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

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by

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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

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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

Liu, Q., Harrington, D., Kohen, J. L., Vemulpad, S., Jamie, J. F., 2006. Bactericidal and cyclooxygenase inhibitory diterpenes from *Eremophila sturtii*. Phytochemistry 67(12), 1256-1261.

Brouwer, N., **Liu, Q.**, Harrington, D., Kohen, J., Vemulpad, S., Jamie, J., Randall, M., Randall, D., 2005. An ethnopharmacological study of medicinal plants in New South Wales. Molecules 10(10), 1252-1262.

Wanandy, S., Brouwer, N., **Liu, Q.**, Mahon, A., Cork, S., Karuso, P., Vemulpad, S., Jamie, J., 2005. Optimisation of the fluorescein diacetate antibacterial assay. Journal of Microbiological Methods 60(1), 21-30.

Conference Abstracts

Liu, Q., Wanandy, S., Harrington, D., Kohen, J. L., Vemulpad, S., Jamie, J. F., Ethnopharmacological studies of Australian Aboriginal medicinal plants. The Fifth International Chemical Congress of Pacific Basin Societies (Pacifichem), Honolulu, the United States of America, December 2005.

Liu, Q., Harrington, D., Kohen, J. L., Vemulpad, S., Jamie, J. F., *Eremophila sturtii* – an ethnopharmacological investigation of an Australian Aboriginal medicinal plant. Royal Australian Chemical Institute 2005 Natural Products Annual Symposium, Sydney, Australia, September 2005.

Liu, Q., Wanandy, S., Harrington, D., Kohen, J. L., Vemulpad, S., Jamie, J. F., Chemical and biological investigations of Australian Aboriginal medicinal plants. Royal Australian Chemical Institute Connect 2005 International Conference, Sydney, Australia, July 2005.

Liu, Q., Dzeha, T., Brouwer, N., Harrington, D., Flower, M., Hunter, J., Kohen, J. L., Vemulpad, S., Jamie, J. F., Maclean (Yaegl) Land Council, Research Partnerships in Aboriginal Bush Medicine. Royal Australian Chemical Institute 2004 Natural Products Group Annual Symposium, Lismore, Australia, September 2004.

Brouwer, N., **Liu, Q.**, Harrington, D., Kohen, J. L., Vemulpad, S., Jamie, J. F., Ethnopharmacological study of Medicinal Plants in New South Wales. International Conference of Indigenous Knowledge and Bioprospecting, Sydney, Australia, April 2004.

Table of Contents

List of Fugures

List of Tables

List of Schemes

List of Abbreviations

$[\alpha]_D$	Specific Optical Rotation
1/10 BPYN	Bacterial growth media containing 10 mM BES buffer, peptone 0.2%, yeast extract 0.1% and NaCl 0.1% (w/v)
^{13}C NMR	Carbon Nuclear Magnetic Resonance Spectroscopy
^{1}H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
2D NMR	Two-Dimensional Nuclear Magnetic Resonance Spectroscopy
BES	*N,N*-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid
BuOH	*n*-Butanol
CFU	Colony Forming Unit
COSY	(Proton – Proton) Correlation Spectroscopy
COX	Cyclooxygenase
DEPT	Distortionless Enhancement by Polarisation Transfer
DMSO	Dimethyl Sulphoxide
EtOAc	Ethyl acetate
FDA	Fluorescein diacetate
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
HREIMS	High Resolution Electron Impact Ionisation
HSQC	Heteronuclear Single Quantum Correlation
IR	Infrared
LO	Lipoxygenase
LREIMS	Low Resolution Electron Impact Ionisation
LT	Leukotriene
m.p.	Melting Point
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration

MS	Mass Spectrometry
NCCLS	National Committee for Clinical Laboratory Standards
nOe	Nuclear Overhauser effect
PG	Prostaglandin
r.p.m.	Revolution per Minute
ROESY	Rotating Frame Overhauser Effect Spectroscopy
TLC	Thin Layer Chromatography
UV	Ultraviolet