An Ethnopharmacological Study of Medicinal Plants of the Kamilaroi and Muruwari Aboriginal Communities in Northern New South Wales

A thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

at

MACQUARIE UNIVERSITY

by

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July, 2006
Declaration

The work presented in this thesis has not been submitted, either in whole or in part, for a higher degree to any other university or institution, and to the best of my knowledge is my own and original work, except as acknowledged in the text.

Qian Liu

July 2006
Acknowledgements

I would like to thank my supervisors Dr Joanne Jamie, Dr Subramanyam Vemulapad and Dr James Kohen for involving me in this multidisciplinary project and letting me participate in all the different aspects of it, and for their support throughout this study.

I also would like to give my thanks to all the members of the Indigenous Bioresource Research Group at Macquarie University: David Harrington, John Hunter, Mathew Flower, Nynke Brouwer and Thomas Dzeha, and Professor Donna Craig and Chris Jones at the Centre for Environmental Law, for their collaboration and help during this study. I am very grateful to Mr and Mrs Roy and June Barker for sharing their customary medicinal plant knowledge with me. I also wish to thank Ms Alison Downing at the Herbarium of Macquarie University for identifying all the plant specimens.

My sincere acknowledgement goes to Professor Jianmin Yue at Shanghai Institute of Materia Medica, Chinese Academy of Sciences, for extending his research facilities to me for a part of this study, and for his support and guidance during my stay in his laboratory. I also want to thank his group members, especially Associate Professor Sheng-Ping Yang, for the helpful discussions on my research work during my overseas study.

I am very thankful for all those people who trained me on all of the instrumentation in the university. I thank Dr Andrew Piggott for the instructions on operating 2D NMR, Dr Isla Hains for the LCMS, Rama Nimmagadda and Mark Tran for the GCMS and Keith Tonkin and Thulas Jeyendra for the IR and polarimeter.
I made a few very good friends during my study at Macquarie University in Sydney. Since I was away from my family, these friends have been an important part of my life. I would like to express my sincere gratitude to Mr Priambudi Kosim-Satyaputra for his always wise suggestions on the problems I encountered in my work and daily life. My special thanks go to Mr Simone Ciampi who proof read the first chapter of this thesis and helped to improve my English expressions. My thanks also go to Ms Ning Xu, Ms Qiang Xu, Ms Hong Yu, Dr Yabai He, Mr Hua Liu and Mr Dayong Jin, for their friendship and for always being there to cheer me up during my difficult times.

I give my heartfelt thanks to my parents, my parents-in-law, my husband and my daughter, for their love, care and patience. They sacrificed a lot for me during these three years while I was away from home for my PhD study. I couldn’t have finished this study without their support and encouragement.

Finally, I thank the Australian government and Macquarie University for providing me with scholarships for this study.
Abstract

The overall objective of this study was to isolate and identify biologically active compounds from Australian medicinal plants with the assistance of customary (traditional and contemporary) medicinal knowledge of Aboriginal communities in northern New South Wales. This study consisted of three interrelated aspects, namely ethnobotanical research, biological studies, and bioassay-guided isolation and characterisation of bioactive constituents from Australian Aboriginal medicinal plants.

An ethnobotanical study of Australian medicinal plants used by the Kamilaroi and Muruwari Aboriginal communities was conducted with the cooperation of members of these communities. The customary medicinal plant knowledge of these two communities, along with scientific research data from published sources, of a total of 35 plants and 2 customary remedies were obtained through interviews and literature studies, and were documented as a database. The ethnobotanical database contributed to the preservation of customary medicinal knowledge of these communities. A series of educational activities were also conducted for Indigenous students as part of the relationship development and benefit sharing with Aboriginal communities in northern New South Wales. The ethnobotanical data were also used as a guide for targeted biological and chemical studies of two Australian medicinal plants, *Eremophila sturtii* and *Exocarpos aphyllus*.

Anti-inflammatory and antimicrobial assays were employed in this study for the evaluation of the biological activities of the selected medicinal plants according to their customary medicinal uses, and were applied throughout the bioactivity-oriented isolation of bioactive agents from these medicinal plants. The biological study also included optimisation and
validation of a fluorescence-based antibacterial assay, the fluorescein diacetate (FDA) assay, to make it suitable for the screening of medicinal plants for antibacterial activity. Antimicrobial and anti-inflammatory activities of *Eremophila sturtii* and *Exocarpos aphyllus* were revealed in this biological study.

Bioassay-guided fractionations of these Aboriginal medicinal plants led to the isolation of two novel compounds, 3,8-dihydroxyserrulatic acid and serrulatic acid, and six known compounds, β-sitosterol, sesamin, 3,6-dimethoxy-5,7-dihydroxyflavone, betulin, betulinic acid and oleanolic acid. The structures of the isolated compounds were elucidated using nuclear magnetic resonance (NMR) and mass spectrometric (MS) techniques. Both novel compounds demonstrated antibacterial activity against *Staphylococcus aureus* and anti-inflammatory activity against cyclooxygenase-1 and cyclooxygenase-2 (COX-1 and COX-2). All known compounds demonstrated anti-inflammatory activity against COX-1, COX-2 and 5-lipoxygenase (5-LO). The biological activities of these compounds were consistent with the customary medicinal applications of these Aboriginal medicinal plants. This is the first time that any of these compounds have been isolated from *Eremophila sturtii* and *Exocarpos aphyllus*. 
List of Publications


Conference Abstracts


Table of Contents

Declaration iii
Acknowledgements v
Abstract vii
List of Publications ix
Table of Content xi
List of Figures xvi
List of Tables xvii
List of Schemes xviii
List of Abbreviations xix

Chapter 1. Introduction

1.1. Aims and scope of this study 1
1.2. Plants for human healthcare 2
1.3. Medicinal plants and drug discovery 4
   1.3.1. Ethnobotany and ethnopharmacology 6
   1.3.2. Ethnobotanical approach in drug discovery 7
   1.3.3. Antimicrobial and anti-inflammatory agents from medicinal plants 9
      1.3.3.1. Anti-inflammatory agents 9
      1.3.3.2. Antimicrobial agents 11
   1.4. Ethnobotanical and ethnopharmacological research in Australia 13

Chapter 2. An Ethnobotanical Study with the Kamilaroi and Muruwari Aboriginal Communities and Relationship Building 17

2.1. Introduction 17
2.2. Ethnobotanical study of medicinal plants of Kamilaroi and Muruwari Aboriginal communities 19
2.2.1. Literature study of plants of the Kamilaroi Aboriginal community 22
2.2.2. Interviews with elders of the Muruwari Aboriginal community 22
2.2.3. Ethnobotanical database for the Kamilaroi and Muruwari Aboriginal communities 25
2.2.4. Plants selected for biological and chemical investigations 29
  2.2.4.1. Ethnobotanical research on Eremophila sturtii 30
  2.2.4.2. Ethnobotanical research on Exocarpos aphyllus 33

2.3. Approaches towards ensuring best ethical practices and benefit sharing 35
  2.3.1. Relationship Building 35
  2.3.2. Contributions to Aboriginal communities’ education 38

2.4. Conclusions and future directions 44

Chapter 3. Biological Assay Methods and Optimisation 47

3.1. Introduction 47

3.2. Inflammation mechanisms and anti-inflammatory assays 48
  3.2.1. Cyclooxygenase pathway 51
  3.2.2. Lipoxygenase pathway 53
  3.2.3. Targeted enzymes and anti-inflammatory assays 55
    3.2.3.1. COX inhibitor screening assay 56
    3.2.3.2. 5-LO inhibitor screening assay 58

3.3. Microbial infections and the need for new antimicrobial agents 59
  3.3.1. Infectious diseases 59
  3.3.2. Drug resistance 60
  3.3.3. Targeted microorganisms and causes of infections 61

3.4. Optimisation of the fluorescein diacetate (FDA) antibacterial assay 63
  3.4.1. Growth curve of E. coli 65
  3.4.2. Optimising the incubation time 68
  3.4.3. Optimising the inoculum density 70
  3.4.4. Validation of the optimised FDA antibacterial screening assay 72
  3.4.5. Studies on solvent effects 73

3.5. Broth microdilution method for Candida albicans 75
3.6. Possible interferences in the determination of MIC values of medicinal plant substances by the FDA assay 77

3.7. Resazurin antibacterial assay 79

3.8. Conclusions and future directions 82

3.9. Experimental 83

3.9.1. Reagents and equipment 83
3.9.2. Microorganisms and inoculum preparation 83
3.9.3. Medium preparation for antimicrobial assays 84
3.9.4. Growth of *E. coli* in 1/10 BPYN and Mueller-Hinton broth 84
3.9.5. Growth curve of *E. coli* in 1/10 BPYN with or without gentamicin 85
3.9.6. Determination of inoculum density 85
3.9.7. Optimised FDA assay procedure 86
3.9.8. Resazurin assay procedure 86
3.9.9. Validation of the optimised FDA assay 87
3.9.10. Broth microdilution assay procedure 87

Chapter 4. Ethnopharmacological study of *Eremophila sturtii* 89

4.1. Introduction 89

4.2. General review of *Eremophila* species 90

4.3. Bioassay-guided chemical and biological investigations of *E. sturtii* 96

4.3.1. Antimicrobial and anti-inflammatory activities of the crude extract and fractions of *E. sturtii* 96
4.3.2. Characterisation of the novel bioactive compounds 103
4.3.3. Biological activities of the novel compounds 109
4.3.4. Biological activities of known compounds 113
4.3.5. Antimicrobial activity of customary preparation of *E. sturtii* 114

4.4. Comparison of the FDA and resazurin antibacterial assays 115

4.5. Conclusions and future directions 116

4.6. Experimental 118

4.6.1. General 118
4.6.2. Plant material 119
4.6.3. Extraction and isolation

4.6.3.1. Isolation of 3,8-dihydroxyserrulatic acid (4.1) 119
4.6.3.2. Isolation of serrulatic acid (4.2) 120
4.6.3.3. Isolation of β-sitosterol (4.3) 121
4.6.3.4. Isolation of sesamin (4.4) 122
4.6.3.5. Isolation of 3,6-dimethoxy-5,7-dihydroxyflavone (4.5) 122

4.6.4. Preparation of customary decoction 123

4.6.5. Antimicrobial assays 123

4.6.6. Anti-inflammatory assays 124

4.6.6.1. Cyclooxygenase inhibitor screening assay 124
4.6.6.2. Lipoxygenase inhibitor screening assay 125

Chapter 5. Ethnopharmacological study of Exocarpos aphyllus 127

5.1. Introduction 127

5.2. General review of Exocarpos species 128

5.3. Prior studies of chemical constituents and antibacterial activity of E. aphyllus 132

5.4. Bioassay-guided investigation of bioactive constituents of Exocarpos aphyllus 134

5.4.1. Characterisation of antimicrobial components of E. aphyllus 134
5.4.2. Isolation and characterisation of anti-inflammatory compounds of E. aphyllus 138

5.4.2.1. Anti-inflammatory activity of pure compounds 144

5.5. Conclusions and future directions 147

5.6. Experimental 148

5.6.1. General 148
5.6.2. Plant material 148
5.6.3. Extraction and isolation 148
5.6.3.1. Betulin (5.1) 150
5.6.3.2. Betulinic acid (5.2) 150
5.6.3.3. Oleanolic acid (5.3) 151
5.6.4. Separation of the n-butanol fraction 151
5.6.5. Detection of phenolic compounds 152
Chapter 6. General Conclusions

Appendices

Appendix 1. The ethnobotanical database for the Kamilaroi and Muruwari Aboriginal communities

Appendix 2. $^1$H and $^{13}$C NMR of 3,8-dihydroxyserrulatic acid (4.1)

Appendix 3. $^1$H and $^{13}$C NMR of serrulatic acid (4.2)


Appendix 6. Journal article in Molecules (2005)

References
List of Figures

Figure 2.1. Kamilaroi country 21
Figure 2.2. Kamilaroi and Muruwari Aboriginal communities in northern New South Wales 21
Figure 2.3. Ruby Saltbush: *Enchylaena tomentose* R. Br. 24
Figure 2.4. Nardoo: *Marsilea hirsute* R. Br. 24
Figure 2.5. *Eremophila sturtii* at Lightning Ridge, New South Wales from where the plant specimens were collected 32
Figure 2.6. Collected specimen of *Exocarpos aphyllus* 34
Figure 2.7. Media coverage of the Yarrawarra workshop 37
Figure 2.8. Media coverage of the Macquarie University Open Day 2005 40
Figure 2.9. Media coverage of the Chemistry Road Show at the Casino High School 42
Figure 2.10. Media coverage of the Chemistry Road Show at the Maclean High School 43
Figure 3.1. The arachidonic acid cascade through the cyclooxygenase pathway 50
Figure 3.2. The arachidonic acid cascade through the 5-lipoxygenase pathway 54
Figure 3.3. Reaction scheme for the COX inhibitor screening assay. 58
Figure 3.4. Fluorescein diacetate hydrolysis. 64
Figure 3.5. Growth of *E. coli* in 1/10 BPYN medium and Mueller-Hinton broth. 66
Figure 3.6. Effect of incubation time on the fluorescence produced by *E. coli*. 67
Figure 3.7. Difference in fluorescence levels produced from FDA by *E. coli* with or without the presence of gentamicin. 69
Figure 3.8. Solvent effects on bacterial growth. 74
Figure 3.9. Reduction of resazurin to resorufin and hydroresorufin. 79
Figure 4.1. HMBC correlations of the aromatic moiety of compound 4.1. 104
Figure 4.2. COSY correlations of the proton (δH 4.07) of compound 4.1. 104
Figure 4.3. nOe correlations of compound 4.1. 106
Figure 5.1. Fruit of *Exocarpos aphyllus*. 128
List of Tables

Table 2.1. Summary of plants documented in the ethnobotanical database with medicinal information from Kamilaroi and Muruwari communities and literature. 27

Table 3.1. Comparison of MIC results from FDA assay and NCCLS broth microdilution method. 73

Table 3.2. Comparison of MIC results from the FDA and resazurin assays. 81

Table 4.1. Antibacterial activities of the ethanol crude extract and partition fractions of *E. sturtii*. 98

Table 4.2. COX inhibitory activities of *E. sturtii* crude extract and partition fractions. 99

Table 4.3. Antibacterial activities of column chromatography fractions of ethyl acetate fraction of *E. sturtii*. 100

Table 4.4. NMR data assignments of compounds 4.1 and 4.2. 107

Table 4.5. Minimum bactericidal concentrations (MBCs) and minimum inhibitory concentrations (MICs) of compound 4.1 and 4.2 against *S. aureus*. 110

Table 4.6. Inhibitory activities of compound 4.1 and 4.2 against inflammation pathway enzymes. 110

Table 4.7. Anti-inflammatory activity of β-sitosterol (4.3), sesamin (4.4) and 3,6-dimethoxy-5,7-dihydroxyflavone (4.5). 114

Table 5.1. Antimicrobial activity of the crude extract and partition fractions of *E. aphyllus*. 135

Table 5.2. Antibacterial activity of fractions from the *n*-butanol partition of *E. aphyllus*. 136

Table 5.3. Cyclooxygenase inhibitory activity of the crude extract and partition fractions of *E. aphyllus*. 139

Table 5.4. 5-Lipoxygenase inhibitory activity of the crude extract and partition fractions of *E. aphyllus*. 139

Table 5.5. Anti-inflammatory activity of column chromatography fractions of the ethyl acetate partition of *E. aphyllus*. 140

Table 5.6. 13C NMR data assignments of compounds 5.1, 5.2 and 5.3. 143

Table 5.7. Anti-inflammatory activities of pure compounds isolated from *E. aphyllus*. 145
List of Schemes

**Scheme 3.1.** Enzyme immunoassay in the COX inhibitor screening assay. 57

**Scheme 4.1.** Bioassay-guided fractionation of *Eremophila sturtii*. 102

**Scheme 5.1.** Bioassay-guided fractionation of *E. aphyllus*. 141
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>$[\alpha]_D$</td>
<td>Specific Optical Rotation</td>
</tr>
<tr>
<td>1/10 BPYN</td>
<td>Bacterial growth media containing 10 mM BES buffer, peptone 0.2%, yeast extract 0.1% and NaCl 0.1% (w/v)</td>
</tr>
<tr>
<td>$^{13}$C NMR</td>
<td>Carbon Nuclear Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>$^1$H NMR</td>
<td>Proton Nuclear Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>2D NMR</td>
<td>Two-Dimensional Nuclear Magnetic Resonance Spectroscopy</td>
</tr>
<tr>
<td>BES</td>
<td>$N,N$-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid</td>
</tr>
<tr>
<td>BuOH</td>
<td>$n$-Butanol</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Unit</td>
</tr>
<tr>
<td>COSY</td>
<td>(Proton – Proton) Correlation Spectroscopy</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>DEPT</td>
<td>Distortionless Enhancement by Polarisation Transfer</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl Sulphoxide</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>FDA</td>
<td>Fluorescein diacetate</td>
</tr>
<tr>
<td>HMBC</td>
<td>Heteronuclear Multiple Bond Correlation</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>HREIMS</td>
<td>High Resolution Electron Impact Ionisation</td>
</tr>
<tr>
<td>HSQC</td>
<td>Heteronuclear Single Quantum Correlation</td>
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<tr>
<td>IR</td>
<td>Infrared</td>
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<tr>
<td>LO</td>
<td>Lipoxygenase</td>
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<tr>
<td>LREIMS</td>
<td>Low Resolution Electron Impact Ionisation</td>
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<tr>
<td>LT</td>
<td>Leukotriene</td>
</tr>
<tr>
<td>m.p.</td>
<td>Melting Point</td>
</tr>
<tr>
<td>MBC</td>
<td>Minimum Bactericidal Concentration</td>
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<tr>
<td>MIC</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectrometry</td>
</tr>
<tr>
<td>NCCLS</td>
<td>National Committee for Clinical Laboratory Standards</td>
</tr>
<tr>
<td>nOe</td>
<td>Nuclear Overhauser effect</td>
</tr>
<tr>
<td>PG</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>r.p.m.</td>
<td>Revolution per Minute</td>
</tr>
<tr>
<td>ROESY</td>
<td>Rotating Frame Overhauser Effect Spectroscopy</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
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<tr>
<td>UV</td>
<td>Ultraviolet</td>
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