20+ UMF honey solutions ranged from 0.5% to 12.5% with catalase under aerobic condition.

Samples	Concentration		Replications						Average	SEM
	%	mg/ml	1	2	3	4	5	6		
AC0.5-aerobic	0.5	5	0.75	-1.27	0.84	-0.45	-5.20	-1.81	-1.19	2.04
AC1-AERO	1	10	5.43	11.22	2.08	9.05	11.76	-1.81	6.29	4.93
AC2.5-aerobic	2.5	25	18.17	21.95	10.27	28.48	22.17	19.23	20.05	5.46
AC5-aerobic	5	50	36.56	32.78	29.30	31.65	32.58	34.12	32.83	2.22
AC7.5-aerobic	7.5	75	30.95	24.48	68.53	63.24	35.11	54.52	35.37	26.08
AC10-aerobic	10	100	72.85	75.20	18.48	76.63	78.55	75.00	66.12	21.37
AC12.5-aerobic	12.5	125	77.26	79.14	4.52	80.84	83.10	80.84	67.62	28.27

One-way ANOVA: Manu20+cat_aero versus conc.

```
Source  DF     SS    MS      F      P
conc.    6  27069  4512  16.45  0.000
Error   35   9601   274
Total   41  36671
S = 16.56   R-Sq = 73.82%   R-Sq(adj) = 69.33%
                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
 0.5   6   -1.19   2.23  (-----*----)
 1.0   6    6.29   5.40     (-----*----)
 2.5   6   20.05   5.98           (----*-----)
 5.0   6   32.83   2.43                (----*-----)
 7.5   6   46.14  18.36                     (----*-----)
10.0   6   66.12  23.41                             (----*-----)
12.5   6   67.62  30.97                              (----*-----)
                         ------+---------+---------+---------+---
                               0        25        50        75
Pooled StDev = 16.56
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of conc.
Individual confidence level = 99.64%
conc. =  0.5 subtracted from:
conc.   Lower  Center  Upper  --------+---------+---------+---------+-
 1.0   -22.41    7.48  37.37                (----*-----)
 2.5    -8.65   21.24  51.12                  (-----*-----)
 5.0     4.14   34.02  63.91                     (-----*-----)
 7.5    17.44   47.33  77.22                       (-----*-----)
10.0    37.42   67.31  97.20                           (-----*-----)
12.5    38.92   68.81  98.70                            (-----*-----)
                              --------+---------+---------+---------+-
                                    -50         0        50       100
conc. =  1.0 subtracted from:
conc.   Lower  Center  Upper  --------+---------+---------+---------+-
 2.5   -16.13   13.76  43.64                 (-----*-----)
 5.0    -3.34   26.54  56.43                     (-----*-----)
 7.5     9.96   39.85  69.74                        (-----*-----)
10.0    29.94   59.83  89.72                            (-----*-----)
12.5    31.44   61.33  91.21                            (-----*-----)
                              --------+---------+---------+---------+-
                                    -50         0        50       100
conc. =  2.5 subtracted from:
conc.   Lower  Center  Upper  --------+---------+---------+---------+-
 5.0   -17.10   12.79  42.67                   (-----*-----)
 7.5    -3.79   26.09  55.98                     (-----*-----)
10.0    16.19   46.07  75.96                         (-----*-----)
12.5    17.68   47.57  77.46                          (-----*----)
                              --------+---------+---------+---------+-
                                    -50         0        50       100
conc. =  5.0 subtracted from:
conc.   Lower  Center  Upper  --------+---------+---------+---------+-
 7.5   -16.58   13.31  43.19                   (-----*-----)
10.0     3.40   33.29  63.17                       (-----*-----)
12.5     4.90   34.79  64.67                       (-----*-----)
                              --------+---------+---------+---------+-
                                    -50         0        50       100
conc. =  7.5 subtracted from:
conc.   Lower  Center  Upper  --------+---------+---------+---------+-
10.0    -9.91   19.98  49.87                   (-----*-----)
12.5    -8.41   21.48  51.36                   (-----*-----)
                              --------+---------+---------+---------+-
                                    -50         0        50       100
conc. = 10.0 subtracted from:
conc.   Lower  Center  Upper  --------+---------+---------+---------+-
12.5   -28.39    1.50  31.38                (-----*-----)
                              --------+---------+---------+---------+-
-50           0         50         100
```


Individual Value Plot of Manu20+cat_aero vs conc.
Percentage of inhibition
80
60
40
20
0
0.5
1.0
2.5
5.0
7.5
10.0
12.5
conc.


Residual Plots for Manu20+cat_aero
Normal Probability Plot
Percent
99
90
50
10
1
-50
-25
0
25
50
Residual
Versus Fits
Residual
20
0
-20
-40
-60
0
15
30
45
60
Fitted Value
Histogram
Frequency
20
15
10
5
0
-60
-40
-20
0
20
Residual
Versus Order
Residual
20
0
-20
-40
-60
1
5
10
15
20
25
30
35
40
Observation Order

Polynomial Regression Analysis: Manu20+cat_aero versus conc.

The regression equation is

Manu20+cat_aero = - 6.390 + 11.03 conc. - 0.3106 conc.**2

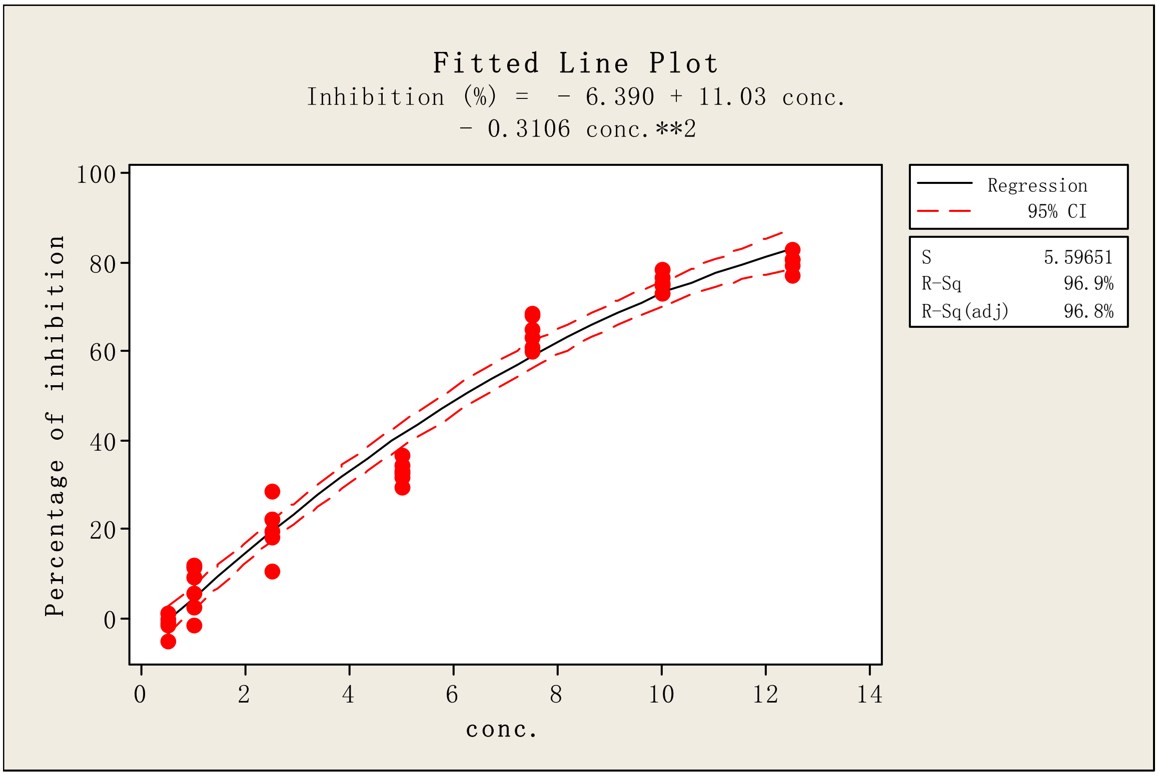
S = 5.59651   R-Sq = 96.9%   R-Sq(adj) = 96.8%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	2	36816.3	18408.1	587.73	0.000
Error	37	1158.9	31.3		
Total	39	37975.2			

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	36091.6	728.14	0.000
Quadratic	1	724.7	23.14	0.000



MIC	%	mg/ml
MIC 50	6.1921	61.92
MIC 90	15.5335	155.34
MIC 100	17.8330	178.33

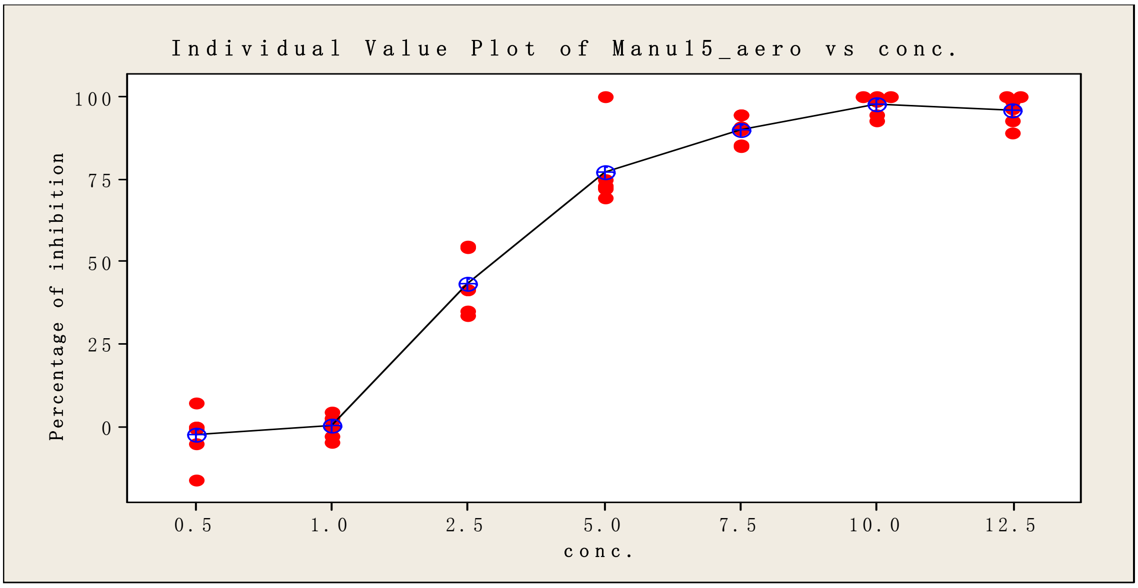
B3-3 Manuka honey 15+ UMF under aerobic condition

Table 3: Percentage of inhibition of *P. acnes* in the Comvita Manuka honey 15+ UMF honey solutions ranged from 0.5% to 12.5% under aerobic condition.

Samples	Concentration		Replications						Average	SEM
	%	mg/ml	1	2	3	4	5	6		
B0.5-aerobic	0.5	5	-16.31	6.97	-0.50	-0.68	-5.20	-0.45	-2.70	7.06
B1-AERO	1	10	4.37	1.61	-4.93	-0.45	-3.17	2.26	-0.05	3.20
B2.5-aerobic	2.5	25	41.27	33.62	34.86	41.58	54.75	54.41	43.42	8.43
B5-aerobic	5	50	72.31	100.00	69.48	72.85	74.76	72.60	77.00	10.40
B7.5-aerobic	7.5	75	84.89	90.95	90.11	85.43	94.66	94.82	90.14	3.93
B10-aerobic	10	100	92.53	100.00	100.00	98.51	100.00	94.43	97.58	2.99
B12.5-aerobic	12.5	125	92.85	100.00	96.31	100.00	98.64	89.07	96.15	4.02

One-way ANOVA: Manu15_aero versus conc.

```
Source  DF       SS       MS       F      P
conc.    6  70078.9  11679.8  243.42  0.000
Error   35   1679.4     48.0
Total   41  71758.3
S = 6.927   R-Sq = 97.66%   R-Sq(adj) = 97.26%
                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
 0.5   6   -2.70   7.73  (-*-)
 1.0   6   -0.05   3.50   (-*-)
 2.5   6   43.42   9.24                (*-)
 5.0   6   77.00  11.39                           (-*-)
 7.5   6   90.14   4.31                               (-*-)
10.0   6   97.58   3.28                                  (-*)
12.5   6   96.15   4.40                                 (-*-)
                         ---+---------+---------+---------+------
                            0        30        60        90

Pooled StDev = 6.93
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of conc.
Individual confidence level = 99.64%
conc. =  0.5 subtracted from:
conc.   Lower  Center   Upper  --------+---------+---------+---------+-
 1.0    -9.86    2.64   15.14                 (-*--)
 2.5    33.61   46.11   58.61                        (-*-)
 5.0    67.20   79.70   92.20                            (-*-)
 7.5    80.34   92.84  105.34                              (-*--)
10.0    87.78  100.28  112.77                                (-*-)
12.5    86.34   98.84  111.34                               (-*--)
                               --------+---------+---------+---------+-
                                     -60         0        60       120

conc. =  1.0 subtracted from:
conc.   Lower  Center   Upper  --------+---------+---------+---------+-
 2.5    30.97   43.47   55.97                       (-*-)
 5.0    64.55   77.05   89.55                            (-*-)
 7.5    77.70   90.20  102.70                              (-*-)
10.0    85.13   97.63  110.13                               (-*-)
12.5    83.70   96.20  108.70                               (-*-)
                               --------+---------+---------+---------+-
                                     -60         0        60       120

conc. =  2.5 subtracted from:
conc.   Lower  Center   Upper  --------+---------+---------+---------+-
 5.0    21.08   33.58   46.08                    (-*-)
 7.5    34.23   46.73   59.23                      (-*-)
10.0    41.66   54.16   66.66                       (-*-)
12.5    40.23   52.73   65.23                       (-*-)
                               --------+---------+---------+---------+-
                                     -60         0        60       120

conc. =  5.0 subtracted from:
conc.   Lower  Center   Upper  --------+---------+---------+---------+-
 7.5     0.64   13.14   25.64                 (-*-)
10.0     8.08   20.58   33.08                  (-*--)
12.5     6.65   19.15   31.65                  (-*-)
                               --------+---------+---------+---------+-
                                     -60         0        60       120

conc. =  7.5 subtracted from:
conc.   Lower  Center   Upper  --------+---------+---------+---------+-
10.0    -5.06    7.44   19.94                (-*-)
12.5    -6.50    6.00   18.50                (-*-)
                               --------+---------+---------+---------+-
                                     -60         0        60       120

conc. = 10.0 subtracted from:
conc.    Lower  Center  Upper  --------+---------+---------+---------+-
12.5    -13.93   -1.43  11.07                (-*-)
                               --------+---------+---------+---------+-
                                     -60         0        60       120
```



The regression equation is

Manu15_aero = - 22.24 + 31.72 conc. - 2.823 conc.**2 + 0.08355 conc.**3

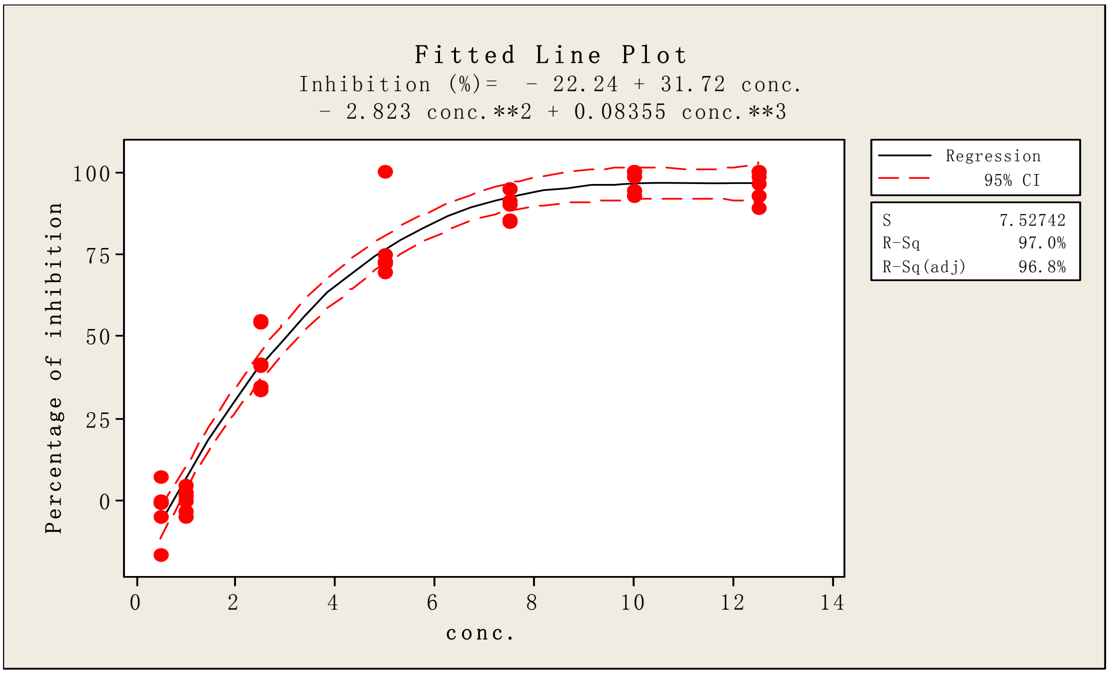
S = 7.52742 R-Sq = 97.0% R-Sq(adj) = 96.8%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	69605.1	23201.7	409.47	0.000
Error	38	2153.2	56.7		
Total	41	71758.3			

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	57748.9	164.89	0.000
Quadratic	1	11349.6	166.42	0.000
Cubic	1	506.6	8.94	0.005



MIC	%	mg/ml
MIC50	3.0135774	72.23979728
mic90	4.4177584	92.23961046
mic100	14.889814	122.2399849

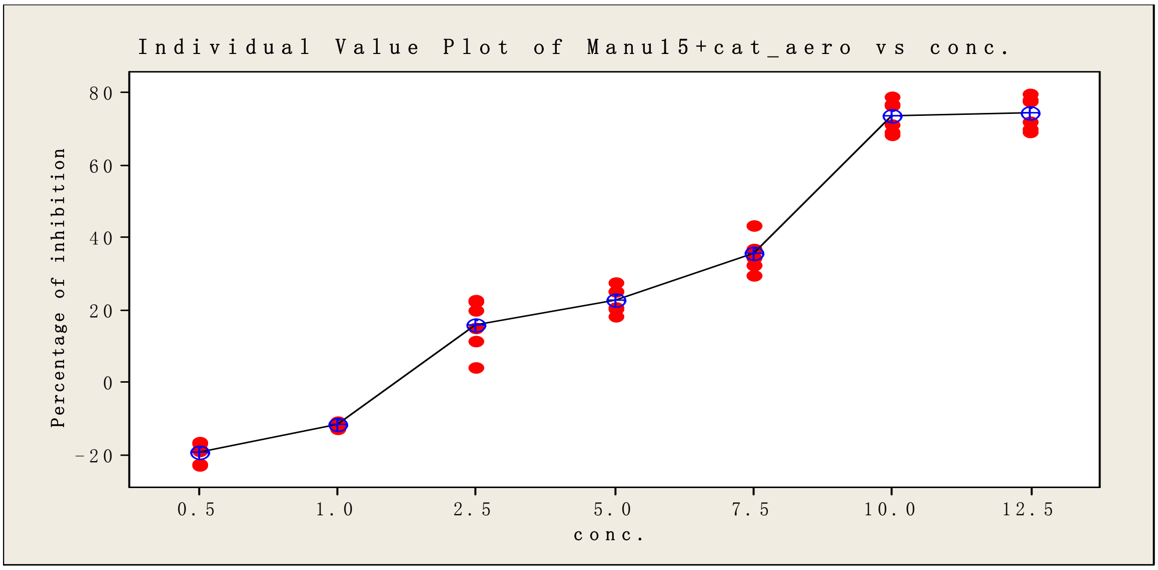
B3-4 Manuka honey 15+ UMF+catalase under aerobic condition

TableB3-4: Percentage of inhibition of *P. acnes* in the Comvita Manuka honey 15+ UMF honey solutions ranged from 0.5% to 12.5% with catalase under aerobic condition.

	Concentration		Replications						Average	SEM
Samples	%	mg/ml	1	2	3	4	5	6		
BC0.5-aerobic	0.5	5	-16.92	-18.73	-22.92	-16.74	-19.00	-23.08	-19.57	2.57
BC1-AERO	1	10	-11.49	-10.86	-12.53	-11.99	-11.31	-13.10	-11.88	0.76
BC2.5-aerobic	2.5	25	22.31	22.58	11.20	19.66	14.98	3.89	15.77	6.67
BC5-aerobic	5	50	27.31	20.27	18.21	25.07	25.02	20.54	22.74	3.24
BC7.5-aerobic	7.5	75	36.65	36.40	43.21	29.41	32.13	34.39	35.37	4.30
BC10-aerobic	10	100	69.19	71.18	68.42	76.56	78.89	76.36	73.43	4.01
BC12.5-aerobic	12.5	125	69.03	69.66	71.88	77.87	77.62	79.48	74.26	4.20

One-way ANOVA: Manu15+cat_aero versus conc.

```
Source  DF       SS      MS       F      P
conc.    6  49700.5  8283.4  422.49  0.000
Error   35    686.2    19.6
Total   41  50386.8
S = 4.428   R-Sq = 98.64%   R-Sq(adj) = 98.40%

                             Individual 95% CIs For Mean Based on
                             Pooled StDev
Level  N     Mean  StDev  --------+---------+---------+---------+-
 0.5   6  -19.566  2.812  (*-)
 1.0   6  -11.882  0.829     (*)
 2.5   6   15.769  7.306             (*)
 5.0   6   22.738  3.547               (-*)
 7.5   6   35.366  4.716                    (*)
10.0   6   73.431  4.392                                (*-)
12.5   6   74.257  4.601                                 (*)
                          --------+---------+---------+---------+-
                                  0        30        60        90
Pooled StDev = 4.428
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of conc.
Individual confidence level = 99.64%
conc. =  0.5 subtracted from:
conc.  Lower  Center   Upper  ---------+---------+---------+---------+
 1.0   -0.31    7.68   15.67                    (-*)
 2.5   27.35   35.34   43.33                         (-*-)
 5.0   34.31   42.30   50.29                           (*-)
 7.5   46.94   54.93   62.92                             (-*-)
10.0   85.01   93.00  100.99                                     (-*)
12.5   85.83   93.82  101.81                                     (-*)
                              ---------+---------+---------+---------+
                                     -50         0        50       100
conc. =  1.0 subtracted from:
conc.  Lower  Center  Upper  ---------+---------+---------+---------+
 2.5   19.66   27.65  35.64                         (-*)
 5.0   26.63   34.62  42.61                          (-*-)
 7.5   39.26   47.25  55.24                             (*-)
10.0   77.32   85.31  93.30                                    (-*-)
12.5   78.15   86.14  94.13                                     (*-)
                             ---------+---------+---------+---------+
                                    -50         0        50       100
conc. =  2.5 subtracted from:
conc.  Lower  Center  Upper  ---------+---------+---------+---------+
 5.0   -1.02    6.97  14.96                     (*-)
 7.5   11.61   19.60  27.59                       (-*-)
10.0   49.67   57.66  65.65                               (-*)
12.5   50.50   58.49  66.48                               (-*)
                             ---------+---------+---------+---------+
                                    -50         0        50       100
conc. =  5.0 subtracted from:
conc.  Lower  Center  Upper  ---------+---------+---------+---------+
 7.5    4.64   12.63  20.62                      (-*)
10.0   42.70   50.69  58.68                              (*-)
12.5   43.53   51.52  59.51                              (*-)
                             ---------+---------+---------+---------+
                                    -50         0        50       100
conc. =  7.5 subtracted from:
conc.  Lower  Center  Upper  ---------+---------+---------+---------+
10.0   30.08   38.07  46.06                           (-*)
12.5   30.90   38.89  46.88                           (-*)
                             ---------+---------+---------+---------+
                                    -50         0        50       100
conc. = 10.0 subtracted from:
conc.  Lower  Center  Upper  ---------+---------+---------+---------+
12.5   -7.16    0.83   8.82                     (*-)
---------+---------+---------+---------+
                                    -50         0        50       100
```



Polynomial Regression Analysis: Manu15+cat_aero versus conc.

The regression equation is

Manu15+cat_aero = - 21.94 + 11.93 conc. - 0.5148 conc.**2 + 0.01596 conc.**3

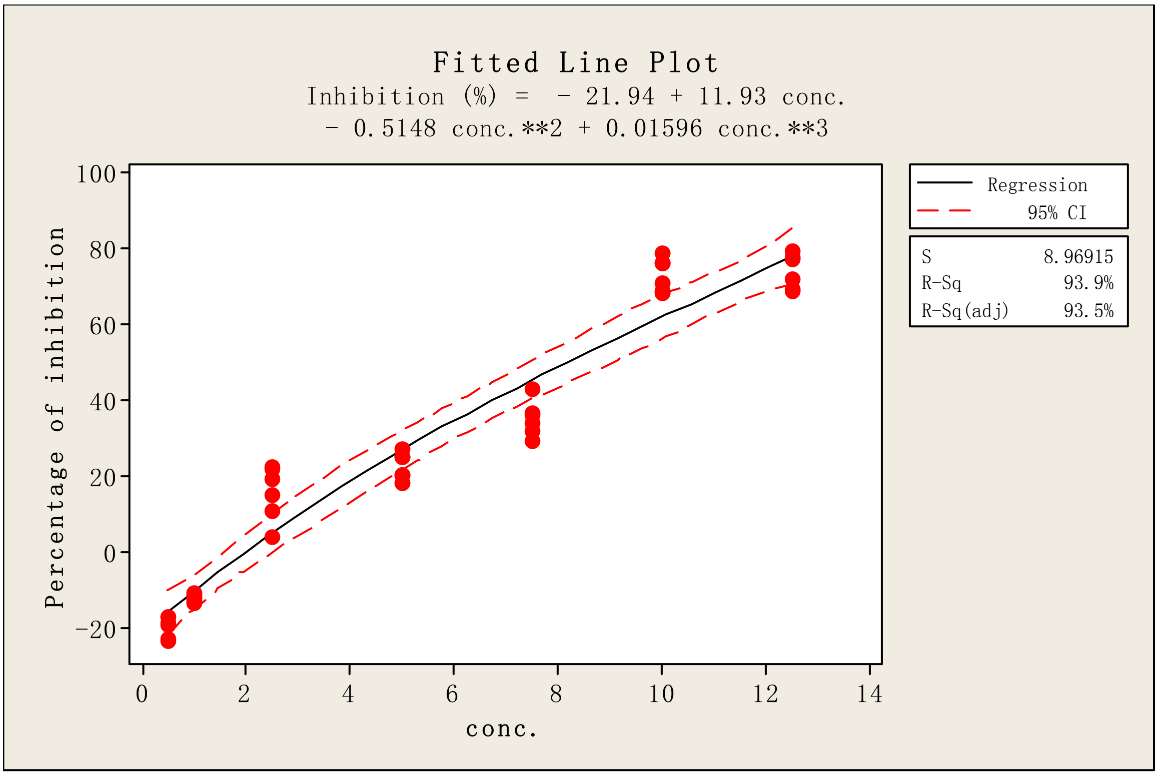
S = 8.96915   R-Sq = 93.9%   R-Sq(adj) = 93.5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	47329.8	15776.6	196.12	0.000
Error	38	3056.9	80.4		
Total	41	50386.8			

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	46981.4	551.85	0.000
Quadratic	1	330.0	4.18	0.048
Cubic	1	18.5	0.23	0.634



MIC	%	mg/ml
MIC 50	8.1894044	81.89
MIC 90	14.291725	140.29
MIC 100	15.66932	156.69

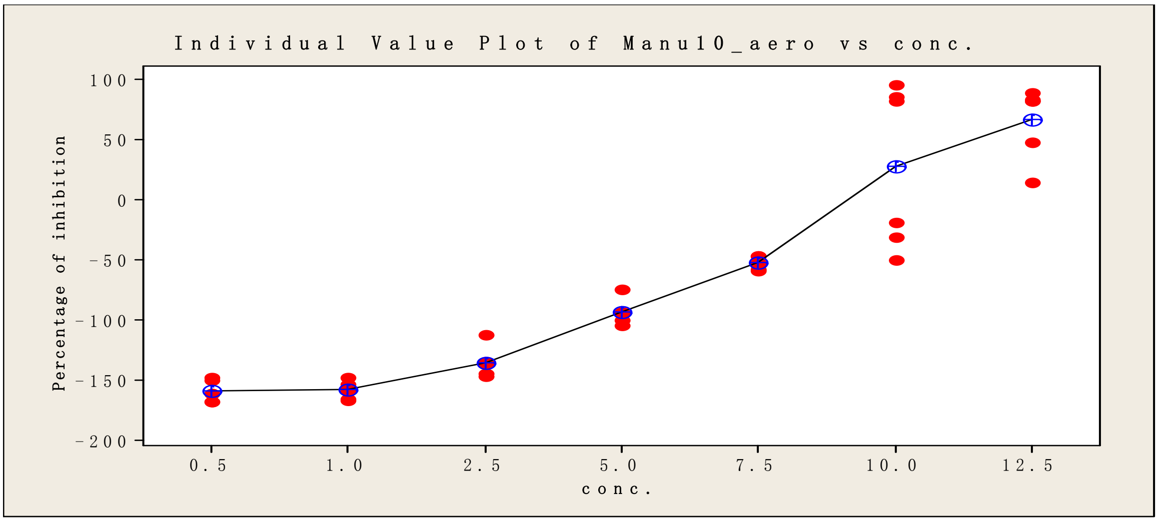
B3-5 Manuka honey 10+ UMF under aerobic condition

Table B3-5: Percentage of inhibition of *P. acnes* in the Comvita Manuka honey 10+ UMF honey solutions ranged from 0.5% to 12.5% under aerobic condition.

Samples	Concentration		Replications						Average	SEM
	%	mg/ml	1	2	3	4	5	6		
C0.5-aerobic	0.5	5	-168.64	-151.18	-161.38	-148.87	-161.54	-168.78	-160.06	7.72
C1-AERO	1	10	-148.87	-167.74	-159.84	-154.07	-156.79	-166.74	-159.01	6.69
C2.5-aerobic	2.5	25	-135.34	-145.02	-137.56	-137.04	-147.81	-112.74	-135.92	11.30
C5-aerobic	5	50	-74.89	-101.13	-105.36	-94.57	-94.00	-95.48	-94.24	9.55
C7.5-aerobic	7.5	75	-54.16	-58.60	-46.97	-59.10	-47.06	-52.53	-53.07	4.86
C10-aerobic	10	100	-51.02	-19.19	-31.27	85.09	82.06	94.95	26.77	61.43
C12.5-aerobic	12.5	125	14.50	83.12	47.85	88.53	82.24	82.26	66.42	26.80

One-way ANOVA: Manu10_aero versus conc.

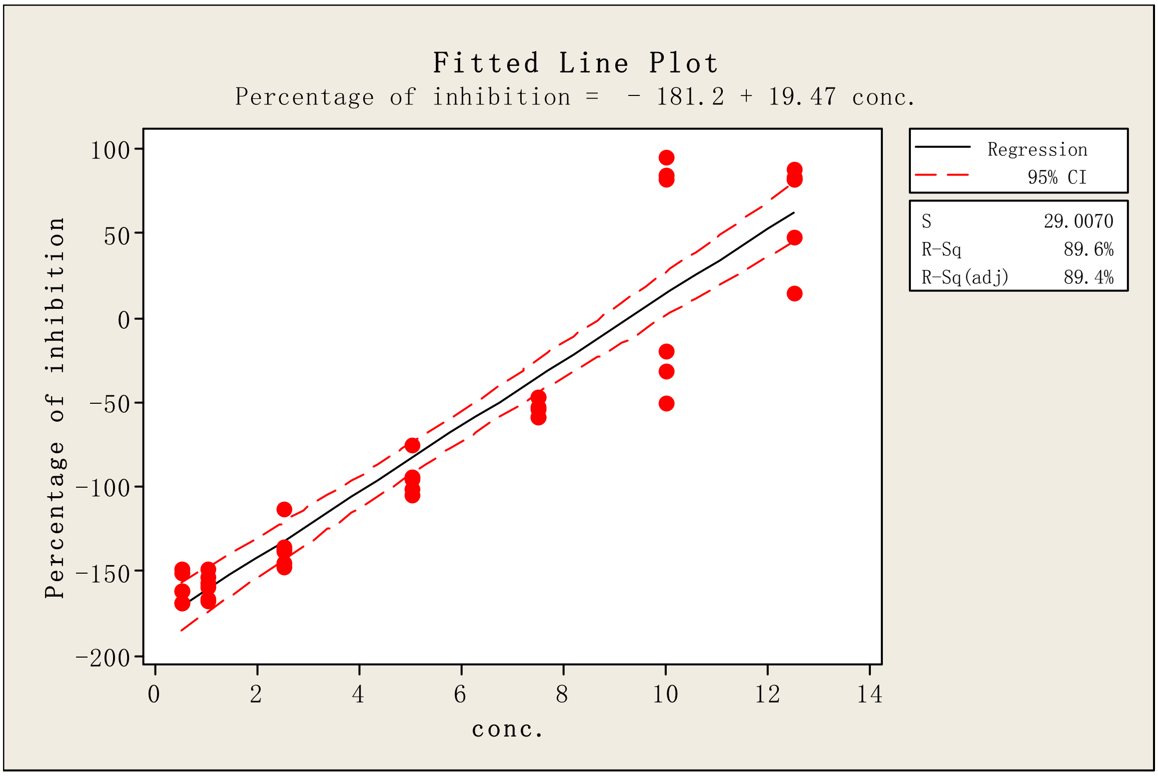
```
Source  DF      SS     MS      F      P
conc.    6  295054  49176  59.29  0.000
Error   35   29029    829
Total   41  324083
S = 28.80   R-Sq = 91.04%   R-Sq(adj) = 89.51%
                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level  N     Mean  StDev  ------+---------+---------+---------+---
 0.5   6  -160.06   8.46  (--*---)
 1.0   6  -159.01   7.33  (--*---)
 2.5   6  -135.92  12.38     (---*--)
 5.0   6   -94.24  10.46           (---*--)
 7.5   6   -53.07   5.32                 (--*---)
10.0   6    26.77  67.29                            (---*--)
12.5   6    66.42  29.36                                  (--*---)
                          ------+---------+---------+---------+---
                             -140       -70         0        70
Pooled StDev = 28.80
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of conc.
Individual confidence level = 99.64%
conc. =  0.5 subtracted from:
conc.    Lower  Center   Upper  --------+---------+---------+---------+-
 1.0    -50.91    1.06   53.02                 (--*---)
 2.5    -27.82   24.15   76.12                  (---*--)
 5.0     13.86   65.83  117.79                     (--*---)
 7.5     55.03  106.99  158.96                        (--*---)
10.0    134.87  186.84  238.80                             (--*---)
12.5    174.51  226.48  278.45                                (--*---)
                                --------+---------+---------+---------+-
                                     -150         0       150       300
conc. =  1.0 subtracted from:
conc.    Lower  Center   Upper  --------+---------+---------+---------+-
 2.5    -28.88   23.09   75.06                  (---*--)
 5.0     12.80   64.77  116.74                     (--*---)
 7.5     53.97  105.94  157.91                        (--*---)
10.0    133.81  185.78  237.75                             (--*---)
12.5    173.46  225.43  277.39                                (--*--)
                                --------+---------+---------+---------+-
                                     -150         0       150       300
conc. =  2.5 subtracted from:
conc.    Lower  Center   Upper  --------+---------+---------+---------+-
 5.0    -10.29   41.68   93.65                   (---*--)
 7.5     30.88   82.85  134.81                      (---*--)
10.0    110.72  162.69  214.66                           (---*--)
12.5    150.37  202.33  254.30                              (--*---)
                                --------+---------+---------+---------+-
                                     -150         0       150       300
conc. =  5.0 subtracted from:

conc.    Lower  Center   Upper  --------+---------+---------+---------+-
 7.5    -10.80   41.17   93.14                   (---*--)
10.0     69.04  121.01  172.98                         (--*---)
12.5    108.69  160.66  212.62                           (---*--)
                                --------+---------+---------+---------+-
                                     -150         0       150       300
conc. =  7.5 subtracted from:
conc.   Lower  Center   Upper  --------+---------+---------+---------+-
10.0    27.87   79.84  131.81                     (--*---)
12.5    67.52  119.49  171.45                        (--*--)
                               --------+---------+---------+---------+-
                                    -150         0       150       300
conc. = 10.0 subtracted from:
conc.    Lower  Center  Upper  --------+---------+---------+---------+-
12.5    -12.32   39.65  91.61                  (---*--)
                               --------+---------+---------+---------+-
-150         0       150       300
```



Regression Analysis: Manu10_aero versus conc.
The regression equation is
Manu10_aero = - 181.2 + 19.47 conc.
S = 29.0070 R-Sq = 89.6% R-Sq(adj) = 89.4%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	290427	290427	345.17	0.000
Error	40	33656	841		
Total	41	324083			



MIC	%	mg/ml
MIC50	11.8768	118.768

MIC90	13.9291	139.291
MIC100	14.4427	144.427

B3-6 Manuka honey 10+ UMF+catalase under aerobic condition

Table B3-6Percentage of inhibition of *P. acnes* in the Comvita Manuka honey 10+ UMF honey solutions ranged from 0.5% to 12.5% with catalase under aerobic condition.

Samples	Concentration		Replications						Average	SEM
	%	mg/ml	1	2	3	4	5	6		
CC0.5-aerobic	0.5	5	-131.33	-163.57	-161.24	-131.67	-163.80	-255.20	-167.81	41.54
CC1-AERO	1	10	-131.33	-163.57	-161.24	-163.57	-161.67	-153.39	-155.80	11.47
CC2.5-aerobic	2.5	25	-119.73	-157.92	-0.90	-133.98	-140.61	-112.29	-110.90	51.32
CC5-aerobic	5	50	-93.08	-115.02	-131.56	-137.19	-118.33	-130.45	-120.94	14.64
CC7.5-aerobic	7.5	75	-72.26	-49.68	-122.17	-116.11	-112.90	-23.98	-82.85	37.04
CC10-aerobic	10	100	-11.54	-74.57	-91.56	86.47	83.51	-77.08	-14.13	74.47
CC12.5-aerobic	12.5	125	72.53	73.55	9.93	90.11	88.39	78.42	68.82	27.18

One-way ANOVA: Manu10+cat_aero versus conc.

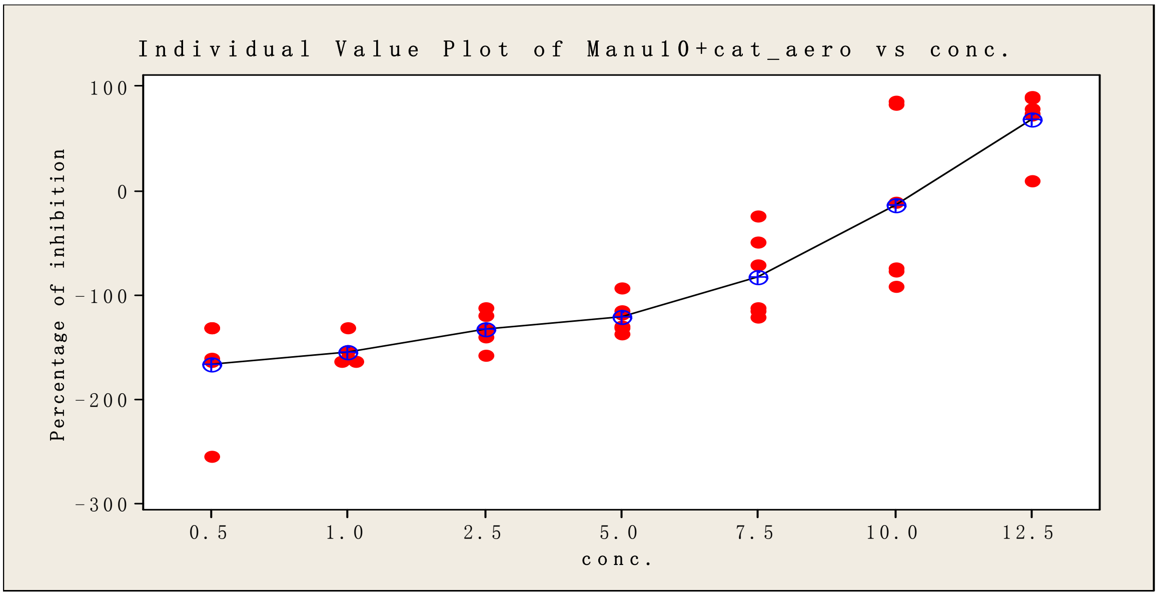
```
Source  DF      SS     MS      F      P
conc.    6  262568  43761  24.94  0.000
Error   34   59648   1754
Total   40  322216
S = 41.88   R-Sq = 81.49%   R-Sq(adj) = 78.22%

                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean  StDev  -----+---------+---------+---------+----
 0.5   6  -167.81  45.50  (---*---)
 1.0   6  -155.80  12.56   (----*---)
 2.5   5  -132.90  17.92      (---*----)
 5.0   6  -120.94  16.04        (---*---)
 7.5   6   -82.85  40.58            (----*---)
10.0   6   -14.13  81.57                     (---*----)
12.5   6    68.82  29.77                               (----*---)
                          -----+---------+---------+---------+----
                             -160       -80         0        80
```

Pooled StDev = 41.88
Tukey 95% Simultaneous Confidence Intervals

```
All Pairwise Comparisons among Levels of conc.
```

Individual confidence level = 99.64%

```
conc. =  0.5 subtracted from:
conc.   Lower  Center   Upper  ---------+---------+---------+---------+
 1.0   -63.74   12.01   87.76                (----*---)
 2.5   -44.55   34.90  114.35                 (----*----)
 5.0   -28.88   46.87  122.62                  (----*----)
 7.5     9.20   84.95  160.71                     (---*----)
10.0    77.93  153.68  229.43                        (----*---)
12.5   160.88  236.63  312.38                             (----*----)
                               ---------+---------+---------+---------+
                                     -160         0       160       320

conc. =  1.0 subtracted from:
conc.   Lower  Center   Upper  ---------+---------+---------+---------+
 2.5   -56.55   22.89  102.34                (----*----)
 5.0   -40.89   34.86  110.61                 (----*----)
 7.5    -2.80   72.95  148.70                    (----*---)
10.0    65.92  141.67  217.42                        (----*----)
12.5   148.87  224.62  300.37                             (----*----)
                               ---------+---------+---------+---------+
                                     -160         0       160       320

conc. =  2.5 subtracted from:
conc.   Lower  Center   Upper  ---------+---------+---------+---------+
 5.0   -67.48   11.97   91.41                (----*----)
 7.5   -29.39   50.05  129.50                  (----*----)
10.0    39.33  118.78  198.22                      (----*----)
12.5   122.28  201.73  281.18                            (----*----)
                               ---------+---------+---------+---------+
                                     -160         0       160       320

conc. =  5.0 subtracted from:
conc.   Lower  Center   Upper  ---------+---------+---------+---------+
 7.5   -37.66   38.09  113.84                  (---*----)
10.0    31.06  106.81  182.56                      (----*---)
12.5   114.01  189.76  265.51                           (----*----)
                               ---------+---------+---------+---------+
                                     -160         0       160       320

conc. =  7.5 subtracted from:
conc.   Lower  Center   Upper  ---------+---------+---------+---------+
10.0    -7.03   68.72  144.47                  (---*----)
12.5    75.92  151.67  227.42                       (---*----)
                               ---------+---------+---------+---------+
                                     -160         0       160       320

conc. = 10.0 subtracted from:
conc.   Lower  Center   Upper  ---------+---------+---------+---------+
12.5     7.20   82.95  158.70                  (----*----)
---------+---------+---------+---------+
                                     -160         0       160       320
```



Polynomial Regression Analysis: Manu10+cat_aero versus conc.

The regression equation is

Manu10+cat_aero = - 169.4 + 12.71 conc. - 0.997 conc.**2 + 0.1216 conc.**3

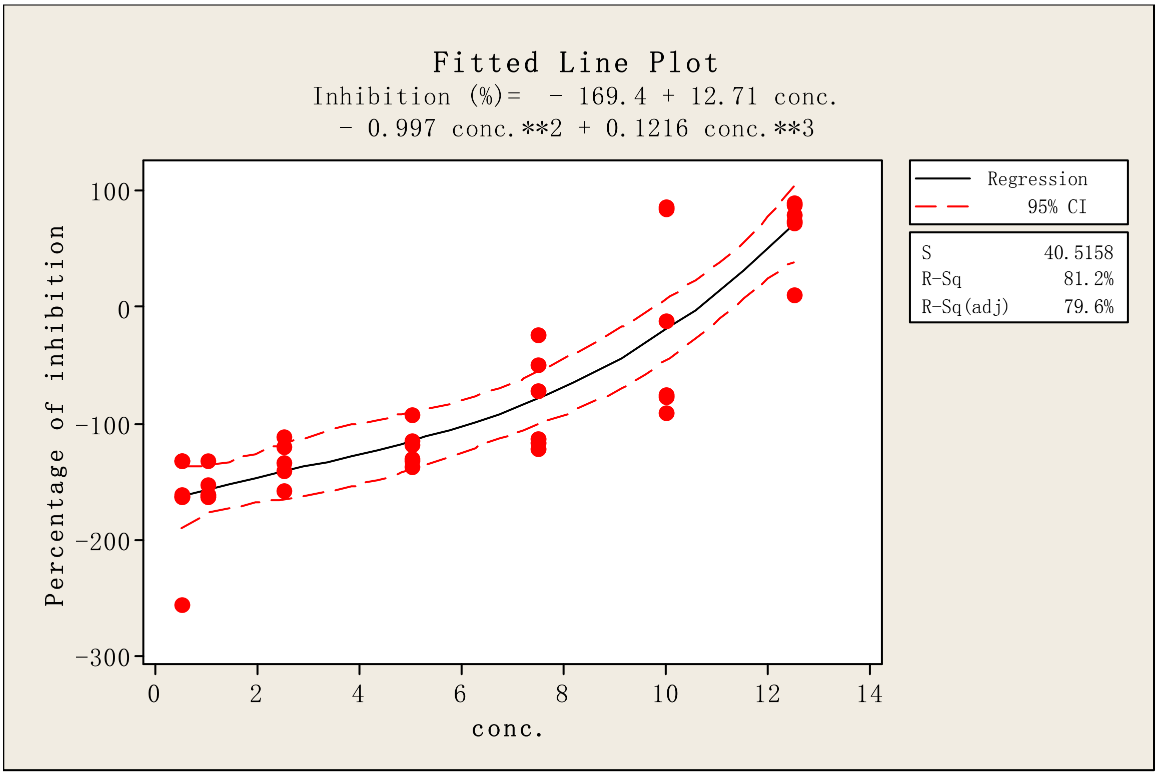
S = 40.5158   R-Sq = 81.2%   R-Sq(adj) = 79.6%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	261479	87159.7	53.10	0.000
Error	37	60737	1641.5		
Total	40	322216			

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	245331	124.44	0.000
Quadratic	1	15119	9.30	0.004
Cubic	1	1029	0.63	0.434



manuka honey 10	%	mg/ml
MIC50	12.01	120.10
MIC90	12.91	129.1
MIC100	13.11	131.1

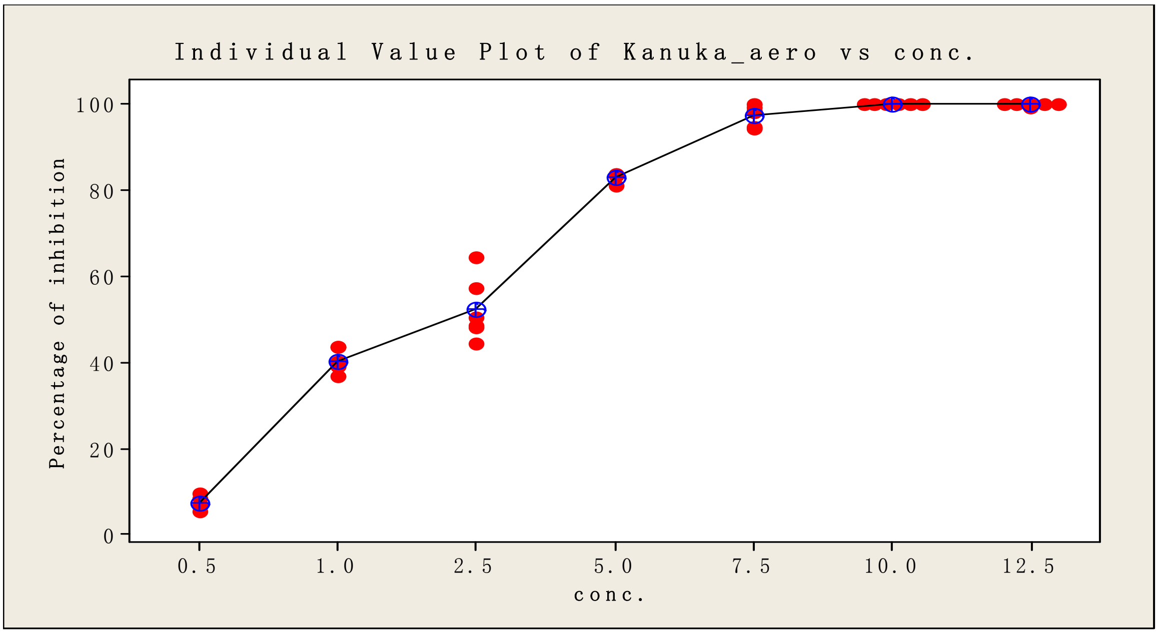
B3-7 Kanuka honey under aerobic condition

Table 7: Percentage of inhibition of *P. acnes* in the Kanuka honey solutions ranged from 0.5% to 12.5%e under aerobic condition.

Samples	Concentration		Replications						Average	SEM
	%	mg/ml	1	2	3	4	5	6		
K0.5-aerobic	0.5	5	8.51	5.14	9.50	6.79	6.33	6.56	7.14	1.45
K1-AERO	1	10	36.65	38.98	43.71	43.44	38.91	40.05	40.29	2.53
K2.5-aerobic	2.5	25	48.64	48.03	44.48	64.48	50.25	57.08	52.16	6.68
K5-aerobic	5	50	83.71	83.28	81.18	83.89	-75.79	-83.71	28.76	76.77
K7.5-aerobic	7.5	75	94.32	94.71	100.00	98.05	99.34	98.42	97.47	2.19
K10-aerobic	10	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00
K12.5-aerobic	12.5	125	100.00	99.16	100.00	100.00	100.00	100.00	99.86	0.31

One-way ANOVA: Kanuka_aero versus conc.

```
Source  DF       SS      MS       F      P
conc.    6  46684.8  7780.8  727.11  0.000
Error   33    353.1    10.7
Total   39  47037.9
S = 3.271   R-Sq = 99.25%   R-Sq(adj) = 99.11%

                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 0.5   6    7.14   1.59  (*)
 1.0   6   40.29   2.78              (*)
 2.5   6   52.16   7.32                   (*)
 5.0   4   83.01   1.25                               (*-)
 7.5   6   97.47   2.40                                     (*)
10.0   6  100.00   0.00                                      (*)
12.5   6   99.86   0.34                                      (*)
                         --------+---------+---------+---------+-
                                25        50        75       100
Pooled StDev = 3.27
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of conc.
Individual confidence level = 99.64%
conc. =  0.5 subtracted from:
conc.  Lower  Center  Upper  ---+---------+---------+---------+------
 1.0   27.22   33.15  39.08                     (-*)
 2.5   39.09   45.02  50.95                        (*)
 5.0   69.25   75.88  82.51                              (*-)
 7.5   84.41   90.34  96.27                                 (*)
10.0   86.93   92.86  98.79                                 (-*)
12.5   86.79   92.72  98.65                                 (-*)
                             ---+---------+---------+---------+------
                              -50         0        50       100
conc. =  1.0 subtracted from:
conc.  Lower  Center  Upper  ---+---------+---------+---------+------
 2.5    5.94   11.87  17.80               (*-)
 5.0   36.09   42.72  49.35                     (-*)
 7.5   51.25   57.18  63.11                        (*-)
10.0   53.78   59.71  65.64                         (*)
12.5   53.64   59.57  65.50                         (*)
                             ---+---------+---------+---------+------
                              -50         0        50       100
conc. =  2.5 subtracted from:
conc.  Lower  Center  Upper  ---+---------+---------+---------+------
 5.0   24.22   30.85  37.48                    (*)
 7.5   39.38   45.31  51.24                       (*)
10.0   41.91   47.84  53.77                       (-*)
12.5   41.77   47.70  53.63                       (-*)
                             ---+---------+---------+---------+------
                              -50         0        50       100

conc. =  5.0 subtracted from:
conc.  Lower  Center  Upper  ---+---------+---------+---------+------
 7.5    7.83   14.46  21.09                (*)
10.0   10.36   16.99  23.61                (*-)
12.5   10.22   16.85  23.48                (*-)
                             ---+---------+---------+---------+------
                              -50         0        50       100
conc. =  7.5 subtracted from:
conc.  Lower  Center  Upper  ---+---------+---------+---------+------
10.0   -3.40    2.53   8.46              (-*)
12.5   -3.54    2.39   8.32              (*-)
                             ---+---------+---------+---------+------
                              -50         0        50       100
conc. = 10.0 subtracted from:
conc.  Lower  Center  Upper  ---+---------+---------+---------+------
12.5   -6.07   -0.14   5.79             (*)
---+---------+---------+---------+------
                              -50         0        50       100
```



Polynomial Regression Analysis: Kanuka_aero versus conc.

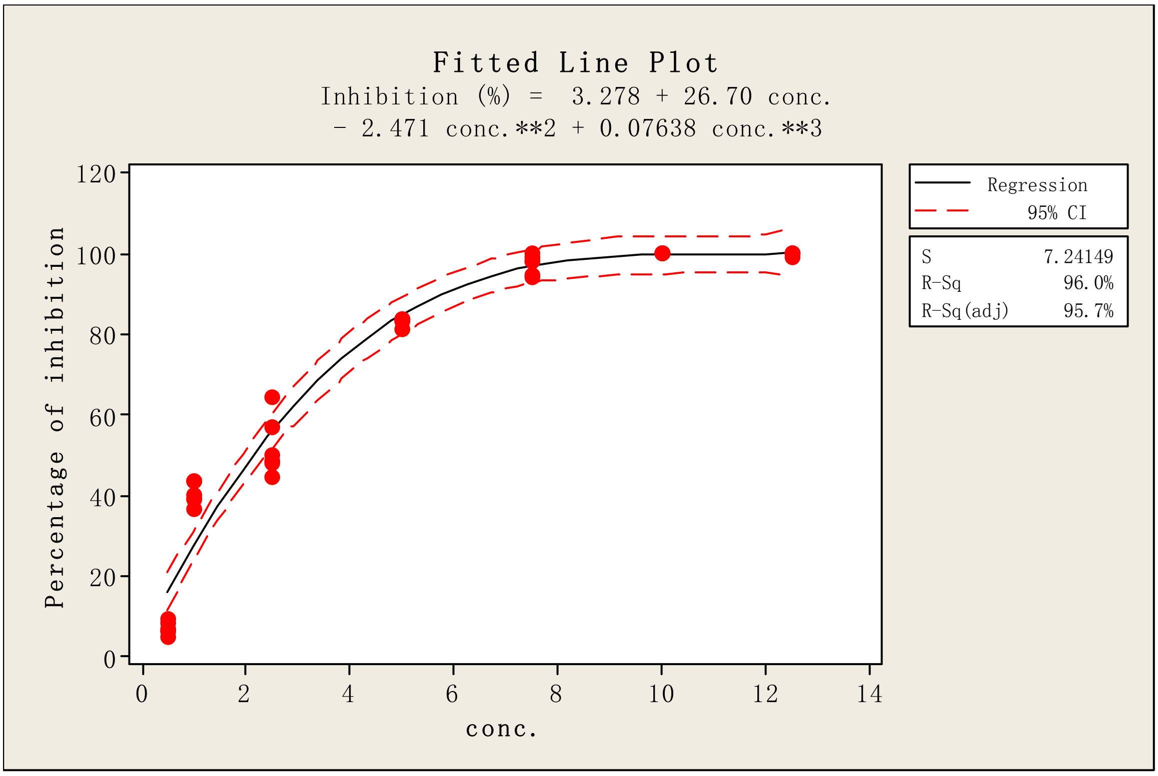
The regression equation is
Kanuka_aero = 3.278 + 26.70 conc. - 2.471 conc.**2 + 0.07638 conc.**3
S = 7.24149 R-Sq = 96.0% R-Sq(adj) = 95.7%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	45150.1	15050.0	287.00	0.000
Error	36	1887.8	52.4		
Total	39	47037.9			

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	37884.4	157.27	0.000
Quadratic	1	6864.2	110.94	0.000
Cubic	1	401.5	7.66	0.009



MIC	%	mg/ml
MIC50	2.15	21.5
MIC90	5.81	58.1
MIC100	12.33	123.3

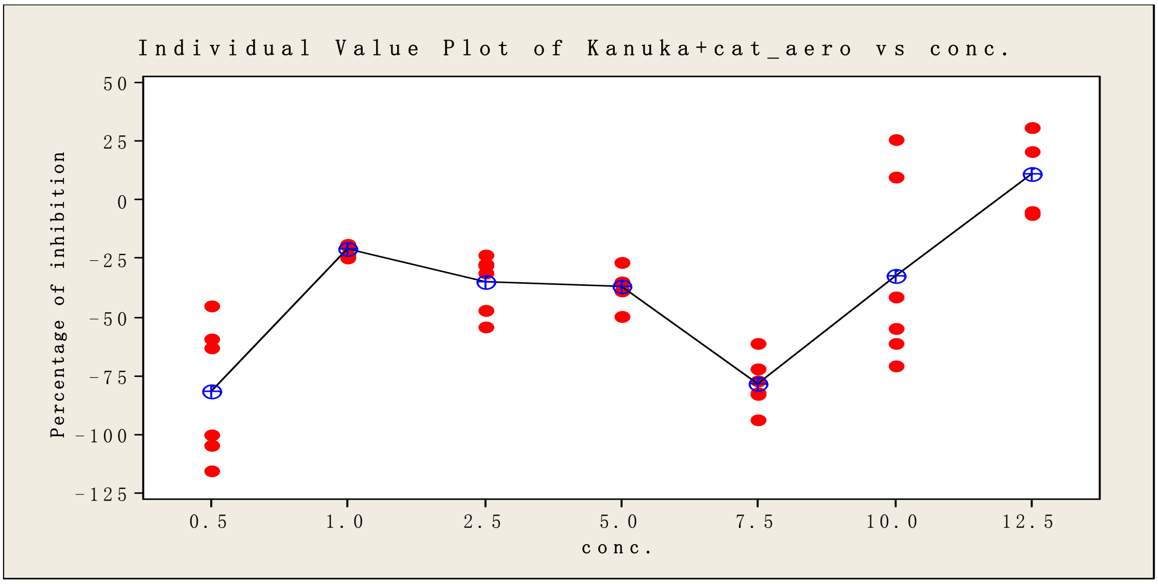
B3-8 Kanuka honey with catalase under aerobic condition

Table 8: Percentage of inhibition of *P. acnes* in the Kanuka honey solutions ranged from 0.5% to 12.5% with catalase under aerobic condition.

Samples	Concentration		Replications						Average	SEM
	%	mg/ml	1	2	3	4	5	6		
KC0.5-aerobic	0.5	5	-45.50	-116.06	-63.21	-100.23	-104.75	-59.50	-81.54	26.46
KC1-AERO	1	10	-18.85	-18.62	-24.43	-19.00	-19.23	-23.53	-20.61	2.40
KC2.5-aerobic	2.5	25	-27.35	-30.79	-23.28	-28.01	-53.96	-47.40	-35.13	11.37
KC5-aerobic	5	50	-26.61	-35.86	-49.73	-35.75	-38.57	-34.71	-36.87	6.84
KC7.5-aerobic	7.5	75	-72.26	-61.27	-83.12	-93.85	-77.13	-82.22	-78.31	10.07
KC10-aerobic	10	100	-41.40	-70.77	-54.68	10.23	-61.47	25.72	-32.06	36.72
KC12.5-aerobic	12.5	125	-5.34	-4.48	-6.11	20.93	31.36	31.00	11.23	16.89

One-way ANOVA: Kanuka+cat_aero versus conc.

```
Source  DF     SS    MS      F      P
conc.    6  37699  6283  14.01  0.000
Error   35  15701   449
Total   41  53400
S = 21.18   R-Sq = 70.60%   R-Sq(adj) = 65.56%
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 0.5   6  -81.54  28.98  (----*----)
 1.0   6  -20.61   2.63                 (----*----)
 2.5   6  -35.13  12.45             (----*----)
 5.0   6  -36.87   7.49            (----*----)
 7.5   6  -78.31  11.03   (----*----)
10.0   6  -32.06  40.22              (----*----)
12.5   6   11.23  18.50                          (----*----)
                         --------+---------+---------+---------+-
                               -70       -35         0        35
Pooled StDev = 21.18
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of conc.
Individual confidence level = 99.64%
conc. =  0.5 subtracted from:
conc.   Lower  Center   Upper  --------+---------+---------+---------+-
 1.0    22.71   60.93   99.15                      (-----*----)
 2.5     8.19   46.41   84.63                    (-----*----)
 5.0     6.45   44.67   82.89                    (----*-----)
 7.5   -34.98    3.24   41.45              (----*-----)
10.0    11.26   49.48   87.70                     (----*-----)
12.5    54.55   92.77  130.99                           (----*-----)
                               --------+---------+---------+---------+-
                                     -70         0        70       140
conc. =  1.0 subtracted from:
conc.   Lower  Center   Upper  --------+---------+---------+---------+-
 2.5   -52.74  -14.52   23.70                (-----*----)
 5.0   -54.48  -16.26   21.96                (-----*----)
 7.5   -95.91  -57.70  -19.48          (-----*----)
10.0   -49.67  -11.45   26.77                 (----*-----)
12.5    -6.38   31.84   70.05                       (-----*----)
                               --------+---------+---------+---------+-
                                     -70         0        70       140
conc. =  2.5 subtracted from:
conc.   Lower  Center  Upper  --------+---------+---------+---------+-
 5.0   -39.96   -1.74  36.48                (-----*----)
 7.5   -81.39  -43.17  -4.96          (-----*----)
10.0   -35.15    3.07  41.29                 (----*-----)
12.5     8.14   46.36  84.58                       (-----*----)
                              --------+---------+---------+---------+-
                                    -70         0        70       140
conc. =  5.0 subtracted from:
conc.   Lower  Center  Upper  --------+---------+---------+---------+-
 7.5   -79.66  -41.44  -3.22           (----*-----)
10.0   -33.41    4.81  43.03                 (-----*----)
12.5     9.88   48.10  86.31                       (-----*----)
                              --------+---------+---------+---------+-
                                    -70         0        70       140
conc. =  7.5 subtracted from:
conc.  Lower  Center   Upper  --------+---------+---------+---------+-
10.0    8.03   46.24   84.46                       (-----*----)
12.5   51.31   89.53  127.75                             (-----*----)
                              --------+---------+---------+---------+-
                                    -70         0        70       140
conc. = 10.0 subtracted from:
conc.  Lower  Center  Upper  --------+---------+---------+---------+-
12.5    5.07   43.29  81.51                      (----*-----)
--------+---------+---------+---------+-
                                   -70         0        70       140
```



Regression Analysis: Kanuka+cat_aero versus conc.

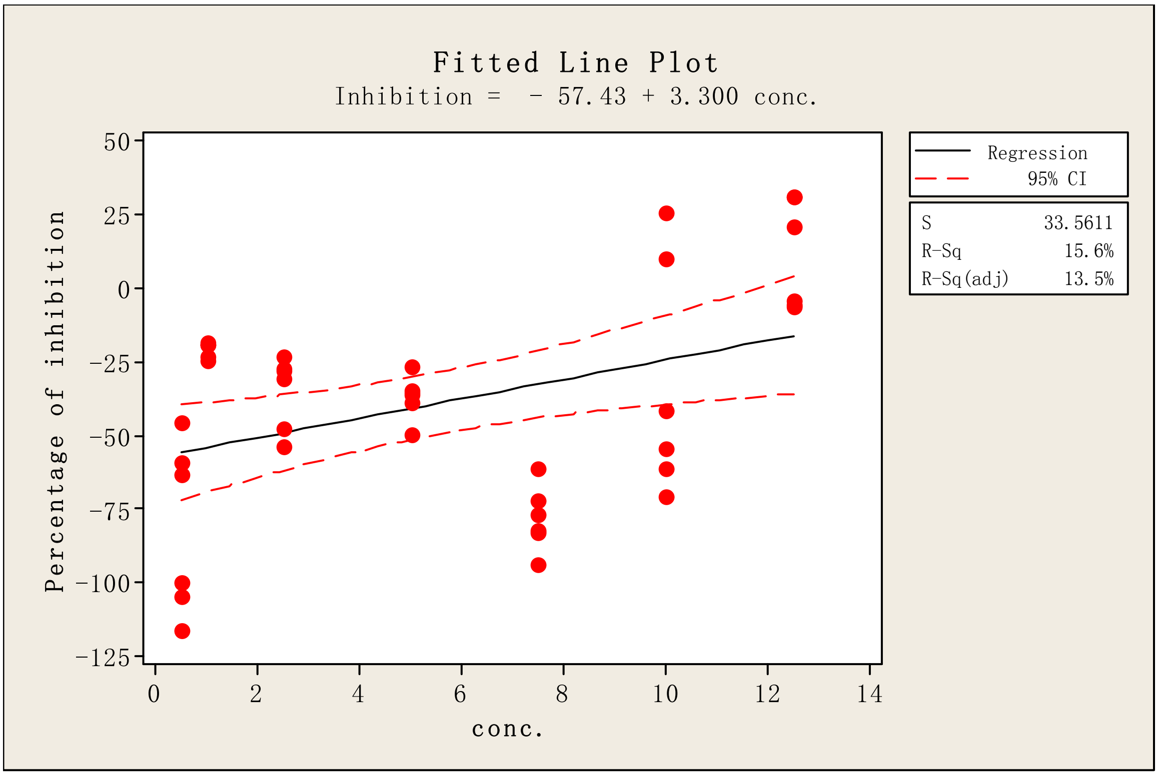
The regression equation is

Kanuka+cat_aero = - 57.43 + 3.300 conc.

S = 33.5611 R-Sq = 15.6% R-Sq(adj) = 13.5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	8346.4	8346.37	7.41	0.010
Error	40	45053.9	1126.35		
Total	41	53400.2			



Kanuka+cat	%	mg/ml
MIC50	28.5077	285.08
MIC90	49.6381	496.38
MIC100	54.9208	549.21

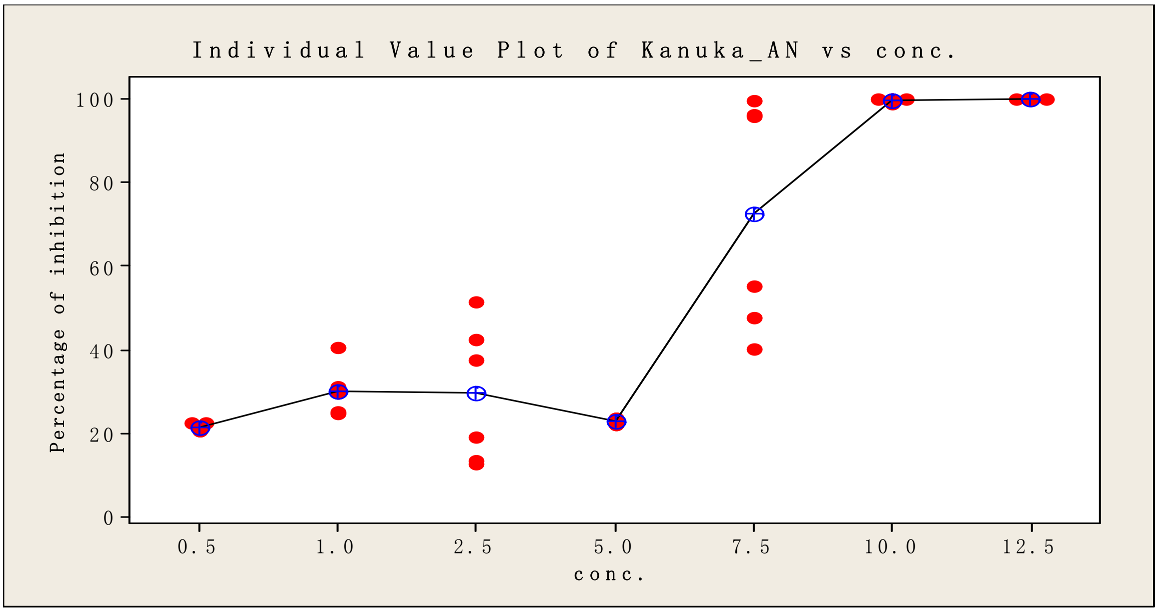
B3-9 Kanuka honey under anaerobic condition

Table B3-9 Percentage of inhibition of *P. acnes* in the Kanuka honey solutions ranged from 0.5% to 12.5% under anaerobic condition.

Samples	Concentration		Replications						Average	SEM
	%	mg/ml	1	2	3	4	5	6		
K0.5-AN	0.5	5	22.24	20.86	20.48	22.24	20.37	21.00	21.20	0.77
K1-AN	1	10	40.28	29.75	24.51	29.39	30.95	24.95	29.97	5.21
K2.5-AN	2.5	25	42.27	51.48	37.28	19.07	13.48	12.63	29.37	15.04
K5-AN	5	50	23.33	21.84	23.67	22.94	23.61	22.05	22.91	0.72
K7.5-AN	7.5	75	55.13	47.73	39.89	99.61	96.19	95.72	72.38	25.21
K10-AN	10	100	100.02	98.85	100.00	100.00	99.84	100.00	99.78	0.42
K12.5-AN	12.5	125	100.00	99.81	100.00	100.00	99.80	99.86	99.91	0.09

One-way ANOVA: Kanuka_AN versus conc.

```
Source  DF     SS    MS      F      P
conc.    6  46608  7768  50.90  0.000
Error   35   5341   153
Total   41  51949
S = 12.35   R-Sq = 89.72%   R-Sq(adj) = 87.96%
                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N   Mean  StDev  ------+---------+---------+---------+---
 0.5   6  21.20   0.84  (---*----)
 1.0   6  29.97   5.70      (---*---)
 2.5   6  29.37  16.47      (---*---)
 5.0   6  22.91   0.79   (---*---)
 7.5   6  72.38  27.62                     (---*---)
10.0   6  99.78   0.46                                (---*---)
12.5   6  99.91   0.10                                (---*---)
                        ------+---------+---------+---------+---
                             25        50        75       100
Pooled StDev = 12.35
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of conc.
Individual confidence level = 99.64%
conc. =  0.5 subtracted from:
conc.    Lower  Center   Upper  -------+---------+---------+---------+--
 1.0    -13.52    8.77   31.06                (--*---)
 2.5    -14.12    8.17   30.46                (--*---)
 5.0    -20.58    1.71   24.00               (--*---)
 7.5     28.89   51.18   73.47                       (---*--)
10.0     56.30   78.59  100.88                           (---*---)
12.5     56.42   78.71  101.01                           (---*---)
                                -------+---------+---------+---------+--
                                     -60         0        60       120
conc. =  1.0 subtracted from:
conc.    Lower  Center  Upper  -------+---------+---------+---------+--
 2.5    -22.89   -0.60  21.69                (---*---)
 5.0    -29.36   -7.07  15.22               (---*---)
 7.5     20.12   42.41  64.70                       (---*---)
10.0     47.52   69.81  92.10                            (---*--)
12.5     47.65   69.94  92.23                            (---*--)
                               -------+---------+---------+---------+--
                                    -60         0        60       120
conc. =  2.5 subtracted from:
conc.    Lower  Center  Upper  -------+---------+---------+---------+--
 5.0    -28.75   -6.46  15.83               (---*---)
 7.5     20.72   43.01  65.30                       (---*---)
10.0     48.13   70.42  92.71                            (---*--)
12.5     48.25   70.54  92.84                            (---*--)
                               -------+---------+---------+---------+--
                                    -60         0        60       120
conc. =  5.0 subtracted from:
onc.   Lower  Center  Upper  -------+---------+---------+---------+--
 7.5   27.18   49.47  71.76                        (--*---)
10.0   54.59   76.88  99.17                            (---*---)
12.5   54.72   77.01  99.30                            (---*---)
                              -------+---------+---------+---------+--
                                   -60         0        60       120
conc. =  7.5 subtracted from:
conc.   Lower  Center  Upper  -------+---------+---------+---------+--
10.0     5.11   27.41  49.70                    (---*--)
12.5     5.24   27.53  49.82                    (---*--)
                              -------+---------+---------+---------+--
                                   -60         0        60       120
conc. = 10.0 subtracted from:
conc.    Lower  Center  Upper  -------+---------+---------+---------+--
12.5    -22.16    0.13  22.42               (---*---)
-------+---------+---------+---------+--
                                    -60         0        60       120
```



Regression Analysis: Kanuka_AN versus conc.

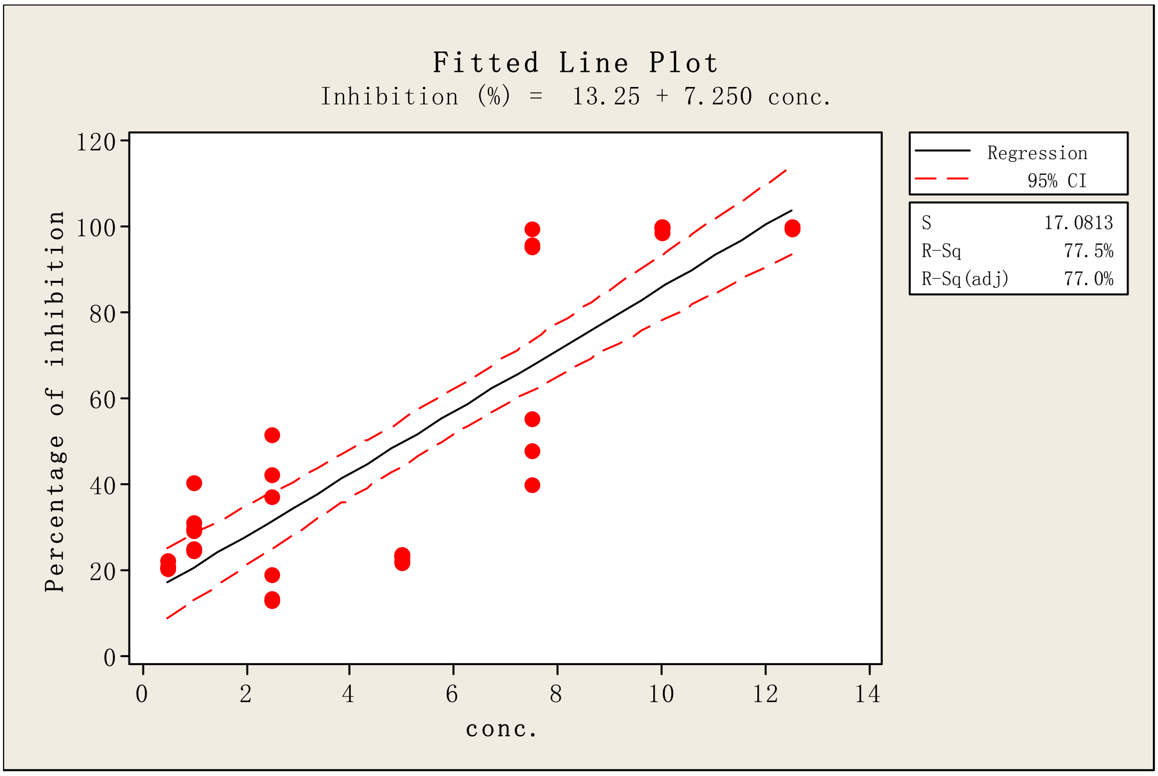
The regression equation is

Kanuka_AN = 13.25 + 7.250 conc.

S = 17.0813   R-Sq = 77.5%   R-Sq(adj) = 77.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	40278.6	40278.6	138.05	0.000
Error	40	11670.8	291.8		
Total	41	51949.4			



Kanuka	%	mg/ml
MIC50	5.0690	50.69
MIC90	10.5862	105.862
MIC100	11.9655	119.66

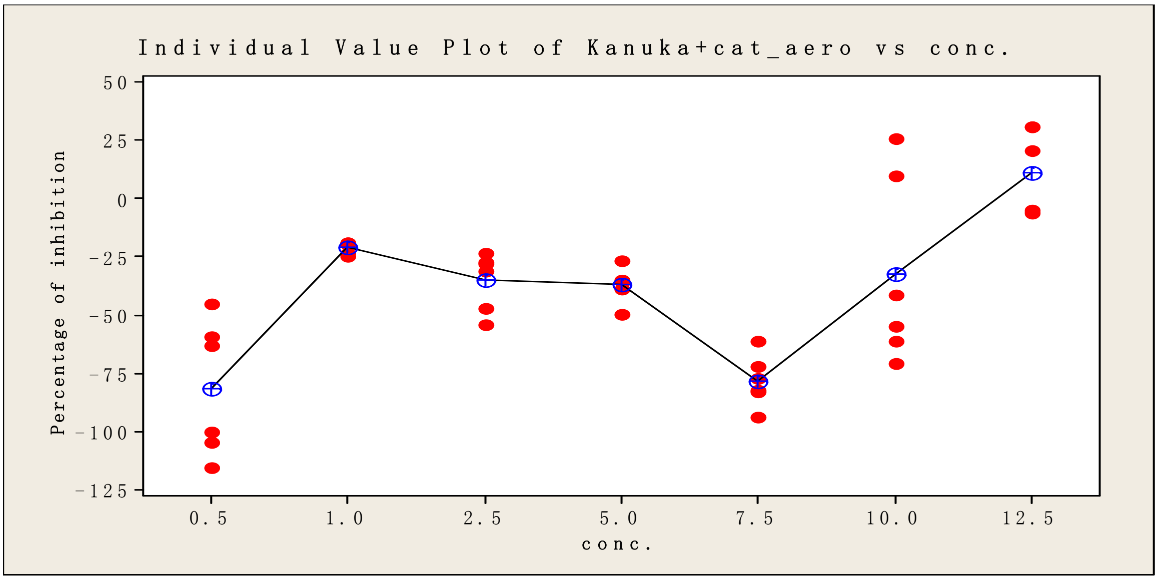
B3-10 Kanuka honey with catalase under anaerobic condition

Table B3-10 Percentage of inhibition of *P. acnes* in the Kanuka honey solutions ranged from 0.5% to 12.5% with catalase under aerobic condition.

Samples	Concentration		Replications						Average	SEM
	%	mg/ml	1	2	3	4	5	6		
KC0.5-aerobic	0.5	5	-45.50	-116.06	-63.21	-100.23	-104.75	-59.50	-81.54	26.46
KC1-AERO	1	10	-18.85	-18.62	-24.43	-19.00	-19.23	-23.53	-20.61	2.40
KC2.5-aerobic	2.5	25	-27.35	-30.79	-23.28	-28.01	-53.96	-47.40	-35.13	11.37
KC5-aerobic	5	50	-26.61	-35.86	-49.73	-35.75	-38.57	-34.71	-36.87	6.84
KC7.5-aerobic	7.5	75	-72.26	-61.27	-83.12	-93.85	-77.13	-82.22	-78.31	10.07
KC10-aerobic	10	100	-41.40	-70.77	-54.68	10.23	-61.47	25.72	-32.06	36.72
KC12.5-aerobic	12.5	125	-5.34	-4.48	-6.11	20.93	31.36	31.00	11.23	16.89

One-way ANOVA: Kanuka+cat_aero versus conc.

```
Source  DF     SS    MS      F      P
conc.    6  37699  6283  14.01  0.000
Error   35  15701   449
Total   41  53400
S = 21.18   R-Sq = 70.60%   R-Sq(adj) = 65.56%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 0.5   6  -81.54  28.98  (----*----)
 1.0   6  -20.61   2.63                 (----*----)
 2.5   6  -35.13  12.45             (----*----)
 5.0   6  -36.87   7.49            (----*----)
 7.5   6  -78.31  11.03   (----*----)
10.0   6  -32.06  40.22              (----*----)
12.5   6   11.23  18.50                          (----*----)
                         --------+---------+---------+---------+-
                               -70       -35         0        35
Pooled StDev = 21.18
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of conc.
Individual confidence level = 99.64%

conc. =  0.5 subtracted from:
conc.   Lower  Center   Upper  --------+---------+---------+---------+-
 1.0    22.71   60.93   99.15                      (-----*----)
 2.5     8.19   46.41   84.63                    (-----*----)
 5.0     6.45   44.67   82.89                    (----*-----)
 7.5   -34.98    3.24   41.45              (----*-----)
10.0    11.26   49.48   87.70                     (----*-----)
12.5    54.55   92.77  130.99                           (----*-----)
                               --------+---------+---------+---------+-
                                     -70         0        70       140

conc. =  1.0 subtracted from:
conc.    Lower  Center   Upper  --------+---------+---------+---------+-
 2.5    -52.74  -14.52   23.70          (-----*----)
 5.0    -54.48  -16.26   21.96          (-----*----)
 7.5    -95.91  -57.70  -19.48    (-----*----)
10.0    -49.67  -11.45   26.77           (----*-----)
12.5     -6.38   31.84   70.05                 (-----*----)
                                --------+---------+---------+---------+-
                                      -70         0        70       140

conc. =  2.5 subtracted from:
conc.    Lower  Center  Upper  --------+---------+---------+---------+-
 5.0    -39.96   -1.74  36.48            (-----*----)
 7.5    -81.39  -43.17  -4.96     (-----*----)
10.0    -35.15    3.07  41.29             (----*-----)
12.5      8.14   46.36  84.58                   (-----*----)
                               --------+---------+---------+---------+-
                                     -70         0        70       140

conc. =  5.0 subtracted from:
conc.    Lower  Center  Upper  --------+---------+---------+---------+-
 7.5    -79.66  -41.44  -3.22       (----*-----)
10.0    -33.41    4.81  43.03             (-----*----)
12.5      9.88   48.10  86.31                   (-----*----)
                               --------+---------+---------+---------+-
                                     -70         0        70       140

conc. =  7.5 subtracted from:
conc.   Lower  Center   Upper  --------+---------+---------+---------+-
10.0     8.03   46.24   84.46                   (-----*----)
12.5    51.31   89.53  127.75                         (-----*----)
                               --------+---------+---------+---------+-
                                     -70         0        70       140

conc. = 10.0 subtracted from:
conc.   Lower  Center  Upper  --------+---------+---------+---------+-
12.5     5.07   43.29  81.51                  (----*-----)
--------+---------+---------+---------+-
                                    -70         0        70       140
```



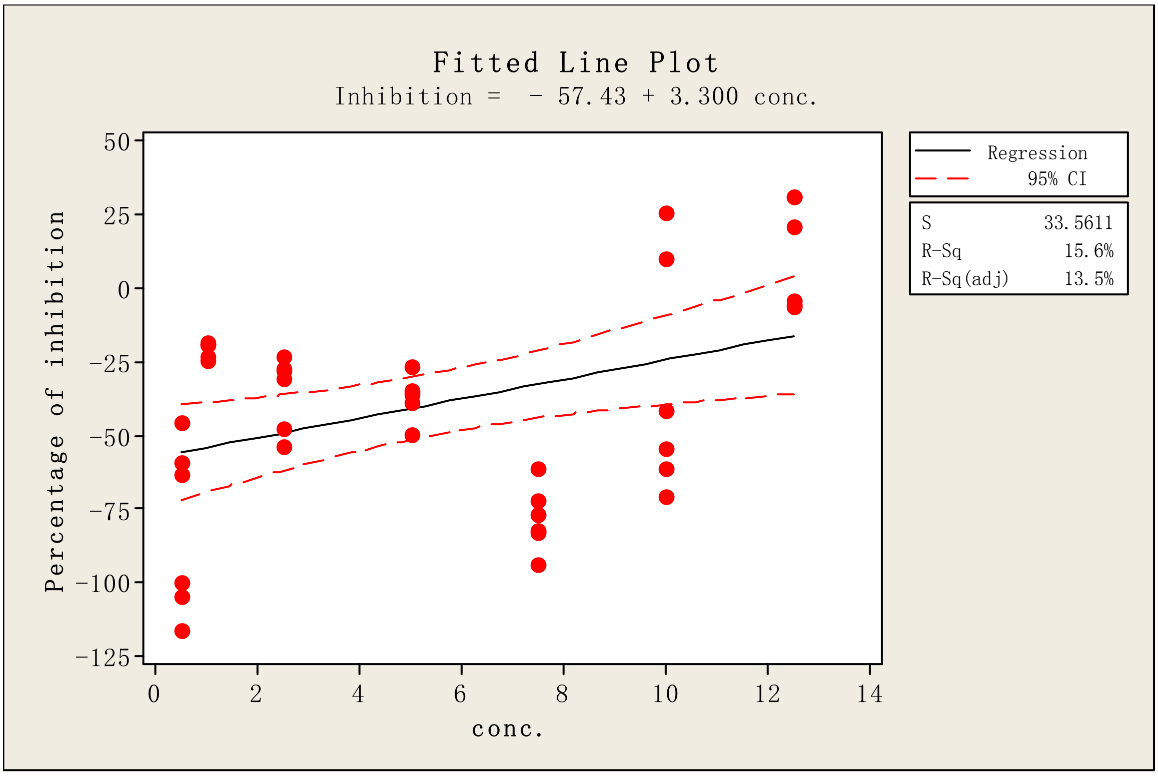
Regression Analysis: Kanuka+cat_aero versus conc.

The regression equation is
Kanuka+cat_aero = - 57.43 + 3.300 conc.

S = 33.5611   R-Sq = 15.6%   R-Sq(adj) = 13.5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	8346.4	8346.37	7.41	0.010
Error	40	45053.9	1126.35		
Total	41	53400.2			



Kanuka+cat	%	mg/mL
MIC50	28.5077	285.08
MIC90	49.6381	496.38
MIC100	54.9208	549.21

B.4 Screen natural bioactives using disc diffusion method

B4-1 Zone of inhibitions (mm) caused by Manuka tree oil and Green tea extracts

Two-Sample T-Test and CI: MTO100, MTO50

```
        N   Mean  StDev  SE Mean
MTO100  4  21.22   4.61      2.3
MTO50   4  12.48   3.50      1.7
Difference = mu (MTO100) - mu (MTO50)
Estimate for difference:  8.75
95% CI for difference:  (1.31, 16.19)
T-Test of difference = 0 (vs not =):
         T-Value = 3.02  P-Value = 0.029  DF = 5
```

One-way ANOVA: GTE100, GTE50, GTE25

```
Source  DF      SS      MS      F      P
Factor   2  220.81  110.40  57.60  0.000
Error   18   34.50    1.92
Total   20  255.31
S = 1.384   R-Sq = 86.49%   R-Sq(adj) = 84.99%
                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level   N    Mean  StDev  ----+---------+---------+---------+-----
GTE100  8  17.500  0.984                              (--*---)
GTE50   8  12.938  1.891               (--*---)
GTE25   5   9.210  0.819  (----*---)
                          ----+---------+---------+---------+-----
                            9.0      12.0      15.0      18.0
```

Pooled StDev = 1.384

Tukey 95% Simultaneous Confidence Intervals

Individual confidence level = 98.00%

```
GTE100    subtracted from:

GTE50   -6.330  -4.563  -2.795           (---*--)
GTE25  -10.305  -8.290  -6.275    (---*---)
                              -+---------+---------+---------+--------
                             -10.0      -5.0       0.0       5.0
GTE50 subtracted from:
                              Lower  Center   Upper

-+---------+---------+---------+--------
GTE25  -5.742  -3.727  -1.713            (---*---)
                              -+---------+---------+---------+--------
                             -10.0      -5.0       0.0       5.0
```

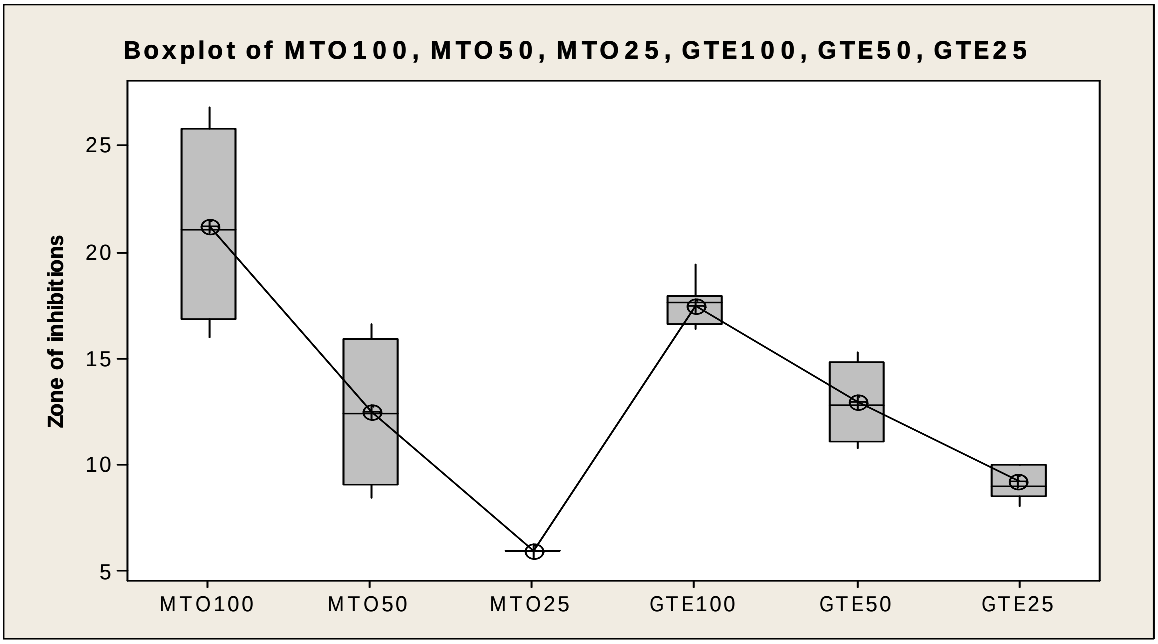


Table 4-1 Summary of different between variable bioacitves treatments

	MTO100	MTO50	MTO25	GTE100	GTE50	GTE25
MTO100						
MTO50	P<0.05					
MTO25	P<0.05	P<0.05				
GTE100	P>0.05	P<0.05	P<0.05			
GTE50	P<0.05	P>0.05	P<0.05	P<0.05		
GTE25	P<0.05	P>0.05	P>0.05	P<0.05	P<0.05	

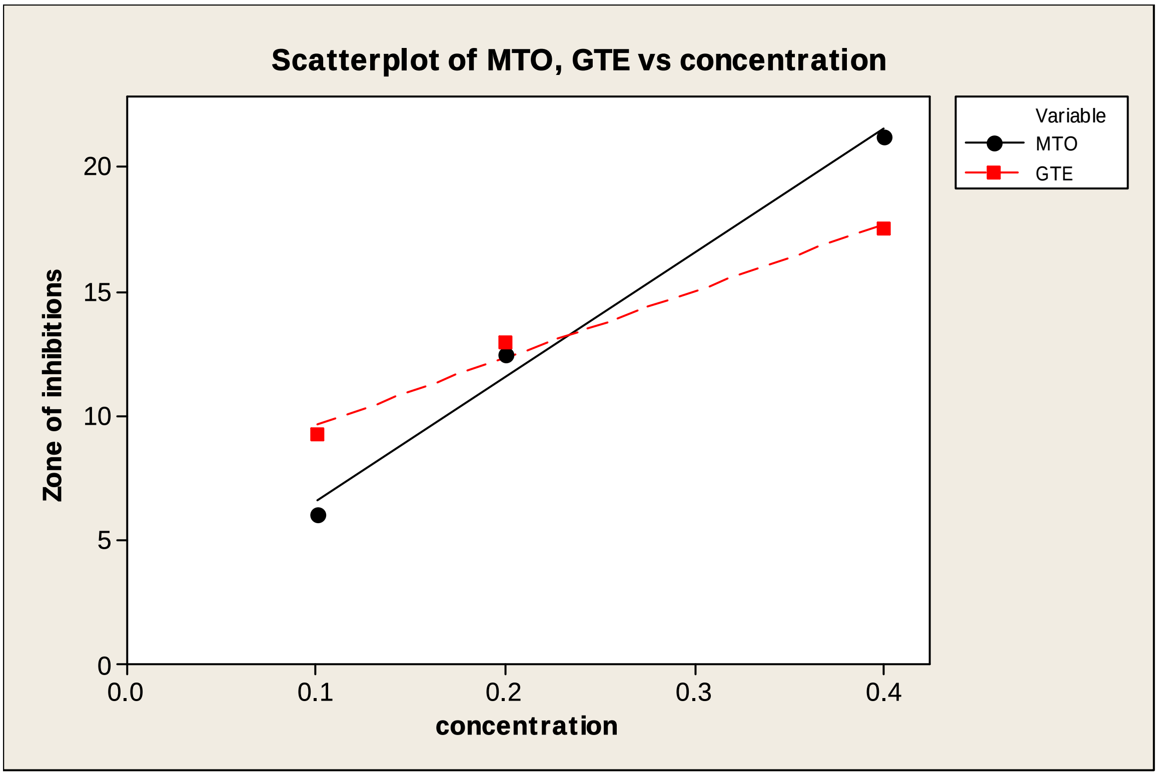
One-way ANOVA: MTO100, MTO50, MTO25, GTE100, GTE50, GTE25

```
Source  DF     SS     MS      F      P
Factor   5  690.21  138.04  27.58  0.000
Error   27  135.14    5.01
Total   32  825.35
S = 2.237   R-Sq = 83.63%   R-Sq(adj) = 80.59%
                       Individual 95% CIs For Mean Based on
                       Pooled StDev
Level   N   Mean  StDev  ---+---------+---------+---------+------
MTO100  4  21.225  4.615                         (---*----)
MTO50   4  12.475  3.500             (----*----)
MTO25   4   6.000  0.000  (----*----)
GTE100  8  17.500  0.984                      (--*--)
GTE50   8  12.938  1.891              (--*--)
GTE25   5   9.210  0.819        (---*----)
                         ---+---------+---------+---------+------
                          5.0      10.0      15.0      20.0
```

Pooled StDev = 2.237
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.51%

```
MTO100 subtracted from:
        Lower   Center    Upper
+---------+---------+---------+---------
MTO50   -13.594   -8.750   -3.906        (----*----)
MTO25   -20.069  -15.225  -10.381   (----*----)
GTE100   -7.920   -3.725    0.470            (---*---)
GTE50   -12.482   -8.287   -4.093         (---*---)
GTE25   -16.610  -12.015   -7.420      (----*----)

+---------+---------+---------+---------
                              -20       -10        0       10
MTO50 subtracted from:
        Lower  Center   Upper
+---------+---------+---------+---------
MTO25   -11.319  -6.475  -1.631          (----*---)
GTE100    0.830   5.025   9.220                   (---*---)
GTE50    -3.732   0.463   4.657               (---*----)
GTE25    -7.860  -3.265   1.330            (----*---)

+---------+---------+---------+---------
                            -20       -10        0       10
MTO25 subtracted from:
       Lower  Center   Upper
+---------+---------+---------+---------
GTE100   7.305  11.500  15.695                      (----*---)
GTE50    2.743   6.938  11.132                   (---*---)
GTE25   -1.385   3.210   7.805                (---*----)

+---------+---------+---------+---------
                           -20       -10        0       10
GTE100 subtracted from:
       Lower  Center   Upper
+---------+---------+---------+---------
GTE50   -7.987  -4.563  -1.138            (--*---)
GTE25  -12.195  -8.290  -4.385         (---*---)

+---------+---------+---------+---------
                           -20       -10        0       10
GTE50 subtracted from:
      Lower  Center  Upper
+---------+---------+---------+---------
GTE25  -7.632  -3.727  0.177            (---*---)
                        +---------+---------+---------+---------
                         -20       -10        0       10
```

B4-2 Correlations between zone of inhibition vs concentrations of GTE and MTO



Pearson correlation of zone of inhibition and concentration of MTO = 0.902

`P-Value = 0.000`

Pearson correlation of zone of inhibition and concentration of GTE = 0.921

`P-Value = 0.000`

B-5 Screen natural bioacitves using spectrophotometric assay to determine the MICs

B5-1 Growth of *P. acnes* in propolis solution

One-way ANOVA: p-100-24, p-50-24, p-25-24, p-12.5-24, p-6.25-24

```
Source  DF      SS      MS      F      P
Factor   4  0.19953  0.04988  11.12  0.000
Error   25  0.11213  0.00449
Total   29  0.31165
S = 0.06697   R-Sq = 64.02%   R-Sq(adj) = 58.27%
                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level      N    Mean    StDev  ------+---------+---------+---------+---
p-100-24   6  0.19179  0.04793  (----*-----)
p-50-24    6  0.26784  0.02116       (-----*----)
p-25-24    6  0.29224  0.02965          (----*-----)
p-12.5-24  6  0.33960  0.06832              (-----*-----)
p-6.25-24  6  0.43804  0.11889                       (-----*----)
                           ------+---------+---------+---------+---
                              0.20      0.30      0.40      0.50
Pooled StDev = 0.06697
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.29%
p-100-24 subtracted from:
            Lower   Center    Upper  ----+---------+---------+---------+-----
p-50-24   -0.03741  0.07605  0.18952           (-----*----)
p-25-24   -0.01301  0.10046  0.21392            (-----*-----)
p-12.5-24  0.03435  0.14782  0.26128               (----*-----)
p-6.25-24  0.13279  0.24626  0.35972                   (----*-----)
                                     ----+---------+---------+---------+-----
                                      -0.20      0.00      0.20      0.40
p-50-24 subtracted from:
            Lower   Center    Upper  ----+---------+---------+---------+-----
p-25-24   -0.08906  0.02440  0.13787          (----*-----)
p-12.5-24 -0.04170  0.07176  0.18523            (-----*----)
p-6.25-24  0.05674  0.17020  0.28367                (-----*----)
                                     ----+---------+---------+---------+-----
                                      -0.20      0.00      0.20      0.40
p-25-24 subtracted from:
            Lower   Center    Upper  ----+---------+---------+---------+-----
p-12.5-24 -0.06610  0.04736  0.16082           (----*-----)
p-6.25-24  0.03234  0.14580  0.25926               (----*-----)
                                     ----+---------+---------+---------+-----
                                      -0.20      0.00      0.20      0.40
p-12.5-24 subtracted from:
            Lower   Center    Upper  ----+---------+---------+---------+-----
p-6.25-24 -0.01502  0.09844  0.21190             (-----*-----)
                                     ----+---------+---------+---------+-----
                                      -0.20      0.00      0.20      0.40
```

Table B5-1 Summary for Tukey's comparisons

	p-100	P-50	p-25	p-12.5
P-100				
P-50	p>0.05			
P-25	p>0.05	p>0.05		
P-12.5	p<0.05	p>0.05	p>0.05	
P-6.25	p<0.05	p<0.05	p<0.05	p>0.05

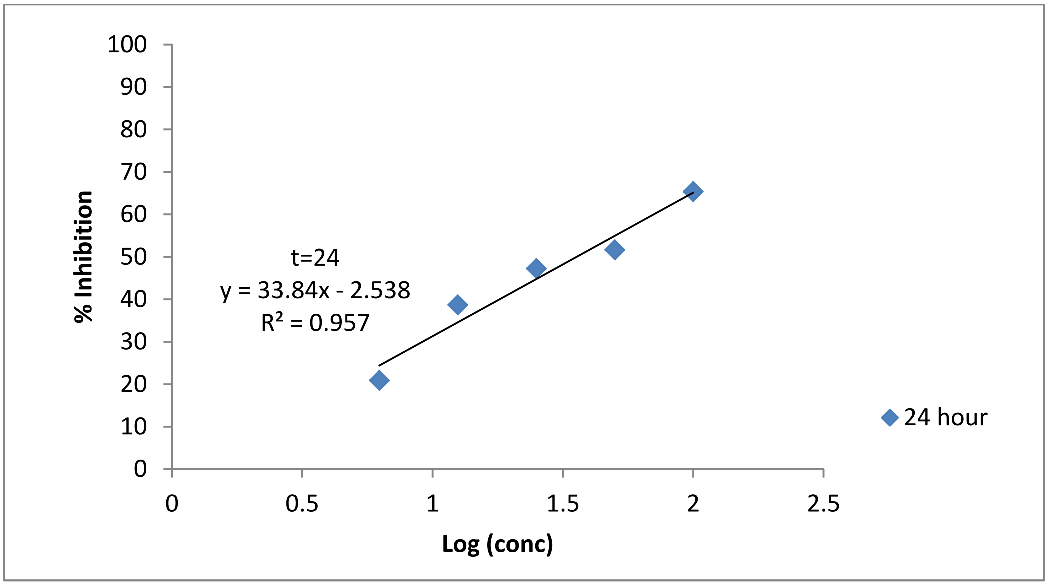


Figure B5-1 Percentage of incubation vs log of concentration of propolis solution

TIME	Calculate 90% inhibition	
	log MIC $_{90}$	MIC $_{90}$
t=24	2.734574	542.7183

B5-2 Growth of *P. acnes* in MTO solution

One-way ANOVA: mto-100-24, mto-50-24, mto-25-24, mto-12.5-24, mto-6.25-24

```
Source  DF     SS     MS    F     P
Factor   4  0.1577  0.0394  3.11  0.066
Error   10  0.1267  0.0127
Total   14  0.2844
S = 0.1126   R-Sq = 55.45%   R-Sq(adj) = 37.63%
Level       N   Mean   StDev
mto-100-24   2  0.1207  0.0212
mto-50-24    1  0.0537      *
mto-25-24    4  0.1387  0.0827
mto-12.5-24  4  0.2148  0.1190
mto-6.25-24  4  0.3657  0.1452
           Individual 95% CIs For Mean Based on Pooled StDev
Level         +---------+---------+---------+---------
mto-100-24          (--------*--------)
mto-50-24      (------------*-----------)
mto-25-24              (-----*-----)
mto-12.5-24               (------*-----)
mto-6.25-24                     (-----*------)
             +---------+---------+---------+---------
           -0.20     0.00      0.20      0.40
Pooled StDev = 0.1126
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.18%
mto-100-24 subtracted from:
             Lower   Center   Upper  --------+---------+---------+---------+-
mto-50-24    -0.5202  -0.0669  0.3863      (----------*-----------)
mto-25-24    -0.3024   0.0181  0.3386          (-------*-------)
mto-12.5-24  -0.2264   0.0941  0.4147            (-------*-------)
mto-6.25-24  -0.0755   0.2450  0.5655               (-------*-------)
                                     --------+---------+---------+---------+-
                                         -0.40     0.00      0.40      0.80
mto-50-24 subtracted from:
             Lower  Center   Upper  --------+---------+---------+---------+-
mto-25-24    -0.3288  0.0850  0.4988         (---------*---------)
mto-12.5-24  -0.2527  0.1611  0.5749           (---------*---------)
mto-6.25-24  -0.1018  0.3119  0.7257              (----------*---------)
                                    --------+---------+---------+---------+-
                                        -0.40     0.00      0.40      0.80
mto-25-24 subtracted from:
             Lower  Center   Upper  --------+---------+---------+---------+-
mto-12.5-24  -0.1856  0.0761  0.3378            (------*-----)
mto-6.25-24  -0.0348  0.2269  0.4886               (------*-----)
                                    --------+---------+---------+---------+-
                                      -0.40     0.00      0.40      0.80
mto-12.5-24 subtracted from:
             Lower  Center   Upper  --------+---------+---------+---------+-
mto-6.25-24  -0.1108  0.1509  0.4126              (------*-----)
                                    --------+---------+---------+---------+-
                                        -0.40     0.00      0.40      0.80
```

Table B5-2 Summary for Tukey's comparisons

	Mto-100	Mto-50	Mto-25	MTO-12.5	Mto-6.25
Mto-100					
Mto-50	p>0.05				
Mto-25	p>0.05	p>0.05			
MTO-12.5	p>0.05	p>0.05	p>0.05		
Mto-6.25	p>0.05	p>0.05	p<0.05	p>0.05	

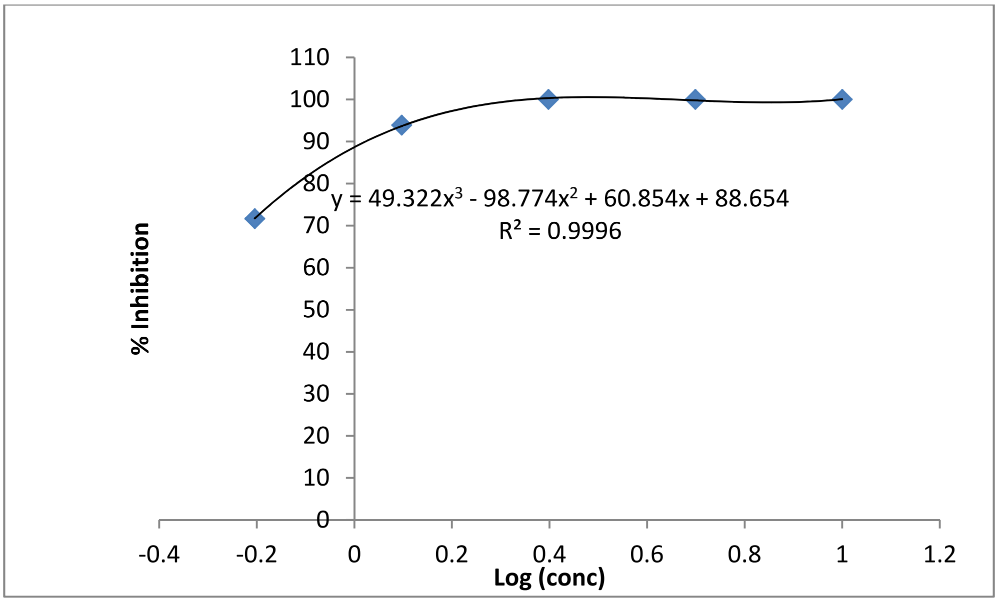


Figure B5-2 Percentage of incubation vs log of concentration of MTO solution

Table B5-2 MIC $_{90}$ of MTO

TIME	Calculate 90% inhibition	
	log MIC $_{90}$	MIC $_{90}$
t=24	0.324694	2.111999

B5-3 Growth of *P. acnes* in OLE solution

One-way ANOVA: ole-100, ole-50, ole-25, ole-12.5, ole-6.25

```
Source  DF      SS      MS      F      P
Factor   4  0.48238  0.12060  65.56  0.000
Error   15  0.02759  0.00184
Total   19  0.50998
S = 0.04289   R-Sq = 94.59%   R-Sq(adj) = 93.15%
                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level     N     Mean    StDev  -------+---------+---------+---------+--
ole-100   4  -0.05939  0.02899  (--*--)
ole-50    4   0.12882  0.04919            (--*--)
ole-25    4   0.21475  0.04901                (--*--)
ole-12.5  4   0.29410  0.05037                     (--*--)
ole-6.25  4   0.39820  0.03159                          (---*--)
                           -------+---------+---------+---------+--
                              0.00      0.15      0.30      0.45
Pooled StDev = 0.04289
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.25%
ole-100 subtracted from:
          Lower   Center    Upper  -----+---------+---------+---------+----
ole-50    0.09449  0.18821  0.28192                  (---*--)
ole-25    0.18043  0.27414  0.36786                    (---*---)
ole-12.5  0.25977  0.35348  0.44720                       (---*---)
ole-6.25  0.36388  0.45759  0.55131                           (--*---)
                               -----+---------+---------+---------+----
                                -0.25      0.00      0.25      0.50
ole-50 subtracted from:
           Lower   Center    Upper  -----+---------+---------+---------+----
ole-25    -0.00778  0.08593  0.17965              (--*---)
ole-12.5   0.07156  0.16528  0.25899                 (---*--)
ole-6.25   0.17567  0.26939  0.36310                    (---*---)
                                -----+---------+---------+---------+----
                                  -0.25      0.00      0.25      0.50
ole-25 subtracted from:
           Lower   Center    Upper  -----+---------+---------+---------+----
ole-12.5  -0.01437  0.07934  0.17306             (---*---)
ole-6.25   0.08974  0.18345  0.27717                 (--*---)
                                -----+---------+---------+---------+----
                                  -0.25      0.00      0.25      0.50

ole-12.5 subtracted from:
          Lower   Center    Upper  -----+---------+---------+---------+----
ole-6.25  0.01039  0.10411  0.19783             (---*---)
                               -----+---------+---------+---------+----
                                 -0.25      0.00      0.25      0.50
```

Table B5-3 Summary for Tukey's comparisons of OLE

	OLE-100	OLE-50	OLE-25	OLE-12.5
OLE-100				
OLE-50	SS			
OLE-25	SS	SS		
OLE-12.5	SS	SS	SS	
OLE-6.25	SS	SS	SS	SS

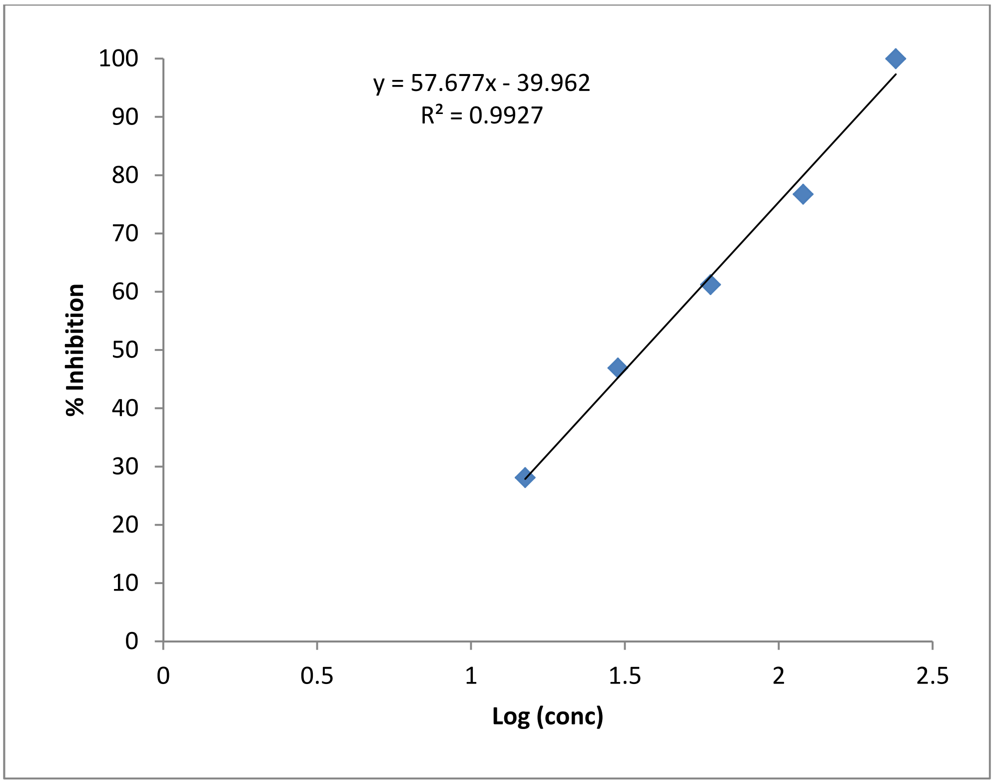


Figure B5-3 Percentage of incubation vs log of concentration of OLE solution

Table B5-3 MIC $_{90}$ of OLE

TIME	Calculate 90% inhibition	
	log MIC $_{90}$	MIC $_{90}$
t=24	2.253273	179.173

B5-4 Growth of *P. acnes* in Lavender oil solution

One-way ANOVA: LO-100, LO-50, LO-25, LO-12.5, LO-6.25

```
Source  DF      SS       MS      F      P
Factor   4  0.38406  0.09602  30.63  0.000
Error   15  0.04702  0.00313
Total   19  0.43108
S = 0.05599   R-Sq = 89.09%   R-Sq(adj) = 86.18%
                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level    N    Mean    StDev  --------+---------+---------+---------+-
LO-100   4  0.08601  0.08346  (---*---)
LO-50    4  0.16522  0.08446      (---*---)
LO-25    4  0.29626  0.01523             (---*---)
LO-12.5  4  0.39323  0.01964                  (---*---)
LO-6.25  4  0.45924  0.03095                      (---*---)
                         --------+---------+---------+---------+-
                              0.15      0.30      0.45      0.60
Pooled StDev = 0.05599
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons
Individual confidence level = 99.25%
LO-100 subtracted from:
          Lower   Center    Upper  -------+---------+---------+---------+--
LO-50    -0.04313  0.07921  0.20155              (----*----)
LO-25     0.08790  0.21024  0.33258                   (---*----)
LO-12.5   0.18488  0.30722  0.42955                      (----*----)
LO-6.25   0.25089  0.37323  0.49556                         (----*----)
                               -------+---------+---------+---------+--
                                   -0.25      0.00      0.25      0.50
LO-50 subtracted from:
         Lower   Center    Upper  -------+---------+---------+---------+--
LO-25    0.00869  0.13103  0.25337                (----*----)
LO-12.5  0.10566  0.22800  0.35034                    (----*----)
LO-6.25  0.17167  0.29401  0.41635                       (----*----)
                              -------+---------+---------+---------+--
                                  -0.25      0.00      0.25      0.50
LO-25 subtracted from:
          Lower   Center    Upper  -------+---------+---------+---------+--
LO-12.5  -0.02537  0.09697  0.21931               (----*----)
LO-6.25   0.04064  0.16298  0.28532                  (----*---)
                               -------+---------+---------+---------+--
                                   -0.25      0.00      0.25      0.50
LO-12.5 subtracted from:
          Lower   Center    Upper  -------+---------+---------+---------+--
LO-6.25  -0.05633  0.06601  0.18835              (----*----)
                               -------+---------+---------+---------+--
                                   -0.25      0.00      0.25      0.50
```

Table B5-4 Summary for Tukey's comparisons of OLE

	LO-100	LO-50	LO-25	LO-12.5
LO-100				
LO-50	SS			
LO-25	SS	SS		
LO-12.5	SS	SS	SS	
LO-6.25	SS	SS	SS	SS

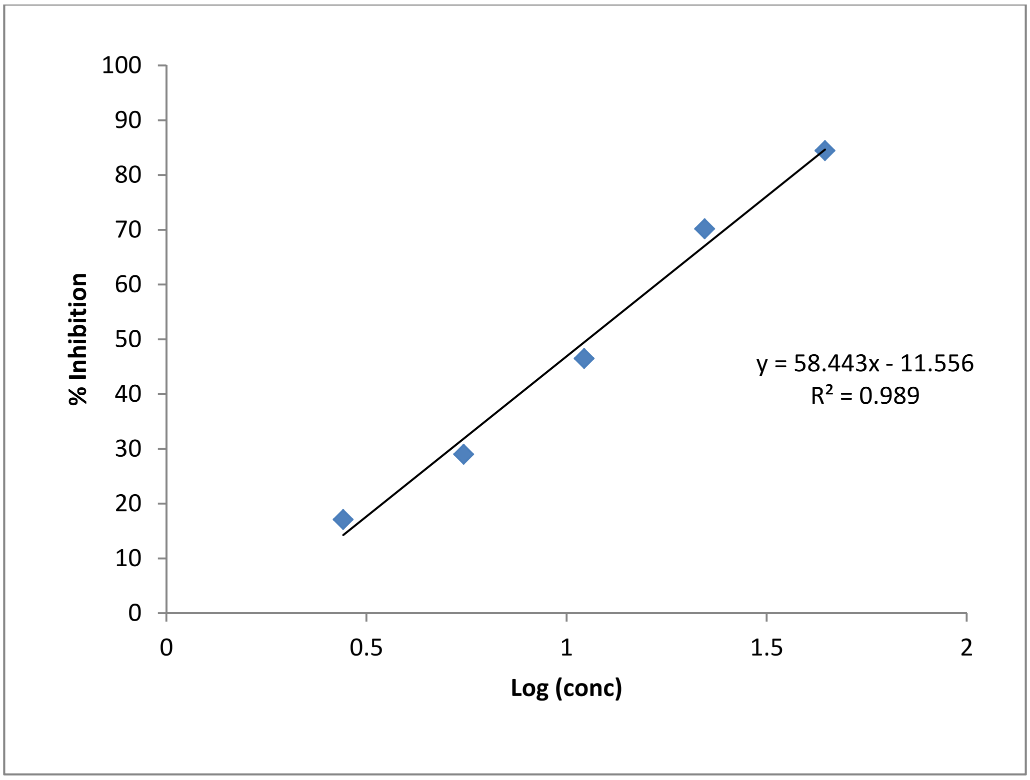


Figure B5-4 Percentage of incubation vs log of concentration of Lavender oil solution

Table B5-4 MIC $_{90}$ of Lavender oil

TIME	Calculate 90% inhibition	
	log MIC $_{90}$	MIC $_{90}$
t=24	1.737693137	54.66296

Appendix C: Material specification

C1 Chemical characteristics of honey

C1-1 Chemical characteristic of honeys for determining the antibacterial activity of honeys

Table C1-1 The phenolic acids, dihydroxyaceton and methylglyoxal (mg/kg) of honeys

Type	methyl syringate	iso methyl syringate (1)	iso methyl syringate (2)	4-methoxy benzoic acid	2-methoxy benzoic acid	Syringic acid	iso syringic acid	Gallic acid	phenyllactic acid	4-methoxy phenyllactic acid	dihydroxyaceton (DHA)	methylglyoxal (MGO)
Kanuka (2/2/5/1)	230	0.6	49.5	8	0.25	0.9	21.4	< 0.2	1784	not quantified	< 10	9.4
Manuka 10+ UMF	72.1	1.9	<0.1	0.4	4.5	0.4	20.6	0.9	732	10.6	1020	439
Manuka 15+ UMF	50.9	6.8	2.9	<0.1	21.7	0.5	60.1	0.3	1340	0.9	3580	656
Manuka 20+ UMF	109	7.6	1.1	<0.1	28.7	0.3	66.4	0.3	1740	3.6	4410	834

Table C1-2 The amount of H_2O_2 in honeys

Honeys	**H_2O_2 Rate (uM/hr) normalised to 10%w/v**
Manuka 20+ UMF	52.49
Manuka 15+ UMF	66.52
Manuka 10+ UMF	41.19
Kanuka	59.99

C1-2 Chemical analysis of Manuka honey 10+ UMF used in the cream emulsion

Hill Laboratories
BETTER TESTING **BETTER RESULTS**

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Hamilton 3240, New Zealand

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Fax +64 7 858 2001
Email mail@hill-labs.co.nz
Web www.hill-labs.co.nz

ANALYSIS REPORT

Client:	Comvita New Zealand Ltd	**Lab No:**	863278 SPv1
Contact:	Ralf Schlothauer	**Date Registered:**	28-Jan-2011
	C/- Comvita New Zealand Ltd	**Date Reported:**	18-Feb-2011
	Private Bag 1	**Quote No:**	34662
	TE PUKE 3153	**Order No:**	33096
		Client Reference:	33096
		Submitted By:	Comvita New Zealand Ltd

Sample Type: Sugars and Sugar Confectionery

Sample Name:		Bruce Manuka	Joanna 10+			
Lab Number:		863278.1	863278.2			
Individual Tests						
Dihydroxyacetone*	mg/kg	1,587	1,838	-	-	-
Methylglyoxal Analysis						
Methylglyoxal	mg/kg	198	607	-	-	-
Phenolic's Analysis (4 compounds)						
2-Methoxybenzoic Acid*	mg/kg	10.1	9.5	-	-	-
4-Methoxyphenyllactic Acid*	mg/kg	18.1	380	-	-	-
Methyl Syringate*	mg/kg	24	66	-	-	-
Phenyllactic Acid*	mg/kg	640	910	-	-	-

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Sugars and Sugar Confectionery

Test	Method Description	Default Detection Limit	Samples
Dihydroxyacetone*	Aqueous extraction, derivatisation, analysis by LC-MS. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	-	1-2
Methylglyoxal Analysis	Aqueous dilution, derivatisation, SPME-GC analysis. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	-	1-2
Phenolic's Analysis (4 compounds)*	Aqueous extraction, SPE cleanup, analysis by LC-MS/MS. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	-	1-2

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

Jason Clague BSc(Tech)
Technologist - Food & Bioanalytical Division

CERTIFICATE OF ANALYSIS FROM COMVITA

PRODUCT: Bulk UMF® 12+ Manuka Honey
CODE: 3198A
BATCH NO: 16673697
MANUFACTURING DATE: 10. 2010
EXPIRY DATE: 11. 2014
DESCRIPTION: New Zealand Golden Brown Medical Grade Active UMF®*
DATE OF ISSUE: 3 November 2011

ANALYSIS	METHOD	SPECIFICATION	RESULTS
Colour	LM02/01	Light to Medium Gold	Medium Gold
Moisture (%)	AOAC 969.38 refractometer	20 max	20.0
Non Peroxide Activity (% phenol)	University of Waikato Honey Assay method	>12	14.2
Total Count-Bacteria cfu/g	BP	<500	150
Yeast & Mould cfu/g	BP	<100	<10
Heavy metals (ppm) (Quarterly tested)	Acid Digest / CPMS	<20	<0.01
Multi-residue screening (Quarterly tested)	GC-MS (US-EPA protocols)	No residues detected	ND

*Issued as sample dispensed in 1kg honey jar.

Signed:______________________________________

Luke Jellett
Quality Systems Coordinator

COA-3198A-01

C2 Characteristic of bioactives

C2-1 Propolis

BULK STARTING MATERIAL SPECIFICATION / QC FORM

Q A Number 16524625
(Record QA number here)

Code	1299A
Version	Version 004
Date	11.03.2009
MFGPro Name	PROP PWD 80%
Synonyms	80% W/W Pure NZ Propolis
Description	Propolis collected from beehives, which is extracted with 80% ethanol, dried and standardised with food grade free flow agent. The powder is used in the manufacture of various propolis based products.

Test Method	Ref. Test Method	Results	
INWARDS GOODS CHECKS			
Approved Supplier	Check item is from correct supplier	PF	Nutrizeal - Ph 03 544 5610
Original Producer	Check original producer is correct	PF	NZ Bees
Supplier's Batch Number	Record	7454	Record Supplier's Batch Number
Date received	Record	2/11/10	Record date received at Comvita
Quantity received	Record	123 kg	Record quantity received at Comvita
Packing slip	Attach	PF	Packing slip is attached to back of QC form
Supplier COA	Attach	PF	Supplier COA is attached to back of QC form
MFGPro label check completed	Record	PF	MFGPro label is correct
Packaging	Information	Information	Original container. 2kg heat sealed foil pouch.
Storage	Information	Information	Store in a sealed container in a cool (5-15ºC) dry place. Avoid compaction of powder or exposure of the powder to air.
SENSORY ATTRIBUTES			
Appearance	Visual	complies	Fine, free flowing powder without lumps.
Colour	Visual	complies	Light beige to mid brown
Odour	Smell sample	complies	Characteristic odour of Propolis without taints of foreign matter.
ANALYTICAL & PHYSICAL SPECIFICATIONS			
Propolis Solids (% w/w)	Record result from CoA	80	80.0 min (quantified by input)
Lead Content (ppm = mg/kg)	Record result from CoA	0.6	25.0 max
Lead Code	LM09/01	L1	L1 is < 1ppm, L2 is <9ppm, L3 is <20ppm. *Enter result into MFGPro "Grade" category when releasing powder
Particle size (microns)	425 micron sieve Record result from CoA	<425	100% passes through sieve
Moisture content (%)	BP Record result from CoA	0	2.0 max
Foil Bag Seal	Visual	complies	Check foil bags are firmly sealed - no tears splits or inadequate sealing.
FLAVONOID SPECIFICATIONS: Amount of compound % if sample at 100% wt/wt:			
Flavonoids	HPLC	NOTE: Not a release specification. Not a license requirement. File Cawthorn results on I:\Quality Control\Stability, Tests and Investigation Records\Flavonoids Spreadsheet	18 min
Dihydroflavonoids (as % of total flavonoids) (%)	HPLC		50 min
Chrysin (%)	HPLC		2 min
Galangin (%)	HPLC		3 min
Pinocembrin (%)	HPLC		4 min
MICROBIOLOGICAL SPECIFICATIONS			
Standard Plate Count (cfu/g)	Record COA result	<100	<1,000

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02/11/2010 12:39, Page 1 of 2

BULK STARTING MATERIAL SPECIFICATION / QC FORM Q A Number ______________ (Record QA number here)		**Code**	**1299A**
		Version	Version 004
		Date	11.03.2009
		MFGPro Name	**PROP PWD 80%**
		Synonyms	80% W/W Pure NZ Propolis
Yeasts and Moulds (cfu/g)	Record COA result	<10	<100
Enterobacteriaceae cfu/g	Record COA result	<10	≤100
E.coli (/g)	Record COA result	ND	Not detected
Salmonella (/10g)	Record COA result	ND	Not detected
RELEASE CHECKS			
MSDS (refer to for first aid)		NO	No
Re-examine after expiry		N/A	Yes
Retention sample	Take, file and log	Yes	1 x 50g sample pot
Date of manufacture	Record	11/10/2010	Date
Use By date	Record	11/10/2015	5 years from date of manufacture
Quality Pass	If all components of this specification meet standard required	Pass	Pass or Fail
Released By	Record	L.G	Name
Released Date	Record	03/11/10	Date
Notes			

Effective from 1 December 2009

[1]STATEMENT FOR THE TRANSFER OF EXPORT BEE PRODUCTS BETWEEN EXPORT PREMISES

Consignor name *(coming from):* Nutrizeal Ltd, RMP identifier code:NZEAL1 Transport identifier code:PBT6	Consignee name *(going to)* :Comvita NZ Ltd. RMP identifier code:COMVITA20

Consignment details

Description of Goods	Quantity & Package Type	Identified as (code number)	Processing Dates	Net Weight
Freeze Dried Propolis Powder (80%w/w)	12 cartons @ 10kg 1 part carton @ 3kg	7454	11th Oct 10	123kg

The bee product described herein;		Yes	No
(a)	Meets the requirements of the "Apiarist and Beekeeper Statement for the Harvest of Honey and other Bee Products for Human Consumption"	☑	☐
(b)	In the case of honey:		
	(i) meets the requirements of one or more of options 2-6 of the Food (Tutin in Honey) Standard 2008,	☐	☑
	(ii) has been sampled and tested to meet the requirements of the Food (Tutin in Honey) Standard 2008 and is compliant with the relevant tutin limit	☐	☑
(c)	Is suitable for human consumption	☑	☐
(d)	Is of New Zealand origin	☑	☐
(e)	Has always been processed, stored or handled within a registered risk management programme under the Animal Products Act 1999	☑	☐
(f)	Meets the requirements of, and is eligible for export to the European Union	☑	☐
(g)	Is eligible for the countries listed below (meets requirements as notified by way of OMAR):		

Additional required declarations here (example: PDB statement, eligible countries)[2].

PDB inclusion rate in finished product is EU Eligible

Source Documents **e.g. Statement for Transfer, or Eligibility Document**	RMP	Date
Available on file at COMVITA New Zealand Ltd	COMVITA20	24 Oct 10

If you have answered "No" to any statements (a) to (f) provide further details for affected product, where required (including the product name, date, and code).

I declare that all the statements made in this document are true and correct. I am aware that the details provided will be received and retained by the processor and may be provided to the New Zealand Food Safety Authority for the general administration of the Animal Products Act 1999. I consent to that happening.

RMP Operator Signature: pp J A Steele Date: 21 / 10 / 10

RMP Operator Name: Julie Steele

Note: It is an offence under section 127 of the Animal Products Act 1999 to provide false or misleading information in this transfer statement and under the Privacy Act 1993 you have certain rights of access to and correction of personal information held about you.

[1] The consignor must send the original signed paper transfer document to the consignee. The consignee must hold the original transfer statement on file.

[2] Where for the purposes of compliance with the Food (Tutin in Honey) Standard 2008, the apiarist(s) or beekeeper(s) operate primary processing RMPs or FSPs, enter the primary processing RMP/FSP identifiers in this box.

Nutrizeal Limited, 379 Appleby Highway, PO Box 3205, Richmond 7050, New Zealand, Phone +64 3 544 5610, Facsimile +64 3 544 5611
http://www.nutrizeal.com

nutrizeal
Nutraceutical innovation and manufacture

Packing Slip

PRODUCT	–	**Freeze Dried Propolis Powder (~80%)** 12 Cartons each containing 10.0kg 1 Carton containing 3.0kg Total Order: 123kg
DOC'S ENCLOSED	–	Packing Slip Certificate of Analysis Bee Transfer
LOT NUMBER	–	7454
ORDER NUMBER	–	26399
PRODUCT CODE	–	PROFPO1.0
SUPPLIED TO	–	Comvita NZ Ltd Wilson Road South Te Puke

Verified
Michéle Buckner
Logistics and Dispensing

Approved
Nigel Gray
Operations & Logistics Manager

Date 26 October 2010

Certified ISO 9001 by BVQi

Filname: Comv261010.pac
Printdate: 26/10/2010

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PO Box 3205, Richmond 7050
Nelson, New Zealand
Phone +64 3 544 5610
Facsimile +64 3 544 5611
www.nutrizeal.com

nutrizeal
Nutraceutical innovation and manufacture

CERTIFICATE OF ANALYSIS

PRODUCT: 80% Propolis Powder

BATCH: 7454

QUANTITY: 12 cartons @ 10 kg
1 carton @ 3 kg
Total weight 123 kg

PACK DATE: 11 October 2010

DESCRIPTION: A free flowing fine beige powder of less than 425 microns.

SOURCE: Extracted from New Zealand Propolis with 80% Ethanol.
NOTE; Food grade free flow agents added.
80% Ethanol Extracted Propolis
15% Tricalcium Phosphate
5% Sipernat

TYPICAL COMPOSITION:

Parameter	Specification	Result

TYPICAL ANALYSIS:

Moisture content	max of 2%	0 %
Particle size Parameter	< 425 microns	< 425 microns

MICROBIAL CONTENT:

	Specification	Result
APC	$<10x10^3$ cfu/g	<100
Y & M	< 100 cfu/g	<10 /g
Enterobacteriaceae	< 100 cfu/g	< 10/g
E.Coli	Not Detected/g	Not Detected
Salmonella	Not Detected/g	Not Detected
Lead	< 25mg/kg	0.6/kg

INTENDED USE: Dietary supplement. Nutritional ingredient in edible powder preparations.

STORAGE RECOMMENDATIONS: Store in sealed containers in a cool (5 - 15^{0}C) dry place.
Avoid compaction of powder or exposure of the powder to air.
Shelf life can be extended by blending powder with excipients (or other diluents).

The product to which this certificate relates is free from genetically engineered organisms.
All product has been manufactured in compliance with Nutrizeal's HACCP (Hazard and Critical Control Point) regime which requires compliance with the Risk Management Programme as registered by the NZFSA. I certify that testing has been conducted such that this certificate is a true representation of the product to which it applies. Amended 28/04/10 to include original test results.

AUTHORISED BY: Hamish Tijsen	**COMPLIANCE MANAGER**
SIGNED: [signature]	**DATE:** 29 Oct 2010

Ref:Comv211010coa.doc

C2-2 Olive leaves extracts

PRODUCT SPECIFICATION SHEET

PURPOSE: To define the product's general and formulation specifications and the tests to be applied by the contract manufacturer(s) to every batch of this product.		
RESPONSIBILITY: Quality Assurance Manager		
	Product Name	Olive Leaf Extract - Pure 1:1
	Product Code	PURE OLE
	Common Name	Pure 1:1 Olive Leaf Extract
	Latin Name	*Olea europaea* extract
	Product Manufacturer[1]	Olive Leaf Australia Pty Ltd
	Plant Part	Leaf
	Origin	Australia
1.0	GENERAL SPECIFICATION	
1.1	Dosage Form	Bulk ingredient for multipurpose oral liquids
1.2	Physical appearance	Characteristic herbal dark brown liquid
1.3	Odour	Fruity and earthy
1.4	Flavour	Bitter and earthy
1.5	Solubility	Soluble in water
1.6	Extracting solvent	Potable water
1.7	Assay (Oleuropein mg/mL)[1]	Minimum 8.0
1.8	Assay test method	HPLC RD for content
1.9	Total viable count[1]	Less than 1000 cfu/mL (Micro Method 7)
1.10	Total yeast & mould[1]	Less than 1000 cfu/mL (Micro Method 7)
1.11	Heavy metal content	Not more than 20ppm (monitored annually)
1.12	Storage conditions	Store below 5°C. Away from sunlight.
1.13	Product shelf-life	Unstable - requires preservation prior to release for sale to consumers
1.14	Packaging	200 litre Food Grade Polyethylene Lined Steel Drum, 25 litre and 5 litre HDPE Food Grade Plastic containers with tamper-evident screw caps.
1.15	Label	n/a - bulk sale

[1] Sub-manufacturer(s): Conmac Laboratories

DOCUMENT #	F1 QA 003 PURE OLE page 1	ISSUE DATE	15.11.10
VERSION #	2010	REVIEW DATE	November 2012

2.0 FORMULATION SPECIFIATION						
	Approved Name	% v/v	Amount (mg/mL)	Label claim (mg/mL)	Release and Expiry Limits	Standard
	ACTIVE INGREDIENT					
2.1	*Olea europaea* (Olive) leaf extract liquid conc. (2g fresh leaf per mL in 100% water)	100	1000		QBI[2]	OLA[3]
	EQUIV. *Olea europaea* (Olive) leaf fresh			2000		
	EQUIV. *Olea europaea* (Olive) leaf dry			1000		
	EQUIV. Oleuropein			≥8.0	≥7.2	
	TOTAL	100				

[1] Sub-manufacturer(s): Conmac Laboratories
[2] QBI: Quantified By Input
[3] OLA: Olive Leaf Australia

Date: November 2010

Prepared by: Amy Gibson (QA Manager)

Amy Gibson.

Manufacturer:
Olive Leaf Australia Pty Ltd
767 Bischoffs Rd, Coominya,
Queensland, 4311, AUSTRALIA

Phone: +61 7 5424 6771
Fax: +61 7 5424 6772
Email: oliveleaf@olea.com.au
Web 1: www.envirolea.com
Web 2: www.olea.com.au

DOCUMENT #	F1 QA 003 PURE OLE page 2	ISSUE DATE	15.11.10
VERSION #	2010	REVIEW DATE	November 2012

C2-3 Lavender oil

Australian Botanical Products

Specialists in the chemistry of essential oils

MASTER COPY IF IN RED

CERTIFICATE OF ANALYSIS

PRODUCT NAME: LAVENDER OIL 38/40 NATURAL
PRODUCT CODE: ESLAVR38N

BATCH NO: P85328
DATE OF MANUFACTURE: 21/10/2010
EXPIRY DATE: OCTOBER 2015
RETEST DATE: APRIL 2015

TEST	MIN	MAX	UNITS	STANDARD	RESULTS
APPEARANCE				MOBILE LIQUID	MOBILE LIQUID
COLOUR				COLOURLESS TO PALE YELLOW	ALMOST COLOURLESS
CLARITY				CLEAR, NO FOREIGN MATTER	CLEAR
ODOUR				CHARACTERISTIC, FLORAL, HERBACEOUS	CHARACTERISTIC
SPECIFIC GRAVITY @ 20 DEG C	0.870	0.900			0.886
REFRACTIVE INDEX @ 20 DEG C	1.448	1.470			1.459

DOCUMENT NO: P85328 REV 1
PREPARED BY: VP DATE: 22/10/2010
RELATES TO PRODSPEC (ESLAVR38N) REV 1

DOCUMENT AUTHORISED BY: DATE: 25/10/10.

BRONSON & JACOBS
NEW ZEALAND DIVISION

Please Note: This information is offered as a guide only. Customers are advised to conduct their own testing to ascertain desirable results. Expiry date data refers to internal standards and assumes appropriate storage conditions. At the discretion of the user, the expiry date may be extended if the product still conforms to specification at the retest date.

Australian Botanical Products Pty Ltd
Enterprise No 15586 TGA Licence No 1380 A.C.N. 006 782 529
39 Melverton Dr, Hallam, Victoria, 3803 Australia
Telephone: +61 3 9796 4833 Facsimile: +61 3 9796 4966
Email : info@abp.com.au Website: www.abp.com.au

Safety Data Sheet

1. IDENTIFICATION OF THE MATERIAL AND SUPPLIER

Product Name: **OIL OF LAVENDER**

Other name(s): Lavender Oil; Lavender Oil 90992; Oil of Lavender 38/40; Oil of Lavender Spike; Oil of Lavender USP/NF; Oil of Lavender Australia; Lavender Oil Organic; Lavender Oil BP

Recommended Use: Essential oil.

Supplier: Bronson & Jacobs Pty Ltd
Street Address: Level four, 123 Carlton Gore Road
Newmarket, Auckland
New Zealand
Telephone Number: +64 9 309 2528
Facsimile: +64 9 0508 366 364
Emergency Telephone: 0 800 734 607 (ALL HOURS)

2. HAZARDS IDENTIFICATION

Not classified as a Dangerous Good under NZS 5433:2007 Transport of Dangerous Goods on Land.

Classified as hazardous according to criteria in the HS (Minimum Degrees of Hazard) Regulations 2001.

Subclasses:
Subclass 3.1 Category D (low hazard) - Flammable Liquids.
Subclass 6.1 Category E - Substances which are acutely toxic.
Subclass 6.3 Category B - Substances that are mildly irritating to the skin.
Subclass 6.4 Category A - Substances that are irritating to the eye.

Hazard and Precautionary Information:
Danger.

Combustible liquid. May be fatal if swallowed and enters airways. Causes mild skin irritation. Causes eye irritation. Keep out of reach of children. Read Safety Data Sheet before use. Keep away from flames and hot surfaces. No smoking. Wash hands thoroughly after handling. Wear protective gloves/protective clothing/eye protection/face protection. IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Do NOT induce vomitting. If skin irritation occurs: Get medical advice/attention. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention. In case of fire: Use normal foam, dry agent (carbon dioxide, dry chemical powder) for extinction. Store in a well-ventilated place. Keep cool. Store locked up. In case of a substance that is in compliance with a HSNO approval other than a Part 6A (Group Standards) approval, a label must provide a description of one or more appropriate and achievable methods for the disposal of a substance in accordance with the Hazardous Substances (Disposal) Regulations 2001. This may also include any method of disposal that must be avoided.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Components	CAS Number	Proportion	Risk Phrases

3. COMPOSITION/INFORMATION ON INGREDIENTS

Lavender oil	8000-28-0	100%	R38, R52/53, R65

4. FIRST AID MEASURES

Inhalation:
Remove victim from area of exposure - avoid becoming a casualty. Remove contaminated clothing and loosen remaining clothing. Allow patient to assume most comfortable position and keep warm. Keep at rest until fully recovered. Seek medical advice if effects persist.

Skin Contact:
If skin or hair contact occurs, immediately remove any contaminated clothing and wash skin and hair thoroughly with running water. If swelling, redness, blistering or irritation occurs seek medical assistance.

Eye Contact:
If in eyes, wash out immediately with water. In all cases of eye contamination it is a sensible precaution to seek medical advice.

Ingestion:
Rinse mouth with water. If swallowed, do NOT induce vomiting. Give a glass of water. Never give anything by the mouth to an unconscious patient. Get to a doctor or hospital quickly.

Medical attention and special treatment:
Treat symptomatically. Delayed pulmonary oedema may result.

5. FIRE FIGHTING MEASURES

Hazards from combustion products:
Combustible liquid. On burning will emit toxic fumes, including those of oxides of carbon .

Precautions for fire fighters and special protective equipment:
Fire fighters to wear self-contained breathing apparatus and suitable protective clothing if risk of exposure to vapour or products of combustion.

Suitable Extinguishing Media:
Normal foam, dry agent (carbon dioxide, dry chemical powder).

6. ACCIDENTAL RELEASE MEASURES

Emergency procedures:
Shut off all possible sources of ignition. If contamination of sewers or waterways has occurred advise local emergency services.

Methods and materials for containment and clean up:
Slippery when spilt. Avoid accidents, clean up immediately. Wear protective equipment to prevent skin and eye contact and breathing in vapours. Work up wind or increase ventilation. Contain - prevent run off into drains and waterways. Use absorbent (soil, sand or other inert material). Collect and seal in properly labelled containers or drums for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling: Avoid skin and eye contact and breathing in vapour, mists and aerosols.

Safety Data Sheet

Conditions for safe storage: Store in a cool, dry, well ventilated place and out of direct sunlight. Store away from sources of heat or ignition. Store below 20°C. Store away from incompatible materials described in Section 10. Keep containers closed when not in use - check regularly for leaks.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Occupational Exposure Limits: No value assigned for this specific material by the New Zealand Occupational Safety and Health Service (OSH).

Engineering controls:
Use in well ventilated areas. If inhalation risk exists: Use with local exhaust ventilation or while wearing organic vapour/particulate respirator. Keep containers closed when not in use.

Personal Protective Equipment:
The selection of PPE is dependant on a detailed risk assessment. The risk assessment should consider the work situation, the physical form of the chemical, the handling methods, and environmental factors.

OVERALLS, SAFETY SHOES, SAFETY GLASSES, GLOVES.

Wear overalls, safety glasses and impervious gloves. If risk of inhalation exists, wear organic vapour/particulate respirator meeting the requirements of AS/NZS 1715 and AS/NZS 1716.
Always wash hands before smoking, eating, drinking or using the toilet. Wash contaminated clothing and other protective equipment before storage or re-use.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical state:	Clear Mobile Liquid
Colour:	Colourless to Pale Yellow / Green
Odour:	Characteristic Lavender
Odour Threshold:	Not available
Solubility:	Partially miscible with water.
Specific Gravity:	0.875 - 0.888
Relative Vapour Density (air=1):	Not available
Vapour Pressure (20 °C):	Not available
Flash Point (°C):	71 - 76
Flammability Limits (%):	Not available
Autoignition Temperature (°C):	Not available
Melting Point/Range (°C):	Not available
Boiling Point/Range (°C):	Not available
pH:	Not available
Viscosity:	Not available
Partition Coefficient:	Not available

10. STABILITY AND REACTIVITY

Chemical stability:	Stable under normal conditions.
Conditions to avoid:	Avoid exposure to heat, sources of ignition, and open flame.
Incompatible materials:	Incompatible with strong acids and oxidising agents .
Hazardous decomposition products:	Oxides of carbon.
Hazardous reactions:	Hazardous polymerisation will not occur.

11. TOXICOLOGICAL INFORMATION

No adverse health effects expected if the product is handled in accordance with this Safety Data Sheet and the product label. Symptoms or effects that may arise if the product is mishandled and overexposure occurs are:

Ingestion:	Swallowing can result in nausea, vomiting and central nervous system depression. If the victim is showing signs of central system depression (like those of drunkeness) there is greater likelihood of the patient breathing in vomit and causing damage to the lungs. Breathing in vomit may lead to aspiration pneumonia (inflammation of the lung).
Eye contact:	A mild eye irritant.
Skin contact:	Contact with skin will result in irritation.
Inhalation:	Breathing in mists or aerosols may produce respiratory irritation.

Long Term Effects:
Repeated or prolonged use may defat the skin and produce dermatitis. (1)

Toxicological Data:
Dermal LD50 (rabbit): >5,000 mg/kg (2)
SKIN: Mild irritant (rabbit). (2)

12. ECOLOGICAL INFORMATION

Ecotoxicity	Avoid contaminating waterways.
Aquatic toxicity:	Harmful to aquatic organisms. May cause long term adverse effects in the aquatic environment.

13. DISPOSAL CONSIDERATIONS

Disposal methods:
Refer to local government authority for disposal recommendations. Dispose of material through a licensed waste contractor. Normally suitable for incineration by an approved agent.

14. TRANSPORT INFORMATION

Safety Data Sheet

14. TRANSPORT INFORMATION

Road and Rail Transport
Not classified as a Dangerous Good under NZS 5433:2007 Transport of Dangerous Goods on Land.

Marine Transport
Not classified as Dangerous Goods by the criteria of the International Maritime Dangerous Goods Code (IMDG Code) for transport by sea; NON-DANGEROUS GOODS.

Air Transport
Not classified as Dangerous Goods by the criteria of the International Air Transport Association (IATA) Dangerous Goods Regulations for transport by air; NON-DANGEROUS GOODS.

15. REGULATORY INFORMATION

Classification:
Classified as hazardous according to criteria in the HS (Minimum Degrees of Hazard) Regulations 2001.

Subclasses:
Subclass 3.1 Category D (low hazard) - Flammable Liquids.
Subclass 6.1 Category E - Substances which are acutely toxic.
Subclass 6.3 Category B - Substances that are mildly irritating to the skin.
Subclass 6.4 Category A - Substances that are irritating to the eye.

16. OTHER INFORMATION

(1) Supplier Material Safety Data Sheet; 10/ 2002.
(2) `Registry of Toxic Effects of Chemical Substances'. Ed. D. Sweet, US Dept. of Health & Human Services: Cincinatti, 2010.

This safety data sheet has been prepared by SH&E Shared Services, Orica.

Reason(s) for Issue:
Revised Primary SDS
Addition/Change of synonymous name(s)
Change in Hazardous Substance Classification
Alignment to HSNO requirements
Change in Personal Protection Requirements
Addition of PPE pictogram(s)

Safety Data Sheet

This SDS summarises to our best knowledge at the date of issue, the chemical health and safety hazards of the material and general guidance on how to safely handle the material in the workplace. Since Bronson & Jacobs Pty Ltd cannot anticipate or control the conditions under which the product may be used, each user must, prior to usage, assess and control the risks arising from its use of the material.

If clarification or further information is needed, the user should contact their Bronson & Jacobs representative or Bronson & Jacobs Pty Ltd at the contact details on page 1.

Bronson & Jacobs Pty Ltd's responsibility for the material as sold is subject to the terms and conditions of sale, a copy of which is available upon request.

Bronson and Jacobs incorporating the businesses of Woods and Woods and Keith Harris.

Safety Data Sheet

This SDS summarises to our best knowledge at the date of issue, the chemical health and safety hazards of the material and general guidance on how to safely handle the material in the workplace. Since Bronson & Jacobs Pty Ltd cannot anticipate or control the conditions under which the product may be used, each user must, prior to usage, assess and control the risks arising from its use of the material.

If clarification or further information is needed, the user should contact their Bronson & Jacobs representative or Bronson & Jacobs Pty Ltd at the contact details on page 1.

Bronson & Jacobs Pty Ltd's responsibility for the material as sold is subject to the terms and conditions of sale, a copy of which is available upon request.

Bronson and Jacobs incorporating the businesses of Woods and Woods and Keith Harris.

C2-4 Manuka tree oil

TAIRAWHITI PHARMACEUTICALS LTD

PO Box 124
Te Araroa, East Cape
New Zealand
Telephone/Fax +64 - 6 - 8644824
E - mail taipharm@xtra.co.nz
Website: http://www.manuka-oil.com

CERTIFICATE OF ANALYSIS

Date : February 2011

PRODUCT NAME: TAIRAWHITI MANUKA OIL

BOTANICAL NAME: LEPTOSPERMUM SCOPARIUM OIL*

BATCH NUMBER: M011.1

MANUFACTURED: January 2011

EXPIRY DATE: December 2014

PHYSICAL DATA :

Relative Density $20^{O}/20^{O}$ C	: 0.9765
Refractive Index 20^{O} C	: 1.5020
Flash point, ^{O}C >	: 98^{O} C

OTHER INFORMATION

GAS CHROMATOGRAPHIC ANALYSIS : see attached GC.

CHEMICAL COMPOSITION : TRIKETONE CONTENT

Triketones	- Flavesone	: }	
	- Leptospermone	: }	Combined total : 29.5 %
	- Iso-Leptospermone	: }	
	- Grandiflorone	: }	

Note: Triketone % Composition shown is determined by % peak area from GC - FID analysis. It should be noted that determination of Triketones % composition from GC- MS analysis will indicate a concentration of triketones up to 30 % higher than the GC-FID figure shown. ie a GC-FID figure of 24 % is comparable to a GC-MS figure of 31 %

BULK STARTING MATERIAL SPECIFICATION / QC FORM		Code	1697A
Q A Number Q41987 (Record QA number here)		Version	Version 002
		Date	02.09.2010
		MFGPro Name	MANUKA OIL 100%
		Synonyms	Tairawhiti Manuka Oil, manex Manuka Oil
		Supplier Item Number	Name
		Description	Manuka oil extracted from the Manuka tree leaves (*Leptospermum scoparium*). Used in the manufacture of manuka oil in Almond Oil Base and propolis soap.
Test Method	**Ref. Test Method**	**Results**	
INWARDS GOODS CHECKS			
Approved Supplier	Check item is from correct supplier	RL	Tairawhiti Pharmaceuticals Ltd
Supplier's Batch Number	Record	M011.1	Record Supplier's Batch Number
Date received	Record	4/4/11	Record date received at Comvita
Quantity received	Record	5 kg's	Record quantity received at Comvita
Packing slip	Attach	RL	Packing slip is attached to back of QC form
Supplier COA	Attach	RL	Supplier COA is attached to back of QC form
MFGPro label check completed	Record	RL	MFGPro label is correct
Packaging	Information		Original container. Aluminium container with screw cap.
Storage	Information		Store in well closed container protected from heat, light and at cool temperature (<18°C)
SENSORY ATTRIBUTES			
Appearance	Visual	LG	Oily liquid
Colour	Visual	LG	Clear yellow
Odour	Sensory	LG	Characteristic odour of Manuka Oil without taints.
ANALYTICAL & PHYSICAL SPECIFICATIONS			
Relative Density @ 20°C	Record COA result	0.9765	0.9550 - 0.9900
Flash Point (°C)	Record COA result	98	98.0 min
Refractive Index	Record COA result	1.5020	1.4960 - 1.5060
Triketone Content (%)	Record COA result	29.5 %	Flavesone, Leptospermone, Iso-Leptospermone combined total of 24% minimum
Melting Point (°C)	Information		25 max
Boiling Point (°C)	Information		230 - 285
MICROBIOLOGICAL SPECIFICATIONS			**Not required**
RELEASE CHECKS			
QC sampling	LM09/01	LG	Yes. Refer to Lab Manual
Re-examine after expiry		N/A	Yes
Retention Sample	Take, file and log	LG	1 x 25 ml
Date of manufacture	Record	Jan 2011	Date
Use By date	Record	July 2012	18 months from date of manufacture
Quality Pass	Record	Pass	Pass or Fail

The document is current on the date printed. It is the responsibility of the user to check that any hard copy in use is the current version.
04/04/2011 11:04, Page 1 of 2

BULK STARTING MATERIAL SPECIFICATION / QC FORM		Code	1697A
Q A Number ______________ (Record QA number here)		Version	Version 002
		Date	02.09.2010
		MFGPro Name	MANUKA OIL 100%
		Synonyms	Tairawhiti Manuka Oil, manex Manuka Oil
Released By	Record	L.G	Name
Released Date	Record	04/04/11	Date
Notes			

C2-5 Green tea extracts (Minimum of 60% catechins)

the nature network®

Martin Bauer Group

June 16, 2010

Comvita New Zealand Ltd.
Tony Wright
Private Bag 1
Te Puke
New Zealand
+64 7 533 1426

<u>Green Tea Extract Samples</u>

Dear Tony:

As per your request, enclosed please find samples of green tea extracts for your evaluation. Product specification sheets are attached for your reference.

Article #	Product Description
285039	100% green tea extract, cold water soluble, minimum of 15% catechins, unmilled powder
285090	100% green tea extract, hot water soluble, minimum of 30% catechins, minimum of 10% EGCG, minimum of 5% caffeine, unmilled powder
285127	100% green tea extract, hot water soluble, minimum of 60% catechins, minimum of 30% EGCG, unmilled powder

Normal use level for these extracts would be 0.45 grams per 150 mls. of water. For your application you will need to see what impact the tea has on the oil and determine what works for your final product.

Article 285039 does not have a specification for EGCG, but the normal range for EGCG would be between 6 and 8%.

Please let me know if you would require additional information or samples.

Sincerely,

Gary G. Vorsheim
Director of Extract Sales

Martin Bauer Inc. • 300 Harmon Meadow Blvd. • Suite 510 • Secaucus, New Jersey 07094 USA • + 1 201 659 3100 the nature network®

Plantextrakt

Product Specification

Article-No.:	**285127**	Edition:	6
Article:	**Green tea powdered extract "min. 60% catechins"**		
		valid from:	16.06.09

Raw material	Greentea
Extraction solvent	Demineralised drinking water
Preservation	None
Legal status	Food
Product description	Green tea powdered extract "min. 60% catechins" is a spraydried, regarding catechins enriched extract, made from green tea.
Appearance	Brown, homogenous powder
Taste and smell	Strong in adstringency
Solubility	Soluble in demineralised water (0.12%/90°C)

Parameter	**Limits**
Identification	
Sensory examination, SOP 103980	Conform
Tests and Assay	
Loss in mass, according to ISO 7513-1990, SOP 304480	<= 5 %
Catechins, calculated with reference to the dried drug,(HPLC), according to ISO/WD CD 14502-2 mod., SOP 609550:	
Caffeine	>= 5 %
Total catechins	>= 60 %
(-)-Epigallocatechingallate EGCG	>= 30 %

Product Specification

Article-No.:	**285127**	Edition: 6
Article:	**Green tea powdered extract "min. 60% catechins"**	
		valid from: 16.06.09

Bulk density, SOP 305600 — 400 - 650 g/1000 ml

Safety Data

Pesticides, §64 LFGB L00.00-34	Pesticide residues of the raw material comply with the maximum limits of Regulation (EC) No. 396/2005, including annexes and successive updates.

Microbiology, according to the requirements of FIP (PHARM.ACTA HELV.51,Nr.3(1976)), SOP 803014:

Aerobic bacteria, SOP 300001	<= 1,000 CFU/g
Moulds, SOP 300010	
Yeasts, SOP 300020	
Yeasts and moulds, SOP 300030	<= 100 CFU/g
Enterobacteria, SOP 300040	<= 100 CFU/g
Escherichia coli (absence test), SOP 300052	Absent /g
Pseudomonas aeruginosa, SOP 300060	Absent /g
Staphylococcus aureus, SOP 300072	Absent /g
Salmonella (PCR), SOP 300102	Absent /25 g

Remarks

Pesticides	Scope of testing Phytolab phytrace P5.3
Shelf life	Best before 24 months, store at a dry place at 15 - 20° C, protected from light, unopened in the original container.
Notice	The product should not be stored together with intensive smelling products.

Dr. B. Klier
Head of quality control

R. Zenger
Product development

C3 Brain heart infusion broth

(http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=CM1135&c=UK&lang=EN)
Code: CM1135
A highly nutritious infusion medium recommended for the cultivation of streptococci, Neisseria and other fastidious organisms.

Formula	**gm/litre**
Brain infusion solids	12.5
Beef heart infusion solids	5.0
Proteose peptone	10.0
Glucose	2.0
Sodium chloride	5.0
Disodium phosphate	2.5
pH 7.4 ± 0.2 @ 25°C	

Directions
Dissolve 37g in 1 litre of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes.

Description
A versatile liquid infusion medium which is suitable for the cultivation of streptococci, *Neisseria* and other fastidious organisms, this medium is recommended for blood culture work and, with the additions described below, for the isolation and cultivation of pathogenic fungi.

Oxoid Brain Heart Infusion Broth is essentially a buffered infusion broth, giving similar results to the brain dextrose broths originally employed for the cultivation of streptococci[1], and for the cultivation of dental pathogens[2]. The addition of 0.1% agar will serve to reduce convection currents and so create conditions of varying oxygen tension which favour the growth and primary isolation of aerobes and anaerobes[3], while even easily cultivated organisms show improved growth[4].

Oxoid Brain Heart Infusion Broth was used in a test for the pathogenicity of streptococci[5,6] and the same medium was enriched with ascitic fluid for the cultivation of gonococci[7]. It is especially useful as a growth and suspension medium for staphylococci which are to be tested for coagulase production; Newman[8] employed a similar medium for this purpose in an investigation of food poisoning caused by dairy products.

A satisfactory medium for blood culture can be prepared by adding 1g of agar per litre of Brain Heart Infusion Broth. Ensure that the agar is uniformly distributed in the sterile broth before dispensing into bottles. More conveniently, add one Agar Tablet (CM0049) to each 100ml of Brain Heart Infusion and sterilize by autoclaving for 15 minutes at 121°C. Cool to 60-70°C and mix gently to ensure uniform distribution of the agar.

Tubes of Oxoid Brain Heart Infusion Broth which are not used the same day as sterilized should be placed in a boiling water bath for several minutes to remove absorbed oxygen, and cooled rapidly without shaking, just before use.

Further supplements to improve the recovery of organisms from blood can be added before sterilization or aseptically post-sterilization. Co-enzyme1 (NAD), penicillinase and p-amino benzoic acid are examples. Brain Heart Infusion Broth supplemented with yeast extract, haemin and menadione was consistently better in producing heavy growth of five species of *Bacteroides* than three standard anaerobic broths. Furthermore, microscopy of overnight cultures showed normal morphology in Brain Heart Infusion Broth but abnormal morphology in the three anaerobic broths[9].

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store tubed or bottled medium in the dark and below 20°C.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder.
Prepared medium: Straw coloured solution.

Quality control

Positive controls	**Expected results**
Streptococcus pneumoniae ATCC® 6303*	Turbid growth
Candida albicans ATCC® 10231*	Turbid growth
Negative control	
Uninoculated medium	No change

C4 Fastidious anaerobe agar

Fastidious Anaerobe Agar (F.A.A.)

LAB 90

Description

A primary isolation medium capable of growing most clinically significant anaerobes. Developed by Lab M, comparisons have shown this medium to be superior to other formulations as a primary isolation medium for fastidious organisms. The peptones included have been chosen for maximum growth stimulation. Starch and sodium bicarbonate act as de-toxification agents whilst haemin encourages pigment production in *Porphyromonas melaninogenicus*. Specific growth promoting agents are Cysteine for *Fusobacterium necrophorum, Propionibacterium acne* and *Bacteriodes fragilis*, arginine for *Eubacterium* spp. soluble pyrophosphate for *Porph. gingivalis* and *Porph. asaccharolyticus*. Pyruvate helps neutralise hydrogen peroxide and is also utilised by *Veillionella* spp. as an energy source. Vitamin K and sodium succinate provide essential growth factors for some anaerobes as does the 0.1% glucose. The low level of glucose prevents the production of high levels of acids and alcohols which would inhibit colonial development.

Formula	g/litre
Peptone mix	23.0
Sodium chloride	5.0
Soluble starch	1.0
Agar No. 2	12.0
Sodium bicarbonate	0.4
Glucose	1.0
Sodium pyruvate	1.0
Cysteine HCl monohydrate	0.5
Haemin	0.01
Vitamin K	0.001
L-Arginine	1.0
Soluble pyrophosphate	0.25
Sodium succinate	0.5

Method for reconstitution

Weigh 46 grams of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C then aseptically add 5-10% of sterile defibrinated horse blood, mix well and pour into Petri dishes. This medium can be made selective for various species of anaerobes by the addition of appropriate selective cocktails e.g.

Gram negative anaerobes X090, X290

Non-sporing anaerobes X091, X291

Actinomyces spp. X092

Clostridium difficile X093

Appearance: Red due to addition of blood. The blood will darken (reduce) because of the presence of reducing agents.

pH: 7.2 ± 0.2

Minimum Q.C. organisms: *B. fragilis*
P. anaerobious

Storage of Prepared Medium: Plates – up to 7 days at 2-8°C in the dark.

Inoculation: Surface plating, streaking out to single colonies.

Incubation: 37°C anaerobically with 10% CO_2 for 48 hours to 5 days.

Growth Characteristics				
organism	colony size (mm)	shape & surface	colour	other
B. fragilis	1.0-2.0	CV.E.G.	Grey	
C. perfringens	1.0-2.0	CV.E.G.	Grey	'Target' haemolysis (non haemolytic)
F. necrophorum	1.0-2.0	CV.E.G.(D)	trans- parent	(grey) (haemolytic)
Porphyromonas asaccharolyticus	1.0-2.0	CV.E.G.	Grey/Brown	(clearing)
B. ureolyticus	0.5	F.E.D.	translucent	pitting
Prop. acne	0.5	CV.E.G.	White	
Pept. anaerobius	0.5-2.0	CV.E.G.	White/Grey	
A. israeli	0.5-1.0	CV.E.G.	White	('molar tooth')(smooth)

References

Brazier, J.S. (1986). Yellow fluorescence of Fusobacteria Letters in Applied Microbiol. 2: 124-126.

Brazier, J.S. (1986). A note on ultra violet red fluorescence of anaerobic bacteria in vitro. J. Appl. Bact. 60: 121-126.

Eley, A., Clarry, T., Bennett, K.W. (1989). Selective and differential medium for isolation of *Bacteriodes ureolyticus* from clinical specimens. European Journal of Clinical Microbiology, Infectious

Diseases. 8: 83-85.

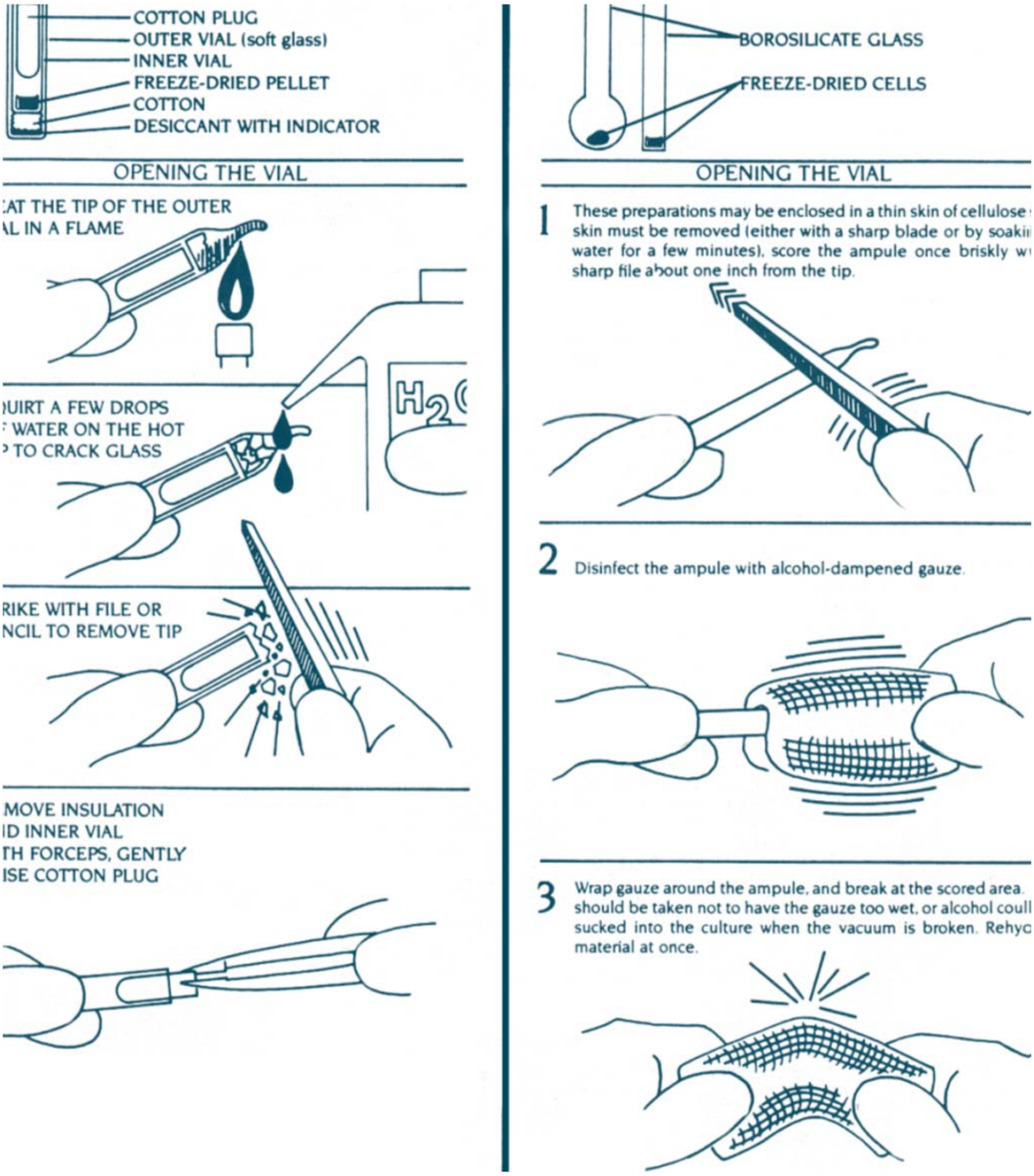
Wade W. Griffiths, M. (1987). Comparison of Media for cultivation of subgingival bacteria. J. Dent. Res. 66: no. 4 abstract 334.

Heginbotham M., Fitzgerald T.C., and Wade W.G. (1990). Comparison of solid media for the culture of anaerobes. J. Clin. Path. 43: 253-256.

MMv1 09/06

C5 Retriving *P. acnes* cultures

(http://www.atcc.org/Portals/1/Pdf/rfdc.pdf retrieved on 3 June 2010)



C6 Emulsifier: Olivem® 1000

B&T COMPANY
creative users only

OLIVEM®1000

TECHNICAL DATA SHEET

01. PRODUCT AND COMPANY IDENTIFICATION

Trade Name	:	**OLIVEM®1000**
Applications	:	non ionic, not ethoxylated self-emulsifying system derived from olive oil for O/W creams and lotions
INCI Name	:	CETEARYL OLIVATE, SORBITAN OLIVATE
CAS Number	:	85116-80-9 / 92202-01-2
EINECS Number	:	285-532-3 / 296-033-5
Other CAS Number	:	(348616-34-2 / 223706-40-9) (92797-33-6 / 97358-61-7) (2278-96-3 / 97358-61-7)
NICNAS (Y/N)	:	Y
Legislative Approval	:	world-wide
Company	:	B & T S.r.l. - Via E. Rossi ,60 20043- Arcore (MI) Italy Phone: +39 039 6180667 - Fax: +39 039 6188946

02. SPECIFICATION

Form @ 20°C	:	waxy solid in flakes
Odour	:	slight, characteristic
Colour	:	white-ivory
Active Substance (%)	:	99.0 – 99.9
Water Content (%)	:	1.00 max.

03. TYPICAL VALUES

Acid Value (mgKOH/g)	:	10.0 max.
Saponification Value (mgKOH/g)	:	100 - 120
Melting Range	:	65° - 75°C
pH of the gel (5% in water)	:	5 - 7
Colour (Gardner on the melted product)	:	3 - 5
Additives and preservatives	:	none

04. SOLUBILITY

Soluble	:	in alcohols and organic solvents
Dispersible	:	in vegetal and mineral oils, propylene glycol, glycerin and water

05. SHELF-LIFE

5 years stored unopened into original containers at a temperature between 5°C and 35°C

Revision: EP004-12-09

Appendix D: APSEA assessments of psychological and social effects of acne (Ayer and Burrows, 2009)

Questions 1-6: tick the most appropriate answer to each question

In the past week:

1. Worrying thoughts have gone through my mind
   a) A great deal of time
   b) A lot of time
   c) From time to time, not often
   d) Only occasionally

2. I can still feel at ease and relaxed
   a) Definitely
   b) Usually
   c) Not often
   d) Not at all

3. I feel restless, as I have to be on the move
   a) Very much indeed
   b) Quite a lot
   c) Not very much
   d) Not at all

At this moment:

4. I like what I look like in photographs
   a) Not at all
   b) Sometimes
   c) Very often
   d) Nearly all the time

5. I wish I looked better
   a) Not at all
   b) Sometimes
   c) Very often
   d) Nearly all the time

6. On the whole I am satisfied with myself
   a) Strongly disagree
   b) Disagree
   c) Agree
   d) Strongly agree

Questions 7-15: read the following questions carefully and put a line at the point that most accurately represents how you feel

7. I still enjoy the things I used to do

Never 0 10 all the time

8. I am more irritable than usual

Never 0 10 all the time

9. I feel that I am useful and needed

Never 0 10 all the time

How has your skin condition limited the following activities or made them more difficult or awkward or less enjoyable since you have had acne?

10. Going shopping

Not at all 0 10 all the time

11. Going out socially to meet friends from the home

Not at all 0 10 all the time

12. Going away for week ends, holidays and outings

Not at all 0 10 all the time

13. Eating out

Not at all 0 10 all the time

14. Using public changing rooms, swimming pools

Not at all 0 10 all the time

15. Do you think your appearance will interfere with your chances of future employment?

Strongly disagree 0 10 Strongly agree