

An Ethnopharmacological Study of Australian  
Indigenous Medicinal Plants Used in Dermatological  
and Wound Healing Remedies

A thesis submitted in fulfilment for the degree of

**Doctor of Philosophy**

**University of New England, Armidale, Australia**

**by**

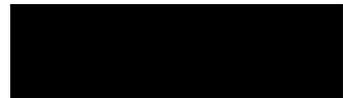
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## **Declaration**

I certify that the substance of this thesis has not been submitted, either in whole or in part, for a higher degree at any other university or institution, and, that to the best of my knowledge, all assistance received in preparing this work and all sources used have been acknowledged.



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November, 2011

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## Abstract

In this study, relevant *in vitro* biological activities were evaluated in extracts of Australian medicinal plants used in indigenous traditional medicine for the treatment of wounds, sores and other dermatological conditions. A survey of Australian medical ethnobotanical literature was conducted in order to identify suitable species and plant parts for inclusion in the study and to collect data for systematic and quantitative analyses of Australian indigenous medicinal plant use. Thirty-seven aqueous extracts were obtained following traditional methods of preparation from various parts of 23 plant species representing 15 families. Sequential solvent extracts (hexane-dichloromethane-methanol) were also prepared using material from 5 species of *Eremophila* to enable comparison of bioactivity with corresponding traditional aqueous preparations. Extracts were screened using *in vitro* assays for anti-microbial activities, anti-oxidant capacity via DMPD decolourisation and Oxygen Radical Absorbance Capacity (ORAC) assays, P388D1 murine lymphoblast cytotoxicity, and inhibition of prostaglandin E<sub>2</sub> from calcium ionophore-stimulated murine fibroblasts. Total phenolic content of the aqueous preparations was also estimated using a Folin-Ciocalteu type assay.

Anti-bacterial activity was assessed using a broth microdilution technique to measure minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). A number of the aqueous preparations were found to exhibit a broad spectrum of anti-bacterial activity (i.e. were active against both Gram-negative and Gram-positive organisms) and retained activity at MIC/MBC levels representing concentrations well below that produced using traditional extraction techniques. These included the expressed fruit juice of *Cochlospermum fraseri* (MIC: 0.20 - 25.00 mg · mL<sup>-1</sup>; MBC: 0.80 - 50.00 mg · mL<sup>-1</sup> representing 0.002 - 0.53× original concentration of 95.2 mg · mL<sup>-1</sup>), a decoction of leaves from *Eucalyptus tetradonta*, (MIC: 0.06 - 4.00 mg · mL<sup>-1</sup>; MBC: 0.50 - 4.00 mg · mL<sup>-1</sup>,

representing 0.01 - 0.74× the original concentration of 5.4 mg · mL<sup>-1</sup>), and a decoction of *Buchanania obovata* trunk inner bark (MIC: 0.50 - 16.00 mg · mL<sup>-1</sup>; MBC: 2.00 - 8.00 mg · mL<sup>-1</sup>, representing 0.03 - 0.78× the original concentration of 20.4 mg · mL<sup>-1</sup>). The *C. fraseri* juice and *B. obovata* inner bark preparations also exhibited the highest levels of phenolic content among the aqueous preparations (11947 - 18189 ppm gallic acid equivalents) and antioxidant capacity as measured in Trolox equivalents (TE), in DMPD assays (46.7 - 60.9 mM TE). Highest ORAC values among the preparations were found for the *C. fraseri* juice (68.2 mM TE) and a decoction of *Erythrophleum chlorostachys* trunk inner bark (19.8 mM TE). The majority of preparations tested did not exhibit *in vitro* cytotoxicity over the range of concentrations assayed. Extracts exhibiting notable P388D1 cytotoxicity included a decoction of *Callitris intratropica* inner bark (IC<sub>50</sub>: 29.4 µg · mL<sup>-1</sup>) and extracts of *Crinum angustifolium* bulbs (IC<sub>50</sub>: 28.4 -58 µg · mL<sup>-1</sup>).

Major use agreement values (MUA), an index used to evaluate the degree of agreement between specific medicinal uses cited by independent ethnobotanic studies, were determined for each of the medicinal preparations examined using data collected in the survey of ethnobotanic literature. MUA values were compared with the results of assays for biological activity, to determine whether any relationship existed between the degree of agreement for specific medicinal uses cited, and any activities observed. Higher MUA values representing citations referring to treatment of wounds and dermal lesions were found to be associated with higher levels of total phenolic content, *in vitro* antimicrobial activity and P-388 murine lymphoblast cytotoxicity among the preparations examined. These findings indicate that phenolic compounds may be of considerable relevance in Australian medicinal plant species and preparations used in the treatment of wounds and dermal lesions, and further studies are required to confirm this finding and to identify whether any specific class or species of phenolic compound predominates in this group of plants.

In previous work, two diterpenes of the serrulatane class, serrulat-14-en-7,8,20-triol (**1**) and serrulat-14-en-3,7,8,20-tetraol (**2**), were identified as the principal anti-bacterial constituents in extracts of the medicinal plant *Eremophila duttonii*. In this study, an attempt was made to isolate and purify the serrulatane diterpenes from a dichloromethane extract of *E. duttonii* leaves, to assay for further bioactivities and use as standards in order to determine whether either compound could be detected in traditional aqueous preparations using this species. As in previous work, partially purified fractions containing both compounds exhibited inhibitory and bactericidal activity against Gram-positive bacteria. MIC against 3 Gram-positive organisms for fractions containing the triol (**1**) ranged from 8 - 15  $\mu\text{g} \cdot \text{mL}^{-1}$  and MBC from 8 - 30  $\mu\text{g} \cdot \text{mL}^{-1}$ . MIC of fractions containing the tetraol (**2**) ranged from 60 - 120  $\mu\text{g} \cdot \text{mL}^{-1}$  and MBC from 120-480  $\mu\text{g} \cdot \text{mL}^{-1}$ . Fractions containing **1** and **2** also exhibited high levels of cytotoxicity against P-388 lymphoblasts and HEP-G1 cell lines (**1**:  $\text{IC}_{50}$ , 7.7 - 13.7  $\mu\text{g} \cdot \text{mL}^{-1}$ ; **2**:  $\text{IC}_{50}$ , 11.2 - 17.2  $\mu\text{g} \cdot \text{mL}^{-1}$ ) and antioxidant capacity in lipophilic ORAC assays (**1**:  $10919 \pm 854 \mu\text{mol TE/g}$ ; **2**:  $13610 \pm 989 \mu\text{mol TE/g}$ ). In traditional aqueous decoctions of this species, only the serrulatane tetraol (**2**) was detected. Attempts to estimate quantities of **2** in traditional preparations were complicated by the finding that this compound appeared to be unstable as products of decomposition were found to appear in purified samples following a period of storage. For this reason, an accurate determination of quantity in the traditional preparations could not be obtained with certainty and were reported as tentative estimations (i.e. around 40-68  $\mu\text{g mL}^{-1}$ , corresponding to 0.6 - 0.7% of extract dry weights) based on estimated purity of standards at the time the assays were performed.

Overall, the organic solvent extracts tended to exhibit higher potency and a higher rate of 'positive hits' in biological assays when compared with corresponding traditional aqueous preparations. These results would support the argument that non-polar organic solvent

extracts may be unsuitable for certain investigations (e.g. in seeking to explain or validate medicinal plant use in the 'traditional' context), or that such studies should be followed up with appropriate investigations into the pharmacognosy of traditional preparations where possible.

In this study, evidence was found which showed a high degree of intraspecific phytochemical variation in volatile oil fractions of one of the species examined (*Eremophila longifolia*). A preliminary study was undertaken to examine chemical variation in volatile oils of this species, resulting in identification of three novel chemotypes (isomenthone, karahanaenone and borneol chemotypes) occurring in western regions of the state of NSW. Previous studies had indicated the possibility of volatile oils of this species containing the toxic phenylpropanoid safrole. Interestingly, no safrole or other phenylpropanoids were detected in any of the specimens examined in this study. When traditional decoctions were prepared using material representative of each of the chemotypes identified, significant differences in biological activity and phenolic content were apparent. This species is frequently mentioned in the ethnobotanical literature of Australia and widespread anecdotal evidence exists which suggests that preparations using this species are frequently and routinely employed by contemporaneous indigenous Australian people. Further work may be warranted to characterise the extent of phytochemical variation in this species, and to carry out pharmacognostic studies of preparations and material in areas where the species is currently used for medicinal purposes. The work undertaken in this study is novel in that biological activity or chemistry has not previously been investigated in extracts obtained following traditional methods of preparation, from the majority of species collected. This work is also, to the best of the author's knowledge, the first instance where relevant biological activities have been investigated in Australian medicinal plants used specifically for dermatological conditions and wounds.

## **Publications arising from this thesis**

Smith, J., Tucker, D., Alter, D., Watson, K. and Jones, G. (2010). "Intraspecific variation in essential oil composition of *Eremophila longifolia* F. Muell.(Myoporaceae): Evidence for three chemotypes." *Phytochemistry* **71**(13): 1521-1527.

Smith, J. E., Tucker, D., Watson, K. and Jones, G. L. (2007). "Identification of antibacterial constituents from the indigenous Australian medicinal plant *Eremophila duttonii* F. Muell. (Myoporaceae)." *Journal of Ethnopharmacology* **112**(2): 386-393.

## **Conference Abstracts**

Smith, J. (2009). Accepted oral presentation: *Screening of some Australian indigenous medicinal plants for in vitro biological activities relevant to wound healing*. International Evidence Based Complementary Medicine Conference, University of New England, Armidale, March 2009.

Smith, J., Tucker, D., Jones, G. and Watson, K. (2005). Poster Presentation (P415): *Characterisation and Evaluation of Antibacterial Activity of a New Type of Essential Oil from Eremophila longifolia*. 53rd Annual Congress of the Gesellschaft für Arzneipflanzenforschung (GA) and joint congress with the Societa Italiana di Fitochimica (SIF), Florence, Italy, 21-25 August 2005.

Smith, J., Tucker, D., Jones, G. and Watson, K. (2005). Poster Presentation (P407): *Isolation and Identification of Antimicrobial Compounds from the Australian Medicinal Plant Eremophila duttonii (Scrophulariaceae)*. 53rd Annual Congress of the Gesellschaft für Arzneipflanzenforschung (GA) and joint congress with the Societa Italiana di Fitochimica (SIF), Florence, Italy, 21-25 August 2005.

## List of Abbreviations and Acronyms

AAPH	2,2'-azobis (2-methyl) propionamidine dihydrochloride
Abs	Absorption
ACM	Australian Collection of Microorganisms
ACN	Acetonitrile
APCI	Atmospheric-Pressure Chemical Ionization
APNI	Australian Plant Name Index
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
aq.	Aqueous
AU	Absorption Units
BA	Blood Agar
BBIz	Broadband Inverse NMR Probe, Z-Gradient
bp	Before Present (Years)
CLSI	Clinical and Laboratory Standards Institute (formerly NCCLS)
COSY	Correlation Spectroscopy (2D NMR)
CPP	Centre for Phytochemistry and Pharmacology
CVPNT	Checklist of the Vascular Plants of the Northern Territory
DCM	Dichloromethane
DMEM	Dulbecco's Modified Eagle Medium
DMPD	N,N-dimethyl-p-phenylenediamine
DMS	Degrees, Minutes, Seconds (Geospatial coordinates)
DMSO	Dimethyl sulfoxide
EIA	Enzyme Immunoassay
EPE	Estimated Position Error (Geospatial coordinates)
ESI	Electrospray Ionisation
EtOAc	Ethyl Acetate
EtOH	Ethanol
FBS	Foetal Bovine Serum
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GMB	Mueller-Hinton Agar with 2% Glucose and 0.5 µg/mL Methylene Blue

HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
HSQC	Heteronuclear Single Quantum Coherence (2D NMR)
IC <sub>50</sub>	Concentration Effecting 50% Inhibition
IBRA	Interim Biogeographic Regionalisation of Australia
LC-MS	Liquid Chromatography-Mass Spectrometry
LPS	Lipopolysaccharide
MBC	Minimum Bactericidal Concentration
MeOH	Methanol
MHA	Mueller-Hinton Agar
MHz	Megahertz
MIC	Minimum Inhibitory Concentration
MS	Mass Spectrum
MW	Molecular Weight
NA/NB	Nutrient Agar/Nutrient Broth
NAED	North American Ethnobotanic Database
NCCLS	National Committee for Clinical Laboratory Standards
NSAID	Non-Steroidal Anti-Inflammatory Drug
NSW	New South Wales
NMR	Nuclear Magnetic Resonance
NT	Northern Territory
ORAC	Oxygen Radical Absorbance Capacity
PC	Principal Component
PCA	Principal Component Analysis
PCA	Plate Count Agar
PGE <sub>2</sub>	Prostaglandin E <sub>2</sub>
PLC	Preparative Thin Layer Chromatography
ppm	Parts Per Million
PYE	Peptone-Yeast Extract
RCF	Relative Centrifugal Force
Rf	Retention Factor
RI	Relative Retention Index/Retention Indices
RMCD	Randomised methylated cyclodextrin
ROS/RNS	Reactive Oxygen/Nitrogen Species

Rt	Retention Time
SCU	Southern Cross University
SPSS	Statistical Package for the Social Sciences
TE	Trolox Equivalent
TFA	Trifluoroacetic Acid
TLC	Thin Layer Chromatography
TSB	Tryptone Soya Broth
TSSA	Tryptone Soya Sloppy Agar
UNE	University of New England
UV	Ultra-Violet
$V_e$	Elution Volume
YEP	Yeast Extract Peptone

## Table of Contents

Chapter 1: Introduction.....	1
1.1 Introduction.....	1
1.1.1 Overview of present study .....	1
1.2 Previous ethnopharmacological investigations of Australian medicinal plants.....	5
1.2.1 Previous studies examining anti-inflammatory activities .....	6
1.2.2 Previous studies examining antimicrobial activities .....	7
1.3 Review of Australian medical ethnobotanical literature.....	10
1.3.1 Regression residual analyses of medicinal plants of the Northern Territory .....	15
1.3.2 Regression residual analyses of Australian medicinal plants used to treat dermatological complaints .....	18
1.3.3 Use consensus and major use agreement in the Australian ethnobotanic literature.....	31
1.3.4 Plant species used to treat dermatological conditions.....	44
1.3.5 Conclusions.....	53
1.4 Dermal lesions and wound healing.....	57
1.4.1 Dermal lesions.....	57
1.4.2 Physiological and biochemical events in wound healing.....	58
1.4.3 <i>In vitro</i> tests for wound healing potential of medicinal plant extracts .....	60
1.4.4 Bioassays employed in the study .....	63
Chapter 2: Materials and Methods.....	65
2.1 General methods and experimental procedures .....	65
2.1.1 Selection of plant species.....	65
2.1.2 Collection of plant material.....	65
2.2 Extraction procedures .....	66
2.2.1 Sources and general methods followed in preparation of traditional type aqueous extracts .....	66
2.3 Chromatographic vouchering of traditional aqueous preparations .....	73
2.4 Assessment of antimicrobial activity of extracts .....	74
2.4.1 General methods for assessment of antimicrobial activity.....	75
2.4.2 Disc diffusion assays for assessment of antimicrobial activity of extracts .....	79
2.4.3 Microdilution assays for assessment of antimicrobial activity of extracts.....	80
2.5 Estimation of antioxidant capacity of extracts.....	85
2.5.1 Estimation of antioxidant capacity by DMPD decolourisation assay .....	85
2.5.2 Estimation of reducing capacity/total phenolic content by Folin-Ciocalteu reagent ....	88
2.5.3 Estimation of antioxidant capacity by oxygen radical absorbance capacity (ORAC) assays .....	90

2.6	Estimation of cytotoxicity against P388D1 lymphoblasts with ATPlite assay.....	94
2.7	Screening for prostaglandin E <sub>2</sub> inhibitory activity.....	96
2.8	Methods and procedures followed for the isolation of serrulatane diterpenes from <i>Eremophila duttonii</i> .....	99
2.8.1	Column chromatography.....	99
2.8.2	Thin layer chromatography of column chromatography fractions.....	100
2.8.3	Detection of serrulatane diterpenes in traditional aqueous preparations of <i>E. duttonii</i> by LC-MS .....	102
2.8.4	Purification of serrulat-14-en-3,7,8,20-tetraol by semi-preparative HPLC .....	102
2.9	Methods and procedures followed for the characterisation of novel <i>Eremophila longifolia</i> essential oil chemotypes .....	105
2.9.1	Collection of plant material.....	105
2.9.2	Isolation of volatile oils.....	106
2.9.3	Gas Chromatography-Mass Spectrometry .....	106
2.9.4	Multivariate statistical analyses .....	108
2.9.5	Assessment of antimicrobial activity in <i>Eremophila longifolia</i> essential oils .....	108
2.10	Statistical analyses.....	110
Chapter 3: Antimicrobial activity in Australian indigenous medicinal plant extracts used for dermatological and ophthalmic conditions .....		111
3.1	Introduction.....	111
3.2	Traditional preparations .....	125
3.3	Antimicrobial activity of traditional type preparations and extracts - Results and discussion .....	131
3.3.1	Disc diffusion assays.....	133
3.3.2	Microtitre plate broth dilution screening assays .....	134
3.3.3	Minimum inhibitory concentrations (MIC) of traditional type extracts .....	139
3.3.4	Minimum bactericidal concentrations (MBC) of traditional type extracts .....	140
3.3.1	Comparison of antibacterial activity of traditional aqueous preparations and sequential organic solvent extractions of <i>Eremophila</i> species.....	143
3.3.2	Antimicrobial activity of traditional type preparations and previous studies .....	145
3.3.3	Antibacterial activity and records of traditional use .....	149
3.3.4	Major use agreements and antibacterial activity of traditional preparations.....	152
3.4	General conclusions.....	156
Chapter 4: Phenolic content, antioxidant capacity and anti-inflammatory activity in Australian indigenous medicinal plant preparations.....		163
4.1	Introduction.....	163
4.1.1	Antioxidant activity and wound healing .....	163

4.1.2	Measurement of antioxidant capacity .....	166
4.1.3	Phenolic and polyphenolic compounds in plants .....	167
4.1.4	PGE <sub>2</sub> inhibition and cytotoxicity assays .....	172
4.2	Results and Discussion .....	174
4.2.1	Antioxidant capacity and total phenolic content of extracts .....	174
4.2.2	Total phenolic content of traditional aqueous preparations by Folin-Ciocalteu reagent assay .....	179
4.2.3	Antioxidant capacity of traditional preparations by DMPD decolourisation assay ....	185
4.2.4	Antioxidant capacity of traditional preparations by ORAC assay .....	185
4.2.5	Correlation between total phenolic content and antioxidant capacity assays .....	186
4.2.6	Antioxidant capacity in MeOH extracts of <i>Eremophila</i> species and comparisons with corresponding traditional preparations.....	192
4.2.7	Comparison of traditional use records and MUA <sub>TMP</sub> values with antioxidant capacity and phenolic content .....	193
4.2.8	PGE <sub>2</sub> inhibitory activity of traditional preparations.....	201
4.2.9	Results of P388D1 cytotoxicity screening assays .....	206
4.2.10	PGE <sub>2</sub> inhibition, P388D1 cytotoxicity and MUA <sub>TMP</sub> .....	209
4.2.11	Major use agreement and PGE <sub>2</sub> inhibitory activity.....	211
4.3	General discussion and conclusions.....	213
Chapter 5: Isolation of serrulatane diterpenes from <i>Eremophila duttonii</i> .....		227
5.1	Introduction.....	227
5.1.1	Ethnopharmacology and phytochemistry of <i>E. duttonii</i> .....	228
5.1.2	Serrulatane diterpenes exhibiting antimicrobial activity.....	230
5.1.3	Outline of present study .....	233
5.2	Results.....	234
5.2.1	Fractionation of <i>E. duttonii</i> extracts with column chromatography.....	234
5.2.2	Detection of serrulatane diterpenes in traditional aqueous preparations of <i>E. duttonii</i> by HPLC/LC-MS .....	236
5.2.3	Quantitative assay of serrulat-14-en-3,7,8,20-tetraol in traditional aqueous preparations of <i>E. duttonii</i> by HPLC/LC-MS .....	239
5.2.4	Antimicrobial activity .....	242
5.3	Discussion and conclusions .....	246
5.3.1	Antimicrobial activity of serrulatane diterpenes .....	246
5.3.2	Antibacterial activity of <i>E. duttonii</i> traditional aqueous preparations and serrulatane content.....	250
5.3.3	Antioxidant, cytotoxic and PGE <sub>2</sub> inhibitory activities .....	252
Chapter 6: Novel essential oil chemotypes of <i>Eremophila longifolia</i> .....		258

6.1	Introduction and background .....	258
6.1.1	Outline of present study .....	259
6.1.2	Description and medical ethnobotany .....	260
6.1.3	Previous studies on ethnopharmacology and bioactivity .....	263
6.1.4	Previous work on volatile oil composition.....	263
6.1.5	Examination of antimicrobial activity of <i>E. longifolia</i> essential oils .....	264
6.2	Results.....	266
6.2.1	Essential oil composition .....	266
6.2.2	Principal component analysis.....	272
6.2.3	Antimicrobial activity of <i>E. longifolia</i> essential oils.....	274
6.3	Discussion and conclusions .....	277
6.3.1	Karahanaenone in <i>E. longifolia</i> essential oils .....	282
6.3.2	Conclusions.....	283
Chapter 7: General Conclusions .....		287
7.1	General discussion and conclusions.....	287
7.1.1	Analysis of records of Australian indigenous medicinal plants use.....	288
7.1.2	Use of traditional aqueous preparations in the study and comparison with non-polar organic solvent extracts.....	290
7.1.3	Biological activity and major use agreement.....	293
7.1.4	Serrulatane diterpenes in traditional aqueous preparations of <i>E. duttonii</i> .....	298
7.1.5	Novel essential oil chemotypes of <i>Eremophila longifolia</i> .....	300
Appendix I: Survey of Ethnobotanical literature (supplementary data) .....		ii
Appendix II: Notes on traditional aqueous preparations and HPLC voucher chromatograms .....		xxii
Appendix III. Column chromatography fractions, miscellaneous information and publications .....		1

## List of Figures

<b>Figure 1.1</b>	Regression residual analysis of medicinal plants and all available plants of the Northern Territory by family.....	21
<b>Figure 1.2</b>	Regression analysis of species used for dermatological complaints and medicinal plants of entire dataset by family.....	27
<b>Figure 1.3</b>	Number of species and use reports within 16 use categories from 29 Australian ethnobotanical reports .....	40
<b>Figure 1.4</b>	Number of species and use reports describing dermatological sub-categories in 29 Australian ethnobotanical reports.....	46
<b>Figure 1.5</b>	Regression analysis by use category of species used for dermatological complaints and medicinal plants of entire dataset.....	51
<b>Figure 2.1</b>	Photographs illustrating steps followed during the preparation of an aqueous 'traditional type' extraction .....	70
<b>Figure 2.2</b>	Scheme for formation of DMPD radical cation.....	86
<b>Figure 2.3</b>	Scheme for use of Folin-Ciocalteu reagent to detect phenolic compounds by reducing ability.....	89
<b>Figure 2.4</b>	Representation of typical ORAC fluorescence decay curve illustrating net area under the curve (AUC).....	91
<b>Figure 2.5</b>	Luciferase/D-luciferin reaction scheme.....	94
<b>Figure 3.1</b>	Bar chart showing the means with standard error bars of the concentration of three traditional extraction methods .....	128
<b>Figure 3.2</b>	Bar chart showing the means with standard error bars of the yield of three traditional extraction methods.....	128
<b>Figure 4.1</b>	Polyphenolics typical of various structural classes for which biological activity has been found.....	169
<b>Figure 4.2</b>	Scheme illustrating major elements of the arachadonic acid/eicosanoid pathway.....	173
<b>Figure 4.3</b>	Bar chart showing means with standard error bars for total phenolic content (ppm GAE extract) of traditional preparations grouped by plant part extracted.....	180
<b>Figure 4.4</b>	Bar chart showing means with standard error bars for total phenolic content (ppm GAE) of traditional preparations grouped by method of extraction .....	180
<b>Figure 4.5</b>	Bar chart showing means with standard error bars for total phenolic content (mg GAE/g FW extract) of traditional preparations grouped by plant part extracted .....	181
<b>Figure 4.6</b>	Bar chart showing means with standard error bars for total phenolic content (mg GAE/g FW) of traditional preparations grouped general method of extraction.....	181
<b>Figure 4.7</b>	Bar chart showing total phenolic content of extracts representative of three separate chemotypes of <i>E. longifolia</i> .....	185

<b>Figure 4.8</b>	Scatter plot showing relationship between Folin-Ciocalteu total phenolic content assay and DMPD TE values (mM) in traditional preparations.....	186
<b>Figure 4.9</b>	Scatter plot showing relationship between Folin-Ciocalteu total phenolic content assay and ORAC values .....	187
<b>Figure 4.10</b>	Scatter plot showing relationship between DMPD TEAC and ORAC assay values.....	187
<b>Figure 4.11</b>	Bar chart showing comparison of antioxidant capacity in traditional medicinal preparations representative of three separate chemotypes of <i>E. longifolia</i> .....	190
<b>Figure 4.12</b>	Extracts exhibiting significant inhibition of PGE <sub>2</sub> formation in calcium ionophore stimulated 3T3 fibroblasts.....	204
<b>Figure 4.13</b>	Extracts exhibiting significant positive increase in PGE <sub>2</sub> formation in unstimulated 3T3 fibroblasts.....	204
<b>Figure 4.14</b>	Cytotoxicity dose-response curves for selected extracts against P388D1 murine lymphoblasts.....	208
<b>Figure 5.1</b>	Basic serrulatane skeleton with serrulat-14-en-7,8,20-tetraol (1) and serrulat-14-en-3,7,8,20-triol (2) identified in extracts of <i>Eremophila duttonii</i> .....	229
<b>Figure 5.2</b>	Serrulatane type diterpenes and o-naphthoquinones exhibiting antimicrobial activity isolated from <i>Eremophila</i> species .....	231
<b>Figure 5.3</b>	Biologically active pseudopterosins isolated from the soft coral <i>Pseudopterosin elisabethae</i> and the anti-mycobacterial diterpene leubethanol from <i>Leucophyllum frutescens</i> .....	232
<b>Figure 5.4</b>	Yield (gm) of fractions eluted from separation of <i>E. duttonii</i> secondary DCM extract with normal phase silica gel column chromatography. ....	235
<b>Figure 5.5</b>	Thin layer chromatograms of <i>E. duttonii</i> secondary DCM extract column chromatography fractions.....	235
<b>Figure 5.6</b>	Analytical HPLC separations of <i>E. duttonii</i> secondary DCM extract column chromatography fractions containing serrulatanes 1 and 2 .....	236
<b>Figure 5.7</b>	HPLC separations of <i>E. duttonii</i> traditional aqueous decoctions alongside column chromatography fractions containing compounds 1 and 2, and secondary DCM extract.....	238
<b>Figure 5.8</b>	Chromatogram representing 1 cycle of semi preparative purification of serrulat-14-en-3,7,8,20-tetraol.....	239
<b>Figure 5.9</b>	HPLC chromatograms of semi-preparative sub-fractions 18-16 to 18-19 .....	239
<b>Figure 5.10</b>	Separation of <i>E. duttonii</i> aqueous preparation (extract SM010TD).....	240
<b>Figure 5.11</b>	Assay for purity of serrulatane tetraol in fraction 17-18 performed immediately following purification by semi-preparative HPLC.....	241

<b>Figure 5.12</b> Assay for purity of serrulatane tetraol in fraction 17-18 (sample used as standard during quantitative assays) performed five days following purification by semi-preparative HPLC.....	241
<b>Figure 5.13</b> Cytotoxicity dose response curves for serrulatane triol (1) and tetraol (2) with curcumin (as reference drug) against murine P388D1 cell line .....	245
<b>Figure 5.14</b> Cytotoxicity dose response curves for serrulatane triol (1) and tetraol (2) with curcumin (as reference drug) against human HepG2 cell line .....	246
<b>Figure 6.1</b> Map (expanded from inset) showing collection localities of <i>E. Longifolia</i> specimens in western NSW and extent of IBRA bioregions covered .....	261
<b>Figure 6.2</b> Variation in leaf and stem morphology of <i>Eremophila longifolia</i> specimens.....	269
<b>Figure 6.3</b> Constituents identified in volatile oils of <i>E. longifolia</i> .....	271
<b>Figure 6.4</b> Factor loadings of variables (A), and Score plot (B) of oil samples by bioregion on axes 1 and 2 derived from principal component analysis (PCA) on chemical composition of <i>E. longifolia</i> volatile oils .....	273
<b>Figure 6.5</b> Examples of MIC microdilution tests and corresponding subcultures for MBC .....	276
<b>Figure 6.6</b> HPLC voucher separations of <i>E. longifolia</i> traditional aqueous decoctions representative of three chemotypes .....	281
<b>Figure 7.1</b> HPLC chromatogram of <i>Eremophila alternifolia</i> traditional decoction of leaves with tertiary methanol extract of same material for comparison.....	292

## List of Tables

<b>Table 1.1</b>	List of Australian ethnobotanical surveys included in dataset .....	13
<b>Table 1.2</b>	Proportion of flora with documented records of medicinal use for the Northern Territory and the entire Australian continent .....	14
<b>Table 1.3</b>	High and low use families identified by regression residual analysis of medicinal plants of the Northern Territory .....	20
<b>Table 1.4</b>	Six highest ranking and five lowest ranking families of the NT medicinal flora with comparison to published data of seven other regions .....	22
<b>Table 1.5</b>	High and low use families identified by regression analysis of dermatological use category .....	26
<b>Table 1.6</b>	Cultural agreement ratio (CAR) values and frequently cited species for use categories in Australian ethnobotanical records .....	39
<b>Table 1.6</b>	Table 1.7 Cultural agreement ratio (CAR) values and frequently cited species for subcategories of the dermatological uses and complaints category .....	45
<b>Table 1.8</b>	Regression analysis of secondary use categories within dermatological category .....	50
<b>Table 2.1</b>	10-95% 30 min Acetonitrile-water gradient timetable used for voucher chromatograms of traditional aqueous preparations .....	74
<b>Table 2.2</b>	Maintenance media, assay media and growth conditions for microorganisms .....	78
<b>Table 2.3</b>	Stepped gradient elution for separation of <i>E. duttonii</i> extracts with normal phase column chromatography .....	100
<b>Table 3.1</b>	Extracts prepared after traditional extraction methods examined in the study .....	112
<b>Table 3.2</b>	Plant species collected and summary of medical ethnobotany .....	114
<b>Table 3.3</b>	Major use agreement values (MUA <sub>TMP</sub> ) for three categories of use in 32 plant derived medicinal preparations .....	124
<b>Table 3.4</b>	Sequential extractions of <i>Eremophila</i> spp. leaves with hexane, DCM, and MeOH .....	125
<b>Table 3.5</b>	Traditional type aqueous preparations active in disc diffusion assays .....	134
<b>Table 3.6</b>	Broth microdilution screening assays - antibacterial activity against six bacterial species .....	137
<b>Table 3.7</b>	Minimum inhibitory (MIC) and bactericidal concentrations (MBC) of traditional type preparations against six bacterial species .....	141
<b>Table 3.8</b>	Disc diffusion assay of <i>Eremophila</i> sp. solvent extracts with traditional decoctions against <i>Candida albicans</i> .....	143
<b>Table 3.9</b>	Screening of Solvent extracts at 0.5 mg· ml <sup>-1</sup> using broth microdilution assay .....	144

<b>Table 3.10</b>	Comparison between antibacterial activity of traditional aqueous preparations and that reported by Barr <i>et al.</i> (1993) for refluxed EtOH extracts of equivalent parts and species.....	146
<b>Table 3.11</b>	Major use agreement values for traditional preparations with antimicrobial potency .....	151
<b>Table 3.12</b>	Proportion of extracts inhibiting growth of Gram-positive bacteria by groupings greater and less than median MUA <sub>TMP</sub> values with Pearson's Chi-square and Fisher's exact tests for association.....	153
<b>Table 3.13</b>	Proportion of extracts with bactericidal activity against Gram-positive bacteria by groupings greater and less than median MUA <sub>TMP</sub> values with Pearson's Chi-square and Fisher's exact tests for association.....	153
<b>Table 4.1</b>	Total phenolic content and antioxidant capacity of 36 traditional aqueous preparations. ....	175
<b>Table 4.2</b>	Total phenolic content and antioxidant capacity of 36 traditional aqueous preparations (values expressed as equivalents per unit weight of plant material extracted).....	177
<b>Table 4.3</b>	Correlation between total phenolic content assay and measurements of antioxidant capacity performed on traditional medicinal preparations .....	188
<b>Table 4.4</b>	Pearson correlation coefficient ( <i>r</i> ) of total phenolic content against antimicrobial potency.....	192
<b>Table 4.5</b>	ORAC values (μmol TE/g) for tertiary MeOH extracts of <i>Eremophila</i> species and corresponding traditional aqueous preparations .....	192
<b>Table 4.6</b>	Major use agreement values for preparations (MUA <sub>TMP</sub> ) with antioxidant capacity and total phenolic assay data.....	194
<b>Table 4.7</b>	Proportion of extracts with phenolic content ≥665 ppm GAE by groupings greater than, and less than median MUA <sub>TMP</sub> values with Pearson's Chi-square and Fisher's exact tests for association.....	198
<b>Table 4.8</b>	Mean total phenolic content for groupings of preparations greater than, and less than 33% MUA <sub>TMP</sub> .....	198
<b>Table 4.9</b>	Proportion of extracts with ORAC values ≥ 6.0 mM TE, by groupings greater than, and less than median MUA <sub>TMP</sub> values with Pearson's Chi-square and Fisher's exact tests for association.....	199
<b>Table 4.10</b>	Mean TE (ORAC) of preparations greater than, and less than 33% MUA <sub>TMP</sub> .....	199
<b>Table 4.11</b>	Proportion of extracts with DMPD assay values > 4.0 mM TE, by greater than, and less than median MUA <sub>TMP</sub> values with Pearson's Chi-square and Fisher's exact tests for association.....	200
<b>Table 4.12</b>	Mean TE (DMPD) of preparations greater than, and less than 33% MUA <sub>TMP</sub> .....	200

<b>Table 4.13</b>	Inhibition of PGE <sub>2</sub> formation in calcium ionophore stimulated and unstimulated 3T3 fibroblasts by traditional aqueous preparations.....	203
<b>Table 4.14</b>	Percentage inhibition of PGE <sub>2</sub> in calcium ionophore stimulated 3T3 fibroblasts by tertiary MeOH extracts of <i>Eremophila</i> species and corresponding traditional aqueous preparations .....	205
<b>Table 4.15</b>	P388D1 cytotoxicity (IC <sub>50</sub> , mg · mL <sup>-1</sup> ) and major use agreement values (MUA <sub>TMP</sub> ) for aqueous preparations. ....	207
<b>Table 4.16</b>	Cytotoxicity for P388D1 murine lymphoblasts by tertiary MeOH extracts of <i>Eremophila</i> species and corresponding traditional aqueous preparations.....	209
<b>Table 4.17</b>	Proportion of extracts exhibiting P388D1 cytotoxicity (IC <sub>50</sub> <200 mg/mL) by groupings greater and less than median MUA of preparations (MUA <sub>TMP</sub> ) .....	211
<b>Table 4.18</b>	Mean IC <sub>50</sub> (µg · mL <sup>-1</sup> ), P388D1 cytotoxicity of preparations greater and less than 50% MUA <sub>TMP</sub> .....	211
<b>Table 4.19</b>	Major use agreement values of preparations with percentage inhibition of PGE <sub>2</sub> formation in ionophore stimulated 3T3 fibroblasts .....	212
<b>Table 4.20</b>	Proportion of extracts exhibiting inhibition of PGE <sub>2</sub> formation in ionophore stimulated fibroblasts by groupings greater and less than median MUA of preparations (MUA <sub>TMP</sub> ) .....	213
<b>Table 4.21</b>	Mean inhibition of PGE <sub>2</sub> formation in ionophore stimulated fibroblasts of preparations greater than, and less than 33% MUA <sub>TMP</sub> .....	213
<b>Table 5.1</b>	Quantitative assay of serrulat-14-en-3,7,8,20-tetraol (2) in traditional aqueous decoctions of <i>E. duttonii</i> leaves.....	242
<b>Table 5.2</b>	Minimum inhibitory and minimum bactericidal concentrations against Gram-positive bacteria for fractions containing serrulatane diterpenes (1) and (2) and aqueous preparations with concentration of (2) at inhibitory/bactericidal dilutions of traditional preparations.....	243
<b>Table 5.3</b>	ORAC values (µmol/TE/g), PGE <sub>2</sub> Inhibition (% inhibition at two concentrations) and P388D1/HepG2 cytotoxicity IC <sub>50</sub> values (µg·mL <sup>-1</sup> ) determined for fractions containing serrulatane diterpenes 1 and 2 .....	245
<b>Table 5.4</b>	Summary of reported MIC/MBC values against <i>Staphylococcus aureus</i> for serrulatanes and naphthoquinones isolated from <i>Eremophila</i> species .....	247
<b>Table 6.1</b>	Collection localities of <i>E. longifolia</i> specimens in western NSW.....	260
<b>Table 6.2</b>	Volatile oil composition of <i>Eremophila longifolia</i> specimens.....	270
<b>Table 6.3</b>	Variables used for principal component analysis and loading for principal components 1 and 2.....	272

<b>Table 6.4</b>	Minimum inhibitory concentration ( $MIC_{p-INT}$ ) and minimum bactericidal concentration ( $MBC_{SIAS}$ ) for essential oils of three <i>Eremophila longifolia</i> chemotypes.....	275
<b>Table 6.5</b>	Disc diffusion assays of <i>Eremophila longifolia</i> essential oil chemotypes against <i>Candida albicans</i> .....	277

