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

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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 July 2006

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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.

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Conference Abstracts

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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.

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List of Abbreviations

| [α] _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 | <i>n</i> -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 |