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

submitted in partial fulfilment of the requirements for the degree of

Master of Food Technology

Massey University Albany, New Zealand

Qiong Wu

June 2011

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.

ACKNOWLEDGEMENTS

It has been a great experience for me to pursue my post-graduate study in Food Technology at Massy University. This thesis would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this study.

I would like to thank Dr. Tony Mutukumira, my supervisor from Massey University, who has believed me and gave me unlimited support and guidance throughout the whole process of my study life. I would also like to thank, Dr Jonathan Stephens, Bilge Thompson, and Dr Ralf Schlothauer from Comvita New Zealand Ltd, who gave me this great opportunity to work on a commercial orientated project, unlimited supervision, and invaluable contribution. I also acknowledge Rachel Liu for her assistance in organising laboratory equipment and purchasing testing materials for the experiments. I also would like thank Chirag Ahuja, Comvita New Zealand Ltd, for helping me to prepare bioactives solutions. Finally, my special thanks go to Comvita New Zealand Ltd and Technology New Zealand for providing financial support.

I am thankful to have Lei Jiao as my partner, who brings love, happiness and encouragement in my life and study, whose advice and support helped me accomplish everything. Most of all, I would like to thank my parents for their unconditional love.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

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
Ɵ	=	Diameter