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**An analysis of polyphenolic  
blackcurrant (*Ribes nigrum*) extracts  
for the potential to modulate  
allergic airway inflammation**

A thesis presented in partial fulfilment  
of the requirements for the degree of

**Master of Science**  
**in**  
**Nutritional Science**

at Massey University, Palmerston North,  
New Zealand.

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2009



## **Statement of originality**

'I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the qualification of any other degree or diploma of a university or other institution of higher learning, except where due acknowledgement is made in the acknowledgements'.

Signed.....

Date.....



## **Abstract**

The allergic disease of asthma is characterized by an infiltration of inflammatory cells to the lung, a process co-ordinated by T-helper (TH) cells. The TH2 cytokine Interleukin (IL)-4 promotes infiltration of eosinophils to sites of inflammation. Eosinophil-selective chemoattractant cytokines (eg. eotaxins) are synthesized by lung epithelial cells. Eotaxin-3 is expressed at high levels in the asthmatic lung, predominantly after IL-4 stimulation. Eotaxin-3 is therefore a marker of inappropriate airway inflammation.

Polyphenolic (PP) compounds found in high concentrations in berries may have beneficial effects in inflammatory conditions. Plant and Food Research produced high-PP extracts of blackcurrant (BC) cultivars that were tested for inflammation modulating effects.

Since high doses of PPs have been shown to cause cell death, we tested two BC cultivars at a range of concentrations in a cell viability (WST-1) assay.

While no toxic effects were attributable to the BC extracts (1-50 $\mu$ g/ml), a dose-related trend in cell death was observed and therefore 10 $\mu$ g/ml was chosen for further experiments.

Ten BC cultivars were compared for efficacy by measuring eotaxin-3 production in IL-4 stimulated human lung epithelial (A549) cells *in vitro*. Cells were incubated with BC extracts (10µg/ml) and IL-4 (10ng/ml) for 24 hours. The supernatants were then quantified for eotaxin-3 levels by an enzyme-linked immunosorbent assay (ELISA). All ten BC extracts reduced eotaxin-3 levels after stimulation with IL-4, and six BC extracts were effective by statistically significant levels ( $P < 0.05$ ), (BC cultivars -01, -02, -03, -05, -09 & -10). Of those, BC extracts of four cultivars demonstrated a reduction of more than 65% from the IL-4 stimulated control. In addition, a positive trend in inflammation modulation vs. one anthocyanin (ACN) in the BC extracts was shown.

This study has demonstrated the beneficial inflammation modulatory effects of polyphenolic BC extracts, which could be related to cyanidin 3-O-rutinoside content. These results may have therapeutic potential for asthma.

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## Table of Contents

Statement of originality		i
Abstract		ii
Acknowledgements		iv
List of figures		v
List of tables		v
Abbreviations		vi
<b>Chapter 1</b>		
<b>1.</b>	<b>Introduction</b>	<b>1</b>
1.1.	<b>Biochemical and Physiological requirements of food</b>	<b>1</b>
1.1.1.	Metabolic burden	3
1.1.2.	Immune system signalling	4
1.1.3.	Response to metabolic burden	6
1.1.4.	Health benefits of fruit consumption	7
1.1.5.	Flavonoid phytochemicals in fruit	8
1.1.6.	Polyphenolic flavonoids and anthocyanins (ACNs)	10
1.1.7.	Bioavailability of polyphenolics	12
1.1.8.	Modulation of inflammatory conditions	15
1.2.	<b>Berryfruit</b>	<b>16</b>
1.2.1.	Berries and health	17
1.2.2.	Polyphenolic berryfruit extracts	18
1.2.3.	Blackcurrant cultivars	18
1.3.	<b>Physiology of asthma</b>	<b>19</b>
1.3.1.	Lung physiology	19
1.3.2.	Asthma	20
1.4.	<b>Biochemical and cellular responses during asthma</b>	<b>22</b>
1.4.1.	Pulmonary alveolar signalling	23
1.4.2.	Inflammatory cytokines and chemokines	23
1.4.3.	Regulation of cytokine signalling	25
1.4.4.	Chemokines	29
1.4.5.	Eotaxins	30
1.5.	<b>Potential effects of fruit and polyphenolics on asthma</b>	<b>32</b>
1.5.1.	Antioxidant properties/Anti-inflammatory properties	33
1.6.	<b>Objectives</b>	<b>38</b>

## Chapter 2

<b>2.</b>	<b>Materials and methods</b>	<b>39</b>
<b>2.1.</b>	<b>Cell culture</b>	<b>39</b>
2.1.1.	Routine cell line maintenance	39
2.1.2.	Experimental conditions	41
<b>2.2.</b>	<b>Interleukin 4 (IL-4)</b>	<b>41</b>
<b>2.3.</b>	<b>Cytotoxicity</b>	<b>42</b>
2.3.1.	Cell toxicity assay	42
<b>2.4.</b>	<b>Co-incubation of berryfruit (BC) extracts and IL-4</b>	<b>43</b>
2.4.1.	Blackcurrant extracts	43
2.4.2.	Dimethyl sulfoxide (DMSO)	44
2.4.3.	Co-incubation of blackcurrant extracts with IL-4	45
<b>2.5.</b>	<b>Pre-incubation</b>	<b>45</b>
<b>2.6.</b>	<b>Eotaxin-3 detection by specific ELISA</b>	<b>45</b>
2.6.1.	ELISA procedure	47
<b>2.7.</b>	<b>Statistical analysis</b>	<b>49</b>
2.7.1.	Standard curves and linear regression	50
2.7.2.	Analysis of variance	51

## Chapter 3

<b>3.</b>	<b>Results</b>	<b>52</b>
<b>3.1.</b>	<b>Optimisation experiments</b>	<b>52</b>
3.1.1.	Eotaxin-3 standard curve	52
3.1.2.	Activity of tetramethylbenzidine (TMB)	54
3.1.3.	A549 cell number optimisation	54
3.1.4.	Cell supernatant for ELISA assay	55
<b>3.2.</b>	<b>IL-4 cytokine challenge to cells</b>	<b>56</b>
3.2.1.	Dose response	56
3.2.2.	Incubation time-course for IL-4	58
<b>3.3.</b>	<b>Cytotoxicity</b>	<b>59</b>
3.3.1.	Cell toxicity of selected blackcurrant cultivars	60

<b>3.4.</b>	<b>Co-incubation with IL-4</b>	<b>62</b>
3.4.1.	Blackcurrant extract dose response	62
3.4.2.	Dimethyl sulfoxide (DMSO) effects	63
3.4.3.	All blackcurrant cultivars co-incubated with IL-4	65
<b>3.5.</b>	<b>Pre-incubation</b>	<b>66</b>
3.5.1.	Pre-incubation of selected BC cultivars	67
<b>3.6.</b>	<b>Comparison of co- and pre-incubation treatments</b>	<b>68</b>
<b>3.7.</b>	<b>Influence of ACNs on inflammation modulation</b>	<b>69</b>
3.7.1.	Four major ACNs in BC extracts	69
3.7.2.	Total ACN glycosides in BC extracts	72
 <b>Chapter 4</b>		
<b>4.</b>	<b>Discussion and conclusions</b>	<b>74</b>
<b>4.1.</b>	<b>Interpretation of the results</b>	<b>74</b>
4.1.1.	Effects of polyphenolic (PP) BC extracts	74
4.1.2.	Eotaxin-3 release by stimulated lung epithelial cells	75
4.1.3.	Cytotoxicity, and effects of DMSO	77
4.1.4.	Antioxidant and modulatory properties of PPs	79
4.1.5.	Cellular environment and RONS	81
4.1.6.	Fruit, polyphenolics and asthma	83
4.1.7.	Anthocyanins	86
<b>4.2.</b>	<b>Conclusions</b>	<b>88</b>
<b>4.3.</b>	<b>Limitations and suggestions for future improvements</b>	<b>89</b>
4.3.1.	Tissue culture limitations	89
4.3.2.	Blackcurrant cultivars	90
<b>References</b>		<b>92</b>
<b>Appendices</b>		<b>99</b>
<b>1.</b>	<b>Working spreadsheet for IL-4 incubation time course</b>	<b>100</b>
<b>2.</b>	<b>Cytotoxicity assay raw data</b>	<b>101</b>
<b>3.</b>	<b>Blackcurrant cultivar raw data</b>	<b>102</b>

## List of figures

<b>Figure 1.1</b>	Signal transduction processes	5
<b>Figure 1.2</b>	Synthesis of polyphenolics	9
<b>Figure 1.3</b>	HPLC of blackcurrant polyphenolic extract	19
<b>Figure 1.4</b>	Overview of the asthma inflammation reaction	22
<b>Figure 1.5</b>	Critical events in the cellular inflammatory response	26
<b>Figure 1.6</b>	Intracellular cytokine regulation	28
<b>Figure 1.7</b>	Structure of chemokines	30
<b>Figure 2.1</b>	Light micrograph of A549 cells	40
<b>Figure 2.2</b>	ELISA technique for quantification of eotaxin-3	46
<b>Figure 2.3</b>	Format of experimental design	49
<b>Figure 3.1</b>	Eotaxin-3 standard curve	52
<b>Figure 3.2</b>	Evaluation of tetramethylbenzidine (TMB)	54
<b>Figure 3.3</b>	Response to IL-4 by cell number	55
<b>Figure 3.4</b>	Cell supernatant dilutions for ELISA detection	56
<b>Figure 3.5</b>	Determination of optimum IL-4 concentration	57
<b>Figure 3.6</b>	Time course for IL-4 incubation with A549 cells	58
<b>Figure 3.7</b>	Positive control for cytotoxicity assay WST-1	60
<b>Figure 3.8</b>	Cytotoxicity of blackcurrant (BC) extracts	61
<b>Figure 3.9</b>	Comparison of BC extract doses	62
<b>Figure 3.10</b>	Co-incubation of DMSO and IL-4	63
<b>Figure 3.11</b>	Comparison of DMSO control with a BC extract	64
<b>Figure 3.12</b>	All BC extracts co-incubated with IL-4	65
<b>Figure 3.13</b>	Pre-incubation of BC cultivars	67
<b>Figure 3.14</b>	Comparison of BC extract treatments	68
<b>Figure 3.15</b>	Cyanidin glucoside vs. eotaxin-3	70
<b>Figure 3.16</b>	Cyanidin rutinoside vs. eotaxin-3	70
<b>Figure 3.17</b>	Delphinidin glucoside vs. eotaxin-3	71
<b>Figure 3.18</b>	Delphinidin rutinoside vs. eotaxin-3	71
<b>Figure 3.20</b>	Total glucosides vs. eotaxin-3	72
<b>Figure 3.21</b>	Total rutinosides vs. eotaxin-3	73
<b>Figure 4.1</b>	Structure of cyanidin 3-O-rutinoside	88

## List of tables

Table 3.1	Measures of accuracy of eotaxin-3 standard curve	53
Table 3.2	Most effective inflammation modulating BC cultivars	66
Table 3.3	Anthocyanin levels in BC cultivars	69

## Abbreviations

ACN	Anthocyanins
APC	Antigen-presenting cells
BALF	Bronchoalveolar lavage fluid
BC	Blackcurrant
BSA	Bovine serum albumin
CCR	CC chemokine receptor
CVD	Cardiovascular disease
Cy-glu	Cyanidin 3-O-glucoside
Cy-rut	Cyanidin 3-O-rutinoside
DMSO	Dimethyl sulfoxide
Dp-glu	Delphinidin 3-O-glucoside
Dp-rut	Delphinidin 3-O-rutinoside
EGCG	Epigallocatechin gallate
ELISA	Enzyme-linked Immunosorbent assay
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
IgE	Immunoglobulin E
IL	Interleukin
iNOS	Inducible NO synthase
LPS	Lipopolysaccharide
NO	Nitric oxide
OONO <sup>-</sup>	Peroxynitrite
PBS	Phosphate buffered saline
PP	Polyphenolic
RONS	Reactive oxygen and nitrogen species
RDA	Recommended daily allowance
STAT	Signal transducer activator of transcription
TH	Thymus helper (cell)
TLR	Toll-like receptor
TMB	Tetramethylbenzidine

