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The pharmaceutical and nutraceutical potential of the halophytic plant *Carpobrotus rossii*

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Statement of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

Adam Douglas Pirie 5th June 2014

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Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University. All animal experiments conducted in this thesis were done under the approval of the University of Tasmania's Animal Ethics Committee; approval numbers A0010751 and A0011684 respectively.

Statement of Co-Authorship

Given that this thesis is presented as a series of papers, either published, in press or submitted, statements of co-authorship are provided for each chapter. Due to this thesis format, some repetition is inevitable.

The following people and institutions contributed to the publication or preparation of the work undertaken as part of this thesis:

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Manuscripts arising from this thesis and statement of contribution

Paper 1. "Ecophysiology of *Carpobrotus rossii* in Tasmania: Linking plant's antioxidant activity with a natural habitat

Chapter 2,

Candidate was the primary author, who in conjunction with authors1-3 contributed to experimental design and development. Authors 1,3 and 13 assisted in the conduction of experiments. Author 10 provided expertise with statistical analysis. Author 3 provided input on data interpretation and presentation. Some of the flavonoid, tannin and DPPH assay field survey data described in this paper were generated as part of author 13's Master's thesis. However, generation of the full dataset and statistical analysis was undertaken during the candidate's PhD candidature.

Paper 2. "Flavonoid and tannin production of Carpobrotus rossii is modulated by environmental conditions"

Chapter 2,

Candidate was the primary author, who in conjunction with authors1-3 contributed to experimental design and development. Authors 1,3 and 13 assisted in the conduction of experiments. Author 10 provided specialist expertise with statistical analysis. Author 3 provided input on data interpretation and presentation. Some of the flavonoid, tannin and DPPH assay field survey data described in this paper were generated as part of author 13's Master's thesis, however generation of the full dataset and statistical analysis was undertaken during the candidate's PhD candidature.

Chapter 3. Low-temperature and ultraviolet B exposure induce separate and structurally unrelated biochemical responses in *Carpobrotus rossii*

Candidate was the primary author, who in conjunction with authors 1-3 contributed to experimental design and development and refinement. Authors 6, 8 and 15 provided specialist instrumentation and analytical skills. Authors 1,2 and 6 provided input on data interpretation. Author 3 provided input on data interpretation and presentation.

Chapter 4. Pirieol A from *Carpobrotus rossii*, a novel spinacetin glycoside containing apiose and HMG moieties.

Candidate was the primary author, who in conjunction with authors 1, 2, 14 and 7 contributed to experimental design and development and refinement. Author 14 undertook the UV spectrum analysis, hydrolysis and comparison to commercial standards, and determination of aglycone structure work described in this chapter (in conjunction with authors 1, 6 and 7), the results of which are reported in her Master's thesis. The candidate propagated the high purity plant and undertook preliminary purification of the extract used to determine the structure of the entire compound. Author 8 undertook the final large-scale fractionation step to produce a high-purity sample for NMR analysis. Authors 7,6 and 1 provided specialist instrumentation, analytical and data interpretation skills.

Chapter 5. Hypolipidaemic effect of crude extract from *Carpobrotus rossii* (pigface) in healthy rats

Candidate was the primary author, who in conjunction with authors 1, 2 and 5 contributed to experimental design and development. Authors 5 and 12 assisted in the conduction of experiments. Authors 6, 9 and 11 provided specialist analytical expertise. Authors 5, 9, 11 and 12 provided input on data interpretation and presentation.

Chapter 6. Flavonoids from Carpobrotus rossii improve glucose clearance in insulin resistant animals

Candidate was the primary author, who in conjunction with authors 1, 2, 4 and 5 contributed to experimental design and development. Author 4 assisted in the conduction of experiments. Authors 9 provided specialist analytical and statistical expertise.

We the undersigned agree with the above stated “proportion of work undertaken” for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

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A selection of *C. rossii* photographs taken by the candidate during the course of their PhD studies

PhD overview

"Metabolic syndrome" refers to the triumvirate of obesity-related, cardiovascular diseases such as hyperlipidaemia, type 2 diabetes, atherosclerosis and hypertension. The worldwide prevalence of these diseases have increased to such an extent that they are now the leading cause of human morbidity and mortality. Metabolic syndrome is characterised by elevated levels of plasma lipids, hyperglycaemia, compromised insulin signalling, excessive production of reactive oxygen species (ROS) and a vasculature that is in a persistently inflamed state. Because of the increasing prevalence of these diseases, considerable research effort has gone into understanding the disease processes and developing appropriate therapies. Two metabolic syndrome targets which have been identified and for which therapeutics have been successfully developed are hyperlipidaemia and hyperglycaemia.

A common target of the lipid-lowering therapies is the HMG-CoA reductase enzyme which catalyses the rate limiting step in the cholesterol synthesis pathway namely the conversion of 3-hydroxy-3-methylglutaric acid coenzyme-A (HMG-CoA) to mevalonate. Statins are the primary class of drugs with this HMG-CoA inhibiting ability. Polyphenolic compounds produced by plants have also been shown to have hypolipidaemic activity by inhibiting HMG-CoA as well as other enzymes involved in the processes of lipid manufacture and delivery to cells. Polyphenolic compounds have also been shown to improve the glucose status of diseased subjects by improving vascular health, improving insulin signalling and glucose uptake into muscle. Of these plant-derived polyphenolic compounds, members of the flavonoid sub-family been shown to be particularly successful in treating both hyperlipidaemia and hyperglycaemia.

Carpobrotus rossii (CR) is a succulent halophyte native to Australia and commonly found growing along the coastal margins of southern Australia. The plant has a history of use by both the indigenous aboriginal population and early Tasmanian settlers. CR was reportedly consumed as a food, to treat gastrointestinal upsets, and applied topically for the treatment of bites and scratches. Preliminary investigations conducted at the University of Tasmania have shown that crude extracts from its leaves inhibit platelet aggregation, inflammatory cytokine release (interleukin-1-beta, tumour necrosis factor-alpha) and lipid oxidation *in vitro* (Geraghty et al., 2011). This activity is believed to be due to the flavonoid compounds that

the plant produces in its leaves. Several of these flavonoids have a known HMG-CoA inhibitor 3-hydroxy-3methylglutaric acid (HMG) present as a substituent (Jager, 2009).The presence of this moiety, in conjunction with the known hypoglycaemic and hypolipidaemic activities of other flavonoids, mean that the consumption of CR flavonoids could potentially improve endothelial health, cardiovascular function and health via a combination of effects related to both their flavonoid and statin properties.

In planta, the primary function of flavonoids appears to be as antioxidants, and their production has been shown to be induced under a suite of conditions which cause the plant to experience oxidative stress. The ROS generation and signalling process *in planta* are complex, and the effect of environmental conditions on a plant's redox status, and hence flavonoid production, is likely to vary between species. The effects of environment on flavonoid product has not been previously investigated for CR. The flavonoid structures described in chapter 4 are extremely complex, and based on informal discussions with an organic chemist familiar with similar compounds, not easily amenable to synthesis. As such, the ability to produce sufficient material and improve the efficiency of their production e.g. increasing biomass or increasing flavonoid concentration by the modification of environmental parameters is a key component of overall CR pharmaceutical and nutraceutical investigations.

This thesis has involved using techniques relevant to the disciplines of pharmacology, organic chemistry and plant physiology. The primary aims were to investigate the pharmaceutical and nutraceutical potential of the flavonoids derived from CR leaves. To do this, several basic questions were addressed, namely:

1. **What effect do environmental conditions have on metabolite production,**
2. **What is the structure of the CR flavonoids,**
3. **Is the consumption of CR leaf derived extracts safe, and,**
4. **Do the CR leaf flavonoids possess pharmacological activity in metabolic syndrome, specifically an improvement in either glycaemic or lipid profile.**

A suite of novel findings which pave the way for further study of this plant are the result of this research. The body of the thesis is presented as a series of articles for publication, of which three are published at the time of thesis submission.

The published articles are as follows:

PIRIE, A. D., DAVIES, N. W., AHUJA, K. D. K., ADAMS, M. J., SHING, C. M., NARKOWICZ, C., JACOBSON, G. A. & GERAGHTY, D. P. 2014. A crude extract from *Carpobrotus rossii* (pigface) lowers cholesterol in healthy rats. *Food and Chemical Toxicology,* 66**,** 134-139.

PIRIE, A., PARSONS, D., RENGGLI, J., NARKOWICZ, C., JACOBSON, G. A. & SHABALA, S. 2013. Modulation of flavonoid and tannin production of *Carpobrotus rossii* by environmental conditions. *Environmental and Experimental Botany,* 87**,** 19-31.

PIRIE, A., SHABALA, S., PARSONS, D., NARKOWICZ, C., JACOBSON, G. & RENGGLI, J. 2011. Ecophysiology of *Carpobrotus rossii* in Tasmania: Linking plant's antioxidant activity with a natural habitat. *Ecological Questions,* 14**,** 91-93.

In addition to these published manuscripts several other articles are currently undergoing the peer-review process.

Due to the multidisciplinary nature of this work, the literature review is quite detailed covering cardiovascular physiology the associated disease processes, plant physiology and organic chemistry, as appropriate. This has been done to ensure that all examiners have sufficient grounding to understand the work described in this thesis when it is outside their area of expertise.

Plant Physiology (Chapters 2 and 3)

The survey investigating flavonoid production by wild CR (77 plants from 16 sites around the coastline of Tasmania) showed that conditions known to induce stress *in planta* were associated with altered flavonoid production. Under conditions of sub- and supra-optimal salinity (<50mM, >100mM NaCl), biomass production was reduced, whilst flavonoid