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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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 (1-50μg/ml), a dose-related trend in cell death was observed and therefore 10μg/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 \textit{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.
Acknowledgements

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## Abbreviations

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<th>Full Form</th>
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<tbody>
<tr>
<td>ACN</td>
<td>Anthocyanins</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen-presenting cells</td>
</tr>
<tr>
<td>BALF</td>
<td>Bronchoalveolar lavage fluid</td>
</tr>
<tr>
<td>BC</td>
<td>Blackcurrant</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CCR</td>
<td>CC chemokine receptor</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>Cy-glu</td>
<td>Cyanidin 3-O-glucoside</td>
</tr>
<tr>
<td>Cy-rut</td>
<td>Cyanidin 3-O-rutinoside</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>Dp-glu</td>
<td>Delphinidin 3-O-glucoside</td>
</tr>
<tr>
<td>Dp-rut</td>
<td>Delphinidin 3-O-rutinoside</td>
</tr>
<tr>
<td>EGCG</td>
<td>Epigallocatechin gallate</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked Immunosorbent assay</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HRP</td>
<td>Horseradish peroxidase</td>
</tr>
<tr>
<td>IgE</td>
<td>Immunoglobulin E</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible NO synthase</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>OONO⁻</td>
<td>Peroxynitrite</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PP</td>
<td>Polyphenolic</td>
</tr>
<tr>
<td>RONS</td>
<td>Reactive oxygen and nitrogen species</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended daily allowance</td>
</tr>
<tr>
<td>STAT</td>
<td>Signal transducer activator of transcription</td>
</tr>
<tr>
<td>TH</td>
<td>Thymus helper (cell)</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TMB</td>
<td>Tetramethylbenzidine</td>
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