Chemical and Biological Studies of Medicinal Plants Used by Chungtia Villagers of Nagaland and Aboriginal People of New South Wales

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A thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

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30 September 2016

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ABSTRACT

This PhD study was based on the ethnomedicinal knowledge of the Chungtia Indigenous community of Nagaland, India and Aboriginal people of New South Wales (NSW), Australia. It follows former investigations of the Macquarie University's Indigenous Bioresources Research Group on first hand documentation of medicinal plants used by the Chungtia villagers and first hand and published accounts of medicinal plants used by Aboriginal people of NSW for the treatment of skin related ailments including sores, wounds and skin infections. The overall objective of this study was to conduct chemical and biological investigations on medicinal plants used for the treatment of skin related conditions by the Chungtia villagers of Nagaland and Aboriginal people of NSW.

A comprehensive literature review on 135 Chungtia medicinal plants documented by first hand interviews was conducted. In addition, an updated literature review on plants used for the treatment of sores, wounds and skin infections was performed. The first hand information of the medicinal plants was compared with published reports of ethnobotanical and ethnomedicinal uses as well as chemical and biological studies worldwide. The review identified eleven medicinal plants used for the treatment of skin related diseases with none or limited reports of chemical and/or biological studies. These therefore have potential for further studies. One of these plants, *Erythrina stricta* Roxb. (Fabaceae), used for the treatment of skin infections, eczema and contact dermatitis by Chungtia villagers, was selected for detailed chemical and biological investigations.

Bark of *E. stricta* was sequentially extracted with *n*-hexane, dichloromethane, ethyl acetate, methanol and water and the extracts were assayed against the bacterial strains methicillin sensitive *Staphylococcus aureus*, methicillin resistant *S. aureus*, multidrug resistant *S. aureus*, antibiotic sensitive *Escherichia coli* and *Pseudomonas aeruginosa* and the fungal strain *Candida albicans*. The most significant antimicrobial activity was observed with the dichloromethane, *n*-hexane and ethyl acetate extracts, with MIC values of 7.81 µg/mL, 125 µg/mL and 125 µg/mL against a methicillin sensitive strain of *S. aureus*. The extracts were also screened for antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging and ferric reducing antioxidant power (FRAP) assay methods. All the extracts showed positive responses to the antioxidant assays. GC-MS analysis of the *n*-hexane extract identified twelve compounds, including the bioactive compounds caryophyllene oxide (16.31%), *β*-caryophyllene (9.06%), *β*-selinene (7.06%), *α*-selinene (6.86%), selin-11-en-4-*α*-ol (6.80%), *α*-copaene (3.31%) and *α*-eudesmol (4.60%). These compounds have been reported for having antimicrobial (*β*-caryophyllene, caryophyllene oxide, *α*-eudesmol, *α*-selinene, *β*-selinene, *α*-copaene and *δ*-cadenine) and anti-inflammatory (*α*-copaene, *β*-caryophyllene, caryophyllene oxide and *δ*-cadenine)

activities. Fractionation of the dichloromethane extract by normal phase silica gel column chromatography yielded eight active fractions that showed antibacterial activity against the methicillin sensitive strain of *S. aureus* with MIC values ranging from 15.6 µg/mL to 1 mg/mL. Purification of the active fractions using different chromatographic techniques led to the isolation of seven antimicrobial and antioxidant compounds; erynone, wighteone, alpinum isoflavone, luteone, obovatin, erythrinassinate B and isovanillin. Erynone was identified as a novel compound and this is the first report of isolation of luteone, obovatin and isovanillin from the genus *Erythrina* and the first report of isolation of wighteone and erythrinassinate B from *E. stricta*.

A literature review of medicinal plants used by Aboriginal people of NSW for skin related ailments was conducted and thirty two plants were identified with none or limited reports on chemical and biological studies. Following this review, *Acacia falcata*, *Acacia implexa*, *Cassytha glabella*, *Eucalyptus haemastoma*, *Hibbertia scandens*, *Smilax glyciphylla*, *Sterculia quadrifida* and *Syncarpia glomulifera* were selected for chemical and biological studies. 70% aqueous ethanolic extracts obtained from the eight selected species were screened for antibacterial and antioxidant activities. Antibacterial activity assays of the extracts were conducted against methicillin sensitive, methicillin resistant and multidrug resistant strains of *S. aureus, E. coli* and *P. aeruginosa* using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] microdilution assay. All the extracts apart from *S. glyciphylla* and *S. quadrifida* possessed antibacterial activity against all three strains of *S. aureus. S. glyciphylla* showed activity against only methicillin sensitive *S. aureus* and *S. quadrifida* did not show any activity against any bacterial strains. *S. glomulifera* was identified with having the most active plant extract with an MIC of 7.81 µg/mL, followed by *E. haemastoma* with MIC 62.5 µg/mL and *A. implexa* with MIC 125 µg/mL. Qualitative and quantitative phytochemical screening of the extracts was also conducted. This is the first report of antibacterial activity of *A. falcata*, *A. implexa*, *C. glabella*, *E. haemastoma*, *H. scandens* and *S. glomulifera* against methicillin resistant strains of *S. aureus*.

Antioxidant activity of the eight NSW plant extracts was evaluated by DPPH free radical scavenging, ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] radical scavenging activity and FRAP assay methods. *E. haemastoma*, *A. falcata* and *A. implexa* possessed high antioxidant activity, with IC_{50} values of 51.99 ± 1.17 µg/mL, 130.20 ± 5.37 and 217.03 ± 3.80, respectively. Qualitative phytochemical screening identified the presence of terpenoids, flavonoids, steroids, alkaloids, saponins, tannins and anthraquinone glycoside classes of compounds within the extracts. The highest amount of total phenolic and condensed tannin contents were found for *E. haemastoma*, followed by *A. implexa*. *Acacia falcata* contained the highest amount of total flavonoid content, with *E.*

haemastoma and *C. glabella* also containing a high flavonoid content. A significant correlation was observed between the antioxidant properties and the total phenolic and flavonoid contents, which suggested that the phenolic and flavonoid type of compounds present in the extracts are the major contributors to their antioxidant properties. This is the first report of antioxidant activity studies for *A. falcata*, *A. implexa*, *C. glabella*, *E. haemastoma*, *H. scandens*, *S. quadrifida* and *S. glomulifera*.

The 70% aqueous ethanolic extract of *S. glomulifera* was partitioned with *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol and water. The partitions were screened for antibacterial activity against methicillin sensitive, methicillin resistant and multidrug resistant strains of *S. aureus*, *E. coli* and *P. aeruginosa*. The *n*-hexane partition showed the greatest activity with an MIC of 7.81 µg/mL against all three strains of *S. aureus*. GC-MS analysis of the *n*-hexane partition identified twenty-four phytochemicals that included the well-known antimicrobial, antioxidant and anti-inflammatory compounds *α*-phellandrene, aromadendrene, *α*-copaene, geranial, globulol, terpinene-4-ol and spathulenol.

The identification of antibacterial and antioxidant activities and bioactive constituents from *E. stricta* and the NSW medicinal plants provides support for their traditional medicinal uses by the Chungtia community of Nagaland and Aboriginal communities of NSW for the treatment of skin related ailments and increases the knowledge on these relatively unexplored plants.

DECLARATION

I declare that the work presented in this thesis has not previously been submitted for a degree, nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

The research presented in this thesis was approved by the appropriate committees of Macquarie University as follows:

Biosafety Committee: Ref: 08/06/LAB (June 2012)

Biohazard Committee: Ref: KAA110412BHA

Human Research Ethics Committee: Ref: 5201200763

Kaisarun Akter (42741920)

30 September 2016

ACKNOWLEDGEMENT

First of all, I would like to express my profound gratitude to my supervisors A/Profs Joanne Jamie and Subramanyam Vemulpad for giving me the opportunity to be a part of your research group. I am really grateful to you for encouraging me to focus on my research and providing invaluable scholarly feedback throughout the thesis and article writing. I would like to thank Joanne, for her supervision, encouragement and guidance throughout all difficult situations. I am truly thankful for her patience, motivation and tremendous academic and personal support throughout the PhD study. I would like to thank Subra, for his support throughout the study, constant guidance and encouragement without which this work would not have been possible. I really feel privileged to have supervisors like you.

I would like to express my heartfelt gratitude to all of our research group members, Teresa Malewska, Jason Smith, Tarannum Naz, Nick Gad and Joanne Packer for their friendship and making the workplace comfortable and pleasant. Thanks also go to Wendy Loa and Ping Yin for helping me with the NMR. I am really grateful to Emma Barnes for her efforts in helping with determination of the structure of the novel compound and providing her valuable feedback on the manuscripts. In addition, my sincere thanks go to Dr Joseph Brophy for assisting me with the GC-MS work at the University of New South Wales and to Dr Russell Barrow of the Australian National University, Canberra for providing the HRMS of the isolated compounds. I am also indebted to A/Prof Paul Prenzler and Mr Daniel Bucio Noble for providing the training on the antioxidant assays. I am grateful to Mr David Harrington for the collection of NSW medicinal plants and associated vouchering work. Special thanks goes to all at Macquarie University who have helped me out over the years. This includes Elsa Mardones, Mark Tran, Tony Wong, Anthony Gurlica, Catherine Wong and Michelle Kang.

This study would not have been possible without the financial support of a Macquarie University Research Excellence Scholarship and the NHMRC grants (488504 and 1028092).

Finally I would like to extend heartfelt thanks to my wonderful family for their support and love. I am grateful to my husband, Mehadi Masud. It would not have been possible to have completed this study without your love, care and inspiration. Thank you for helping me survive all the stressful times during the study and for not letting me give up. I feel privileged to have such an understanding husband. I owe a lot to my eight year old son, Sharonno Tahseen, who has endured a lot of hardship and tolerated so many hours when I could not give my attention to him. I feel gratified to have such an amazing son who showed enormous patience and tolerance throughout my study. I would like to thank my parents, brothers, sister and mother-in-law for providing their endless support. I am sad that my dad, Abdul Quddus Miah, passed away before I finished my study. He was so proud of my higher education studies. I feel proud to have such a loving and supportive family.

LIST OF ABBREVIATIONS

The following abbreviations are used throughout the text:

1D	One dimensional
2D	Two dimensional
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
APCIMS	Atmospheric pressure chemical ionization mass spectrometry
ATCC	American type culture collection
CBD	Convention of Biological Diversity
$CDCl_3$	Deuterated chloroform
CD_3CN	Deuterated acetonitrile
CFU	Colony forming unit
COSY	(Proton-proton) correlation spectroscopy
CSMT	Chungtia Senso Mokokchung Town
CVC	Chungtia Village Council
DMSO	Dimethyl sulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ESIMS	Electrospray ionisation mass spectrometry
EI	Electron impact
FDA	Food and Drug Administration
FID	Flame ionisation detector
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
GLC	Gas liquid chromatography

GC-MS	Gas chromatography-mass spectrometry
HCl	Hydrochloric acid
HMBC	Heteronuclear multiple bond correlation
H_2SO_4	Sulphuric acid
HSQC	Heteronuclear single quantum correlation
HRESIMS	High resolution electrospray ionisation mass spectrometry
IBRG	Indigenous Bioresources Research Group
IC_{50}	Inhibitory concentration capable of inhibiting growth by 50%
IR	Infrared
MBC	Minimum bactericidal concentration
MDRSA	Multidrug resistant *Staphylococcus aureus*
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration
MIQ	Minimum inhibitory quantity
MH	Müller Hinton
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin sensitive *Staphylococcus aureus*
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NCE	New chemical entity
NHMRC	National Health and Medical Research Council
NMR	Nuclear magnetic resonance
NSW	New South Wales
PAR	Participatory action research

PTLC	Preparative thin layer chromatography
ROESY	Rotating frame Overhauser spectroscopy
SGC	Silica gel column
TFC	Total flavonoid content
TLC	Thin layer chromatography
TMS	Tetramethylsilane
TPC	Total phenolic content
TPTZ	2,4,6-Tripyridyl-s-triazine
TROLOX	(±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid
UNESCO	United Nations Educational, Scientific and Cultural Organization
UV	Ultraviolet
VISA	Vancomycin intermediate *Staphylococcus aureus*
VRE	Vancomycin–resistant enterococci

CHAPTER ONE

Introduction

This PhD study involved ethnobotanical, chemical and biological investigations of traditional medicinal plants used by Nagaland people of India and Australian Aboriginal people of New South Wales for skin related ailments. This chapter presents the history of plants as medicines and the importance of plant based natural products in modern healthcare and drug discovery. It also introduces major plant natural product classes of medicinal importance; the need for new antimicrobials and wound healing agents; and the significant contributions of ethnobotanical and ethnopharmacological research to modern healthcare and drug discovery, including the importance of such investigations within Nagaland, India and New South Wales, Australia. The objectives of this PhD study and the overview of the thesis are also provided.

1 Introduction

Plants have been utilised as medicines for thousands of years.[1] From ancient literature to modern scientific records of traditional medicinal knowledge, there is evidence that plants supply the main medicinal source for peoples' healthcare in developing countries.[2] It has been estimated that about 20,000 plant species are used for medicinal purposes throughout the world.[3] According to the World Health Organization (WHO) about ~80% of the world's population in developing countries depends on plants for their primary healthcare.[4]

India has a rich heritage of traditional medicine and traditional healthcare systems that have been flourishing for many centuries.[5] Nagaland is located in North East India and the areas occupied by the Naga tribal community are considered as one of the most biodiverse in this biodiversity hotspot region.[6] Nagaland is comprised 15 tribes. Its rural people and tribes live in remote/forest areas and are still dependent to a great extent on the Indigenous systems of medicine.[7] They thus have a wealth of knowledge of medicinal plants that they have developed through their age-long trialling.[8] Although some ethnobotanical studies on Nagaland medicinal plants have been conducted,[8-10] in most cases the ethnomedicinal publications cited only the names of plants used without going into the details of the methods of use, the quantum of use and other related aspects.[11] Systematic ethnobotanical studies on North East India including Nagaland are very few.

Australian Aboriginal people have over 40,000 years of knowledge of flora and fauna as a source of food, healing agents and other resources.[12] Aboriginal medicinal knowledge has been accumulated over many thousands of years through extensive trialling and observation of the results.[13] Though numerous plant species have been utilised as traditional medicines by the Indigenous Aboriginal peoples of Australia and mostly for the treatment of infectious diseases,[14] the unique Australian medicinal flora are relatively unexplored for their biological and/or chemical studies.[15, 16] Therefore, the study of Australian flora using Aboriginal medicinal knowledge can be a good avenue for drug discovery and development of healthcare products generally.[17]

Microbial infection is a growing health concern worldwide, with skin and wound infections being especially common in Indigenous communities.[18-20] The situation has been complicated by the appearance of multidrug resistant (MDR) pathogens.[21] Increased inflammation and oxidative stress and its role in delaying wound healing is also well documented.[22, 23] Finding new antimicrobials and skin healing agents is thus imperative for reducing the disease burden arising from skin infections and chronic wounds. Traditional medicinal plants used in the treatment of sores, wounds and skin infections are very often indicative of plants containing compounds with antimicrobial

activity[24] and/or wound healing properties.[25] Thus, the study of traditional medicinal plants is an important avenue for identifying plant preparations or pure compounds for the treatment of these conditions.

This project presents the biological and chemical investigations of medicinal plants used by the Ao tribe of the Chungtia Indigenous community of Nagaland, India and of New South Wales medicinal plants used by Australian Aboriginal people for the treatment of skin related ailments. The selections of plants were guided by reported first hand ethnobotanical and ethnopharmacological studies along with previous published chemical and biological investigations.

1.1 Plants for healthcare from ancient to present

For centuries people have used plants for healing. The use of natural products with therapeutic properties is as ancient as human civilisation.[26] More than 80,000 of the globally known 250,000 species of flowering plants have been reported to be used by human civilisations for medicinal purposes.[27] Fossil records date human use of plants as medicines to at least the Middle Paleolithic age some 60,000 years ago.[28] Written records about medicinal plants date back to at least 5000 years to the Sumerians.[29] Egyptian medicine dates from around 2900 BC, but the best known Egyptian pharmaceutical record is the *Ebers Papyrus* dating from 1500 BC. This documents some 700 drugs (mostly plants). The Chinese *Materia Medica* has been extensively documented over the centuries, with the first record dating from 1100 BC (Wu Shi Er Bing Fang, containing 52 prescriptions), followed by works such as the *Shennong Herbal* (~100 BC; 365 drugs), and the *Tang Herbal* (659 AD; 850 drugs). Documentation of the *Indian Ayurvedic system* dates from about 1000 BC (Susruta and Charaka); this system formed the basis for the primary text of *Tibetan medicine*, Gyu-zhi (Four Tantras), translated from Sanskrit during the eighth century AD.[30,31]

Hippocrates (in the late fifth century BC) documented 300 to 400 medicinal plants.[32] In the first century AD, Dioscorides wrote *De Materia Medica*, a medicinal plant catalogue that became the prototype for modern pharmacopoeias. During the Dark Ages, the Arab world continued to explore their own older works and to build upon them. Asian cultures were also busy compiling their individual pharmacopoeias. In the West, the Renaissance years saw a revival of ancient medicine, which was built largely on plant medicines.[33] Plants have not only been found beneficial in the ancient medical system, they are still performing an essential role in contemporary healthcare. The World Health Organization (WHO) has estimated that about 80% of the population in African and Asian countries rely on traditional medicine for their primary healthcare and it can be presumed that a major part of traditional therapy involves active ingredients of plant parts or other plant materials or combinations of them.[4]

1.2 Natural products as a source of novel drugs

During the course of history, natural products have offered a variety of compounds that have extensive applications in the fields of medicine, food and agriculture.[34] Plants are the largest biochemical and pharmaceutical source in the world and are able to generate endless biochemical compounds.[35] Natural products from plants have been the most successful source of potential drug leads. Organic chemists, have long been interested in novel plant phytochemicals (secondary metabolites) and have investigated their chemical properties extensively since the 1850s.[36]

Approximately one-third of the top-selling drugs in the world are natural products or their derivatives.[37] Moreover, natural products are well recognised by the pharmaceutical industry for their broad structural diversity as well as their wide range of pharmacological activities.[38] A survey of clinically available therapeutic agents confirms that a very large percentage of useful drugs either are natural products themselves or, more commonly, have a clearly discernible small molecule natural product connection. For instance, Taxol, rapamycin and vancomycin were originally isolated from natural sources.[39] Natural products have played an important role in the provision of new chemical entities (NCEs). Approximately 24% of NCEs from 1981 to 2014 were natural products or natural product derived. Another 23% of NCEs during this time period were considered natural product mimics, meaning that the synthetic compound was derived from the study of natural products. Combining these categories, research on natural products accounts for approximately 47% of the NCEs reported from 1981-2014.[40]

In 1785, the English physician Withering published his observations on the use of foxglove, *Digitalis purpurea*, for the treatment of heart disorders; this eventually led to the isolation of the cardiotonic agent digoxin.[30] The isolation of the antimalarial drug quinine, from the bark of *Cinchona* species (*e.g.*, *C. officinalis*), was reported in 1820 by the French pharmacists Caventou and Pelletier. The bark had long been used by Indigenous groups in the Amazon region for the treatment of fevers and was first introduced into Europe in the early 1600s for the treatment of malaria. Quinine formed the basis for the synthesis of the commonly used antimalarial drugs chloroquine and mefloquine.[41] Another plant long used in the treatment of fevers in traditional Chinese medicine, *Artemisia annua* (Quinhaosu), has yielded the agents artemisinin and its derivatives artemether and artether, which are effective against strains of malaria resistant to quinine and quinine derivatives.[42] The analgesic used in ancient Mesopotamia (*vide infra*), morphine, was isolated from the opium poppy (*Papaver somniferum*) in 1816 by the German pharmacist Serturner. This isolation laid the basis for alkaloid chemistry and the development of a range of highly effective analgesic agents.[30] Epipodophyllotoxin, which is an isomer of podophyllotoxin, was isolated as the active antitumour agent from the roots of *Podophyllum* species, *Podophyllum peltatum* and *Podophyllum emodi*.[43] These

plants possess a long history of medicinal use by early American and Asian cultures, including for the treatment of skin cancers and warts.[44] Pilocarpine, found in *Pilocarpus jaborandi* (Rutaceae), is an L-histidine-derived alkaloid that has been used as a clinical drug in the treatment of chronic open-angle glaucoma and acute angle-closure glaucoma for over 100 years.[34] In 1994, an oral formulation of pilocarpine was approved by the FDA to treat dry mouth (xerostomia), which is a side effect of radiation therapy for head and neck cancers.[45] Structures of some of these plant based drugs are shown in Figure 1.1.

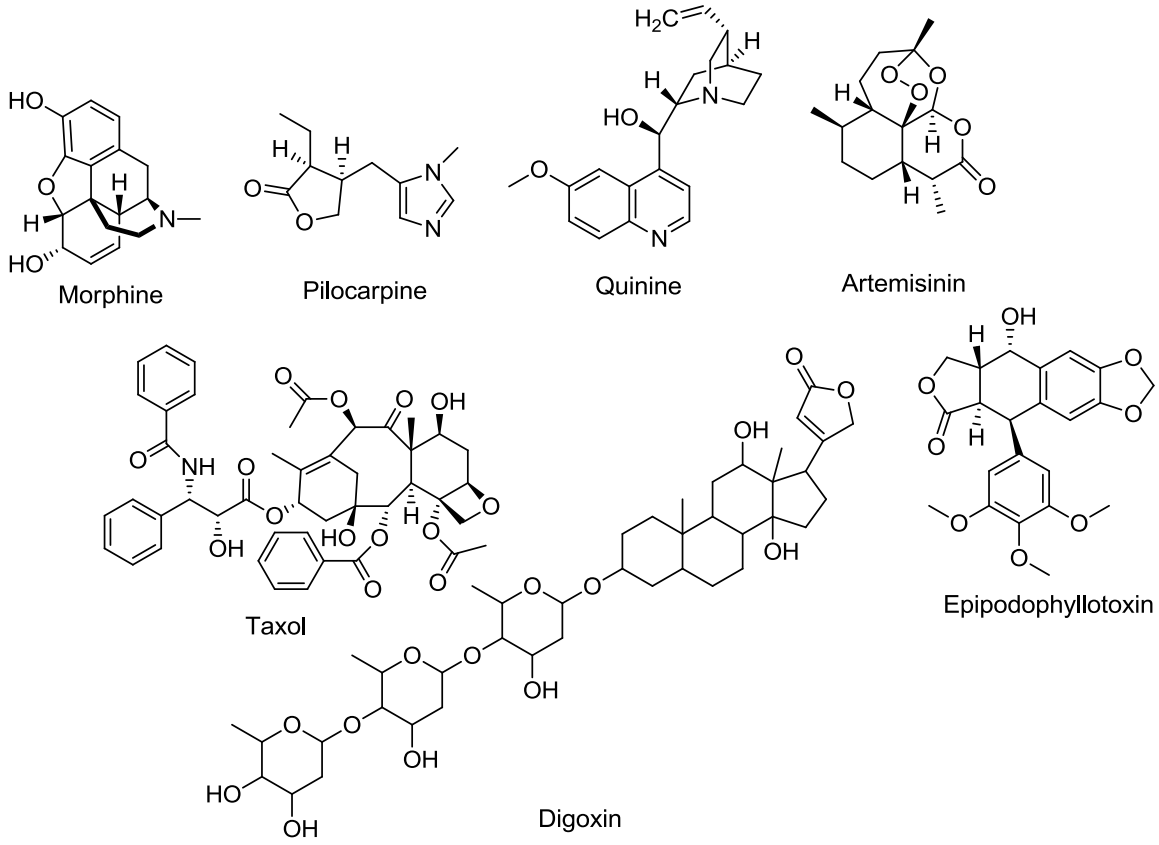

Figure 1.1: Examples of plant based drugs

From 2008–2013, a total of 25 natural product based drugs were approved for marketing worldwide, among which five were classified as natural products, ten as semisynthetic natural products and ten as natural product derived drugs.[46] Structures of lead compounds of some of these drugs are shown in Figure 1.2.

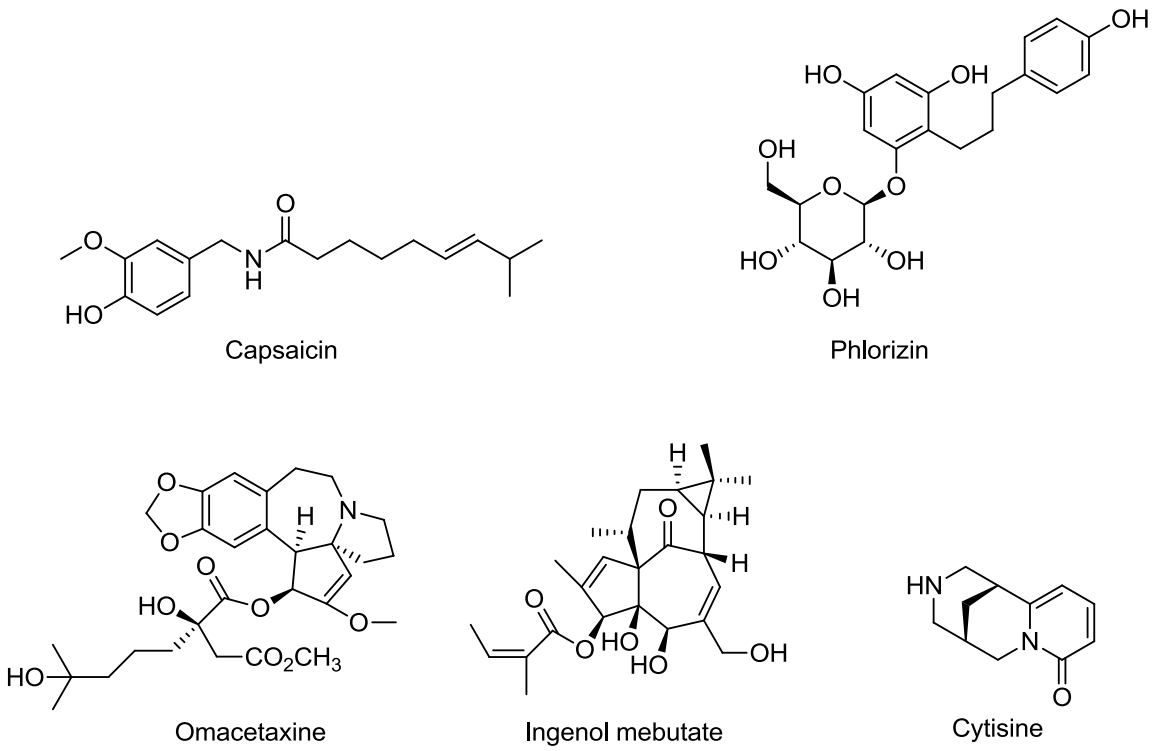

Figure 1.2: Structures of lead compounds of natural product based drugs

1.3 Major classes of secondary metabolites from plants

Metabolites are compounds synthesised by plants for both essential functions, such as growth and development (primary metabolites), and specific functions, such as pollinator attraction or defence against microorganisms and herbivores (secondary metabolites).[47] Collectively, plants produce a remarkably diverse array of over 100,000 low molecular mass secondary metabolites.[48] Before the 1970s, secondary metabolites were regarded as metabolic waste products of plants, without apparent function.[49] The significant role of plant secondary metabolites has been increasingly recognised; for example, in terms of resistance to pests and diseases.[50] Plant secondary metabolites are unique resources for pharmaceuticals, food additives, flavours and other industrial materials.[51]

Plant secondary metabolites are often classified into three major groups: (i) phenolic and polyphenolic compounds, (ii) terpenes and steroids and (iii) alkaloids.[52,53] Due to their large range of biological activities, plant secondary metabolites have been used for centuries in traditional medicine.[54] Some of the represented groups of secondary metabolites that have antimicrobial and antioxidant activities are described below.

1.3.1 Phenolic and polyphenolic compounds

Plant phenolics are generally characterised as aromatic metabolites that possess one or more acidic hydroxyl groups attached to the phenyl (aromatic) ring.[48] Phenolics range from simple, low molecular weight, single aromatic-ringed compounds to large and complex tannins and derived polyphenols. They are commonly conjugated to sugars and organic acids. Phenolics can be classified into two groups: flavonoids and non-flavonoids.

1.3.1.1 Flavonoids

The pigments that colour most flowers, fruits and seeds are flavonoids.[55] Flavonoids are polyphenolic compounds comprising fifteen carbons, with two aromatic rings connected by a three-carbon bridge. They are the most abundant of the phenolics and are found throughout the plant kingdom.[56] The main subclasses of flavonoids are the flavonols, flavones, flavan-3-ols, isoflavones, flavanones and anthocyanidins. The basic flavonoid skeleton can have various substituents. Hydroxyl groups are usually present in the 4′, 5 and 7 positions. The majority of flavonoids exist naturally as glycosides.[57]

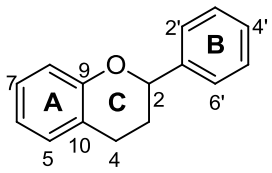

Figure 1.3: General chemical structure of flavonoids

Flavonols are the most widespread of the flavonoids, being dispersed throughout the plant kingdom, with the exception of algae and fungi. Flavonols are most commonly found as *O*-glycosides. Conjugation occurs most frequently at the 3 position of the C ring, but substitutions can also occur at the 5, 7, 4′, 3’ and 5’ positions of the carbon skeleton.[58] Flavones differ from flavonols in the absence of a hydroxyl group at C-3 of the C ring. A wide range of substitution is also possible with flavones including hydroxylation, methylation, *O*- and *C*-alkylation and glycosylation. Flavanones do not have a double bond at the C ring.[52,59] Oxidative rearrangement of flavanones, involving a 2,3–aryl shift, yields an isoflavone.[60] In plants, anthocyanidins occur as glycosylated forms known as anthocyanins. The prevalent sugar moieties are glucose, rhamnose, xylose, galactose, arabinose and fructose. Both mono- and di-glycosides are common, as well as acylated forms. The sugar moiety can be located on carbons 3, 5, 7, 3′ and 5′, with the 3 and 5 positions being dominant. Anthocyanins may exist in a variety of protonated, deprotonated, hydrated and isomeric forms.[61] Flavan-3-ols are characterised by the C

ring, which is a saturated heterocycle with a hydroxyl group at position 4, and by the presence of hydroxyl groups in the A and B rings.[62]

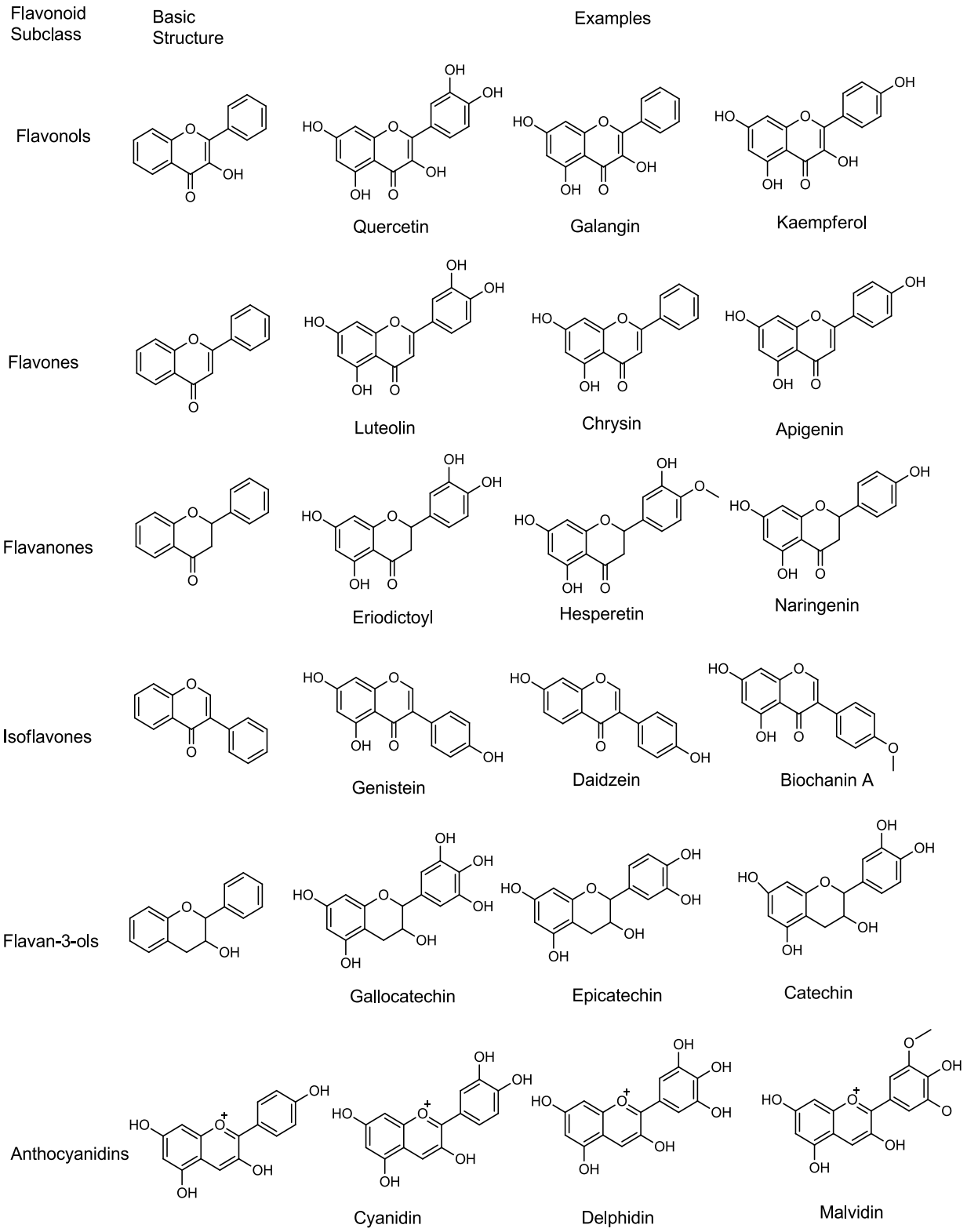

Figure 1.4: Examples of flavonoids

Flavonoids have been reported to demonstrate many useful properties, including anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity,[63,64] antiallergic activity, antioxidant activity,[65] vascular activity and cytotoxic activity.[66] The best described property of almost every group of flavonoids is their ability to act as antioxidants,[67] with flavonols being recognised as the subclass of flavonoids with typically the strongest antioxidant activity. The essential part of the flavonol structure for exerting such activity is the *o*–dihydroxyl structure at the 3′ and 4′ positions of the B ring.[68] Examples of flavonoids are presented in Figure 1.4.

1.3.1.2 Non-flavonoids

The non-flavonoid phenolics include phenolic acids (hydroxybenzoic and hydroxycinnamic acids), stilbenes, coumarins and lignans.[69] Natural phenolic acids, either occurring in the free or conjugated forms, usually appear as esters or amides. Some examples of hydroxybenzoic acids are gallic acid, *p*–hydroxybenzoic acid, protocatechuic acid, vanillic acid and syringic acid, and of hydroxycinnamic acids are ferulic acid, *p*-coumaric acid and caffeic acid (Figure 1.5).[70] Hydroxycinnamic acid derivatives are reported to be more efficient antioxidant agents than benzoic acid derivatives as the double bonds of propenoic acid derivatives play a role in stabilising the radical produced in oxidative processes by resonance.[71] Phenolic acids are reported to demonstrate antioxidant, antibacterial, antitumor, antiallergic and anti-inflammatory activities.[72] The antimicrobial activity of phenolic acids is particularly determined by the number and position of substitutions in the benzene ring, and the saturated chain length;[73] the antimicrobial effect increases with the increasing length of the alkyl chain.[74]

Coumarins comprise a very large class of compounds found throughout the plant kingdom. The basic structure of coumarins is formed by a pyrone–phenyl system (Figure 1.5). Coumarins are subdivided into simple coumarins, furanocoumarins, pyranocoumarins and the pyrone-substituted coumarins.[75] Coumarins are characterised by a variety of oxygenation patterns on the benzopyrone nucleus[76] and display a remarkable array of biochemical and pharmacological action.[77-79] Coumarins are especially reported for antimicrobial, antiviral, antioxidant, anti-inflammatory, anticancer and enzyme inhibition activity.[80]

Stilbenes are low molecular weight phenolics that occur in many plant species.[81] Stilbenes exist as both monomers and increasingly complex oligomers.[82] The monomeric stilbene aglycone's skeleton is relatively simple and comprises of two aromatic rings joined by an ethylene bridge, of which the *trans* (*E*) isomer is the most common configuration (Figure 1.5). The vast majority of naturally occurring stilbenes contain several phenolic groups. They can be prenylated or geranylated in some species and are often glycosylated as well. These oligomeric stilbenes arise from the oxidative coupling

of *trans*-resveratrol or other monomeric stilbene units. Stilbenes are reported to possess activities including antifungal, antibacterial, tyrosinase inhibitory, anticancer and antioxidant.[83-85]

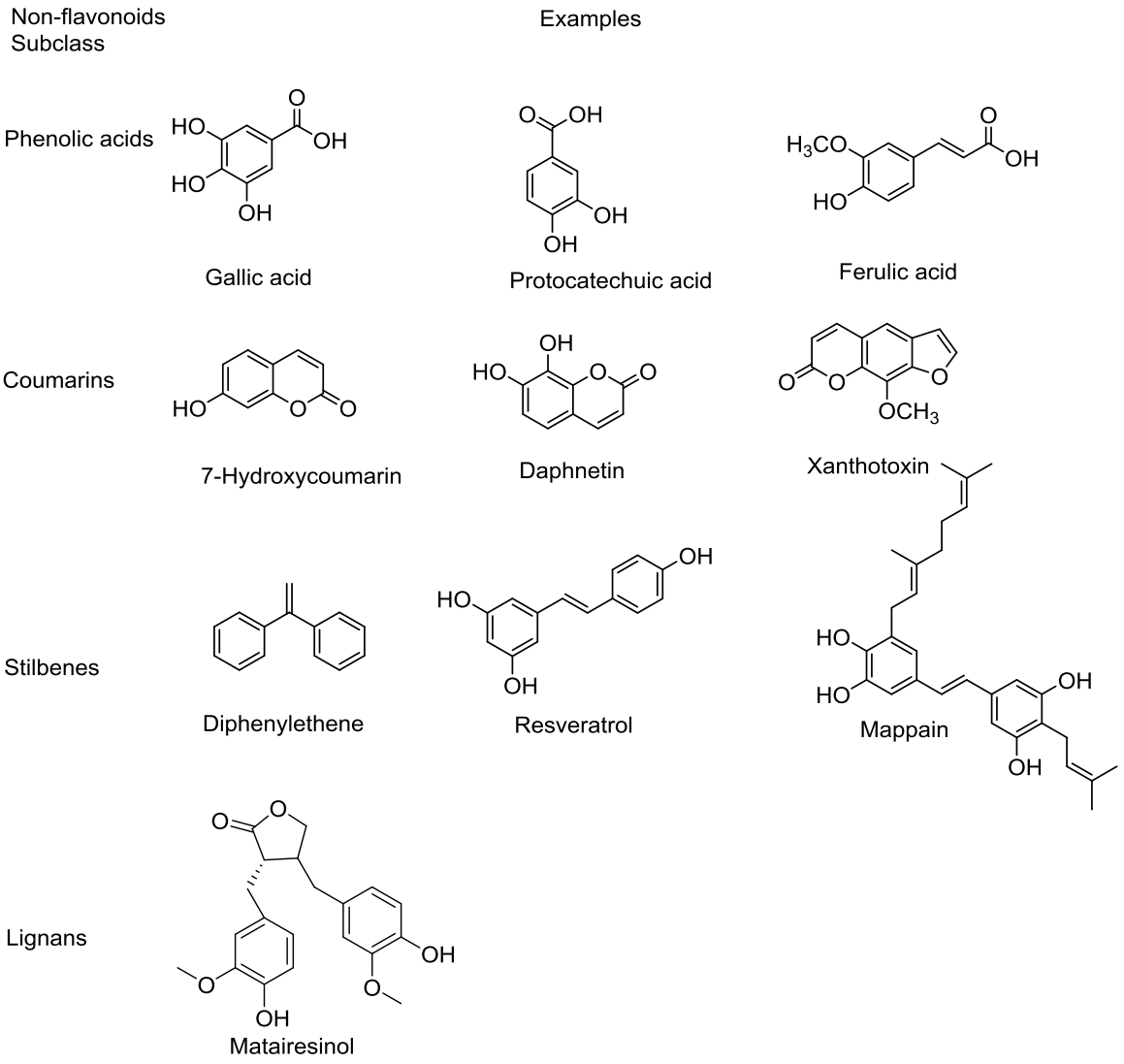

Figure 1.5: Examples of non-flavonoids

Lignans are polymeric compounds that are abundant in wood resins.[86] The skeleton of lignans is a dimer $(C_6\text{-}C_3)_2$ formed by oxidative dimerisation of two phenylpropanoid units linked by the central carbons of their side propane chains in position C-8 and C-8' (Figure 1.5). Lignans can occur in the plant kingdom not only in the dimer form but also in small amounts as trimers (sesquilignans) or tetramers (dilignans); the whole group is designated as oligolignans.[87] Lignans are reported to exhibit activities such as anti HIV-1, antioxidant, cytotoxicity, anticancer, antileishmanial, anti-

inflammatory and cytochrome inhibitory activity.[88] Examples of non-flavonoids are presented in Figure 1.5.

1.3.1.3 Tannins

Tannins are one of the major classes of polyphenols. They are highly hydroxylated polyphenolic compounds of intermediate to high molecular weight. Plant tannins are divided into two major categories, hydrolysable and condensed tannins.[89] Hydrolysable tannins are based on gallic acid, except for brown algae, which are based on phloroglucinol. Hydrolysable tannins are usually multiple esters of gallic acid with D-glucose, and condensed tannins are high molecular weight polymers of flavonoid monomers. Tannins may also be formed by the condensation of flavan derivatives or by polymerisation of quinones (Figure 1.6).[33]

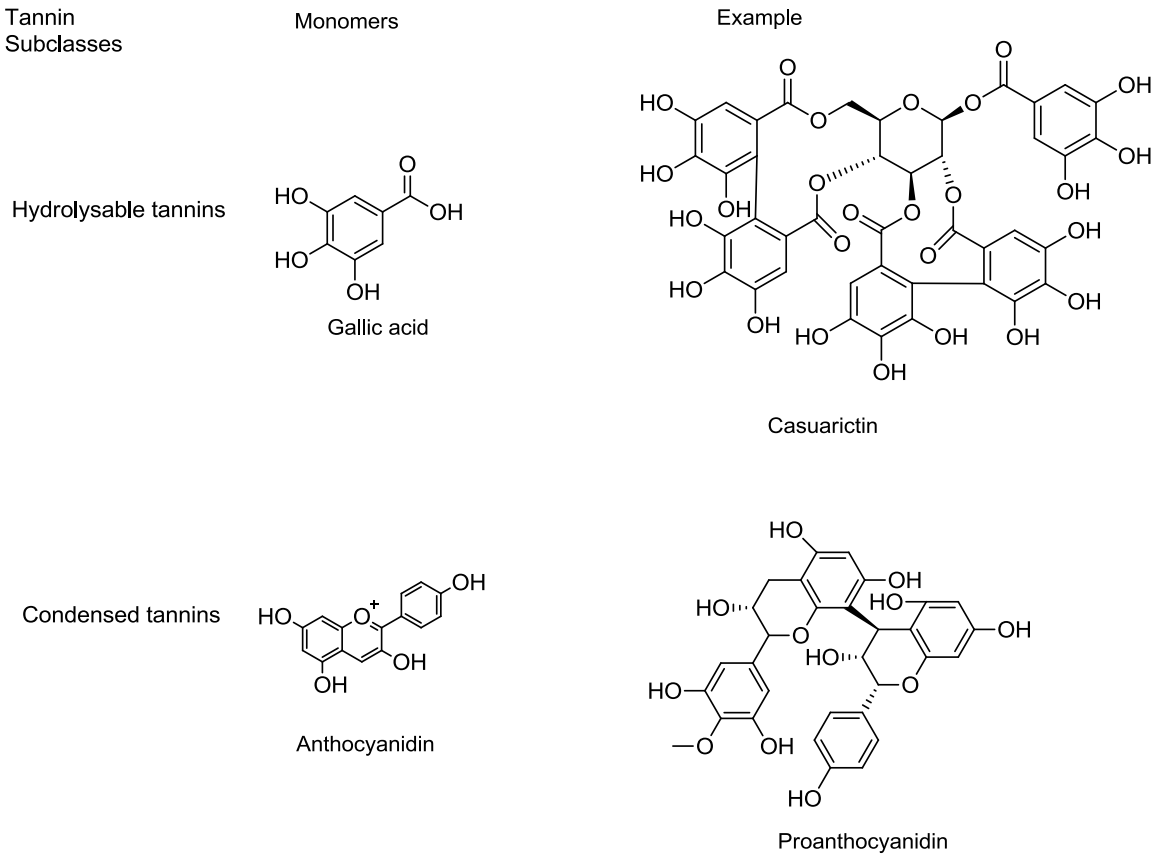

Figure 1.6: Examples of tannins

Tannins have been proposed to exert their biological effects through complexing with metal ions, acting as antioxidants and radical scavenging compounds, and/or through their ability to complex with other molecules, including macromolecules such as proteins and polysaccharides.[90] Due to the high degree of hydroxylation of the aromatic rings, tannins are often good antioxidants. They also act as protective agents against

progression of some diseases such as Alzheimer's or Parkinson's disease.[91] Tannins are reported to inhibit lipid peroxidation, which is especially significant in damage of the cardiovascular system and in addition are well known to possess anti-inflammatory, anticancer, hepatoprotective, antiviral, antibacterial and antiprotozoal activity.[92] Examples of hydrolysable and condensed tannins are shown in Figure 1.6.

1.3.2 Terpenes and steroids

Terpenes are compounds that are built up from isoprene units. Their structures are divisible into C_5 units linked in a head to tail manner. Terpenes can exist as hydrocarbons or have oxygen-containing functional groups such as hydroxy, aldehyde or ketone groups. Terpenoids are classified by the number of isoprene units that they contain, and are referred to as monoterpenoids (C_{10}), sesquiterpenoids (C_{15}), diterpenoids (C_{20}), sesterterpenoids (C_{25}), triterpenoids (C_{30}) and carotenoids (C_{40}). Natural rubber is a polyisoprenoid substance. Steroids are derived from tetracyclic triterpenoids.[93,94] The diverse array of terpenoid structures and functions has provoked increased interest in their commercial use. Monoterpenes are best known as constituents of essential oils, floral scents and the defensive resins of aromatic plants.[95] Terpenoids have been found to be useful in the prevention and therapy of several diseases including cancer, and also to have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties.[94] The anticancer drug Taxol and the antimalarial drug artemisinin are two of the most renowned terpene based drugs. Steroids are widely used as drugs and include anti-inflammatory, contraceptive and anticancer agents.[96] Examples of terpenoids and steroids are given in Figure 1.7.

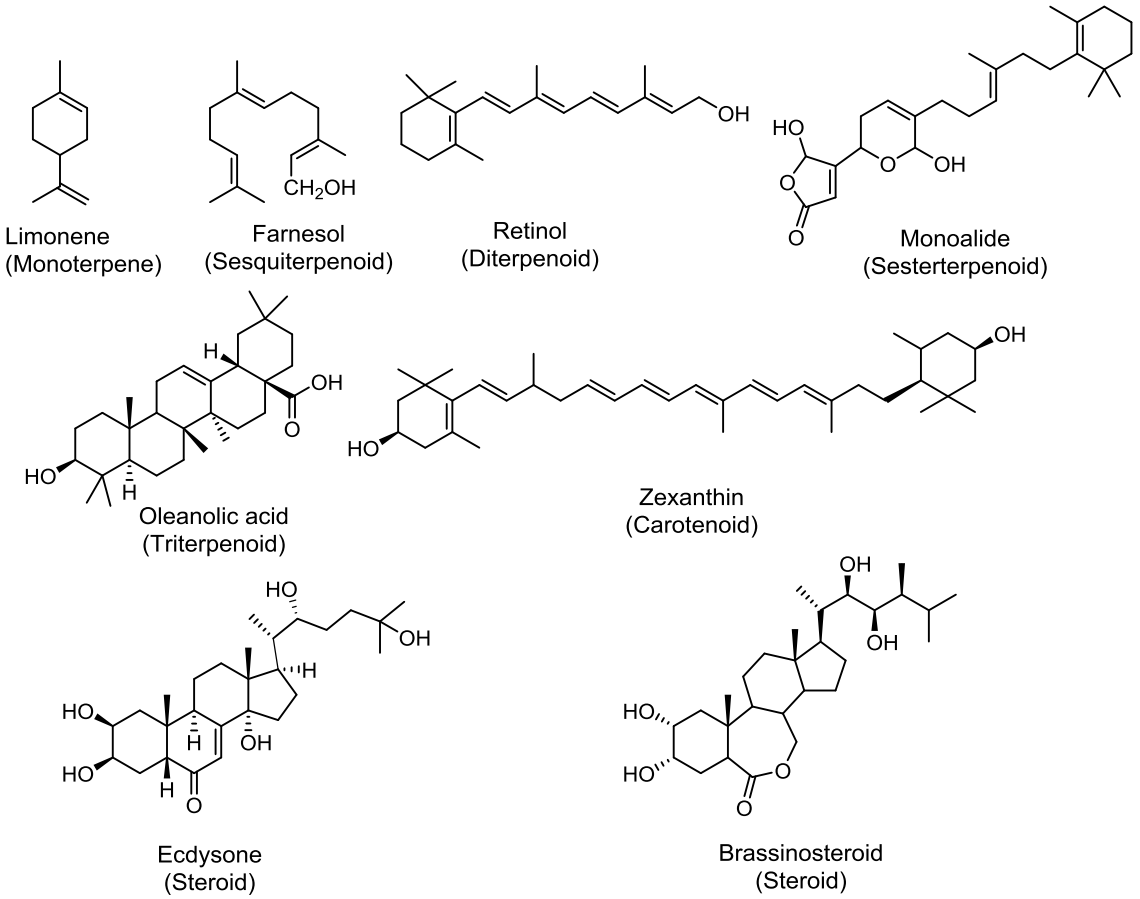

Figure 1.7: Examples of terpenoids and steroids

1.3.3 Alkaloids

Alkaloids are one of the most diverse groups of secondary metabolites found in living organisms and have an array of structural types.[97] Several approaches to the classification of alkaloids are available including chemical, taxonomic, biological and biosynthetic. In the classification of alkaloids on the basis of chemical structure, they are organised based on a common, typically heterocyclic nucleus, such as isoquinoline, indole, quinolone, quinazoline, pyrrolizidine and tropane alkaloids.[98] Alkaloids are one of the most important groups of naturally occurring substances of therapeutic interest.[99] Following the first commercial natural product isolation of morphine in the early years of the 19th century,[100] there was a cascade of successful isolations and discoveries of compounds including the isolation of xanthine (1817), strychnine (1818), atropine (1819), quinine (1820) and caffeine (1820) (Figure 1.3.6).[98] Most alkaloids with biological activity in humans affect the nervous system, particularly the action of the neurotransmitters, for example, acetylcholine, epinephrine, norepinephrine, γ–aminobutyric acid, dopamine and serotonin. Antibiotic activities are common for alkaloids and some are even used as

antiseptics in medicine, for example berberine in opthalmics and sanguinarine in toothpastes.[97] Examples of alkaloids are shown in Figure 1.8.

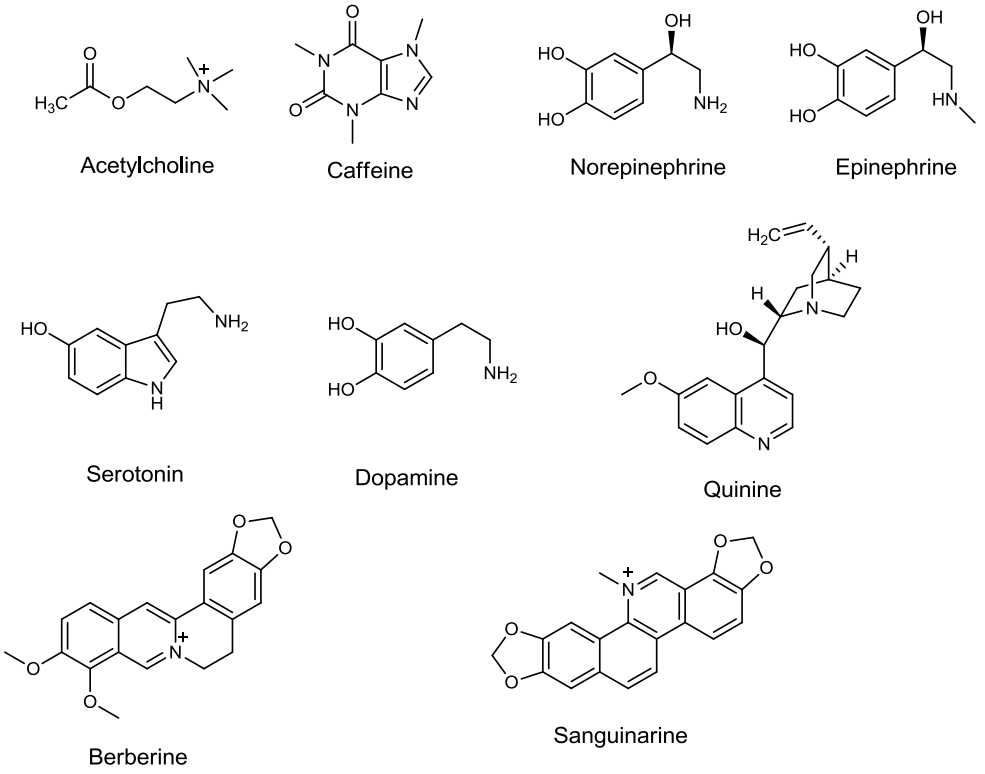

Figure 1.8: Examples of alkaloids

1.4 Importance of discovery of new active agents for wound healing

Chronic wounds are a serious health problem globally.[101] Approximately 6.5 million people suffer from chronic wounds each year in the USA, resulting in annual healthcare costs of > US$25 billion.[101] Non-healing wounds represent a significant cause of morbidity and mortality.[102] Wound healing, as a normal biological process in the human body, is achieved through four precisely and highly programmed phases: hemostasis, inflammation, proliferation and remodelling. For a wound to heal successfully all four phases must occur in the proper sequence and time frame. Many factors can interfere with one or more phases of this process, thus causing improper or impaired wound healing.[103] One of the underlying mechanisms responsible for the failure of chronic wounds to heal is an out-of-control inflammatory response that is self-sustaining.[102] Another most important factor responsible for delayed wound healing is bacteria. All chronic wounds are colonised by bacteria. There is growing evidence that bacteria in chronic wounds live within biofilm communities.[104] Biofilms are inherently protected from host defences and develop resistance to antibiotics.[105] Standard antimicrobial treatments typically fail to eradicate biofilms, which can result in chronic infection.[106] Besides the

bacterial responses, low antioxidant level is another important factor often associated with delayed wound healing.[107] Antioxidants play an important role in all phases of the healing process.[108] When reactive oxygen species (ROS) are overproduced, oxidative stress results in detrimental cytotoxic effects causing delayed wound healing. Thus, elimination of ROS could be an important strategy to improve healing of chronic wounds.[109] Therefore, discovery of new antimicrobials and skin healing agents (including anti-inflammatories and antioxidants) is important for reducing the disease burden due to chronic wounds. In many countries a large number of plants are used by tribal people for the treatment of sores, wounds and skin infections. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms.[110] These phytomedicines are in many cases cheap and also safe.[111] Thus, these plants have an immense potential for the management and treatment of sores, wounds and skin infections and other skin related ailments.

1.4.1 Antimicrobial agents

The global emergence of resistance to antimicrobial drugs is increasingly limiting the effectiveness of current drugs.[112] The overuse, improper dosage and general misuse of antibiotics are major factors in the emergence and dissemination of resistance.[113] Data from the Centers for Disease Control and Prevention (CDC) show fast growing rates of infections due to methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE) and fluoroquinolone-resistant *Pseudomonas aeruginosa*.[114] More people now die of MRSA infection in US hospitals than of HIV/AIDS and tuberculosis combined.[115] The spread of resistant bacteria, leading to untreatable infections, is a major public health threat but the pace of antibiotic discovery to combat these pathogens has slowed down.[116]

In the past thirty years, a limited number of new classes of antibiotics (*e.g.* daptomycin and linezolid) have entered the market and these are only effective against Gram-positive bacterial infections. Recently, the new antibiotic teixobactin has been shown to be effective against a wide range of Gram-positive bacteria including methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin intermediate *S. aureus* (VISA) and vancomycin resistant enterococci (VRE), but further clinical trials are still needed.[117] In the last five years no new anti-Gram negative bacterial agents have been developed, except for some molecular alterations to available antibiotics.[118] Therefore, for improved infection prevention, a sustainable supply of new effective antimicrobial agents is necessary.[119,120]

Invasive fungal infections are also increasingly recognised as a major threat in critically ill adult and paediatric patients, with significant associated morbidity and mortality.[121] The mortality rates for invasive infections with the three most common

species of human fungal pathogens are *Candida albicans* 20–40%; *Aspergillus fumigatus* 50–90%; and *Cryptococcus neoformans* 20–70%.[122] During the last decade rare pathogenic fungi, such as *Aspergillus*, *Fusarium*, *Scedosporium* and *Zygomycetes* species have emerged as a significant cause of infectious mortality in immunocompromised patients.[123,124] The therapeutic options for invasive fungal infections are quite limited and include only three structural classes of drugs: polyenes, azoles and echinocandins.[125] Clinically available antifungal agents have numerous drawbacks such as limited potency and spectrum, drug related toxicity, non-optimal pharmacokinetics and increasing resistance. The growing number of fungal infections, coupled with increasing resistance, has resulted in a need to develop new, more effective antifungal agents.[126]

1.4.2 Antioxidant agents

Reactive oxygen species (ROS), including superoxide anion ($O_2^{\cdot-}$), hydroxyl radical (·OH) and hydrogen peroxide (H_2O_2), are known to be harmful to cells and tissues.[127] A variety of immune cells are attracted to the wound lesion during the inflammation process. While those immune cells secrete pro-inflammatory cytokines, the inflammatory cells, notably neutrophils, also generate large amounts of ROS, which are essential to protect the body against developing an infection. However, when present in excess, this is when ROS can simultaneously damage the surrounding tissues.[128] Various features of wound healing under redox control require a proper balance between oxidative stress and antioxidants. While the normal physiology of wound healing depends on low levels of ROS and oxidative stress, an overexposure to oxidative stress leads to impaired wound healing.[23]

Many studies suggest that ROS-induced damage is also associated with aging, cancer and various degenerative diseases, including cataract and macular degeneration.[129] This damage is in part due to ROS reacting with nucleic acids, proteins and lipids, causing loss of function in cells and tissue damage.[130]

Antioxidants are substances that neutralise oxidants such as free radicals and ROS, or their actions.[131] Antioxidants at the site of injured or stressed cells/tissue may be inactivated and used up.[132] When antioxidant defences are not sufficient, increased ROS and free radical formation is likely to accelerate the damage. Therefore, antioxidant supplementation have been proposed as an adjuvant to reduce the deleterious secondary effects of such free radical or ROS exposure.[133]

In recent years, the search for “natural therapies” for the treatment of wounds and for novel antioxidants has gained momentum. Plants provide a rich source of natural antioxidants and many plants or plant-derived compounds possessing high levels of antioxidant properties also show wound healing activities.[134] Natural antioxidants include phenolic compounds (*e.g.* flavonoids, phenolic acids and tannins), nitrogen containing

compounds (*e.g.* alkaloids, amino acids, peptides and amines), carotenoids, tocopherols or ascorbic acid and its derivatives.[135] Ascorbic acid (vitamin C) is considered to be one of the most powerful and least toxic antioxidant and α-tocopherol (vitamin E) is the best known and most widely used natural antioxidant.[136] Quercetin, myricetin, kaempferol, luteolin and apigenin are known as the most important phenolic types of natural antioxidants.[137] Despite the importance of antioxidants, only two natural products, Medihoney, which contains active *leptospermum* honey, and the polysaccharides β-glucans, have been critically evaluated by the FDA (Food and Drug Administration) and only Medihoney has been evaluated specifically for wound healing.[23] The most widely used antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been restricted recently because of serious concerns about their carcinogenic potential.[138] Therefore, there is a great interest in finding new and safe antioxidants from natural sources.[139]

1.5 Conventional approaches of drug discovery from natural products

Despite the historical successes, many large pharmaceutical companies have decreased the use of natural products in drug discovery screening due to perceived disadvantages of low bioavailability and complexities of natural product chemistry and inherent slowness of natural products research.[140] There is an increasing awarness that the chemical and structural diversity of natural products is a better match to that of successful drugs than the diversity of collections of synthetic compounds.[141,142] Thus, the interest in applying natural chemical diversity to drug discovery research appears to be increasing once again.[143,144]

Successful identification of a new biologically active natural product can be influenced firstly by a clever selection of the species to investigate or secondly by how quickly and effectively a random screening can be conducted. Different approaches have been used by researchers with varying levels of success.[145] The most common and documented approaches include random screening, chemotaxonomic approach and ethnopharmacological approach.[146] This is discussed further below in the context of plant-based natural products.

1.5.1 Random screening

The principle of random screening is to identify a lead compound in the absence of any structural information about active molecules.[147] In random plant selection programmes, plants are collected and screened without regard to their taxonomic affinities, ethnobotanical context or other intrinsic qualities.[148] The National Cancer Institute (NCI) of National Institute of Health, USA, studied about 35,000 plant species for anticancer activity from 1960 to 1980 using this random screening approach. It resulted in two

success stories, which were those of the anticancer drugs paclitaxel (Taxol) and camptothecin.[146]

1.5.2 Chemotaxonomic approach

The chemotaxonomic approach is a potentially useful strategy in plant-derived drug discovery programmes. Chemotaxonomy relies upon the fact that taxonomically related plants often biosynthesise chemically similar secondary metabolites. Certain families are known to be rich in the kind of specific secondary metabolites that tend to be medicinally relevant. For example, active alkaloids are particularly concentrated in Rubiaceae, Solanaceae, Leguminosae, Raunuculaceae, Berberidaceae and Papaveraceae families. Thus, taxonomic correlations can provide useful clues towards drug discovery.[149]

1.5.3 Ethnopharmacological approach

Ethnopharmacology concerns the intersection of medical ethnography and biological studies of therapeutic action *i.e.*, a transdisciplinary exploration that spans the biological and social sciences.[150] Bruhn and Holmstedt defined 'ethnopharmacology' as the "interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by man".[151] The ethnopharmacological approach may reduce the number of plants that need to be screened for drug discovery attempts, resulting in a corresponding greater success rate than by random selection and mass bioscreening.[152]

Ethnopharmacology was initiated by the missionaries, such as the Jesuits in 16th century Latin America, that were interested in the use of pharmacologically active plants.[153] Molecular ethnopharmacology was later established by scientists including Luis Lewin [1850–1929], Carl Hartwich [1851–1917], Alexander Tschirch [1856–1939] and Richard Evans Schultes [1915–2001], who contributed to the study of the chemistry of pharmacologically active plants and their cultural significance.[154] Early ethnopharmacologists made revolutionary discoveries, such as the elucidation of the pharmacological principles in foxglove (*Digitalis* spp.), poppy (*Papaver somniferum* L.), curare (mixture of plants including *Chondodendron tomentosum* Ruiz & Pav. and *Strychnos* species), the finding of the first antimalarial quinine from *Cinchona* species and the elucidation of the pharmacology of psychedelic plants.[154] The ethnopharmacological approach has been and continues to be a particularly valuable strategy for drug discovery.[17,155,156]

The ethnopharmacological approach has been applied in the studies presented in this thesis to discover bioactive constituents from medicinal plants used by the Chungtia tribe of Nagaland, India and Australian Aboriginal people.

1.6 Objectives of this study

The overall objectives of this study were to conduct chemical and biological investigations on medicinal plants used for the treatment of skin related conditions by the Chungtia Indigenous community of Nagaland, India and by Aboriginal people of New South Wales, Australia.

The specific objectives of this project were to:

- Conduct a literature review of 135 medicinal plants documented by ethnobotanical studies on medicinal plants used by the Chungtia villagers of Nagaland, India.
- Undertake phytochemical and biological studies on a selected Chungtia medicinal plant.
- Conduct a literature review on New South Wales (NSW) medicinal plants reported for the treatment of skin related ailments by Aboriginal people of NSW.
- Undertake chemical and biological studies on selected NSW medicinal plants.

1.7 Thesis overview

It was hypothesised that medicinal plants used by Chungtia villagers of Nagaland, India and Aboriginal communities of New South Wales, Australia contain antimicrobial and antioxidant compounds and skin healing properties of value in healthcare and as drug leads. The research described herein was divided into two sections:

Section 1: Chemical and Biological studies on Nagaland Medicinal plants

Chapter 2 contains ethnobotanical studies on medicinal plants used by Chungtia villagers for various ailments followed by an updated literature review on documented medicinal plants used for the treatment of skin infections, sores and wounds.

Chapter 3 provides the rationale for the selection of *Erythrina stricta*, a medicinal plant used by Chungtia villagers, followed by antibacterial and antioxidant screening of crude extracts of this plant and isolation of antibacterial and antioxidant compounds.

Section 2: Chemical and biological studies on NSW Medicinal plants

Chapter 4 provides a literature review on medicinal plants used by the Aboriginal people of NSW for skin related ailments such as sores, wounds and skin infections. This is followed by a justification for the selection of eight medicinal plants (*Acacia implexa*, *Acacia falcata*, *Cassytha glabella*, *Eucalyptus haemastoma*, *Hibbertia scandens*, *Smilax glyciphylla*, *Sterculia quadrifida* and *Syncarpia glomulifera*) for further studies.

Chapter 5 describes detailed chemical and biological studies on the eight selected plants, including qualitative and quantitative phytochemical studies on crude extracts and antioxidant and antibacterial screening of crude extracts.

Chapter 6 provides a summary of the outcomes of the research and future directions.

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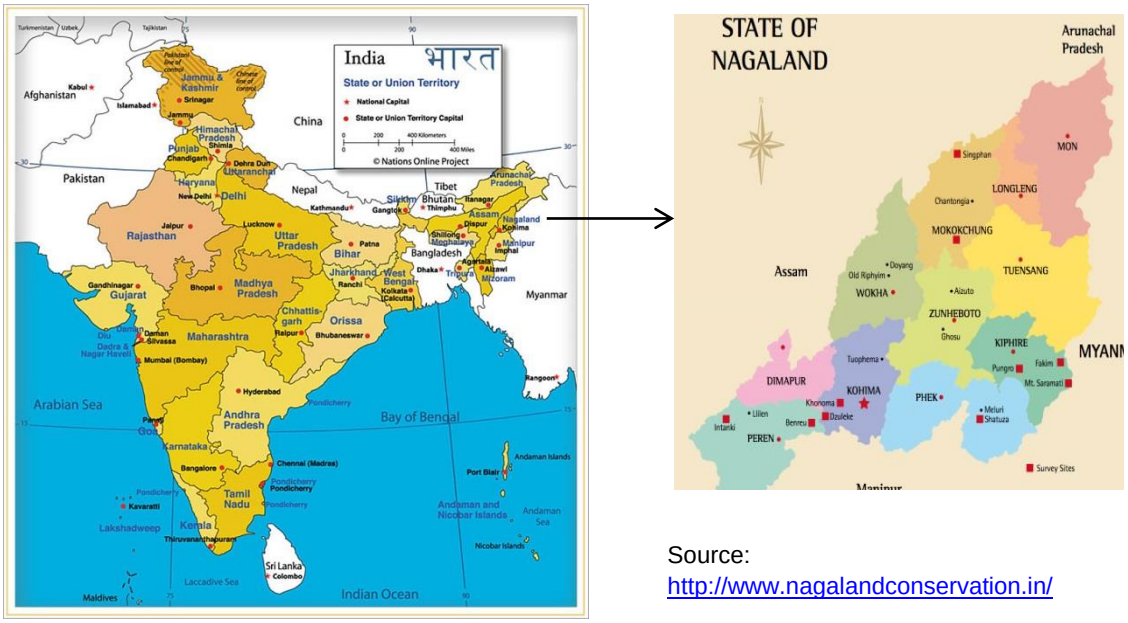
CHAPTER TWO

Review on Chungtia Medicinal Plants

This Chapter introduces Chungtia village of Nagaland as the study area and the best ethical approach adopted in working with Chungtia Elders. A literature review on 135 Chungtia medicinal plants that have been previously ethnobotanically documented is provided. This review was conducted to assist in the identification of Chungtia plants used in the treatment of skin related ailments for further chemical and biological investigations.

2.1 Introduction

Nagaland is situated in North East India, which is a part of the Indo-Burma biodiversity hotspot supporting about 50% of India's biodiversity.[1] Over 90% of the population of Nagaland is rural and is almost entirely tribal, belonging to a broad group, the Nagas.[2] The Nagas consist of a composite of at least 18 major tribes, each with its own language and cultural features.[2,3]



Source:
http://www.nationsonline.org/oneworld/india_map.html

Source:
http://www.nagalandconservation.in/

Figure 2.1: Map of study area

The Nagas led a fairly isolated life until the advent of British colonialisation in the late 19th century.[4] Their livelihoods depended solely on plants and animals. Through this process, they have developed their own traditional knowledge of medicines by experimenting with the available resources (mainly plants) and this knowledge has been passed down orally for generations.[5] Nagaland's rural people and tribes, who live in remote/forest areas, still depend to a great extent on their Indigenous system of medicine.[6]

Chungtia village is an Ao tribal village located in the Mokokchung district of Nagaland. The geography of the Mokokchung district shows six distinct hill ranges. Like for much else of Nagaland,[7] due to modernisation and development of health practices, Chungtia traditional knowledge is gradually being lost. The number of studies that have been undertaken to document and preserve the medicinal plant knowledge in Nagaland

is very limited. As part of a collaborative research project between the Indigenous Bioresources Research Group (IBRG) of Macquarie University (host of this PhD project) and the authorising body of Chungtia Senso Mokokchung Town (CSMT), Nagaland, a study was initiated in 2007 to assist in preserving Chungtia medicinal plant knowledge and to begin to identify plants of medicinal potential for further chemical and biological studies.[5,8] This followed specific requests from Chungtia village Elders for this study to be undertaken by a Nagaland man and IBRG PhD student (Kichu).[8] This initial study involved first hand interviews of the Elders, who are the key custodians of the medicinal plant knowledge, to document their valuable ethnobotanical information of medicinal plants of Chungtia village. This led to the documentation of 135 medicinal plants of Nagaland.[5]

2.2 Establishment of the collaborative research partnership between the IBRG and the CSMT authorising body

2.2.1 Aim of the collaboration

The primary goal of the collaboration established between the IBRG and the CSMT was to assist in the preservation of the valuable traditional medicinal plant knowledge held by the Elders of Chungtia village and to also investigate their relatively unexplored plants used for the treatment of skin related ailments to determine their antimicrobial/antioxidant activities and bioactive compounds aligned with their traditional use.

The Elders of this village were particularly concerned in preserving their traditional medicinal plant knowledge as in recent years the community had lost a number of key Elders who were the custodians of this knowledge.

2.2.2 Establishment of collaborative research partnership

The collaborative research partnership was founded between the IBRG of Macquarie University, Sydney, Australia, and the council of the Chungtia Senso Mokokchung Town (CSMT), Mokokchung, Nagaland, on behalf of the Chungtia villagers.[8] Details of the CSMT has provided in the attached article. This partnership was formalised by a collaborative research agreement that followed the principles set by the Convention of Biological Diversity (CBD) along with the stepwise Participatory Action Research (PAR) methodology of UNESCO[9] and the ethical guidelines of the National Health and Medical Research Council[10] for working with traditional knowledge holders. It was co-developed with the CSMT to ensure that:

- all research took place with the full consent of the CSMT

- all interviewees were informed of the project aims and their participation was entirely voluntary
- ownership of traditional knowledge was respected and confidentiality was maintained concerning any information not in the public domain
- publication of any data was only allowed with the consent of the CSMT
- if any opportunities for commercialisation arose, a process of further negotiation would be undertaken for appropriate benefit sharing with the village community.

2.2.3 First hand medicinal plant knowledge documentation

The CSMT, in consultation with the CVC, invited the Elders of Chungtia village to be interviewed on their first hand knowledge of traditional medicinal plants. They especially encouraged Elders considered to be the key custodians of traditional knowledge to participate, contingent upon their consent. Over a period of two years, researcher Kichu made two field trips to Chungtia village for the interviews and collection of voucher specimens of documented medicinal plants.[8] Initial interviews of ten village Elders including two herbal practitioners were performed in November 2007. The second trip was made in November 2008 and further interviews were carried out with the two herbal practitioners and one of the ten village Elders, who had the most extensive knowledge on their medicinal plants.[8]

2.3 Outcomes of this study

The interviews conducted by Kichu identified that the Chungtia villagers had a strong preference for using medicinal plants for the treatment of many common conditions even though contemporary treatments are available to them. Overall, the first hand interviews resulted in the documentation of 135 medicinal plant species used by the Chungtia villagers. The first hand information of the documented medicinal plants was compared with reported ethnobotanical and ethnomedicinal uses as well as chemical and biological studies worldwide. The literature review of the 135 documented medicinal plants was conducted by Kichu, the author of this thesis, and Masters' student Teresa Malewska and reviewed information in the public domain (journal articles, books, websites and other publicly available materials) up to September 2014. Comparison of the first hand information with the existing literature suggested that the most common uses of the plants were for gastrointestinal complaints, followed by skin infections, cuts, sores and wounds. The outcome of this ethnobotanical study of medicinal plants of Chungtia village was published in the Journal of

Ethnopharmacology. This study identified that thirty-seven plants are used for skin related treatments (*e.g.* cuts and wounds, sores, abscesses and boils, eczema, contact dermatitis) by the Chungtia villagers.[5]

2.3.1 An ethnobotanical study of medicinal plants of Chungtia village, Nagaland, India

A paper titled “An ethnobotanical study of medicinal plants of Chungtia village, Nagaland, India” was published in the *Journal of Ethnopharmacology* (date 26/05/2015).[5]

2.3.2 Author’s contribution to publication

The data contained in this publication is the result of a combined effort from Meyanungsang Kichu, Teresa Malewska and the author. The first hand interviews were conducted by Kichu. The literature review of the 135 medicinal plants and discussions arising from these were conducted by the author of this thesis, Kichu, and Malewska. The overall contribution of the author to the paper was approximately 20%.

Journal of Ethnopharmacology 166 (2015) 5–17

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

An ethnobotanical study of medicinal plants of Chungtia village, Nagaland, India

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ARTICLE INFO

Article history:
Received 17 November 2014
Received in revised form
25 February 2015
Accepted 26 February 2015
Available online 6 March 2015

Keywords:
Ao tribe
Biodiversity
Chemical constituents
Ethical engagement
Ethnopharmacology
Traditional knowledge

ABSTRACT

Ethnopharmacological relevance: Traditional medicinal plant knowledge is an integral and very important part of Indigenous cultures worldwide. For many communities there is a great urgency in recording this knowledge in written form. This is the first ethnobotanical report of medicinal plant knowledge of the Nagaland Ao tribe of Chungtia village and is an important step in the preservation of this culturally and medicinally significant knowledge.
Aim of the study: The aim of the presented work was to perform an ethnobotanical study on plants of medicinal and other significance to the Chungtia villagers of Nagaland, North East India.
Materials and methods: Ethnobotanical data were collected from traditional practitioners and Elders of Chungtia village by means of open group discussions and semi-structured interviews of groups and individuals using questionnaires. The interviews were also recorded in an audio format in the local Mongsen language. The gathered ethnobotanical knowledge was compared with reported ethnobotanical usages worldwide and reported biological properties and phytochemical studies relevant to the Chungtia villagers' applications.
Results: A total of 135 plant species of 69 families and 123 genera were recorded for medicinal and household maintenance applications. Those applications were grouped into 13 categories based on Chungtia villagers' classification system. The families most represented were Asteraceae, Euphorbiaceae and Solanaceae. The most reported uses were for gastrointestinal problems, followed by dermatological problems. The most commonly used plant parts were leaves, followed by fruits and stems and they were most commonly administered as a paste, decoction, infusion, juice or poultice, or taken orally with no preparation. There was strong agreement among the informants as to the usages of the plants (informant consensus factor 0.80–0.91). The use value of 6 for *Cassia floribunda*, *Dolichos lablab*, *Hedyotis scandens*, *Phyllanthus urinaria* and *Rhus javanica* indicated these are the most important species. Forty four of the 135 plants had a fidelity level of 100%.
Conclusion: This study has helped to document and preserve in written format important traditional plant knowledge of 135 plants of the Chungtia villagers, assisting them in the continued preservation of their cultural values.

1. Introduction

Nagaland is located in North East India. It lies within the Indo-Burma (Eastern Himalayas) region, which is one of the world's most biodiverse areas. The biogeography of Nagaland is unique, with its rich reservoir of plant diversity (Mao et al., 2009). The population of Nagaland is close to 2 million, with 90% of the population being Indigenous tribal people, collectively known as the 'Nagas'. There are 15 major tribes that differ in language, culture and traditions.

Until the first half of the 19th century, Nagaland communities lived secluded lives and their livelihoods depended solely on plants and animals. Through this process, they have developed their own traditional knowledge of medicines by experimenting with the available resources (mainly plants) and this knowledge has been passed down orally for generations.

The number of studies that have been undertaken to document and preserve medicinal plant knowledge in Nagaland are few. There is still much to be documented given the nature of the biodiversity of the country and the cultures and traditions of its people. Moreover, the Chungtia village Ao tribe, with which this

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http://dx.doi.org/10.1016/j.jep.2015.02.053
0378-8741/

study is concerned, has not had any of their medicinal knowledge formally documented to date (Pfoze et al., 2014).

This project was established following specific requests from Elders of Chungtia village to assist in the preservation of their medicinal plant knowledge and to undertake studies to determine the bioactive constituents of some of their medicinal plants. The motivation of these villagers to document their knowledge was particularly strong as in recent years this village has lost several key Elders who have been the custodians of significant medicinal knowledge. It is essential for this information to be conserved because of its historical and cultural value. Given that the majority of plant-based medicines have arisen from Indigenous medicinal systems (Fabricant and Farnsworth 2001), the disappearance of this knowledge would also be a significant loss to the wider scientific community and the public.

Herein, we present an ethnobotanical study carried out in Chungtia village as part of a collaborative research partnership between Chungtia village and the Indigenous Bioresources Research Group, Macquarie University, Australia.

2. Materials and methods

2.1. Establishment of the collaborative research partnership and the authorising body

2.1.1. Council of Chungtia Senso Mokokchung Town (CSMT): the authorising body

Over the course of time, Chungtia villagers have migrated and settled in Mokokchung town and at present there are about 230 households, with a population of around 1200 villagers in the town. In order to look after their wellbeing, they have established their own governing body, the Council of Chungtia Senso Mokokchung Town (CSMT).

The CSMT was established in the year 1960 and is the sole governing body of the Chungtia residents. The CSMT executive members, headed by the President, are elected by the Chungtia residents and every resident is a *de facto* member of the CSMT. The CSMT functions on its own, within the jurisdiction of Mokokchung town, and in turn is supervised by the Chungtia Village Council (CVC). The CVC is the main governing body of Chungtia village. The executive members are represented by one member each from the 14 clans. The CVC often seeks help from the CSMT in matters of importance. For this project, the CVC authorised the CSMT to enter into a collaborative research partnership with Macquarie University, on behalf of the Chungtia villagers.

2.1.2. Establishment of the collaborative research partnership

A collaborative research partnership was established between the Indigenous Bioresources Research Group (IBRG) of Macquarie University, Sydney, Australia, and the Council of the CSMT, Mokokchung, Nagaland, on behalf of Chungtia villagers, through the author M. Kichu who is from the same village. Human Research Ethics approval for this project was granted (Ref: HE22JUN2007-R05316) from Macquarie University, Sydney, Australia on 10th of July 2007. This partnership was formalised by a collaborative research agreement that followed the principles of the Convention of Biological Diversity (CBD) (Tuxill and Nabhan 2001) along with the stepwise Participatory Action Research (PAR) methodology of UNESCO (Tuxill and Nabhan 2001) and the ethical guidelines of the National Health and Medical Research Council (NHMRC, 2003) for working with traditional knowledge holders. The agreement was co-developed with the CSMT to ensure that all research took place with the full consent of the CSMT; all interviewees were informed of the project aims and their participation was entirely voluntary; ownership of traditional knowledge was respected and confidentiality was maintained concerning any information not in the public domain.

2.2. Study area and climate

Chungtia village is located within Mokokchung district, Nagaland, India, at an elevation of 896 m above sea level and lies between 25°, 45′ to 26°, 30′ N latitude and 94°, 0′ to 94°, 45′ E longitude. Chungtia inhabitants belong to the Ao tribe. Topographically it is a hilly area, with vegetation predominantly of a semi-evergreen forest type. January and February are the coldest months where temperatures can drop down to 2 °C, whilst the summer months' average temperatures are mild and oscillate between 27 and 32 °C. The region is influenced by a monsoonal climate with a high yearly rainfall that falls over nine months of the year, with an average of 2500 mm. July and August receive the most rainfall.

2.3. Choice of informants

The CSMT, in consultation with the CVC, had the role of inviting Chungtia village Elders (residents of Chungtia village) to be interviewed on their first-hand medicinal plant knowledge. They especially encouraged Elders and herbal practitioners, considered to be the key custodians of traditional knowledge, to participate upon their consent. Four CSMT representatives were selected to guide and oversee the project and 10 informants (2 male herbal practitioners and 8 village Elders including 2 females) were interviewed. Their average age was 75 years. Their highest qualification was matriculation. The herbal practitioners had no specific qualifications or education in the field. They gained their status through the knowledge passed on from their forefathers/ancestors (through word of mouth), practicing on their own and gaining respect from the villagers.

2.4. Ethnobotanical data collection

Over a period of two years (2007–2009), two trips were made to Chungtia village by the author M. Kichu to conduct first-hand interviews and for collection of voucher specimens. The interviews were conducted by means of an open group discussion with the 10 informants and four CSMT representatives present. Informants were asked to talk about the knowledge of the plants in the village, such as where they grow and how they are prepared and used. These interviews were recorded in an audio format in local Mongsen language and also by filling out a structured questionnaire that included the local plant name, local distribution of plants, habitat, seasonal availability, flowering time, medicinal use, preparation and parts used. On a follow up visit, individual interviews were also carried out with three informants (2 traditional herbal practitioners and 1 village Elder) to obtain more detailed information. Prior to the interviews, individual consent forms in English were handed out; when the informant was not English literate, the form was translated and explained in the local Mongsen language by the CSMT representative. All informants were free to withdraw from further participation in the research at any time without having to give a reason and without consequence.

This first-hand information was compared with customary (traditional and contemporary) worldwide ethnobotanical uses reported in the public domain, available biological and chemical details and information related to the plants' reported toxicity and adverse effects. The biological and chemical data search results are presented in Appendix 1 and the toxic and adverse effects are listed in Section 3.9.

2.5. Collection and taxonomic identification of the plants

For the collection of the plants, which was done on the second visit, the informants were asked to accompany the author M. Kichu and CSMT representatives to places where the discussed plants were located. These plants were collected for herbarium preparation following standard procedures (Jain and Rao, 1977). Prepared herbarium specimens of all the plants were identified with the assistance of Prof. N.S. Jamir (Nagaland University), Dr Alemmeren Jamir (Fazl Ali College, Mokokchung), Mr Bendangchuchang Longchar and Miss Wangshikokla Jamir (Nagaland University) and later authenticated by the Botanical Survey of India (BSI), Eastern Circle, Shillong, India. The voucher specimens were deposited at the BSI herbarium for future reference. All plant names were confirmed using http://www.theplantlist.org (accessed 22/01/2015).

2.6. Data analysis

The species were listed in alphabetical order by scientific name, family name, local name, plant part used, mode of preparation, administration, number of informants and use value.

2.6.1. Use value

The use value (UV) is an expression of the relative importance of each plant species used by the informants in the study area. The value was calculated using the formula (Phillips et al., 1994):

$$UV = \Sigma U/n$$

where UV is the use value of the species, U is the number of use reports cited by each informant for the given plant species and n is the total number of informants interviewed for a given plant (Morvin et al., 2014). The UV parameter helps to determine which of the plants are most frequently used for a particular purpose. Thus, UV is high when the plant is mentioned by a large number of informants and low when there are few usages cited.

2.6.2. Informant consensus factor (F_{ic})

To evaluate the level of agreement amongst informants regarding the use of particular plants, the informant consensus factor (F_{ic}) was used. The F_{ic} was calculated using the formula (Heinrich et al., 1998):

$$F_{ic} = (N_{ur} - N_t)/(N_{ur} - 1)$$

where N_{ur} refers to the number of use reports for a particular usage category and N_t refers to the number of taxa used for a particular category by all informants (Morvin et al., 2014). A high F_{ic} value indicates that specific taxa are used for the same purpose by relatively large groups of informants (Morvin et al., 2014).

2.6.3. Fidelity level

Fidelity level (FL) is a measure of which plant species are used most frequently by the informants of the study area as a treatment for a particular ailment category. The FL was calculated using the formula (Friedman et al., 1986):

$$FL(\%) = N_P/N \times 100$$

where N_p is the number of use reports cited for a given species for a particular usage category and N is the total number of use reports cited for any given species. FL=100% means all use reports refer to the same application of the given species, whereas lower FLs mean the plants are used for different purposes.

3. Results and discussion

First-hand information on the uses, preparation and mode of administration of 135 plants by Chungtia villagers was gathered via interviews of two herbal practitioners and eight Elders. Although it was apparent from the interviews that the villagers strongly rely on their traditional knowledge and that it is an important part of their culture, it was also evident that the knowledge was endangered. The causes of this loss are multivalent: several key custodians of the knowledge have died before passing it on; the younger generation are using more Western medicines; and the villagers' general wellbeing is improving due to modernisation of their lifestyle. A lack of awareness regarding the importance of traditional knowledge preservation was noted as a concern by the village Elders.

The first-hand information from the Chungtia villagers regarding the plant usage, preparation and mode of administration of the 135 plants is presented in Table 1. Reported customary uses of these plants, biological activity and bioactive chemical constituents, and the literature cited, are presented in Appendix 1 for brevity.

The literature review presented in Appendix 1 on the 135 plants used by Chungtia villagers compared reported traditional usages of the plants by communities worldwide with the Chungtia applications. Ninety two species were found to be utilised for the same ethnomedicinal or ethnobotanical purposes by other communities. Twelve had no ethnomedicinal or ethnobotanical uses reported by any community other than the first-hand reports by the Chungtia villagers. Out of the 92 species for which customary usages were found to be similar with other communities' reports, 80 were validated by pharmacological research and 55 have been studied for the presence of phytoconstituents aligned with their bioactivities. For the 12 species exclusively reported for their ethnobotanical usages by Chungtia villagers, three have been validated by pharmacological studies. However, no bioactive compounds responsible for the activities have been documented. Detailed analysis of the literature search results are presented below. Relevant references are cited in the Appendix.

3.1. Medicinal plants recorded, their habit and habitat

In this study, a total of 135 plant species from 69 families and 123 genera were identified as having medicinal and domestic value. The most often cited were plants belonging to the Asteraceae family with nine species used (6.6%), followed by the Euphorbiaceae family with seven species (5.1%) and Solanaceae with six species (4.4%). Of all the plants, 68 (50.3%) were woody (tree, shrub, woody vine or liana) and 67 (49.7%) were herbs or herbaceous vines. Seventy eight (57.7%) were collected in the wild, 47 (34.8%) were cultivated and 10 (7.4%) were collected as both wild and cultivated.

3.2. Information regarding the parts used and the preparation modes

In many cases different parts of the same plant as well as the whole plant were used for the management of various ailments. The most frequently cited were leaves (58.5%), followed by fruit/drupes (18.5%), stem (13.3%), roots/rhizomes (11.1%), seeds (7.4%), whole plant (6.6%), flowers (2.9%), sap/latex (2.9%), with the bulbs least used (1.4%). The most preferred modes of preparation were in the form of pastes (27.4%), decoctions (26.6%), infusions (14.0%), juices (5.9%) and poultices (1.4%). Decoction refers to boiling the plant in water and infusion means soaking the plant in cold water. Banana leaves were preferred as the wrapping material for the poultice treatments. In terms of administration, oral consumption was the most utilised followed by topical applications. Preparations in most cases were made from single plants, but some plant mixtures were also recorded.

Table 1
Plants documented from Chungtia village and their reported ethnobotanical usages.

Scientific name (Botanical family), Herbarium accession number (Assam acc. no)	Local name	Habitat[a]	Endemicity[b]	Part used[c]	Recorded uses, preparation and mode of administration by Chungtia villagers	NI[d]	UV[e]
Acacia pennata (Linn.) Willd. (Mimosaceae) (69649)	Zanghi	WD	N	Stem (F)	Stem is crushed into river or creek water to poison fish	3	0.3
Acorus calamus Linn. (Araceae) (69659)	Mukupen	WD	C	Leaves (F)	Leaf decoction is used in bath for the treatment of flu	2	0.2
Adenia trilobata Engl. (Passifloraceae) (69536)	Tenik tepang	WD	E	Leaves (F)	Leaf poultice is bandaged onto the knee to relieve pain	3	0.3
Adhatoda vasica Nees. (Acanthaceae) (69665)	Sungjem wa	CD	N	Leaves (F)	Leaf extract is applied externally for treatment of fever, cold and body ache. Leaves are also used to ward off evil spirits	6	2.4
Albizia chinensis (Osb) Merr. (Mimosaceae) (69660)	Mokokwa	WD	N	Leaves and stem (F)	Leaves or stem bark are crushed into river or creek water to poison fish. Leaves are put in a sack with unripe bananas to assist the ripening process	9	1.8
Albizia lebbeck Linn. Benth. (Mimosaceae) (69677)	Moang	WD	N	Stem (B)	Sack filled with stem bark is crushed with a stone in river for poisoning (killing) fish. For tempering *anok* (machete), the *anok* is wrapped with sun dried stem bark and burned until red hot. It is then immersed in water for 1 s. This results in a hardening of the metal	7	1.4
Albizia lucidior (Skud) Hara (Mimosaceae) (69595)	Sunemtong	WD	N	Roots (F)	Root infusion is applied topically to abscesses and boils	3	0.3
Allium chinense G. Don (Liliaceae) (69679)	Alolasung	CD	N	Bulbs (F)	During fever, the bulbs, roasted in mustard oil, are rubbed on the body. Fresh bulbs are eaten raw for treating high blood pressure. Bulb paste is applied topically to treat spider and snake bites and skin diseases	10	4
Allium hookeri Thw. (Liliaceae) (69628)	Repchalasung	CD	N	Leaves (F)	Leaves are eaten raw for vermifuge	10	1
Allium sativum Linn. (Liliaceae) (69602)	Lasung	CD	C	Bulbs (B)	During high blood pressure, bulbs are kept in the mouth without chewing for half an hour each day. Bulb paste is applied externally for spider and snake bites	10	2
Alstonia scholaris (Linn.) R. Br. (Apocynaceae) (69541)	Loomi	CD	N	Leaves, roots and stem (B)	Decoction of stem, leaves and roots is taken orally to treat gastritis, jaundice and also drunk as a liver tonic	4	1.2
Amaranthus gangeticus Linn. (Amaranthaceae) (69699)	Tsumarlua	CD	N	Leaves (F)	Boiled leaves are consumed to treat indigestion and also taken as a laxative	2	0.4
Aquillaria agallocha Roset. (Thymelaeaceae) (69604)	Sungza	CD, WD	N	Roots and stem (F)	Either an infusion or decoction of the roots or stem is taken orally thrice a day to treat dysentery and malaria	10	2
Artemisia vulgaris Linn. (Asteraceae) (69505)	Chinangchibaza	WD	C	Roots (F)	Root infusion is taken orally to treat dysentery	5	0.5
Artocarpus chaplasha Roxb. (Moraceae) (69688)	Unem	WD	N	Ripe fruits (B)	Eaten either raw or as an infusion taken orally twice a day for liver, kidney and gall bladder problems	4	1.2
Artocarpus heterophyllus Lamk. (Moraceae) (69596)	Polong	CD	N	Sap	Sap is applied topically to treat skin disease	10	1
Asclepias curassavica Linn. (Asclepiadaceae) (69617)	Noklangchang	CD	I	Fruits (F) and leaves (D)	Leaf paste is applied topically for cuts and wounds	4	0.4
Averrhoa carambola L. (Averrhoaceae) (69692)	Jarkona	CD	N	Fruits (F) and leaves (D)	Either fresh fruit or dried leaves or dried fruit made into a powder are consumed during high blood pressure, bladder and intestinal problems	8	2.4
Bambusa tulda Roxb. (Bambusoideae) (69605)	Longme	WD, CD	N	Ash, leaves (B) and roots (F)	Ash is used as dye, leaf decoction is used in bath during common cold and fresh root juice is taken orally as vermifuge	6	1.2
Basella alba Linn. (Basellaceae) (69633)	Latsungen	WD	C	Leaves (F)	Leaves eaten either raw or boiled to treat gastritis and as a laxative	3	0.9
Bauhinia variegata Linn. (Caesalpiniaceae) (69599)	Owepanghef	CD	N	Leaves (F)	Boiled immature leaves are consumed during gastrointestinal problems	1	0.1
Begonia picta Smith (Begoniaceae) (69642)	Tesenlawa	WD	E	Leaves (F)	Leaves are used to cleanse hands by crushing between palms. Leaves are also used in cooking for their sour taste	2	0.4
Brassica oleracea Linn. (Brassicaceae) (CD) (69510) (I)	Pandacobi	CD	I	Foliage (F)	The fresh juice of the foliage is consumed to treat jaundice	1	0.1
Cajanus cajan (Linn.) (Fabaceae) (69672)	Mahajang	CD	N	Leaves (F)	Leaf decoction is consumed to provide relief from fever	2	0.2
Calotropis gigantea Linn. (Asclepiadaceae) (69691)	Kutjak moli	WD	N	Leaves (F)	Leaf poultice is used topically to treat bone dislocation, body pain, sprain and burns	10	4
Cannabis sativa L. (Cannabaceae) (69608)	Ganja	CD	N	Leaves (F)	Leaf decoction is taken orally for stomach ache	3	0.3
Capsicum annum Linn. (Solanaceae) (69658)	Metsu	CD	I	Fruits (F)	Fruits are eaten during loss of appetite, indigestion and to 'purify blood'	2	0.6
Carica papaya Linn. (Caricaceae) (69626)	Kumita	CD	I	Fruits and sap (F)	Decoction of unripe fruit is consumed as liver tonic and to treat gastritis. Boiled unripe fruit is consumed as a laxative. The sap is used as preservative for citrus juices and extracts of other herbs	6	2.4
Cassia floribunda Cav. (Caesalpiniaceae) (69535)	Napongchami	WD	I	Leaves (F)	Warmed leaves are made into a paste and applied externally for fungal infection, eczema, contact dermatitis, allergic reaction, prickly heat and burns. Caution – only for external use	8	6
Catharanthus roseus (Linn.) G. Don (Apocynaceae) (69517)	Supienaro	CD	I	Leaves (F)	Leaf decoction is taken orally for gastroenteritis problems and as a laxative	6	1.2

Table 1 (*continued*)

Scientific name (Botanical family), Herbarium accession number (Assam acc. no)	Local name	Habitat[a]	Endemicity[b]	Part used[c]	Recorded uses, preparation and mode of administration by Chungtia villagers	NI[d]	UV[e]
Celosia cristata L. (Amaranthaceae) (69520)	Alonaro	WD	N	Flowers and leaves (F)	Decoction of flowers is taken orally for urinary tract infection. Leaf paste is applied topically for cuts and wounds	3	0.6
Centella asiatica L. (Apiaceae) (69598)	Longtsukolok	WD	N	Whole plant (F)	Plant is boiled and consumed for gastrointestinal problems	7	0.7
Chrysanthemum indicum L. (Asteraceae) (69529)	Asurongmang	CD	I	Leaves (F)	Leaf paste is applied topically to treat lip scab (angular cheilitis) and scabies	3	0.6
Cissampelos pareira Linn. (Menispermaceae) (69675)	Likhazung	WD	I	Roots (B)	Roots are eaten raw or infusion is taken orally for high blood pressure, malaria, dysentery, piles, gastrointestinal problems and diabetes. Powdered dried roots are used for long term storage	6	4.2
Cissus repens Lam. (Vitaceae) (69537)	Zerebliwa	WD	N	Leaves (B)	Leaf decoction is taken orally for high blood pressure, urinary, spleen and kidney problems	2	0.8
Citrus microcarpa Bunge (Rutaceae) (69618)	Nimbutinga	CD	C	Fruits (F)	Fresh fruit juice is taken judiciously for stomach ache and gas formation (purgative)	10	2
Clerodendron colebrookianum D. Don (Verbenaceae) (69531)	Oremwa	WD, CD	N	Leaves (F)	Boiled leaves are consumed to treat high blood pressure and also eaten as a delicacy. Caution – ingestion of drupe may induce body swelling and vomiting	10	2
Coix lacryma-jobi Linn. (Poaceae) (69662)	Jemur	WD	N	Fruits and leaves (F)	Fruits and leaves are cooked and consumed as vitamin source	7	0.7
Costus speciosus (Koenig ex Retz) JE Smith (Costaceae) (69496)	Aokmejang	WD	N	Stem (F)	Inner stem is chewed for tooth ache and as vermifuge	10	2
Crataeva nurvala Buch-Ham. (Capparaceae) (69690)	Kongkawa	WD	N	Leaves (F)	Warmed leaves are applied externally to relieve pain and body swelling. Boiled leaves are consumed as liver tonic	8	2.4
Croton caudatus Gieseler (Euphorbiaceae) (69664)	Khemetsu koila	WD	N	Leaves and roots (F)	Fresh leaves or roots are chopped into fine pieces and soaked in water overnight and the extract is drunk twice a day for cancer, sinusitis and gastrointestinal problems	1	0.3
Cucurbita pepo L. (Cucurbitaceae) (69682)	Moyamatsu	CD	I	Fruits and leaves (F)	Leaves and fruits are cooked and consumed as vitamin source	10	1
Curculigo capitulata (Lour.) Kuntze (Hypoxidaceae) (69527)	Kurivu	WD	N	Rhizomes (F)	Outer skin of rhizomes is peeled off and soaked in water until it turns slimy and then consumed for gastritis and squeezed into eyes for treating eye infection (dirt, conjunctivitis)	9	1.8
Curanga amara Juss. Syn: *Picria fel-terrae* (Scrophulariaceae) (69538)	Longri	WD, CD	N	Leaves (F)	Fresh leaves are chewed or infusion taken orally to treat dysentery, high blood pressure, food poisoning, gastroenteritis and loss of appetite. Caution – extremely bitter	10	5
Cyclea peltata Diels. (Menispermaceae) (69650)	Tsungrempangmoli	WD	N	Leaves (B)	Leaf decoction is applied topically to abscesses and boils. Leaves also added to bath to ward off evil spirits	6	1.2
Datura stramonium Linn. (Solanaceae) (69528)	Kohima sangjem	WD	C	Leaves (F)	Warmed leaves are applied externally to relieve back pain	5	0.5
Debregeasia longifolia (Burm. f.) Wedd. (Urticaceae) (69504)	Natsulawa	WD	N	Leaves (F)	Leaf decoction is taken orally to treat diabetes, fever and high blood pressure. Boiled leaves are eaten as a delicacy	3	0.9
Dendrocnide sinuata (Bl.) (Urticaceae) (69508)	Zaklojawa	WD	N	Stem (F)	Outer fresh stem is scraped off and the mucilage secreted is applied on fresh cuts and wounds (haemostatic). Caution – produces extremely painful sting	10	1
Diospyros lanceifolia Roxb. (Ebenaceae) (69544)	Urcha	WD	C	Fruits and roots (F)	Fruits or roots are crushed in stream to poison fish	10	1
Dolichos lablab L. (Fabaceae) (69523)	Napakauv	CD	N	Leaves and pods (B)	Cooked pods are consumed to treat diarrhoea, nausea, vomiting and poor appetite. For insect and poisonous spider bites and bee stings, leaf paste is applied topically. Caution – fatal when consumed after a dog bite	8	6
Drymaria cordata (Linn.) Willd. (Caryophyllaceae) (69530)	Pipivula	WD	C	Whole plant (F)	Plant warmed in fire is crushed into a paste and applied topically to treat fungal infection (ringworm), contact dermatitis and lip scab (angular cheilitis). For sinusitis, leaf paste is inserted into the nostril. To deodorise armpits, plant is wrapped in banana leaves and toasted for 5–10 min and then applied to armpits. For ear pain and infection (otitis media), leaves, mustard oil and spider exuvia are pounded, filtered and a few drops instilled into the ear	6	3.6
Dryopteris filix-mas (L.) syn *Cyclosorus parasiticus* Schott (Dryopteridaceae) (69507)	Nachav	WD	C	Whole plant (F)	Whole plant is crushed into the stream to poison fish. Infusion is sprayed as pesticide and insecticide. Leaves are laid down in chicken coop for killing chicken ticks/bugs. Leaf paste is used to treat skin irritation and snake and insect bites	3	1.2
Duabanga grandiflora (Roxb. ex DC.) Walp. (Sonneratiaceae) (69686)	Kisati	CD, WD	N	Stem bark (F)	Fresh bark is scraped off and applied topically to treat skin diseases, cuts and wounds	3	0.6
Elsholtzia blanda Benth. (Lamiaceae) (69524)	Changjang	WD	N	Leaves (B)	Leaf paste is applied to fresh cuts and decoction of leaves is added to bath during cold or fever. Leaf paste is also inserted into the nostril to treat sinusitis	4	1.2
Entada pursaetha DC. (Leguminosae) (69616)	Keling	WD	N	Seeds (B)	Seed extract is used for head wash to treat head lice and dandruff	10	1
Equisetum ramosissimum Desf. Subsp. *Debile* (Equisetaceae) (WD) (69499) (C)	Avpenba	WD	C	Stem (F)	Stem decoction is taken orally for kidney problems	6	0.6
Eryngium foetidum Linn. (Apiaceae) (69668)	Aong thonia	WD	I	Leaves (F)	Leaves are consumed either raw or cooked for indigestion	3	0.3
Erythrina stricta Roxb. (Fabaceae) (69629)	Lochet	WD	N	Stem bark (F)	Bark paste is applied topically to treat contact dermatitis, eczema and skin infections	10	3

Table 1 (*continued*)

Scientific name (Botanical family), Herbarium accession number (Assam acc. no)	Local name	Habitat[a]	Endemicity[b]	Part used[c]	Recorded uses, preparation and mode of administration by Chungtia villagers	NI[d]	UV[e]
Eucalyptus globulus Labill. (Myrtaceae) (69663)	Eucalyptus	CD	I	Leaves (B)	Steam from boiling leaves is inhaled for nasal decongestion	3	0.3
Eupatorium odoratum Linn. (Asteraceae) (69523)	Zasen	WD	I	Leaves (F)	Leaf paste is applied topically to fresh cuts and wounds	10	1
Euphorbia pulcherrima Willd. ex Klotzsch (Euphorbiaceae) (69615)	Muluchangnaro	CD	I	Flowers and leaves (F)	Death may result when flowers or leaves are fed accidentally to pigs	2	0.2
Euphorbia royleana Boiss. (Euphorbiaceae) (69684)	Takterak	CD	N	Milky sap (F)	Milky sap is applied topically to treat skin diseases and body pain	3	0.6
Eurya acuminata DC. (Theaceae) (69612)	Mesetwa	WD	C	Fruits and leaves (F)	Leaf infusion is taken orally to treat dysentery/diarrhoea. Leaf paste is applied topically to cuts and wounds. Fresh fruits are crushed and mixed with water and drunk 3–4 times to treat gas formation	4	1.2
Ficus elastica Roxb. ex Hornem. (Moraceae) (69619)	Ngisa	CD	N	Roots and sap (F)	Root juice or sap is applied topically to snake bite, cuts and wounds	3	0.6
Garcinia cowa Roxb. (Guttiferae) (69631)	Songtula	WD	N	Stem bark and seeds (D)	Decoction of dried bark or seed covers are taken orally to treat dysentery/diarrhoea. Seeds are edible	10	1
Garcinia pedunculata Roxb. Ex Buch.-Ham. (Guttiferae) (69630)	Asong	WD	N	Stem bark and seeds (D)	Decoction of dried bark or seed covers are taken orally to treat dysentery/diarrhoea. Seeds are edible	10	1
Girardinia palmata (Forsk.) Gaud. (Urticaceae) (69661)	Ongpangzakl	WD	N	Leaves (F)	Leaf paste is applied to dog bites. Dogs stung with this plant may die	10	1
Glycine max (L.) Merr. (Fabaceae) (69680)	Alichami	CD	I	Seeds (B)	Roasted seeds are made into powder and taken with tea to treat dysentery	3	0.3
Gmelina arborea Roxb. (Verbenaceae) (69518)	Ekong	WD	N	Drupe (F)	Mesocarp of the drupe is applied topically to treat skin diseases	6	0.6
Gonatanthus pumilus (D. Don) Engler and Krause (Araceae) (69498)	Longtong	WD	N	Leaves and stem (F)	Small quantity of the leaves or stem is mixed with food and given as vermifuge to pigs. Caution – extremely poisonous and death may result if ingested by humans. Rhizomes also cause extreme itching when contacted	8	0.8
Gossypium herbaceum Linn. (Malvaceae) (69611)	Khumpa	CD	I	Roots (F)	Root decoction is taken orally as diuretic	2	0.2
Gynocardia odorata R. Br. (Flacourtiaceae) (69501)	Lamen	WD	N	Leaves (F)	Leaf paste is applied to bee sting and leaves are used to protect from bee sting during honey harvest. Caution – plant growing near drinking water source or channel can contaminate the drinking water and if consumed results in abnormal enlargement of neck	6	0.6
Gynura crepidioides Benth. (Asteraceae) (69506)	Monglibaza	WD	I	Leaves (F)	Leaf decoction is taken orally to treat diabetes	4	0.4
Hedyotis scandens Roxb. (Rubiaceae) (69539)	Termoli	WD	E	Leaves and roots (B)	Leaf paste is applied topically to cuts and wounds and infusion or decoction is taken orally to treat urinary tract infection, piles, gastrointestinal problems and also taken as a laxative. Roots are chewed and applied topically to bee sting	8	6
Hodgsonia macrocarpa (Blume) Cogn. (Cucurbitaceae) (69667)	Assa	WD	E	Leaves and seeds (F)	Leaf paste is used for massaging body pain and roasted seed is consumed as a laxative	6	1.2
Holboellia latifolia Wall. (Lardizabalaceae) (69500)	Mezetsuk	WD	E	Leaves (F)	Foam from crushed leaves is applied topically to burns	5	0.5
Houttuynia cordata Thunb. (Saururaceae) (69532)	Nokna	CD	N	Whole plant (F)	Eaten raw to treat dysentery/diarrhoea, gas formation and as vermifuge	10	3
Ipomoea nil (Linn.) Roth. *Pharbitis nil* (Convolvulaceae) (69678)	Makenchangnaro	CD	C	Flower and leaves (F)	Leaf and flower paste is applied topically to burns	2	0.2
Kalanchoe pinnata (Lam.) Pers. (Crassulaceae) (69515)	Nokchamoli	CD	I	Leaves (F)	Warmed leaf paste is applied topically to treat ringworms, skin diseases and burns	6	1.8
Lagenaria siceraria (Molina) Standl. (Cucurbitaceae)	Aakuf	CD	I	Leaves (F)	Juice extract of leaves is applied topically to treat skin diseases and inflammation	6	1.2
Lantana camara Linn. (Verbenaceae) (69620)	Aiangketba naro	WD	I	Whole plant (F)	Plant decoction is taken orally to treat jaundice, cold and fever	1	0.3
Lasia spinosa (L.) Thwaites (Araceae) (69655)	Turang	WD	N	Leaves and stem (F)	Decoction of stem/leaves is taken orally as vermifuge. Leaf paste is applied topically to treat skin diseases. Young tender leaves are edible	10	2
Lycopersicon esculentum Linn. (Solanaceae) (69654)	Benganatasula	CD	I	Fruits (F)	Juice from unripe fruits is taken orally twice a day to treat urinary problems and kidney pain	3	0.6
Luffa acutangula Linn. (Cucurbitaceae) (69676)	Pokka	CD	N	Flowers, fruits and leaves (F)	Flowers, fruits and leaves are boiled and consumed as a laxative and to aid digestion	6	1.2
Macropanax undulatus (Wall. ex G. Don) Seem. (Araliaceae) (69540)	Semza	WD	N	Leaves (F)	During common cold and high fever leaves are laid down and slept on. Leaves can be seen in many bird nests, particularly flamingo and bulbul	4	0.4
Maesa indica (Roxb.) Wall. (Myrsinaceae) (69514)	Kensametong	WD	N	Leaves (F)	Leaf paste is applied topically to cuts and wounds (haemostatic)	3	0.3
Manihot esculenta Crantz. (Euphorbiaceae) (69695)	Alicha	CD	I	Tubers (B)	Tubers are used with some rice and herbs to produce fermented beer and this is taken for gastrointestinal problems	10	1
Melastoma malabathricum (Melastomataceae) (69652)	Nemna	WD	N	Fruits and leaves (F)	Fruits are edible and leaf paste is applied to cuts and wounds	3	0.3

Table 1 (*continued*)

Scientific name (Botanical family), Herbarium accession number (Assam acc. no)	Local name	Habitat[a]	Endemicity[b]	Part used[c]	Recorded uses, preparation and mode of administration by Chungtia villagers	NI[d]	UV[e]
Melia composite Willd. (Meliaceae) (69669)	Aiet	WD	N	Fruits and leaves (F)	Fruits/leaves are consumed to expel gas from stomach	2	0.2
Mentha cordifolia Opiz ex Fresen. (Lamiaceae) (69683)	Pudina	CD	I	Leaves (F)	Leaf paste is applied topically to fresh cuts and skin diseases. Crushed leaves are inhaled for nostril decongestion	10	2
Mikania cordata (Burm. F.) B. L. Rob. (Asteraceae) (69534)	Indialeelang	WD	I	Leaves and stem (B)	Leaf/stem paste is inserted into the rectum for about 5 min to treat dysentery/diarrhoea and piles. Leaf/stem paste is applied topically to treat skin diseases and cuts. Dried powdered leaves are also used with other plants	10	4
Millettia cinerea Benth. (Fabaceae) (69666)	Suli	WD	E	Roots and vines (F)	Roots are crushed into stream or creek to poison fish. Vine extract is used in massages to relieve body pain	10	2
Mimosa pudica Linn. (Mimosaceae) (69526)	Amidangku-ayaklawa	WD	I	Leaves (F)	Leaf paste is applied topically to treat inflammation and decoction is taken orally for gastrointestinal problems	8	1.6
Mirabilis jalapa Linn. (Nyctaginaceae) (69609)	Chumdangnaro	CD	I	Leaves and roots (F)	Decoction of leaves/roots is taken orally as a diuretic and to treat ear ache	4	0.8
Mussaenda roxburghii Hk. f. (Rubiaceae) (69502)	Andipeernaro	WD	E	Leaves (B)	Fresh leaf paste is applied topically to cuts and wounds. Leaves are also used in combination with other herbs to brew rice beer	10	2
Myrica esculenta Buch.-Ham., ex D. Don (Myricaceae) (69522)	Mediong	CD	N	Fruits and leaves (F)	Fruits are edible and leaf paste is applied to cuts and wounds	10	1
Nephrolepis cordifolia (Davalliaceae) (69623)	Seraenjen	WD	C	Tubers (F)	Tubers are crushed and taken orally with water to treat hiccups and sneezing. They are also used as a diuretic. A few drops of juice extract are inserted into the nostrils to treat sinusitis. Fresh tubers are crushed and applied topically or a slice of tuber is rubbed into the affected areas for the treatment of skin infections	4	1.6
Nerium indicum, Mill. (Apocynaceae) (69674)	Sharonnaro	CD	C	Flowers (F)	Flower decoction is used to kill lice and insects	1	0.1
Ocimum basilicum Linn. (Lamiaceae) (69543)	Nangperra	CD	N	Whole plant (B)	Infusion is taken orally to treat stomach upset and gas formation. It is also added in bath to treat flu	10	2
Oroxylum indicum (Linn.) Vent. (Bignoniaceae) (69637)	Ochamiliau	WD	N	Bark (F)	Decoction is taken orally to treat dysentery and rheumatism	2	0.4
Oxalis acetosella L. (Oxalidaceae) (69607)	Waroetsu	WD	C	Leaves (F)	Eaten raw as diuretic, vermifuge and to treat gas formation and dysentery	2	0.8
Paederia foetida Linn. (Rubiaceae) (69694)	Atsulelang	WD	N	Stem (F)	Decoction is taken orally to treat intestinal problems	6	0.6
Passiflora edulis Sim. (Passifloraceae) (69700)	Entsulashi	CD	I	Leaves (F)	Leaf decoction is taken orally once a day after meal to treat high blood pressure	8	0.8
Phyllanthus emblica, Linn. (Euphorbiaceae) (69671)	Lher	WD, CD	N	Fruits (B)	Fresh or dried fruits are eaten raw or infusion or decoction is taken orally to treat cough, high blood pressure, bladder and kidney problems and also taken as a laxative	10	5
Phyllanthus urinaria Linn. (Euphorbiaceae) (69696)	Asularlir	WD	N	Fruits and leaves (F)	Fruits or leaves are eaten raw to treat fever, kidney pain, jaundice, dysentery and gastrointestinal problems. They are also taken as a laxative	10	6
Physalis alkekengi L. (Solanaceae) (69647)	Entsupilvu	WD	N	Fruits (F)	Eaten raw or infusion is taken orally for kidney and bladder problems	5	1
Piper betel (Piperaceae) (69697)	Patiwa	CD	N	Leaves (F)	Leaf paste is applied topically to cuts and wounds. Fresh leaves are chewed with lime, areca nut and tobacco to treat dental caries	10	2
Plantago erosa Wall. (Plantaginaceae) (69625)	Sangnem	WD	C	Leaves (F)	Boiled leaves are consumed as a laxative	4	0.4
Polygonum hydropiper Linn. (Polygonaceae) (69641)	Nikmeremlawa	WD	C	Leaves (F)	Leaf paste is applied topically to treat fungal infections and skin infections	4	0.8
Prunus persica (L.) Stokes (Rosaceae) (69503)	Mokori	CD	I	Leaves, roots and seeds (F)	Fresh roots are soaked in water overnight and taken orally to treat typhoid. Also used to treat skin related infections. Seed endosperms are consumed to treat dysentery/diarrhoea and leaf extract is applied topically to treat skin diseases (acne)	10	4
Psidium guajava Linn. (Myrtaceae) (69513)	Monaim	CD	I	Leaves (F)	Leaves are chewed and swallowed to treat constipation and dysentery/diarrhoea	10	2
Punica granatum Linn. (Punicaceae) (69621)	Jarem	CD	N	Fruits (D) and leaves (B)	Leaf decoction is taken twice a day before meals to treat dysentery/diarrhoea. Dried fruit cover is also used for the same purpose	10	1
Rhus javanica var. *roxburghiana* (Anacardiaceae) (69603)	Tangma	WD	N	Fruits (B)	Infusion of the fresh or dried fruits (gall) is taken orally once or twice a day to treat gas formation, stomach ache, mushroom poisoning, dysentery, high blood pressure and allergies (rash)	10	6
Rhus roxburghii Hook. f. (Anacardiaceae) (69673)	Jarak	WD	N	Whole plant (F)	Causes contact dermatitis	10	1
Ricinus communis Linn. (Euphorbiaceae) (69516)	Pakawa	WD, CD	N	Leaves and seeds (F)	A quarter of a roasted seed is chewed and swallowed as a laxative. Caution – high dose causes extreme diarrhoea (not advisable for children). Leaves are used for rearing silk worms	10	2
Saccharum officinarum Linn. (Poaceae) (69622)	Mostutong	CD	N	Culms (F)	Juice is taken orally twice a day to treat jaundice	10	1
Scutellaria glandulosa Colebr. (Lamiaceae) (69542)	Yimramoli	WD	N	Whole plant (B)	Plant infusion is taken orally to treat stomach upset and gas formation. Infusion is also used in bath to treat flu	10	3
Solanum indicum Linn. (Solanaceae) (69627)	Ao longkok	CD	N	Fruits and leaves (F)	Unripe fruits are roasted and consumed once or twice a day as anthelminthic (pinworm). Fresh leaves, rice and water are ground, dried, powdered and used as brewing cake for rice beer	10	2

Table 1 (*continued*)

Scientific name (Botanical family), Herbarium accession number (Assam acc. no)	Local name	Habitat[a]	Endemicity[b]	Part used[c]	Recorded uses, preparation and mode of administration by Chungtia villagers	NI[d]	UV[e]
Solanum myriacanthum (Solanaceae) (69597)	Atsu longkok	WD	C	Seeds (F)	Fumes from the burning seeds are channelled into the affected area to treat tooth ache	10	1
Sonerila maculata Roxb. (Melastomaceae) (69509)	Alichang	WD	N	Leaves (F)	Leaf paste is applied topically to treat insect bites and inflammation	4	0.8
Spermacoce scaberrima Blume (Rubiaceae) (69643)	Ongpangentilawa	WD	N	Leaves (F)	Leaf paste is applied immediately to snake bite	4	0.4
Spermacoce poaya Linn. (Rubiaceae) (69698)	Intifada	WD	N	Leaves (B)	Fresh leaves are chewed and swallowed as a laxative. Dried leaf paste pounded with rice grains is used for brewing rice beer	4	0.8
Spilanthes acmella Linn. (Asteraceae) (69639)	Okensencha	WD	I	Flowers (F)	Flowers are chewed 2–4 times a day to treat tooth ache	6	0.6
Spondias pinnata (Linn. F.) Kurz. (Anacardiaceae) (69519)	Pakho	WD	N	Drupes and leaves (F)	Fresh immature leaves are eaten raw to treat gastrointestinal problems. Ripe drupes are eaten raw or the juice is taken orally as a liver tonic and appetiser	10	2
Stereospermum chelonoides (Linn. f.) DC (Bignoniaceae) (69610)	Sengpet	WD	N	Stem (F)	Stem bark paste is applied to treat cuts, wounds and skin diseases. Stem bark paste is also used as an antiseptic and a decoction of stem bark is taken orally to treat allergies	7	1.8
Stixis suaveolens (Roxb.) Pierre (Capparidaceae) (69525)	Aiemaluv	WD	N	Fruits, roots and seeds (F)	Root infusion is taken orally to treat spleen enlargement. Fruits and seeds are edible	10	1
Tagetes erecta, Linn. (Asteraceae) (69670)	Pesunaro	CD	I	Whole plant (F)	Plant infusion is taken orally to treat intestinal problems, rheumatic pain, boils, skin infection and sinusitis	1	0.4
Terminalia chebula Retz. (Combretaceae) (69640)	Ningkha	WD, CD	N	Drupes (B)	Drupes are eaten as a good source of vitamins and to treat stomach ache	10	2
Thunbergia grandiflora Roxb. (Acanthaceae) (69497)	Koktsuli	WD	N	Stem (F)	Fluid from stem is added to the eye to treat eye infection	3	0.3
Tithonia diversifolia (Hemsl.) A. Gray (Asteraceae) (69687)	Zoninaro	CD	C	Leaves (F)	Infusion is taken orally to treat high blood pressure, malaria and leaf paste is applied topically to treat abscesses and body pain	1	0.3
Urtica dioica L. (Urticaceae) (69601)	Zaklutasula	WD	N	Leaves (F)	Leaf paste is applied topically to treat dog and snake bites	10	1
Viola betonicifolia Boj. Ex. baker (Violaceae) (69651)	Hingpangmoli	WD	N	Leaves (F)	Leaf paste is applied to draw out stings or thorns	4	0.4
Wedelia chinensis (Osb.) Merrill (Asteraceae) (69693)	Enze	WD, CD	N	Leaves (F)	Fresh leaves are eaten raw in the form of a salad to treat gastrointestinal problems	10	1
Zanthoxylum acanthopodium DC. (Rutaceae) (69600)	Changpet	WD, CD	N	Leaves (F)	A mixture of leaves with *Rhus javanica* fruit extract is taken orally to treat stomach ache. Leaves soaked in lukewarm water are used in bath to treat flu. Leaf infusion is sprayed on pest infected plants as pesticide	8	2.4
Zanthoxylum rhetsa (Roxb.) DC. (Rutaceae) (69689)	Ongret	WD	N	Seeds (D)	Seeds are crushed in the stream to poison fish. Leaf infusion is sprayed on pest infected plants as pesticide	10	2

[a] CD – cultivated; WD – wild.
[b] E – Himalayan endemic; N – native; C – cosmopolitan; I – introduced.
[c] F – fresh; D – dry; B – both fresh and dry.
[d] NI – number of informants.
[e] UV – use value.

3.3. Usage categories

Table 2 summarises the uses of the plants and classifies them into 13 categories according to the Chungtia villagers' system of classification. From the 135 plants recorded first-hand, 53 (39.2%) have been applied for one usage category and 82 (60.8%) have been used for more than one category. The most common uses of the plants are for gastrointestinal complaints (45.1%), followed by skin infections, cuts, sores and wounds (27.4%), then musculoskeletal problems (11.8%).

3.4. Comparing traditional usages

The first-hand Chungtia use reports comprised 135 species. Ninety two of these plants have been used for the same or very similar purposes by other tribal traditional healers and 12 species (*Adenia trilobata*, *Albizia lucidor*, *Curanga amara*, *Macropanax undulatus*, *Mentha cordifolia*, *Millettia cinerea*, *Oxalis acetosella*, *Rhus roxburghii*, *Scutellaria glandulosa*, *Sonerila maculata*, *Spermacoce scaberrima* and *Spermacoce poaya*) were found to be exclusively applied by Chungtia community members, not being mentioned in any other customary knowledge study.

Interestingly, *Basella alba*, *Carica papaya* and *Catharanthus roseus*, which are used by the Chungtia community for their laxative effects, were reported by other communities as antidiarrhoeal, and *Prunus persica*, used as an antidiarrhoeal by Chungtia villagers, was reported by others as a laxative. *P. persica* leaves contain phytochemicals responsible for both spasmogenic (causing spasm) and spasmolytic (able to relieve spasm) properties, thus the same plant could be successfully used for the treatment of both ailments (Gilani et al., 2000). However, no literature reports have been found concerning spasmogenic or spasmolytic activities of *B. alba*, *C. papaya* and *C. roseus*.

3.5. Comparing pharmacological and phytochemical studies with Chungtia community traditional applications

Gastrointestinal ailments: Forty four out of 61 plants used for gastrointestinal disorders were reported in pharmacological studies as possessing biological activities, supporting the traditional usage by Chungtia villagers. These biological activities consisted of antibacterial, antidiarrhoeal, antiulcer, gastroprotective, hepatoprotective and anthelmintic properties. Phytochemical studies of 40 plants identified compounds with relevant biological activities, also supporting the Chungtia applications.

Table 2
Number of plants and their usage categories.

Usage category	Ethnobotanical/ethnomedicinal applications	No. plants used
Gastrointestinal ailments	Gastritis, indigestion, mushroom poisoning, food poisoning, laxative, gas formation, diarrhoea, nausea, vomiting, poor appetite, dysentery, intestinal problems, stomach ache, stomach upset, loss of appetite, gastroenteritis, spleen problems, spleen enlargement, hiccups, jaundice, liver problems, liver tonic, vermifuge, anthelminthic, typhoid, constipation, vitamin sources	61
Skin related treatments	Skin diseases, abscesses and boils, cuts and wounds, burns, skin irritation, fungal infection, eczema, contact dermatitis, prickly heat, lip scab (angular cheilitis), scabies, allergic reactions, acne, rash, antiseptic, cleansing agents, contact dermatitis, haemostatic	37
Musculoskeletal problems	Knee pain, body ache, body pain, back pain, body swelling, bone dislocation, sprain, rheumatism, inflammation	16
Flu/cold/fever	Flu, cold, fever, sneezing, cough	13
Hypertension	High blood pressure, blood purification	13
Urinary tract infections and kidney and bladder ailments	Gall bladder, bladder problems, piles, diuretic, urinary tract infections, kidney problems, kidney pain	12
Snake/insect/dog bites	Snake bites, spider bite, dog bite, bee sting	12
Eye, ear, nose problems	Sinusitis, nasal congestion, eye infection, ear pain and infection (otitis media), ear ache	11
Dental ailments	Tooth ache, dental caries	4
Diabetes	Diabetes	3
Malaria	Malaria	3
Cancer	Cancer	1
Others	To ward off evil spirits, tempering machete (hardening metal), food preservative, ripening process, dyes, armpit deodorisation	9
	Fish poisoning	7
	Beer fermentation	4
	pesticide and insecticide, head lice	4

The most common property reported with relevance to gastrointestinal complaints was antibacterial activity against bacteria known to cause diarrhoea and stomach cramps (*Enterobacter aerogenes*, *Shigella sonnei*, *Shigella flexneri*, *Escherichia coli* and *Shigella boydii*) (Murray et al., 2013); dysentery (*Shigella dysenteriae*) and cholera (*Vibrio cholerae*) (Murray et al., 2013); gastroenteritis (*Edwardsiella tarda, Vibrio parahaemolyticus, Vibrio mimicus*) (Murray et al., 2013); food poisoning (*Salmonella choleraesuis, Salmonella typhimurium, Salmonella enteritidis, Bacillus cereus, Bacillus subtilis, Clostridium perfringens, Erwinia* spp) (Murray et al., 2013); enteric fever (*Salmonella typhi, Salmonella paratyphi*); and peptic ulcers (*Helicobacter pylori*). Thirty one of the 40 plants were reported as possessing antibacterial activity against at least one of the above bacterial species. Moreover, pharmacological studies identified antidiarrhoeal properties for *Alstonia scholaris*, *Amaranthus gangeticus*, *Capsicum annum*, *Mikania cordata*, *Mimosa pudica*, *Paederia foetida*, *Psidium guajava* and *Punica granatum*; antiulcer properties for *Centella asiatica*, *Cissampelos pareira*, *Citrus macrocarpa*, *M. pudica*, *Oroxylum indicum*, *P. foetida*, *Spondias pinnata, Terminalia chebula* and *Wedelia chinensis*; hepatoprotective properties for *Bauhinia variegata*, *Luffa acutangula*, *P. foetida* and *P. guajava*; gastroprotective properties for *C. macrocarpa*, *O. indicum* and *Phyllanthus urinaria*; anthelmintic properties for *B. variegata*, *C. papaya*, *Eryngium foetidum*, *O. indicum* and *P. foetida*; and increasing gastrointestinal motility properties for *T. chebula*. No reports concerning any kind of bioactivity or phytochemical studies were found for *S. glandulosa* and *S. poaya*.

Skin related treatments: Plants used to treat skin related disorders were the second largest group. Twenty nine species were reported for possessing biological activities relevant to the Chungtia applications. Biological activities relevant to treating skin related ailments included antimicrobial, anti-inflammatory, antioxidant and collagen production. All 29 plants were reported for antimicrobial properties against at least one of the dermatologically relevant pathogens such as the bacteria *Staphylococcus aureus, Streptococcus pyogenes, Staphylococcus epidermidis, Sarcinia lutea, Staphylococcus haemolyticus* and *Pseudomonas aeruginosa* and the fungi *Candida albicans* and *Aspergillus niger* (Murray et al., 2013). Additionally, *A. scholaris*, *Asclepias curassavica*, *Chrysanthemum indicum*, *Drymaria cordata*, *Euphorbia royleana*, *Gmelia arborea*, *Lagenaria siceraria*, *M. pudica*, *P. persica* and *Targetes erecta* have been reported for their anti-inflammatory activity; *Allium chinense*, *Cassia floribunda*, *Celosia cristata*, *Dendrocnide sinuata*, *Ficus elastica*, *Lasia spinosa*, *Myrica esculenta*, *Polygonum hydropiper* and *P. persica* have been found to be antioxidant; and *Artocarpus heterophyllus*, *Duabanga grandiflora*, and *T. erecta* have been shown to support wound healing by increasing collagen production. The phytochemicals responsible for the reported plants activities have been isolated for 20 species.

Musculoskeletal problems: Out of the 16 species used for musculoskeletal problems by the Chungtia villagers, eight plants were reported for possessing pharmacological activities consistent with their applications; *i.e.* analgesic (*Calotropis gigantea, Datura stramonium, L. siceraria* and *M. pudica*), anti-inflammatory (*Crataeva nurvala, D. stramonium, E. royleana, L. siceraria, M. pudica, O. indicum* and *T. erecta*), anti-rheumatic (*D. stramonium*) and anti-nociceptive (*T. erecta*). The phytochemicals responsible for the bioactivities have been isolated for all eight plants. *M. cinerea, S. maculata* and *A. trilobata* have not been reported for their pharmacological or phytochemical properties.

Flu, colds and fever: For the plants used for flu, colds and fever, *Acorus calamus* and *Nephrolepis cordifolia* were found to be antibacterially active against *S. pyogenes* (causes throat infections) and *Klebsiella pneumoniae* (causes lung and lower respiratory tract infections). *A. calamus*, *Adhatoda vasica*, *Elsholtzia blanda*, *Ocimum basilicum* and *Cajanus cajan* were found to be antiviral and *A. vasica* and *D. cordata* antipyretic. *Bambusa tulda, M. undulatus* and *S. glandulosa* have not been reported for pharmacological or phytochemical studies.

Hypertension: Ten plants were reported for possessing relevant biological activities for use in treatment of hypertension. These included cardioprotective (*A. chinense, Averrhoa carambola, C. annum, C. pareira, Cissus repens* and *Rhus javanica*) and antihypertensive (*Allium sativum, Clerodendron colebrookianum, Passiflora edulis* and *Phyllanthus emblica*). All of these plants have also been shown to have compounds with pharmacological properties aligned with their medicinal uses.

Urinary tract infections and kidney and bladder problems: Out of the 12 plants used for urinary, kidney or bladder problems, only

three were found to be validated by pharmacological and phytochemical research. *Gossipium herbaceum* was found to be diuretic, *Hedyotis scandens* was found to be active against *Proteus vulgaris* (causes urinary tract infection) (Murray et al., 2013) and *P. emblica* was reported to possess kidney-protective properties (Tasanarong et al., 2014).

Bites and stings: Twelve plants have been reported by Chungtia Elders as remedies for animal bites and insect stings. Of these, *S. maculata* and *Spermacoce scabberima* were not reported for pharmacological or phytochemical studies, while *A. chinense*, *A. sativum*, *Dolichos lablab* and *H. scandens* have been found to be antimicrobial against *S. aureus, P. aeruginosa, E. coli* and *Streptococcus epidermidis*, and *Urtica dioica* was found to be antibacterial against *S. aureus, E. coli, P. aeruginosa* as well as anti-inflammatory and anti-nociceptive.

Ear, eyes and nose problems: Eleven plants were reported by Chungtia villagers as treatments for ear, eyes and/or nose problems. Seven of these plants were found to possess relevant pharmacological properties. *E. blanda* has been found to be antibacterial and antiviral; *D. cordata, Eucalyptus globulus, M. cordifolia*, *N. cordifolia, T. erecta* and *Thunbergia grandiflora* have been shown to be antibacterial; and *D. cordata, Mirabilis jalapa* and *T. erecta* are anti-nociceptive.

Three plants (*Gynura crepidioides*, *Debregeasia longifolia* and *C. pareira*) are used by the Chungtia community as a treatment for diabetes. *C. pareira* has been shown to possess antidiabetic activity, but the compounds responsible for this activity have not been isolated.

Of the three plants (*Tithonia diversifolia*, *C. pareira* and *Aquillaria agallocha*) used by the Chungtia community to treat malaria, *C. pareira* and *T. diversifolia* have been reported to be active against the malaria causing parasite *Plasmodium falciparum.* The compounds responsible for this activity have not been isolated.

Only one plant, *Croton caudatus*, was reported to be traditionally applied as a treatment for cancer. The plant was reported as possessing anticancer properties but compounds responsible for these have not been isolated.

No relevant pharmacological or phytochemical research has been reported to validate the usage of the seven plants used by the Chungtia community to poison fish.

Four plants have been reported by Chungtia Elders for the treatment of dental disorders such as tooth ache (*Spilanthes acmella*, *Solanum myriacanthum* and *Costus speciosus*) and dental caries (*Piper betel*). *C. speciosus* and *P. betel* have been reported as possessing analgesic activities.

Four species (*Zanthoxylum rhetsa, Zanthoxylum acanthopodium, Nerium indicum* and *Entada pursaetha*) have been used by Chungtia villagers as pesticides and insecticides. None of the plants have been studied for their biological properties or their chemical composition aligned with their ethnobotanical application.

3.6. Use value

The most commonly used plant species were *C. floribunda*, *D. lablab*, *H. scandens*, *P. urinaria* and *R. javanica*, all with use values (UV) of 6 (48 use reports cited by 8 informants). *C. floribunda* is a plant used for a variety of skin related ailments in the form of a paste. *D. lablab* cooked pods and leaves are used for many diseases including gastrointestinal ailments such as nausea and vomiting as well as skin diseases and poisonous bites. *H. scandens* leaf paste is most used for the treatment of cuts and wounds. A decoction of leaves of this plant is used to treat urinary and gastrointestinal problems. Roots of the plant are applied as a remedy for bee sting. *P. urinaria* fruits and leaves and *R. javanica* fruits are used as a treatment for various ailments including gastro, urinary and skin related complaints.

3.7. Informant consensus factor

The reported usages were grouped into 12 categories based on the ethnobotanical application. The F_{ic} values ranged from 0.80 to 0.91, which generally indicated the agreement in the use of these plants in the usage categories between the plants users (Morvin et al., 2014). The highest F_{ic} values were reported for dental disorders (36 use reports, 4 species) and for beer fermentation (34 use reports, 4 species), followed by hypertension (98 use reports, 13 species) and malaria (17 use reports, 3 species). The F_{ic} values for all use categories are presented in Table 3.

3.8. Fidelity level

The fidelity level of each of the plant species was calculated for each usage category (Table 4). The highest fidelity levels (100%) were observed for the plants used within single categories with many use reports (Morvin et al., 2014). Less than three use reports were not considered for this study (Table 4).

Table 3
Informant consensus factor for plants used by Chungtia villagers.

Usage category		No. plants used	No of use reports	F_{ic}
Gastrointestinal ailments		61	422	0.85
Skin related treatments		37	237	0.84
Musculoskeletal problems		16	78	0.80
Flu/cold/fever		13	66	0.81
Hypertension		13	98	0.87
Urinary tract infections and kidney and bladder ailments		12	69	0.83
Snake/insect/dog bites		12	66	0.83
Eye, ear, nose problems		11	49	0.79
Dental disorders		4	36	0.91
Diabetes		3	13	0.83
Malaria		3	17	0.87
Cancer		1	1	N/A
Others	To ward off evil spirits, tempering machete, food preservative, ripening process, dyes, armpit deodorisation	9	60	0.86
	Fish poisoning	7	44	0.86
	Beer fermentation	4	34	0.90
	Pesticides/insecticides	4	22	0.85

The largest number of plants with fidelity level value 100% was observed for the species used for skin related treatments, where 29.7% (11 species out of 37) of the reported plants were used exclusively as a treatment within this category. This was followed by plants used for gastrointestinal disorders with 26% (16 plants out of 61 used exclusively as gastrointestinal disorder treatments).

3.9. Toxicity

Chungtia villagers indicated five plants (*C. colebrookianum*, *Euphorbia pulcherrima*, *Gonatanthus pumilus*, *R. roxburghii* and *Ricinus communis*) that cause toxic or adverse effects such as body swelling, vomiting, contact dermatitis, animal and even human death. A literature search of the 135 plants used by the villagers highlighted a total of 14 species that have adverse effects. This included *E. pulcherrima* and *R. communis*. No toxicity reports were found for *C. colebrookianum*, *G. pumilus* or *R. roxburghii*. Flowers and leaves of *E. pulcherrima* have been reported by the villagers as poisonous when fed to pigs. These plant parts have also been shown to be toxic to ruminants and to contain diterpene esters that cause skin, mucous membranes and gastrointestinal tract irritation (Cortinovis and Caloni 2013). *R. communis* seeds have been used by the villagers as a laxative and they noted caution on its 'extreme diarrhoea effect'. The seeds have also been described in the literature (Aslani et al., 2007) as a source of beneficial medicinal products. This plant, however, is known to contain ricin (Aslani et al., 2007), a highly toxic and potentially fatal compound upon ingestion. Nine of the plants reported in the literature that had not been identified as toxic by the villagers concerned different plant parts. It might be that the plant parts used by the villagers have no toxic effects, but caution with their usage is also warranted. Toxicity reported by the Chungtia villagers and that in the literature is summarised in Table 5.

4. Conclusions

A review was conducted on 135 plant species, comprising 69 families and 123 genera, for their ethnobotanical and ethnomedicinal uses by Chungtia villagers and communities worldwide. Ninety three

Table 4
Fidelity level values for plants by usage category.

Usage category	Plant name	Use	FL (%)
Gastrointestinal ailments	*Allium hookeri*	Vermifuge	100
	Alstonia scholaris	Gastritis, jaundice, liver tonic	100
	Artemisia vulgaris	Dysentery	100
	Catharanthus roseus	Gastroenteritis, laxative	
	Citrus microcarpa	Stomach ache, gas formation	100
	Cucurbita pepo	Vitamin source	100
	Garcinia cowa	Dysentery, diarrhoea	100
	Garcinia pendunculata	Dysentery, diarrhoea	100
	Houttuynia cordata	Dysentery, diarrhoea, gas formation, vermifuge	100
	Luffa acutangula	Laxative, to aid digestion	100
	Paederia foetida	Intestinal problems	100
	Psidium gujava	Constipation, dysentery, diarrhoea	100
	Punica granatum	Dysentery, diarrhoea	100
	Saccharum officinarum	Jaundice	100
	Spondias pinnata	Gastrointestinal problems	100
	Wedelia chinensis	Gastrointestinal problems	100
Skin related treatments	*Albizia lucidor*	Abscesses and boils	100
	Artocarpus heterophyllus	Skin disease	100
	Asclepias curassavica	Cuts and wounds	100
	Cassia floribunda	Fungal infections, eczema, contact dermatitis, allergic reaction, prickly heat, burns	100
	Dendrocnide sinuata	Cuts and wounds	100
	Erythrina stricta	Dermatitis, eczema, skin infections	
	Eupatorium odoratum	Cuts and wounds	100
	Gmelia arborea	Skin diseases	100
	Holboellia latifolia	Burns	100
	Kalanchoe pinnata	Ringworm, skin diseases, burns	
	Myrica esculenta	Cuts and wounds	100
Musculoskeletal problems	*Datura stramonium*	Back pain	100
Flu/cold/fever	*Macropanax undulatus*	Cold and fever	100
Hypertension	*Clerodendron colebrookianum*	High blood pressure	100
	Passiflora edulis	High blood pressure	100
Urinary tract infections and kidney and bladder ailments	*Equisetum ramosissimum*	Kidney problems	100
	Physalis alkekengi	Kidney and bladder problems	100
Snake/insect/dog bites	*Girardinia palmata*	Dog bite	100
	Gynocardia odorata	Bee sting	
	Spermacoce scaberrima	Snake bite	100
	Urtica dioica	Dog and snake bites	100
Eye, ear, nose problems	*Thunbergia grandiflora*	Eye infection	100
Fish poisoning	*Diospyros lanceifolia*	To poison fish	100
Beer fermentation	*Mussaenda roxburghii*	Brew rice beer	50
Dental disorders	*Spilanthes acmella*	Tooth ache	100
Pesticides/insecticides	*Nerium indicum*	Kill lice and insects	100
Diabetes	*Gynura crepidioides*	Diabetes	100
Malaria	*Aquillaria agallocha*	Malaria	50
Cancer	NA		NA

Table 5
Details of the plants surveyed having toxic/adverse effects.

Scientific name	Plant part	Toxic/adverse effects reported by Chungtia community	Toxic/adverse effects reported in literature			
			Toxic effect	Toxic part	Toxic compound/ extract	Citation
Acorus calamus	Leaves	No reports	Carcinogenic, toxic	Rhizomes	β-Asarone	(Rajput et al., 2014)
Adhatoda vasica	Leaves	No reports	Abortifacient/ cytotoxic	Leaves	Vasicine	(Roy et al., 2013)
Calotropis gigantea	Leaves	No reports	Inhibit spermatogenesis/ abortifacient	Roots/ latex	Calotropin	(Gupta et al., 1990; Fahim Kadir et al., 2014; Srivastava et al., 2007)
Carica papaya	Sap and fruits	No reports	Abortifacient/ azoospermia	Seeds	Chloroform extract	(Lohiya et al., 2002)
			Anti-fertility/decline in sperm motility	Bark	Ethanolic extract	(Vij and Prashar, 2014)
Catharanthus roseus	Leaves	No reports	Hypotension, neurotoxicity, anaemia, seizure	Roots, shoots	Vincristine, vinblastine	(Fahim Kadir et al., 2014)
Clerodendron colebrookianum	Leaves	Ingestion of drupe may induce body swelling and vomiting	No reports	No reports	No reports	No reports
No reports						
Costus speciosus	Fruits and leaves	No reports	Anti-fertility/ estrogenic activity	Rhizomes	Diosgenin	(Patel et al., 2012; Pawar and Pawar, 2014)
Crataeva nurvala	Leaves	No reports	Anti-fertility/ estrogenic activity	Stem bark	Ethanolic extract	(Bhaskar et al., 2009)
Datura stramonium	Leaves	No reports	Hallucinogenic/ anticholinergic toxicity	Seeds	Atropine, hyoscyamine, scopolamine	(Roblot et al., 1994; Boumba et al., 2004)
Euphorbia pulcherrima	Flowers and leaves	Death may result when fed accidently to pigs	Poisonous for animals	Aerial parts (eaten fresh)	Diterpene esters	(Cortinovis and Caloni, 2013)
Gonatanthus pumilus	Leaves and stem	Extremely poisonous and death may result if ingested by humans. Rhizome also causes extreme itching on contact	No reports	No reports	No reports	No reports
Gossypium herbaceum	Roots	No reports	Anti-fertility/ azoospermia or oligospermia	Seeds	Gossypol	(Khaleequr et al., 2012)
Manihot esculenta	Tubers	No reports	Neurotoxic	Tubers	Linamarin and lotaustralin	(Rivadeneyra-Domínguez et al., 2013)
Mimosa pudica	Leaves	No reports	Anti-fertility/ gonadotropin and estradiol secretion	Roots	Aqueous roots extract	(Joseph et al., 2013)
Mirabilis jalapa	Leaves and roots	No reports	Abortifacient	Roots	Antiviral protein MAP	(Lim, 2014)
Rhus roxburghii	Whole plant	Causes contact dermatitis	No reports	No reports	No reports	No reports
Ricinus communis	Seeds	High doses causes extreme diarrhoea	Bloody diarrhoea, toxic	Seeds	Ricin	(Ferraz et al., 1999; Fahim Kadir et al., 2014)

different applications by the Chungtia villagers were identified, which were classified into 13 categories. For 92 plants, usages by the Chungtia community were found to be in agreement with other community reports. Twelve plants, namely *A. trilobata*, *A. lucidor*, *C. amara*, *M. undulatus*, *M. cordifolia*, *M. cinerea*, *O. acetosella*, *R. roxburghii*, *S. glandulosa*, *S. maculata*, *S. scaberrima and S. poaya* had no ethnomedicinal or ethnobotanical uses reported by any community other than the first-hand reports by the Chungtia villagers, and only three of these (*C. amara*, *M. cordifolia* and *O. acetosella*) have been investigated for their biological properties. Out of the 92 species for which customary usages were found to be similar with other communities' reports, 80 were validated by pharmacological research and 55 have been studied for the presence of phytoconstituents aligned with their bioactivities.

There was strong agreement among the informants in the usages of the plants (informant consensus factor 0.80–0.91). The use value of 6 for *C. floribunda*, *D. lablab*, *H. scandens*, *P. urinaria* and *R. javanica* indicated these are the most important species. Forty four of the 135 plants had a fidelity level of 100%.

Traditional healers and Elders of Chungtia village possess significant knowledge of medicinal plants and their application for the treatments of many ailments. Studies have shown that many customarily used medicinal plants of the villagers possess important therapeutic value. These findings validate the medicinal usage by Chungtia villagers and highlight the significance of the traditional knowledge of the Chungtia Elders. Further research is warranted on the plant species that are medicinally utilised by Chungtia villagers.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2015.02.053.

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Appendix 1 provides the published supplementary data associated with this article. The supplementary data includes a literature review of the 135 medicinal plants with regard to worldwide ethnobotanical use, biological and phytochemical studies that captures information from the public domain up to September 2014.

2.3.3 Results from literature review of Nagaland medicinal plants

As described in the Introduction (Chapter 1), this PhD study was focused on the identification of antibacterial and antioxidant treatments for skin related conditions including skin infections, sores and wounds. The ethnobotanical documentation, as detailed in the publication, identified thirty seven Chungtia medicinal plants that have been used for the treatment of such skin related conditions. Table 2.1 presents the literature review conducted up to September 2014 of these thirty seven plants extracted from the Journal of Ethnopharmacology publication (supplementary data; Appendix 1 of the thesis), along with the results of a further literature survey up to March 2016 (shown in bold) with reference to agreement of literature with traditional uses by the Chungtia villagers, biological activity relevant to the traditional uses and any phytochemical studies.

Table 2.1: Summary of information gathered through literature review on Chungtia village medicinal plants[5]

Bolded references are from literature review subsequent to date of review within Journal of Ethnopharmacology publication				
Scientific name (Botanical family, Herbarium accession number, Assam Acc. No)	**First hand recorded uses for skin related treatment**	**Agreement of literature with traditional use(s) of Chungtia villagers**	**Literature with reference to biological activity**	**Literature with reference to phytochemical studies**
Albizia lucidior (Skud) Hara (Mimosaceae) (69595)	Root infusion is applied topically to abscesses & boils	None found	None found	None found
Allium chinense G. Don (Liliaceae) (69679)	Bulb paste is applied topically for skin diseases	Bulbs: lower cholesterol levels, improve circulatory system, treat heart asthma & skin diseases[11]	WR Bulbs: cytotoxic, antioxidant, antimicrobial, cardioprotective[11, 12] **Bulbs: insecticidal, antitumour, cardioprotective, cytotoxic, antimicrobial[13, 14]**	WR Bulbs: phytochemical constituents[11] **Bulbs: GC-MS analysis of insecticidal essential oils[14]**
Artocarpus heterophyllus Lamk. (Moraceae) (69596)	Sap is applied topically to treat skin diseases	WR Leaves: treat wounds Latex: boils[15, 16] **Leaves: treats wounds & skin diseases to improve healing process[16, 17]**	WR Fruits: cytotoxic Seeds: antibacterial Leaves: wound healing[18-20] **Sap: antimicrobial** **Leaves: antioxidant, antimicrobial** **Wood: antibacterial, tyrosinase inhibitory activity [21–25]**	WR Fruit: phytochemical composition Seeds: chemical composition[18, 26] **Roots: new anti-respiratory burst active flavanones** **Wood: antibacterial compounds cycloartocarpin, cyanomaclurin & flavonoids[23, 25, 26]**
Asclepias curassavica Linn. (Asclepiadaceae) (69617)	Leaf paste is applied topically for cuts & wounds	Leaves: treat warts, wounds & healing process[27]	WR Aerial parts: anti-inflammatory Whole plant: antibacterial; antiviral Latex: antifungal[27] **Aerial parts: anticancer[28]**	WR Seeds: cardenolide glycosides Leaf: calotropagenin, flavonoid glycosides[27] **Whole plant: cytotoxic cardenolide lactates & cardenolide glycosides[29]**
Begonia picta Smith (Begoniaceae) (69642)	Leaves are used to cleanse hands by crushing between palms	Whole plant: treats cuts & wounds[30] **Whole plant: stops bleeding from cuts & wounds** **Leaves: as poultice to heal sore nipples[31, 32]**	Whole plant: headaches Roots: conjunctivitis[30, 33] **Whole plant: antioxidant[32]**	**Whole Plant: antioxidant flavone glucoside[32]**

Table 2.1 (Continued)

Scientific name (Botanical family, Herbarium accession number, Assam Acc. No)	**First hand recorded uses for skin related treatment**	**Agreement of literature with traditional use(s) of Chungtia villagers**	**Literature with reference to biological activity**	**Literature with reference to phytochemical studies**
Cassia floribunda Cav. (Caesalpiniaceae) (69535)	Warmed leaves are made into a paste & applied externally for fungal infections, eczema, contact dermatitis, allergic reactions, prickly heat & burns	None found	WR Seeds: antioxidant, antidiabetic, antibacterial[34, 35] **Root bark: antiplasmodial[36]**	Seeds: phytochemical & antioxidant activity analysis[34]
Celosia cristata L. (Amaranthaceae) (69520)	Leaf paste is applied topically for cuts & wounds	Whole plant: treat urinary tract infections Leaves: treat sores & wounds[37]	WR Leaves: antiviral, antioxidant, anthelmintic Flowers: antimicrobial[37, 38] **Seeds: immunomodulatory** **Aerial parts: antioxidant, antibacterial** **Leaves: tyrosinase, acetylcholinesterase & butylcholinesterase inhibition** **Flowers: antifungal[39–41]**	WR Leaves: celosianins, kaempferol, quercetin, 4-hydroxy phenethyl alcohol, cochliophilin A[37] **Seeds: hepatoprotective saponin; immunoprotective polysaccharides[41, 42]**
Chrysanthemum indicum L. (Asteraceae) (69529)	Leaf paste is applied topically to treat lip scab & scabies	Flowers: treat boils, itchiness of skin[43] **Flowers: treat infectious diseases[44]**	WR Leaves: antiplasmodial, hepatoprotective, antifungal Flowers: analgesic, antibacterial, anti-inflammatory[43, 45] **Flowers: neuroprotective, antioxidant** **Whole plant: anti-inflammatory [46–48]**	WR Flowers: flavones, flavanone glycosides, terpenoids, polyacetylenes[43] **Flowers: 3,5-diarylpyrazole analogues; GC-MS analysis of essential oils & antioxidant activity analysis[47, 48]**
Cyclea peltata Diels.(Menispermaceae) (69650)	Leaf decoction is applied topically for abscesses & boils	Roots: treat skin disorders[49] **Leaves: treat boils[50]**	WR Whole plant: antibacterial Roots: hepatoprotective[50-52] **Roots: antidiabetic** **Whole plant: antitumour, antidermatophytic** **Leaves: jaundice, stomach pain, asthma & snake poisoning[53–56]**	WR Whole plant: phytochemical & cytotoxic activity analysis[52]
Dendrocnide sinuata (Bl.) (Urticaceae) (69508)	Outer fresh stem is scraped off & the mucilage secreted is applied on fresh cuts & wounds	Roots: treat injury & itchy skin[57]	Leaves: antimicrobial, antioxidant[58] **Roots: analgesic, anti-inflammatory** **Leaves: antioxidant[59, 60]**	None found
Drymaria cordata (Linn.) Willd. (Caryophyllaceae) (69530)	Toasted plants are crushed into a paste & applied topically to treat fungal infection (ringworm), contact dermatitis & lip scabs	Leaves: treat sinusitis[61] **Leaves: treat common cold, cuts & wounds[62]**	Leaves: fever[63] **Leaves: cytotoxic** **Whole plant: antidiabetic** **Aerial parts: antioxidant[64–66]**	WR **Aerial parts: glycosylflavone[64]**

Table 2.1 (Continued)

Scientific name (Botanical family, Herbarium accession number, Assam Acc. No)	**First hand recorded uses for skin related treatment**	**Agreement of literature with traditional use(s) of Chungtia villagers**	**Literature with reference to biological activity**	**Literature with reference to phytochemical studies**
Duabanga grandiflora (Roxb. ex DC.) Walp. (Sonneratiaceae) (69686)	Fresh bark is scraped off & applied topically to skin diseases, cuts & wounds	Bark: treat skin diseases & eczema[7] **Bark: treat skin diseases & eczema[67]**	Leaves: decreasing skin damage, skin whitening, antibacterial[68, 69] **Leaves: antibacterial** **Leaves: skin whitening** **Seeds: anti-inflammation, antiaging[70–72]**	WR Stem: pyranone[73] **Stem Bark: cytotoxic triterpenes** **Leaves: antibacterial fraction[71, 74]**
Elsholtzia blanda Benth. (Lamiaceae) (69524)	Leaf paste is applied to fresh cuts	Aerial parts: treat fever, cholera, skin diseases & inflammation Leaves: used to treat inflamed glands[75] **Aerial parts: treat skin diseases & inflammation[76]**	WR Leaves, roots: antibacterial, antiviral, antioxidant [77–79] **Leaves: antibacterial[71]**	WR Aerial parts: chemical constituents[80, 81] **Aerial parts: GC-MS analysis of essential oils[76]**
Erythrina stricta Roxb. (Fabaceae) (69629)	Bark paste is applied topically to treat contact dermatitis, eczema & skin infections	None found **Bark: treat skin diseases[82]**	WR Stem: spasmolytic, diuretic, anticonvulsant, analgesic, antiviral, antifungal Bark: antibacterial[83, 84] **Leaves: anti-inflammatory, anticataract & cardioprotective** **Bark: antibacterial[82, 85-87]**	Roots: pterocarpan, flavanone, isoflavone, alkaloids, triterpenes[88] **Leaves: phytochemical studies & hypouriacemic activity analysis** **Bark: alpinum isoflavone [89, 90]**
Eupatorium odoratum Linn. (Asteraceae) (69523)	Leaf paste is applied topically to fresh cuts & wounds	WR Leaves: treat cuts & wounds[91] **Leaves: heal cuts & wounds** **Aerial parts: treat burns & wounds[92–95]**	WR Leaves: antifungal, antibacterial[82, 96] **Flowers: antibacterial, larvicidal, insecticidal** **Leaves: antibacterial, insecticidal, antimicrobial[97–101]**	Leaves: phytochemical screening[82, 96] **Leaves: antioxidant & antiadipogenesis flavanone & chalcones** **Flowers: phytochemical studies[99, 102]**
Euphorbia royleana Boiss. (Euphorbiaceae) (69684)	Milky sap is applied topically to treat skin diseases & body pain	Latex: treat skin diseases[103] **Latex: used as antiseptic & germicidal** **Stem: treats joint pain[104, 105]**	WR Latex: antibacterial, anti-inflammatory[82, 106] **Whole plant: antibacterial, antioxidant & antitumor** **Latex: piscicidal & muricidal[107, 108]**	Latex: chemical constituents[107, 109] **Whole plant: phytochemical studies[108]**
Eurya acuminata DC. (Theaceae) (69612)	Leaf paste is applied topically to cuts & wounds	Leaves: treat stomach disorders, dysentery, diarrhoea, cholera & skin diseases[110, 111]	Leaves, stem: antibacterial[112] **Leaves: antioxidant[113]**	None found

Table 2.1 (Continued)

Scientific name (Botanical family, Herbarium accession number, Assam Acc. No)	**First hand recorded uses for skin related treatment**	**Agreement of literature with traditional use(s) of Chungtia villagers**	**Literature with reference to biological activity**	**Literature with reference to phytochemical studies**
Ficus elastica Roxb. ex Hornem. (Moraceae) (69619)	Root juice or sap is applied topically for cuts & wounds	Leaves: treat skin infections & skin allergies[114] **Aerial rootlets, bark: heals wounds, cuts & sores[115, 116]**	WR Leaves: antioxidant, anticancer, antibacterial[117–120]	Leaves: chemical constituents[117] **Root bark: antibacterial amino alcohol derivative** **Leaves: GC-MS analysis of essential oils[121, 122]**
Gmelina arborea Roxb. (Verbenaceae) (69518)	Mesocarp of the drupe is applied topically to treat skin diseases	Roots: treat skin problems[123] **Whole plant: cure snake & scorpion bite, skin diseases** **Fruits: heal serious wounds[124-126]**	WR Stem bark: antibacterial Leaves: anti-inflammatory, antinociceptive[127–129] **Roots: antidiarrheal, anthelmintic, antidiabetic** **Leaves: antioxidant & cytotoxic** **Bark: antioxidant, anticonvulsant, antidiabetic[124, 130–132]**	WR Bark: phytochemical screening Fruits: physicochemical studies[128, 133, 134] **Leaves: antioxidant flavone, flavone glucoside, quercetin derivatives** **Roots: phytochemical studies[135, 136]**
Hedyotis scandens Roxb. (Rubiaceae) (69539)	Leaf paste is applied topically to cuts & wounds	Leaves: treat itches, scabies, eczema Roots: treat gastric problems[137] **Leaves: promote tissue generation in wounds** **Whole plant: treat fungal infection[138, 139]**	WR Whole plant: antibacterial[137]	WR Whole plants: total flavonoids[140] **Whole plant: antiviral phenolic glycosides isolated[141]**
Holboellia latifolia Wall. (Lardizabalaceae) (69500)	Foam from crushed leaves is applied topically to burns	Leaves: cure burns[49] Fruits: treat skin diseases[142]	None found	None found
Ipomoea nil (Linn.) Roth. *Pharbitis nil* (Convolvulaceae) (69678)	Leaf paste is applied topically to burns	None found **Seeds: treat ringworm & skin diseases[143]**	WR Seeds: antimicrobial & antitumour[144]	WR Seeds: phytochemical screening[144] **Stems: phytochemical analysis** **Seeds: phytochemical analysis[145, 146]**
Kalanchoe pinnata (Lam.) Pers. (Crassulaceae) (69515)	Warmed leaf paste is applied topically to burns	Leaves: cure skin diseases, eczema, pruritis[50] **Leaves: treat skin injuries & sprains, eczema, infections, burns & carbuncle[147, 148]**	WR Roots: antibacterial Leaves: antibacterial, diuretic & hepatoprotective[149, 150] **Leaves: antimicrobial, antioxidant, cytotoxic haemolytic** **Stems & roots: antinociceptive, antipyretic[151–153]**	WR Leaves, stems & flowers: flavonoid glycosides[154] Leaves: GC-MS analysis of bioactive ompounds[155]

Table 2.1 (continued)

Scientific name (Botanical family, Herbarium accession number, Assam Acc. No)	**First hand recorded uses for skin related treatment**	**Agreement of literature with traditional use(s) of Chungtia villagers**	**Literature with reference to biological activity**	**Literature with reference to phytochemical studies**
Lagenaria siceraria (Molina) Standl. (Cucurbitaceae)	Juice extract is applied topically to treat skin diseases & inflammation	WR Roots: treat wounds Fruits: skin disorders[156, 157] **Leaves: cure boils** **Fruits: treat skin diseases[158–160]**	WR Fruits: cardioprotective, analgesic & anti-inflammatory Seeds: anticancer Leaves: antimicrobial[161, 162] **Fruits: antioxidant** **Seeds: antioxidant, antibacterial[163, 164]**	WR Fruits: steroids, flavone C-glycosides, triterpenoids, flavonoids[162, 165] **Fruits: phytochemical & antioxidant activity analysis** **Seed oil: phytochemical & antioxidant activity analysis[163, 164]**
Lasia spinosa (L.) Thwaites (Araceae) (69655)	Leaf paste is applied topically to treat skin diseases	Leaves: treat cuts & injuries[2] **Rhizomes: treat skin diseases** **Leaves: treat itches[166, 167]**	WR Whole plant: antioxidant Leaves: antioxidant, cytotoxic & antimicrobial[168, 169] **Stem: antidiabetic** **Leaves: antinociceptive & antihyperlipidemic[170–172]**	WR Whole plant: phytochemical analysis[173]
Maesa indica (Roxb.) Wall. (Myrsinaceae) (69514)	Leaf paste is applied topically to cuts & wounds	None found	Whole plant: antiviral[174] **Bark: antidiabetic** **Leaves: larvicidal, anticaries & gastroprotective[175-178]**	Fruits: new quinone[179]
Melastoma malabathricum (Melastomataceae) (69652)	Leaf paste is applied to cuts & wounds	WR Leaves: treat cuts & wounds[180] **Leaves: treat boils** **Leaves: treat cuts & wounds to stop bleeding** **Roots: wounds[181–183]**	WR Leaves, stems, flowers: antibacterial[180, 184] **Leaves: antidiabetic & antihyperlipidaemic, anticancer, antibacterial, antifungal, antioxidant, anticoagulant & antiulcer[185–189]**	WR Leaves, stems, roots: flavonoids, alkaloids, steroids Flowers: naringenin, kaempferol, kaempferol glucosides[180, 190] **Leaves: cinnamic acid & *p*-hydroxycinnamic acid[188]**
Mentha cordifolia Opiz ex Fresen. (Lamiaceae) (69683)	Leaf paste is applied topically to fresh cuts & skin diseases	None found	Leaves: antimicrobial[191, 192] **Leaves: antityrosinase, antifungal & inhibit development of hypertension[193-195]**	Leaves: flavonoids, sitosterol, lutein[191] **Leaves: analgesic menthalactone[196, 197]**
Mikania cordata (Burm. F.) BL Rob. (Asteraceae) (69534)	Leaf paste is applied topically to treat skin diseases & cuts	WR Leaves: treat cuts, wounds [198] **Leaves: treat cuts, wounds[199, 200]**	WR Leaves: antibacterial, antidiarrhoeal[201-203] **Leaves: antidiarrhoeal[204]**	Leaves: phytochemical properties[201] **Leaves: phytochemical cytotoxicity & analysis[205]**
Mussaenda roxburghii Hk. f. (Rubiaceae) (69502)	Fresh leaf paste is applied topically to cuts & wounds	WR Leaves: cure boils & to brew rice beer[206, 207] **Leaves: treat burning sensation in hands or legs[208]**	WR Leaves: antimicrobial, cytotoxic[209, 210] **Leaves: anti-arthritic, anti-inflammatory, anticancer & thrombolytic[211]**	Aerial parts: antibacterial iridoid[212]

Table 2.1 (Continued)

Scientific name (Botanical family, Herbarium accession number, Assam Acc. No)	**First hand recorded uses for skin related treatment**	**Agreement of literature with traditional use(s) of Chungtia villagers**	**Literature with reference to biological activity**	**Literature with reference to phytochemical studies**
Myrica esculenta Buch.-Ham., ex D. Don (Myricaceae) (69522)	Leaf paste is applied to cuts & wounds	Sap: treats cuts & wounds[213] **Bark: treats sores, itching, skin eruptions[57, 214]**	Fruits: antioxidant & antipyretic Stem bark: antimicrobial[215, 216]	Bark: essential oils Fruits: phytochemical analysis[215, 216] **Leaves: phytochemical analysis[217]**
Nephrolepis cordifolia (Davalliaceae) (69623)	Tubers are rubbed on skin for skin diseases	WR Bulbs, tubers: treats upset stomach & urinary problems Leaves: treat wounds[218, 219] **Fronds: treat wounds[220]**	WR Fronds: antibacterial[221] **Aerial parts: cytotoxic & antimicrobial[222]**	WR Whole plant: phytochemical analysis[223, 224] **Aerial parts: GC-MS analysis of antimicrobial essential oils[222]**
Piper betel (Piperaceae) (69697)	Leaf paste is applied topically to cuts & wounds	WR **Leaves: treat cuts, wounds[225]** **Leaves: treat cuts, stomach-ache, centipede bite & skin diseases[199, 226, 227]**	WR Leaves: analgesic, antimicrobial, anthelmintic[228, 229] **Leaves: anticancer, antitumour, antibacterial, antidiabetic & antimicrobial[230–234]**	WR Leaves: phytochemical screening, chemical composition[228, 235-237] **Leaves: antimicrobial & anti-inflammatory allylpyrocatechol[238]**
Polygonum hydropiper Linn. (Polygonaceae) (69641)	Leaf paste is applied topically to treat fungal infections & scabies	WR Leaves, roots: cure eczema & scabies[239] **Whole plant: treat eczema[240]**	WR Leaves: antibacterial Whole plant: antioxidant, anticholinesterase, anti-inflammatory[241-243] **Whole plant: anti-inflammatory** **Leaves: cytotoxic, antinociceptive, antihyperglycemic[243, 244]**	WR Leaves: confertifolin, saponins[241, 242]
Prunus persica (L.) Stokes (Rosaceae) (69503)	Leaf juice extract is applied topically to treat skin diseases	Leaves: killing maggots, treat skin diseases, ear infections, cough & bronchitis[61] **Seeds: treat skin infection** **Leaves: treat boils & botfly[245, 246]**	WR Leaves, fruits: cardioprotective, spasmolytic, anti-inflammatory & antioxidant[247, 248] **Flowers: prokinetic & anti-inflammatory** **Fruits: antioxidant[249–251]**	WR Fruits: phytochemical & antioxidant activity analysis, quantification of anthocyanin & proanthocyanidins[248, 252] **Fruits: phytochemical analysis[251]**
Stereospermum chelonoides (Linn. f.) DC (Bignoniaceae) (69610)	Bark paste is applied to treat cuts & wounds & skin diseases & used as antiseptic	WR Flowers: treat boils caused by diabetes[253] **Leaves: treat scabies[254]**	WR Stem bark: antimicrobial[253, 255] **Aerial parts: hyperlipidemic** **Roots: anti-inflammatory[256, 257]**	WR Bark: stereochenols, quinones Roots: phytochemical & HPTLC analysis[253, 258, 259] **Roots: lignans isolated[260]**
Tagetes erecta, Linn. (Asteraceae) (69670)	Plant infusion is applied topically to treat boils & skin infections	WR Flowers: treat sores, burns, wounds, ulcers[261] **Leaves: treat cuts & skin allergy[262, 263]**	WR Leaves: antifungal, wound healing Flowers: antinociceptive & anti-inflammatory[264–266] **Flowers: antioxidant & larvicidal** **Stems: antioxidant** **Leaves: antibacterial** **Aerial parts: antibacterial[267-271]**	WR Leaves: essential oil composition Roots: bithienyl compound Flowers: flavonoids[261, 272-274] **Leaves & flowers: GC-MS analysis of bioactive methanol extracts[275]**

2.3.4 Literature review of plants used for skin diseases, cuts and wounds

The literature review of the thirty seven medicinal plants that have been used for treatment of skin related ailments by the Chungtia villagers identified that thirty one out of the thirty seven plants have been reported to be used for similar conditions by other Indigenous communities globally and that thirty five of the plants have been reported to have either antimicrobial, antioxidant or anti-inflammatory acitivities. The review revealed that *Albizia lucidor* and *Holboellia latifolia* have no reports for either any biological or phytochemical studies. Though *Dendrocnide sinuata and Eurya acuminata* have a few reports on biological studies, they have had no phytochemical studies reported. *Begonia picta*, *Cassia floribunda*, *Euphorbia royleana and Mussaenda roxburghii* are widely reported for biological studies but phytochemical studies on these plants are very limited. On the other hand *Erythrina stricta, Myrica esculenta and Prunus persica* have limited reports on biological and phytochemical studies on the plant parts medicinally used by the Chungtia villagers. Thus, the literature review of the ethnobotanically documented medicinal plants led to the identification of eleven plants worthy of further chemical and biological investigations, *i.e. Albizia lucidor, Begonia picta*, *Cassia floribunda*, *Dendrocnide sinuata*, *Erythrina stricta*, *Euphorbia royleana*, *Eurya acuminata*, *Holboellia latifolia*, *Myrica esculenta*, *Mussaenda roxburghii* and *Prunus persica*.

2.4 Conclusion

A thorough literature review was conducted by the author on 135 medicinal plants that had been previously documented through first hand ethnobotanical interviews of Chungtia village Elders. This identified thirty seven plants used by the Chungtia villagers for skin related ailments, including sores, cuts, wounds and skin infections. Of these, *Albizia lucidor* and *Holboellia latifolia* have had no reports on their biological and phytochemical studies, while *Begonia picta*, *Cassia floribunda*, *Dendrocnide sinuata*, *Erythrina stricta*, *Euphorbia royleana, Eurya acuminata*, *Myrica esculenta*, *Mussaenda roxburghii* and *Prunus persica* have limited reports on antimicrobial or other biological studies aligned with their traditional uses as well as limited studies on their phytochemicals. These plants are therefore considered as valuable for further biological and chemical investigations aligned with their traditional uses. Detailed biological and chemical studies on these plants may lead to the identification of new antimicrobial and antioxidant agents.

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CHAPTER THREE

Chemical and Biological Studies of the Nagaland Medicinal Plant *Erythrina stricta* Roxb. (Fabaceae)

This chapter provides a detailed description of the chemical and biological studies undertaken on the Chungtia medicinal plant Erythrina stricta *Roxb. (Fabaceae). This includes antimicrobial and antioxidant screening of crude extracts and isolation of antibacterial and antioxidant compounds.*

3.1 Introduction

As described in Chapter Two, thirty-seven plants used for the treatment of skin related diseases were documented through first hand interviews with Chungtia villagers. An extensive literature review on these plants identified that *Albizia lucidor*, *Begonia picta*, *Cassia floribunda*, *Dendrocnide sinuata, Erythrina stricta*, *Euphorbia royleana, Holboellia latifolia*, *Mussaenda roxburghii, Myrica esculenta*, *Eurya acuminata* and *Prunus persica* have none or limited reports of phytochemical or biological studies aligned with their traditional uses. Antibacterial activity of 70% aqueous ethanolic extracts of *B. picta* (leaves), *C. floribunda* (leaves), *E. stricta* (bark), *L. spinosa* (leaves) and *P. persica* (roots) have been previously determined by our research group and this identified *E. stricta* stem bark as having good antibacterial activity.[1] Thus, based on these findings, *E. stricta* was selected for chemical and biological analyses in this PhD study.

This chapter describes the antimicrobial and antioxidant activities of extracts of *E. stricta* Roxb. (Fabaceae) stem bark and the conduct of phytochemical and biological studies on the most active extract to isolate pure compounds and determine their biological activities.

3.2 Review of literature of *Erythrina stricta*

The *Erythrina* genus is distributed across tropical and subtropical regions of the world and comprises about 110 species. Some of these species have been used as traditional medicines for the treatment of various diseases,[2] including frequent parasitic and microbial infections, inflammation, cancer and wounds.[3] The *Erythrina* genus is well documented as a good source of biologically active compounds, including representatives from the pterocarpan,[4] flavonoid,[5,6] isoflavone,[7] alkaloid[8,9] and saponin[10] structure classes.

E. stricta Roxb. (Fabaceae) is commonly known as the Indian coral tree and is locally known as Lochet. It is a medium sized deciduous tree belonging to the family Fabaceae/Leguminosae and is widely distributed in India, China, Thailand and Vietnam.[11] The bark of *E. stricta* is grey in colour with deeply cracked cork. Branches are armed with white or pale yellow prickles.[12] Leaves of the plant are trifoliate and stalked, the flowers appear in dense clusters in terminal racemes, and the pods are 10 to 13 cm long.[13]

Figure 3.1: *Erythrina stricta* Roxb. (Fabaceae) (photo obtained from Kichu)[1]

The Chungtia villagers use the stem bark paste of *E. stricta* topically to treat contact dermatitis, eczema and skin infections.[14] The Lotha-Naga tribes of Wokha district of Nagaland use the stem bark paste for the treatment of rheumatism, stomach-ache, asthma and dysentery.[15] The Tirunelli and Pakshipathalam villagers of Wayanad district administer the bark juice for one week, daily in the morning, to cure leucorrhoea and excessive thirst.[16] The Pawra tribe of Nandurbar district of Maharashtra crush the stem bark in water and apply locally for lice destruction.[17] In Arunachal Pradesh of North East India, the bark of this plant is used to treat headache.[18] The Indigenous people inhabiting the Mikir Hills of Assam pound the flowers and make a tonic and a lotion made from the burnt wood is applied on facial inflammation.[19] The traditional curers of Assam apply the root paste on gout and root juice mixed with cow's milk is given orally to cure gout.[20] Leaves of this plant have been used in Indian traditional medicinal systems for the treatment of joint pain, earache, eye infections, toothache[11] and colds.[21]

Antibacterial, antioxidant and cytotoxic activities have been reported for some members of the *Erythrina* family.[7,22,23] The roots of *E. stricta* were found to contain antimicrobial, antiplasmodial and cytotoxic compounds.[4] The leaves of this plant are reported to possess xanthine oxidase inhibitory,[11] antioxidant[24] and anti-inflammatory[25] activities and an ethanol extract of the bark has been shown to be active against *Escherichia coli* and *Bacillus subtilis*.[26]

A number of antiplasmodial, antimycobacterial and cytotoxic active compounds have been isolated from the *n*-hexane and dichloromethane extracts of roots of *E. stricta*,

namely, erythrabissin I, erythrabissin II, erystagallin A, 5-hydroxysophoranone, sandwicensin and soyasapogenol.[4] Erythraline, erysodine, erythrinine, erysopine, 11-hydroxyerysodine and 11-hydroxyerysovine have been isolated from the seeds,[27] while a new alkaloid, 11-acetyl erysotrine and a known alkaloid, erythratidinone, have been isolated from an alkaloidal extract of flowers of *E. stricta*.[28] Alpinum isoflavone has been isolated from an *n*-hexane extract,[12] and erysovine, erysodine, 7-methoxy-8-(15-hydroxypentadecyl)-coumarin and hypaphorine have been isolated from an alkaloidal extract of the stem bark (Figure 3.2).[29] There are no reports for biological or chemical studies on the bark of *E. stricta* that are aligned with its traditional uses.

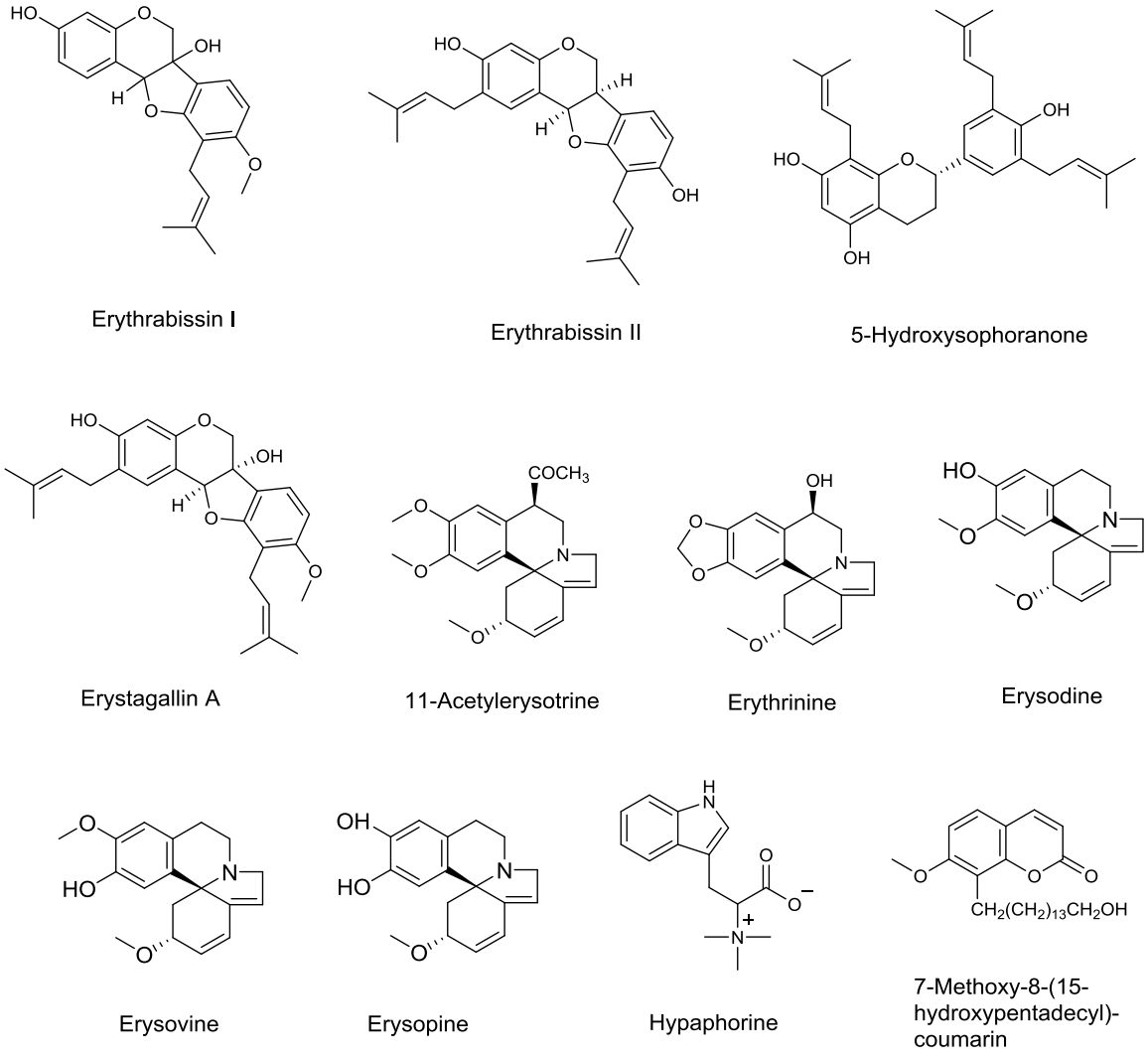

Figure 3.2: Examples of compounds reported from *E. stricta*

3.3 Selection of microorganisms for antimicrobial testing

Methicillin sensitive, methicillin resistant and multidrug resistant strains of *Staphylococcus aureus*, antibiotic senstive *Escherichia coli* and *Pseudomonas aeruginosa*, and the fungus *Candida albicans* were selected for antimicrobial screening of *E. stricta* extracts, fractions and pure compounds. These are common pathogens associated with sores, wounds and skin infections.[30] All microorganisms were obtained from Dr John Merlino (Department of Microbiology, Concord Hospital, Sydney). The use of all microbial strains was approved by the Macquarie University Biosafety committee (KAA110412BHA, 08/06/LAB, Appendix 6).

3.4 Selection of methods for biological testing of extracts, fractions and isolated bioactive compounds

To examine the antimicrobial activity, disc diffusion,[31] MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] microdilution[32] and TLC bioautography[33] assays were chosen. Typically a suite of antioxidant assay methods are reported in the literature for determination of antioxidant activity.[34,35] Among them three complementary antioxidant activity assay methods, DPPH [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)],[36] ferric reducing antioxidant power (FRAP)[37] and ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)][38] were chosen to determine the antioxidant activity. An overview of the selected methods and their complementarity is given below and details of the methods are provided in the inserted article.

3.4.1 Antimicrobial activity study

3.4.1.1 Overview of disc diffusion assay

The disc diffusion method is simple and practical and has been well standardised.[39] This method is versatile in that it is suitable for testing the majority of microbial pathogens and it requires no special equipment.[40] It is the least costly of all susceptibility methods.[39] In this method, agar plates are inoculated with a standardised inoculum of the test microorganism. Filter paper discs (6 mm in diameter), containing the test compound at a desired concentration, are then placed on the agar surface and the plates are incubated under suitable conditions. Generally, the antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured.[41] The results obtained by this method are qualitative.[42]

3.4.1.2 Overview of MTT microdilution assay

The MTT microdilution assay is based on the reduction of the yellow dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by reductases in living cells to produce purple MTT formazan (Figure 3.3).[43] The MIC (minimum inhibitory concentration) is recorded as the lowest concentration where no viability of living cells is observed after incubation (*i.e.* no purple colour, Figure 3.4).[44] The MTT microdilution assay is regarded as a rapid method (being amenable to microtitre plate format) and is cost-effective.[45]

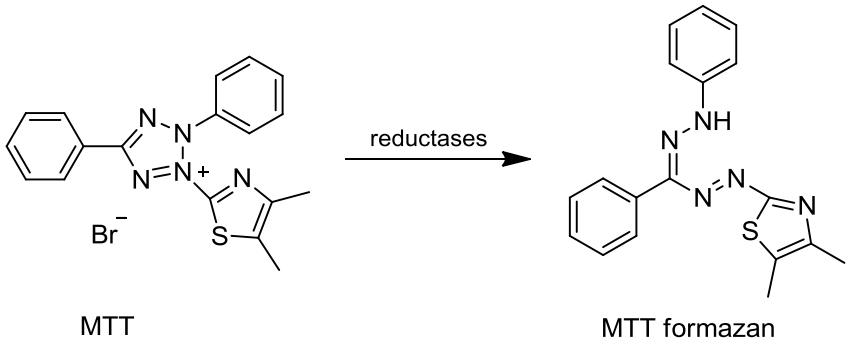

Figure 3.3: Conversion of MTT (yellow) to MTT formazan (purple) by living cells

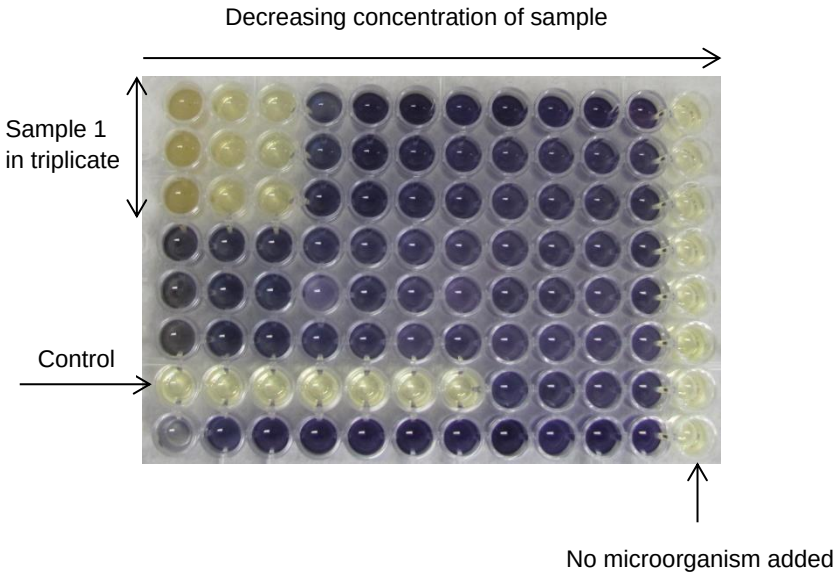

Figure 3.4: MTT microdilution assay plate (MIC is determined as the well with lowest concentration of sample that displayed no yellow to blue colour change of MTT)

3.4.1.3 Overview of TLC bioautography

Thin layer chromatography (TLC) bioautography, as a method to localise antibacterial activity on a chromatogram, has found widespread application in the search for new

antibiotics.[46] TLC bioautographic methods combine chromatographic separation and *in situ* activity determination, facilitating the localisation and targeted isolation of active constituents in a mixture.[47] This method is simple, cheap, time-saving and does not require sophisticated equipment.[48]

In this method a developed TLC plate is dipped in to a suspension of microorganisms growing in a broth and then incubated in a humid atmosphere. The silica surface of the TLC plate covered with the broth medium becomes a source of nutrients and enables growth of the microorganisms directly on it. Inhibition zones of the microorganism growth are formed in the areas where antimicrobial agents are present. Use of MTT for visualisation of these zones results in cream-white spots appearing against a purple background on the TLC plate (Figure 3.5).[48]

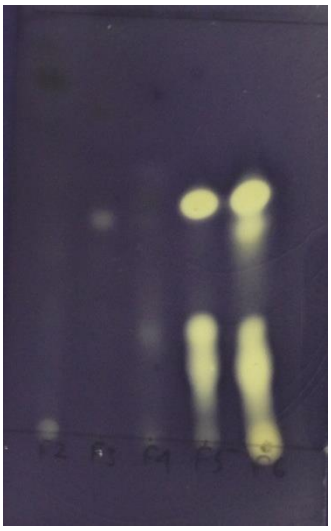

Figure 3.5: A TLC bioautography assay

3.4.2 Antioxidant activity study

3.4.2.1 Overview of DPPH free radical scavenging activity assay

The DPPH method measures the ability of antioxidants to scavenge free radicals. It is a rapid and simple method that gives accurate and repeatable results.[49, 50] In this assay, the purple free radical is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine (Figure 3.6). The reducing ability of antioxidants towards DPPH can be evaluated by monitoring decrease in the absorbance at 517 nm.[35]

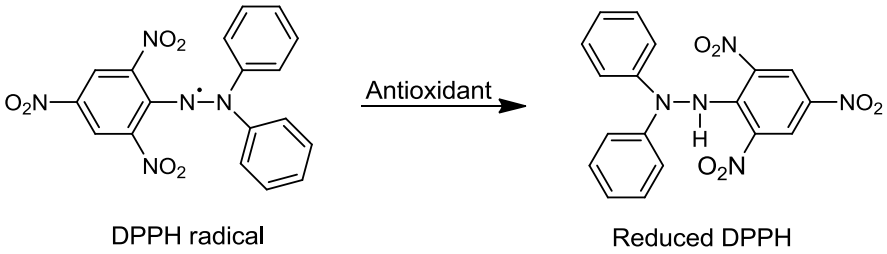

Figure 3.6: Conversion of DPPH radical (purple) to reduced DPPH (pale yellow)

DPPH can only be dissolved in organic media (especially in alcoholic media), not in aqueous media, which is an important limitation when interpreting the role of hydrophilic antioxidants. The steric accessibility of the DPPH$^{\bullet}$ radical is a major determinant of the reaction, since small molecules that have better access to the radical site have relatively higher antioxidant capacity. On the other hand, many large antioxidant compounds that react quickly with peroxyl radicals may react slowly or may even be inert in this assay. The inexistence of DPPH$^{\bullet}$ or similar radicals in biological systems is also a limitation.[51]

3.4.2.2 Overview of ABTS radical cation scavenging activity assay

The ABTS [(2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)] assay has become one of the most widely employed methods for estimating antioxidant activity[52] because of its greater sensitivity than that of the DPPH assay.[53] The ABTS assay has extra flexibility in that it is amenable to different pH levels. ABTS is also soluble in both aqueous and organic solvent and can be used to assess the antioxidant activity in different media to determine both hydrophilic and lipophilic antioxidant capacities of extracts.[53,54] This assay is based on the ability of antioxidant substances to scavenge ABTS$^{\bullet+}$ [55] and is measured by quantifying the decrease of absorbance of the ABTS$^{\bullet+}$ radical at 734 nm when treated with these substances (Figure 3.7).[34]

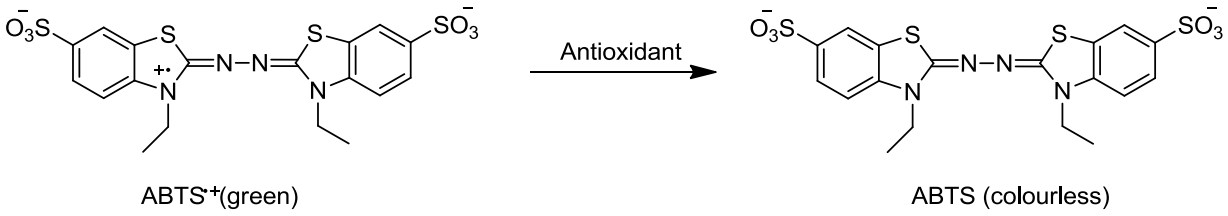

Figure 3.7: Reduction of ABTS radical cation (green) to ABTS (colourless)

A limitation of this method is that the Trolox equivalent antioxidant capacity (TEAC) value measured in this assay characterises the capability of test extracts to react with the $ABTS^{\bullet+}$ radical rather than to inhibit the oxidation process.[56]

3.4.2.3 Overview of ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay is a simple, speedy, inexpensive, and robust method. It does not require specialised equipment and can be performed using automated, semiautomated, or manual methods.[34] It is based on the ability of antioxidants to reduce the yellow ferric tripyridyltriazine complex (Fe(III)-TPTZ) to the blue ferrous complex (Fe(II)-TPTZ) by the action of electron-donating antioxidants (Figure 3.8). The resulting blue colour, measured spectrophotometrically at 593 nm, is taken as linearly related to the total reducing capacity of electron-donating antioxidants.[35]

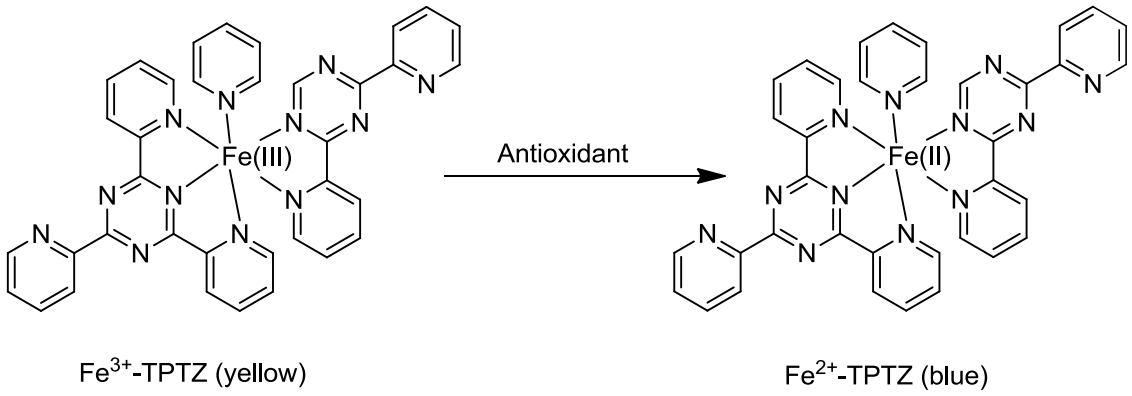

Figure 3.8: Conversion of Fe^{3+}-TPTZ (yellow) to Fe^{2+}-TPTZ (blue)

The FRAP assay relies on the hypothesis that the redox reactions proceed so rapidly that all reactions are complete within 4 minutes, but in fact this is not always true. Therefore, determination of antioxidant capacity by utilizing a fixed end point may not represent a completed reaction.[35] Another limitation of this method is that the measured reducing capacity does not necessarily reflect antioxidant activity.[57] Since the method does not include any oxidisable substrate, no information is provided on the protective properties of the antioxidants. It does not actually measure chain breaking antioxidant activity or preventive antioxidant activity. Also, if the FRAP assay is used to assess in vivo antioxidant status, Fe^{2+} can interact with H_2O_2 to produce hydroxyl radicals.[58] The non-physiological pH value is also a limitation.[59] Moreover, it also can not detect species that act by radical quenching (H transfer), particularly SH containing antioxidants like thiols, such as glutathione and proteins.[34]

3.5 Phytochemical and biological studies of *Erythrina stricta*

3.5.1 *Antimicrobial and antioxidant activity and chemical characterisation of* Erythrina stricta

A paper titled “Antimicrobial and antioxidant activity and chemical characterisation of *Erythrina stricta* Roxb. (Fabaceae)” was published in the *Journal of Ethnopharmacology*.[57]

The phytochemical and biological studies of *E. stricta* were carried out by the author. The structures of the pure compounds were elucidated with the assistance of Drs Emma Barnes, Wendy Loa-Kum-Cheung and Ping Yin. The overall contribution of the author to the paper was 70%.

Journal of Ethnopharmacology 185 (2016) 171–181

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Antimicrobial and antioxidant activity and chemical characterisation of *Erythrina stricta* Roxb. (Fabaceae)

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ARTICLE INFO

Article history:
Received 24 December 2015
Received in revised form
16 February 2016
Accepted 7 March 2016
Available online 8 March 2016

Keywords:
Erythrina stricta
Chemical constituents
Antimicrobial
Antioxidant
Isoflavanone

Chemical compounds studied in this article include:
Alpinum Isoflavone (PubChem CID: 54901393), Isovanillin (PubChem ID: 12127)
Luteone (PubChem CID: 5281797)
Obovatin (PubChem CID: 13940733)
Wighteone (PubChem ID: 5281814)

ABSTRACT

Ethnopharmacological relevance: The bark of *Erythrina stricta* Roxb. (Fabaceae) has been used in Indian indigenous systems as a remedy for rheumatism, stomach-ache, asthma, dysentery, contact dermatitis, eczema and skin infections. However, there have been limited phytochemical or biological studies on the bark of *E. stricta* and there are no studies that align with its traditional medicinal uses.
Aim of the study: The aim of this study was to assess the antimicrobial and antioxidant activity of the stem bark of *E. stricta* to support its topical use in the treatment of contact dermatitis, eczema and skin infections and to isolate and identify any bioactive compounds.
Materials and methods: MTT microdilution and disc diffusion assays were used to determine the antimicrobial activities of *n*-hexane, dichloromethane, ethyl acetate, methanol and water extracts of the bark of *E. stricta*. Column and preparative thin layer chromatography were used for the purification of the dichloromethane extract. The structures of the compounds isolated were elucidated by extensive 1D and 2D NMR spectroscopic techniques and comparison with published data. The antioxidant activities of the extracts were determined by DPPH free radical scavenging and FRAP assays and the antioxidant activity of the pure compounds by dot-blot and DPPH staining methods.
Results: The dichloromethane, ethyl acetate, and *n*-hexane extracts showed the most significant activity with MIC values of 7.8 μg/mL, 125 μg/mL, and 125 μg/mL against a sensitive strain of *Staphylococcus aureus*. The dichloromethane and ethyl acetate extracts also showed significant activity against *Candida albicans* with MIC values of 125 μg/mL and 1 mg/mL respectively. GC-MS analysis of the *n*-hexane extract showed the presence of the antibacterial and antifungal compounds β-caryophyllene, caryophyllene oxide, α-selinene, β-selinene, selin-11-en-4-α-ol, α-copaene and δ-cadenine. Phytochemical studies of the dichloromethane extract led to the isolation of the novel compound erynone (**1**), together with six known compounds; wighteone (**2**), alpinum isoflavone (**3**), luteone (**4**), obovatin (**5**), erythrinassinate B (**6**) and isovanillin (**7**). Luteone (**4**) exhibited the most significant antibacterial activity with minimum inhibitory quantity (MIQ) values of 1.88 μg, 1.88 μg and 3.75 μg, respectively, against sensitive (MSSA) and resistant strains (MRSA and MDRSA) of *S. aureus* using a TLC bioautography assay. Erynone (**1**) exhibited the greatest DPPH free radical scavenging activity.
Conclusions: Seven compounds, including a new chromanone, were isolated from the antimicrobial dichloromethane extract of the stem bark of *E. stricta*. Six of the seven compounds showed antibacterial and/or antioxidant activities. These findings provide support for the customary (traditional and contemporary) use of *E. stricta* bark for the treatment of skin and wound infections.

1. Introduction

India has a rich heritage of customary (traditional and contemporary) medicine use, with traditional healthcare systems having flourished there for many centuries (Mukherjee, 2001). A

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http://dx.doi.org/10.1016/j.jep.2016.03.011
0378-8741/

number of Indian ethnobotanical studies with a good scientific foundation have appeared over the last decade (Ayyanar and Ignacimuthu, 2009; Nagendrappa et al., 2013; Yabesh et al., 2014). However, considering the vast size of the country and its varied flora and ethnic groups, further studies are needed. In particular, the people of the state of Nagaland in North East India, which is a composite of at least 18 major tribes (Bhupathy et al., 2013), possess a wealth of knowledge of medicinal plants, developed through age long, trial and error methods (Jamir et al., 1999). The high percentage of people living a rural and/or tribal lifestyle in this region means there is a high dependency on customary medicines (Dutta and Dutta, 2005). This offers considerable scope for the discovery of new drugs that can be applied to a wider base of people (Rao and Jamir, 1982).

Traditional plant medicines used in the treatment of sores, wounds and skin infections are very often indicative of plants containing compounds with antimicrobial activity (Datta et al., 2011) and/or wound healing properties (Rios and Recio, 2005). Microbial infections continue to be a serious health concern worldwide, with skin and wound infections being especially common in Indigenous communities (Holt et al., 2010; Hotez, 2014; Otter and French, 2010). The situation has been complicated by the appearance of multidrug resistant (MDR) pathogens (Tankeo et al., 2015). The roles of increased inflammation and oxidative stress in delaying wound healing are also well documented (Eming et al., 2007; Fitzmaurice et al., 2011). Finding new antimicrobials and skin-healing agents are thus imperative for reducing the worldwide disease burden arising from skin infections and chronic wounds.

In order to investigate the potential antimicrobial and/or antioxidant activity of Indian customary plant medicine extracts and isolated natural products, an initial ethnobotanical study of Nagaland medicinal plants used by Chungtia villagers was conducted and resulted in the documentation of 135 species (Kichu et al., 2015). Thirty-seven plants were recorded as having been used as skin related treatments. Eleven of these had none or limited reports of antimicrobial or phytochemical studies. Preliminary screening of extracts of these eleven plants against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* identified *Erythrina stricta* stem bark as having good antibacterial activity. Thus, this plant was selected for further chemical and biological studies.

The *Erythrina* genus is distributed across tropical and subtropical regions of the world and comprises of about 110 species. Some of these species have been used as traditional medicines for the treatment of various diseases (Nkengfack et al., 2001), including frequent parasitic and microbial infections, inflammation, cancer, and wounds (Kone et al., 2011). The *Erythrina* genus is well documented as a good source of biologically active compounds, including representatives from the pterocarpan (Rukachaisirikul et al., 2007b), flavonoid (Cui et al., 2007; Kamat et al., 1981), isoflavone (Amir et al., 2011), alkaloid (Ghosal et al., 1971; Ozawa et al., 2011) and saponin (Mbafor et al., 1997) structure classes.

E. stricta, commonly known as the Indian coral tree, is a medium sized deciduous tree belonging to the family Fabaceae/Leguminosae and is widely distributed in India, China, Thailand and Vietnam. Its leaves have been used for the treatment of joint pain, ear ache, eye infections, toothache (Umamaheswari et al., 2009) and colds (Kadavul and Dixit, 2009). In Nagaland, India, the stem bark paste is taken for rheumatism, stomach-ache, asthma and dysentery (Jamir and Takatemjen, 2010) and is used topically for the treatment of various skin-related conditions including contact dermatitis, eczema and skin infections (Kichu et al., 2015).

Antibacterial, antioxidant and cytotoxic activities have been reported for extracts and natural products isolated from some members of the *Erythrina* family (Amir et al., 2011; Pillay et al., 2001; Rukachaisirikul et al., 2007a). The roots of *E. stricta* were found to contain antimicrobial, antiplasmodial and cytotoxic compounds (Rukachaisirikul et al., 2007b). An ethanol extract of the bark has been reported to be active against *E. coli* and *Bacillus subtilis* (Kumar et al., 2011).

In this study, antimicrobial and antioxidant testing of crude extracts of *E. stricta* bark demonstrated that the dichloromethane extract was the most active. Therefore, phytochemical studies were undertaken on this sample to isolate any biologically active natural products. This resulted in the purification of seven compounds, including one with a new structure. Six of these compounds were biologically tested and shown to have antibacterial and/or antioxidant activities. The *n*-hexane fraction, which possessed moderate antimicrobial activity, showed the presence of known antibacterial and antifungal compounds by GC-MS analysis. This study was carried out to support the customary uses of the stem bark of *E. stricta* by the Chungtia villagers of Nagaland.

2. Materials and methods

2.1. Ethics

This research was approved by the Human Research Ethics Committee at Macquarie University (Ref: HE22JUN2007-R05316 and Ref: 5200700334). It was governed by collaborative research agreements that followed the principles of the Convention of Biological Diversity (CBD) along with the stepwise participatory Action Research (PAR) methodology of UNESCO (Tuxill and Nabhan, 2001) and was conducted under the framework of best ethical practice, working in partnership with Indigenous people (NHMRC 2003).

2.2. General experimental procedures

NMR spectra were recorded on either a Bruker AVANCE-400 instrument (^{1}H NMR: 400 MHz, ^{13}C NMR: 100 MHz) or a Bruker AVANCE-600 instrument equipped with a cryoprobe (^{1}H NMR: 600 MHz, ^{13}C NMR: 150 MHz) using TMS as an internal standard. The ^{1}H chemical shifts were referenced to the residual protonated solvent peaks at δ_H 2.49 for DMSO-d_6, at δ_H 1.93 for acetonitrile-d_3 and at δ_H 7.24 for chloroform-*d*. ^{13}C chemical shifts were referenced to the central solvent peaks of bulk solvent at δ_C 39.5 for DMSO-d_6, at δ_C 1.39 for acetonitrile-d_3 and at δ_C 77.0 for chloroform-*d*. *J* values are given in Hz. HRESIMS (high resolution electrospray ionisation mass spectra) of compounds **3**, **4** and **5** were obtained on a Bruker Apex 3 instrument and of compound **1** on a Bruker maXis II™ QTOF mass spectrometer. ESIMS analyses of compounds **2** and **7** were undertaken on a Shimadzu 2010 LC-MS instrument. GC-APCI/MS of compound **6** was carried out on a Shimadzu GCMS-QP5000 instrument. UV–visible spectra were recorded on a CARY 1 Bio spectrophotometer (Varian, USA). IR spectra were recorded on a NICOLET iS10 (Thermo Scientific, USA). Optical rotations were measured using a P-1010 polarimeter (JASCO, Japan). Analytical gas chromatography (GC) was carried out on a Shimadzu GC-17A gas chromatograph with an FID detector. GC-MS analyses were carried out on a Shimadzu QP-5000 gas chromatograph mass spectrometer. Column chromatography was performed with normal phase silica gel 60 (0.040–0.063 mm, Merck, Germany), Sephadex LH-20 (18–111 μm, GE Healthcare Biosciences AB, Sweden), reversed phase silica gel RP-18 (40–63 μm, Merck, Germany) or using a biotage column (25 g silica SNAP cartridge, flow rate 30 mL/min, Biotage® Isolera®). Preparative TLC (PTLC) was carried out using Uniplate preparative TLC plates (Sigma Aldrich, Australia). TLC analyses were performed on fluorescent Merck silica gel F_{254} plates (Germany) or reversed

phase Merck silica gel 60 RP-18 F_{254} plates. All chemical solvents used for extraction and chromatographic separations were of analytical HPLC grade from Merck, Germany. Organic solvents were evaporated using a Buchi rotary evaporator (Switzerland).

2.3. Plant material

The stem bark of *Erythrina stricta* Roxb. (Fabaceae) was collected by Chungtia villagers in Nagaland, India, dried and sent to Dr R. Velmurugan (collaborative research partner in India) for processing. The plant material was washed with clean water, dried, chopped and passed through a micro-pulveriser to provide a powder. The powder was then sieved (130–200 mesh size) and dried under vacuum. This was packed into a plastic bag, sealed in a plastic container and couriered to Australia under the import permit IP12012991 from the Department of Agriculture, Fisheries and Forestry (DAFF). A herbarium specimen was prepared and identified with the assistance of Prof. N.S. Jamir (Nagaland University), Drs Alemmeren (Fazl Ali College, Mokokchung), Bendangchuchang and Wangshikokla (Nagaland University), and later authenticated by the Botanical Survey of India (BSI), Eastern Circle, Shillong, India. A voucher specimen (69629) was deposited at the BSI herbarium.

2.4. Antimicrobial activity

Antimicrobial testing was undertaken against the Gram-positive bacterial strains: methicillin-sensitive *Staphylococcus aureus* (MSSA, ATCC 29213), methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC BAA1026), and a multi-drug resistant *Staphylococcus aureus* (MDRSA, clinical isolate), the Gram-negative bacterial strains: *Pseudomonas aeruginosa* (ATCC-27853) and *Escherichia coli* (ATCC 25922), and the fungal strain *Candida albicans*. All microbial strains were kindly provided by Dr John Merlino (Department of Microbiology, Concord Hospital, Sydney) and the work was approved by the Macquarie University Biosafety Committee (approval reference 08/06/LAB, *KAA110412BHA)*. Mueller Hinton (MH) II broth and MH II agar (Bacto Laboratories Pty Ltd., Australia) were used for the growth of all the bacterial strains and Difco™ Sabouraud dextrose broth and potato dextrose agar (Bacto laboratories Pty Ltd., Australia) were used for the growth of *C. albicans*. Disc diffusion and microdilution assay methods were used for the screening of the crude extracts and column fractions. TLC bioautographic methods were used for the identification of active column fractions and to determine the minimum inhibitory quantity (MIQ) of the pure compounds.

2.4.1. Disc diffusion assay

The disc diffusion method used was based on the Kirby-Bauer assay (Bauer et al., 1966), which is commonly used for the screening of antimicrobial agents. The test samples were dissolved in DMSO at 50 mg/mL. Sterile filter paper discs (6 mm in diameter, Whatman, Maidstone) were impregnated with 20 μL of plant extract (1 mg per disc). DMSO (20 μL) impregnated discs were included as negative controls while vancomycin (antibacterial assay) and miconazole (antifungal assay) (2 μg/disc) impregnated discs were included as positive controls. All discs were air dried for 30 min, then placed on MH II agar plates inoculated with broth cultures at A_{600}=0.08 (bacteria 10^7–10^8 CFU/mL and *C. albicans* 10^5 CFU/mL) (Naz et al., 2016), which were then incubated at 37 °C for 18–20 h. The efficacy of the samples was determined by measuring the diameter of the zone of inhibition of microbial growth.

2.4.2. Microdilution assay

The broth microdilution assay method was performed in duplicate as outlined by Appendino et al. with minor modifications (Appendino et al., 2008). A solution of each sample (10 mg/mL) in 20% aqueous DMSO along with that of a suitable antibiotic (1 mg/mL, vancomycin for Gram positive strains, gentamycin for Gram negative strains and miconazole for fungal strains) were prepared and serially diluted to give a final plant sample concentration of 2–1000 μg/mL and antibiotic concentration of 0.05–100 μg/mL in 96-well clear bottom microtitre plates. Test samples (20 μL) were inoculated with 175 μL of microbial culture (A_{600}=0.08 diluted 100 fold in MH II broth); a sterile broth control was included. A 20% DMSO control was also included and the plates were incubated at 37 °C. After 18 h of incubation 5 μL of a methanolic solution (5 mg/mL) of MTT (3-(4,5-dimethylthiazol-2-yl)–2,5-diphenyl tetrazolium bromide) was added to each well and the plates further incubated at 37 °C for 1 h to determine the minimum inhibitory concentration (MIC). MTT was used as an indicator of where microbial growth reduced the yellow tetrazolium bromide to a violet formazan. MIC was described as the lowest concentration of the test compounds that inhibited visible growth of the microorganisms (the last well showing no colour change of MTT from yellow to blue). The samples that showed any antimicrobial activity in the MTT microdilution assay were assessed for their minimum bactericidal concentration (MBC) against the bacterial strains and minimum fungicidal concentration (MFC) against *C. albicans* by subculturing onto fresh agar plates after the assay (Karaman et al., 2003). Aliquots (5 μL) were taken from yellow (no growth) wells of the MTT test plates and spotted onto MH II agar plates. Plates were incubated overnight at 37 °C. The lowest concentrations showing no growth were identified as the MBCs or MFCs.

2.4.3. TLC bioautographic analysis

The overlay TLC bioautography procedure described by others, with minor modification, was used to detect bioactive fractions and compounds (Moulari et al., 2006; Nostro et al., 2000; Rahalison et al., 1991). For bioautographic analyses of extracts and column fractions, thin layer chromatography (TLC) plates were loaded with 100 μg of each extract and column fraction and air-dried before being developed in mobile phases of varying polarities (*n*-hexane:ethyl acetate 9:1 to 1:9). The plates were air-dried for at least 24 h, placed on a plate and covered with agar medium containing the microorganism (A_{600}=0.08). The plates were incubated at 37 °C for 18 h. After incubation, a methanolic solution (5 mg/mL) of MTT was sprayed over the plate before further incubation for 30 min at 37 °C. Clear zones were indicative of the antibacterial activity of compounds on the TLC plates.

The minimum inhibitory quantity (MIQ) values of the pure compounds were determined using the same (above) method. The experiment was run in triplicate. MIQ values were described as the minimal quantity of the compounds that showed clear zones of inhibition. MIQ values were calculated as follows:

$$\text{MIQ } (\mu g) = C\ (\mu g/\mu L) \times V\ (\mu L)$$

C being the concentration of compound used and V the volume of compound solution applied onto the TLC plate.

2.5. Antioxidant activity

2.5.1. DPPH free radical scavenging activity

Scavenging activity on DPPH was assessed according to the method reported by Adedapo et al., (2008) with minor modification. Briefly, the reaction mixture contained 3 mL of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) in methanol and 200 μL of either test extracts at various concentrations (6.75 –100 μg/mL) or control (DPPH in methanol). The mixtures were vigorously shaken and left to stand for 30 min in the dark. The reduction of DPPH free radical was measured by reading the absorbance at 517 nm. L-

Ascorbic acid was used as a positive control. The percentage of free radical scavenging activity was calculated as follows:

$$\text{Scavenging activity}(\%) = \left[\left(A_{\text{control}} - A_{\text{sample}} \right) / A_{\text{control}} \right] \times 100]$$

The antioxidant activity of the extracts was expressed as an IC_{50}, which was defined as the concentration (μg/mL) of extract required to inhibit the formation of DPPH radicals by 50%.

2.5.2. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out by following the method described by Wang et al., (2013b). The FRAP reagent included 300 mM acetate buffer (3.1 g of CH_3COONa in 16 mL glacial acetic acid), 10 mM of 2,4,6-tris-(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM hydrochloric acid and 20 mM $FeCl_3 \cdot H_2O$ aqueous solution in the ratio of 10:1:1 (v/v). The extracts were prepared at a final concentration of 0.2 mg/mL. Extract solution (400 μL) was mixed with 3 mL of freshly prepared FRAP solution and incubated at 37 °C in a water bath for 30 min. The absorbance of the solutions was measured at 593 nm. Trolox was used as the standard solution to draw the calibration curve in a concentration range of 25–400 μM ($Y=0.0056x+0.0159$, $R^2=0.9993$). The FRAP results were calculated as mg of Trolox equivalent per gram sample. All experiments were done in triplicate.

2.5.3. Evaluation of antioxidant activity of pure compounds by dot-blot and DPPH staining

The dot-blot DPPH assay was carried out according to the method described by Choi et al., (2002). An aliquot (3 μL) of each dilution of each sample was carefully loaded onto a 20 × 20 cm TLC plate (silica gel 60 F254 on an aluminium backing; Merck) and allowed to dry for a few minutes. Aliquots of the same compound were loaded in order of decreasing concentration down the column. The staining of the silica plate was based on the method of Soler-Rivas et al., (2000). The sheet containing the dry spots was placed upside down for 10 seconds in a 0.4 mM DPPH solution in methanol. Stained silica plates that gave a purple background with yellow spots at the location of the drops showed radical scavenger capacity. The intensity of the yellow colour correlates with the amount and radical scavenging nature of the compound.

2.6. Statistical analysis

All results are expressed as means ± standard deviation. Statistical analyses were performed using Microsoft Excel. The IC_{50} values were calculated by regression analysis.

Values with $p < 0.05$ and $p < 0.01$ were considered statistically significant and very significant respectively. The IC_{50} values were compared by paired *t* test (two sided).

2.7. Phytochemical studies

Phytochemical screening of the *n*-hexane, dichloromethane, ethyl acetate, methanol and water extracts for alkaloids, flavonoids, steroids, terpenoids, tannins, carbohydrates and anthraquinone glycosides (Table 2) was determined in accordance with published methods (Aiyegoro and Okoh, 2010; Chew et al., 2011; Roy et al., 2011).

2.8. Extraction and isolation of compounds

The dried, powdered stem bark (1.5 kg) of *E. stricta* was extracted successively with n-hexane (3L × 24 hr × 3), dichloromethane (3L × 24 hr × 3), ethyl acetate (3L × 24 hr × 3), methanol (3L × 24 hr × 3) and water (3L × 24 hr × 3) at room temperature in a shaker incubator (120 rpm) to give 4.2 g, 21.9 g, 7.9 g, 33.9 g, and 23.2 g of each extract, respectively. Since the dichloromethane extract showed the strongest biological activity, it was chosen for further chemical investigations. A portion of this extract (14 g) was dissolved in dichloromethane, loaded onto a normal phase silica gel column and eluted with mixtures of dichloromethane:methanol (100:0 to 80:20), in order of increasing concentration of methanol. Twelve fractions (F1–F12) were collected. The MIC values of these fractions were obtained using an MTT microdilution assay.

As F7 and F8 showed the most activity, they were initially chosen for further analysis. Fraction F8 (1.4 g) was subjected to Sephadex LH-20 column chromatography eluting with chloroform:methanol (1:1) to give five fractions (F8-1 to F8-5). Fraction F8-3 (310 mg) was subjected to Biotage column chromatography (10 g silica SNAP cartridge, flow rate 10 mL/min) eluting with a mobile phase of *n*-hexane:ethyl acetate (10:90 to 50:50), yielding six fractions. Purification of the sub-fraction F8-3-6 (110 mg) using reversed phase silica gel column chromatography eluting with acetonitrile:water (15:85 to 100:0) followed by Sephadex LH-20 column chromatography (chloroform:methanol = 1:1) yielded compound **1** (2.3 mg, 0.00024% dry wt, $R_f=0.38$ [chloroform:methanol = 19.5:0.5]). F7 (2.0 g), from the initial normal phase silica gel column, was subjected to Sephadex LH-20 column chromatography eluting with chloroform:methanol (1:1) to give 7 fractions (F7-1-7). Fraction F7-6 (27 mg) was subjected to Biotage column chromatography (10 g silica SNAP cartridge, flow rate 10 mL/min) eluting with a mobile phase of *n*-hexane:ethyl acetate (5:95 to 60:40) in order of increasing concentration of ethyl acetate. Fractions of 12 mL were collected and analysed by TLC. Fractions 57 and 58 were found to contain pure compound **2** and were combined (4.3 mg, 0.00045% dry wt, $R_f=0.59$ [*n*-hexane:ethyl acetate = 6:4]). Fraction F7-4 (190 mg) was subjected to Biotage column chromatography, eluting with *n*-hexane:ethyl acetate (95:5 to 70:30) in order of increasing concentration of ethyl acetate. Fractions of 12 mL were collected, analysed by TLC and grouped into 12 fractions. Further purification of the first eluting fraction (37 mg) using normal phase preparative TLC with a mobile phase of *n*-hexane:ethyl acetate (7:3) yielded compound **3** (14 mg, 0.0015% dry wt, $R_f=0.55$ [*n*-hexane:ethyl acetate = 6:4]). Another sub-fraction, F8-5 (3.6 mg) was further purified using normal phase preparative TLC eluting with *n*-hexane:ethyl acetate (5:5) to afford **4** (1.4 mg, 0.00015% dry wt, $R_f=0.5$ [*n*-hexane:ethyl acetate = 4.5:5.5]).

Fraction F2 (1.0 g) from the initial normal phase silica gel column was dry loaded onto a silica gel column (90 g) and eluted with mixtures of *n*-hexane:ethyl acetate (100:0 to 70:30). Ninety nine fractions were collected and combined to give six fractions according to their R_f values. Fraction F2-3 (2 mg) was further purified by preparative normal phase TLC (*n*-hexane:ethyl acetate, 9:1) to give compound **5** (0.5 mg, 0.00005% dry wt, $R_f=0.46$ [*n*-hexane:ethyl acetate = 9.5:0.5]). Fraction F2-6 (104 mg) was subjected to Sephadex LH-20 column chromatography eluting with chloroform:methanol (50:50) to give compound **6** (30.4 mg, 0.0032% dry wt, $R_f=0.56$ [*n*-hexane:ethyl acetate = 8:2]).

Fraction F5 (250 mg) from the initial normal phase silica gel column was dry loaded onto a normal phase silica gel column (28 g) and eluted with a mixture of *n*-hexane:ethyl acetate (95:5 to 50:50) in order of increasing concentration of ethyl acetate. Fraction 65 was found to be pure compound **7** (1.4 mg, 0.00015% dry wt, $R_f=0.45$ [*n*-hexane:ethyl acetate = 6:4]). Owing to the low yield of some of the pure compounds, minimum inhibitory quantities (MIQs) against sensitive and resistant strains of *S. aureus* were evaluated by TLC bioautography rather than as MIC values by the MTT microdilution assay.

*2.8.1. Erynone (**1**)*

Brown solid; $[\alpha]_D^{24}-77.2$ (*c* 0.25, CH_3OH); UV (CD_3CN) λ_{max} (log ε) 287 (1.82), 318 (1.27) nm; IR (ATR) 3363, 1614, 1541, 1506, 1489, 1437, 1373, 1260, 1227, 1155, 1034 cm^{-1}; ^1H NMR (600 MHz, CD_3CN): See Table 3; ^{13}C NMR (150.0 MHz, CD_3CN): See Table 3; HRESIMS *m/z* 799.3832 $[M+H]^+$ (calcd 799.3841 for $C_{50}H_{55}O_9$) and 821.3654 $[M+Na]^+$ (calcd 821.3660 for $C_{50}H_{54}NaO_9$ $[M+Na]^+$).

*2.8.2. Wighteone (**2**)*

Pale yellow crystals; ^1H NMR (600 MHz, DMSO-d_6): See Table S1; ^{13}C NMR (150.0 MHz, DMSO-d_6): See Table S1; ESIMS *m/z* 339.5 $[M+H]^+$ and *m/z* 337.5 $[M-H]^-$.

*2.8.3. Alpinum Isoflavone (**3**)*

Yellow needles; ^1H NMR (600 MHz, CD_3CN): see Table S1; ^{13}C NMR (150.0 MHz, CD_3CN): See Table S1; ESIMS *m/z* 335.5 $[M-H]^-$; HRESIMS *m/z* 335.0941 $[M-H]^-$ (calcd 335.0919 for $C_{20}H_{15}O_5$ $[M-H]^-$).

*2.8.4. Luteone (**4**)*

Pale yellow needles; ^1H NMR (600 MHz, CD_3CN): see Table S1; ^{13}C NMR (150.0 MHz, CD_3CN): See Table S1; HRESIMS *m/z* 352.1021 $[M-H]^-$ (calcd 335.1025 for $C_{20}H_{17}O_6$ $[M-H]^-$).

*2.8.5. Obovatin (**5**)*

White amorphous solid; $[\alpha]_D^{24}-63.3$ (*c* 0.06, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): see Table S1; ^{13}C NMR (150.0 MHz, $CDCl_3$): See Table S1; HRESIMS *m/z* 323.1285 $[M+H]^+$ (calcd 323.1283 for $C_{20}H_{19}O_4$ $[M+H]^+$).

*2.8.6. Erythrinassinate B (**6**)*

White powder; ^1H NMR (600 MHz, $CDCl_3$): see Table S2; ^{13}C NMR (150.0 MHz, $CDCl_3$): See Table S2; APCIMS *m/z* 585.80 $[M-H]^-$.

*2.8.7. Isovanillin (**7**)*

White crystalline solid; ^1H NMR (600 MHz, $CDCl_3$): see Table S2; ^{13}C NMR (150.0 MHz, $CDCl_3$): See Table S2; ESIMS *m/z* 151 $[M-H]^-$.

2.9. GC and GC-MS analysis of n-hexane extract

The *n*-hexane extract of *E. stricta* was analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). GC was carried out on a BP-20 column (60 m × 0.25 mm × 0.25 μm). The temperature program was 50 °C (5 min) to 220 °C (15 min) at 3 °C/min with helium as the carrier gas. The temperature of the injector and detector were both set at 220 °C. Two columns were used for GC-MS: (a) a BP-20 column (30 m × 0.35 mm × 0.25 μm, injector temperature 220 °C), programmed from 35 °C to 220 °C at 3 °C/min and (b) a BP-5 column (30 m × 0.25 mm × 0.25 μm, injector temperature 250 °C), programmed from 35 °C (5 min) to 250 °C (15 min) at 5 °C/min or 80 °C (5 min) to 300 °C (30 min) at 10 °C/min, both with helium as the carrier gas. Mass spectra were recorded in electron impact (EI) mode at 70 eV, scanning from 41 to 450 *m/z*. Compounds were identified by their identical GC retention times and retention indices relative to *n*-alkanes and by comparison of their mass spectra with either pure standards or published spectra in the NIST GC-MS library and those in the literature (Adams, 2007; Babushok et al., 2011; Flavornet, 2015; Joulain and König, 1998; Pherobase, 2015; Stenhagen et al., 1974)

3. Results and discussion

3.1. Biological activity of extracts

The crude *n*-hexane, dichloromethane, ethyl acetate, methanol and water extracts of *E. stricta* bark were tested for their antimicrobial activities against methicillin-sensitive *S. aureus* (MSSA, ATCC 29213), methicillin-resistant *S. aureus* (MRSA, ATCC BAA1026), multi-drug resistant *S. aureus* (MDRSA, clinical isolate), *E. coli*, *P. aeruginosa,* and the fungal strain *C. albicans* using disc diffusion and MTT microdilution assays. The strains used were selected as they represent common pathogens associated with wounds, sores and skin infections (Giacometti et al., 2000). The *n*-hexane, dichloromethane and ethyl acetate extracts showed good activity against both the methicillin sensitive and resistant strains of *S. aureus,* with MIC and MBC values ranging from 7.81 to 500 μg/mL. The dichloromethane and ethyl acetate extracts additionally showed prominent antifungal activity against *C. albicans,* with MIC and MFC values ranging from 125 to 1000 μg/mL (Table 1). The greatest activity was recorded for the dichloromethane extract against *S. aureus* (MSSA) with an MIC value of 7.81 μg/mL and a 15 mm zone of inhibition in the disc diffusion assay. The methanol and water extracts were not effective against any of the tested microbial strains at the highest concentration tested (MIC, MBC and MFC values > 1000 μg/mL in all cases). None of the extracts were active against *E. coli* or *P. aeruginosa*. Both the disc diffusion and microdilution assays showed similar levels of activity for all the extracts. The lower polarity extracts were observed to possess higher activity than the more polar ones. According to Rios and Recio (2005), extracts possessing MIC values of 1000 μg/mL or less are considered active and worthy of further investigation. Therefore, the dichloromethane extract was selected for the isolation of its constituent compounds.

Table 1
MIC, MBC and MFC values (μg/mL) of crude extracts.

Extracts	*S. aureus* (MSSA)		*S. aureus* (MRSA)		*S. aureus* (MDRSA)		*C. albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
n-Hexane	125	250	250	250	125	250	na	na
DCM	7.81	62.5	31.25	62.5	31.25	62.5	125	125
EtOAc	125	500	250	500	250	500	1000	1000
MeOH	na	na	na	na	na	na	na	na
Water	na	na	na	na	na	na	na	na
Vancomycin	1.56	1.56	3.12	3.12	1.56	1.56	NT	NT
Muconazole	NT	NT	NT	NT	NT	NT	12.5	12.5

NT: not tested; na: not active; DCM: dichloromethane; EtOAc: ethyl acetate; MeOH: methanol; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; MFC: minimum fungicidal concentration

Table 2
Preliminary phytochemical analysis of crude extracts of *E. stricta* bark.

Phytochemical compounds tested	*n*-Hexane extract	Dichloromethane extract	Ethyl acetate extract	Methanol extract
Flavonoids	−	+	+	−
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Alkaloids	−	−	−	−
Tannins	−	+	+	+
Carbohydrates	+	+	+	+
Anthraquinone glycosides	−	+	−	−

+ = present; − = absent

3.2. Chemistry

3.2.1. Phytochemical studies

Phytochemical studies of the crude extracts of *E. stricta* revealed the major classes of constituents present to be flavonoids, terpenoids, steroids, tannins, carbohydrates and anthraquinone glycosides (Table 2). These classes of phytochemicals are known to possess a variety of biological activities including antibacterial, antifungal, antioxidant, antiviral, anti-inflammatory, antitumor, anticancer, enzyme inhibition, oestrogenic, angiostatic and antidiabetic (Cowan, 1999; Cushnie and Lamb, 2005; Nijveldt et al., 2001; Tringali, 2003; Wang et al., 1998). Thus, the presence of these phytochemicals may be responsible for some of the antimicrobial and antioxidant activities of the extracts, and is in agreement with the customary use of the bark of *E. stricta* for the treatment of skin related diseases such as contact dermatitis, eczema and skin infections.

3.2.2. Isolation and structure elucidation of compounds **1**–**7**

Seven compounds (**1**–**7**, Fig. 1) were isolated from the dichloromethane extract after fractionation using normal phase, size exclusion and preparative thin layer chromatography.

Compounds **2**–**7** were identified as wighteone (**2**) (Lane and Newman, 1986; Lingham et al., 1977), alpinum isoflavone (**3**) (Olivares et al., 1982), luteone (**4**) (Fukui et al., 1973), obovatin (**5**) (Chen et al., 1978; Peralta et al., 2011), erythrinassinate B (**6**) (Wandji et al., 1990) and isovanillin (**7**) (Koorbanally et al., 2000) by comparison of their NMR and MS spectral data with that reported in the literature. The relative configuration at C-2 of obovatin (**2**) was assigned after analysis of the ^{1}H–^{1}H coupling constants in the ^{1}H NMR spectrum of **2**. The optical rotation value obtained for obovatin (**2**) was in agreement with that reported in the literature (Chen et al., 1978; Peralta et al., 2011). A new chromanone, erynone (**1**), was also obtained (Fig. 1) and its structure elucidated by extensive 1D and 2D NMR and HRESIMS data analyses.

From the HRESIMS data, the molecular formula of $C_{50}H_{54}O_9$ was inferred for compound **1**, which corresponds to 24 double bond equivalents. The ^{1}H NMR spectrum of **1** contained 29 resonances (Table 3), including eight aromatic signals (δ_H 6.14, 6.21, 6.42, 6.47, 6.69, 6.79, 6.96, 7.55) that could be assigned in combination with the HSQC data. Fifty resonances were detected in the ^{13}C NMR experiment of **1**, 24 of which were found to be due to quaternary carbons when analysed alongside the HSQC spectrum. A signal at δ_C 196.8 indicated the compound possessed a ketone moiety. Analyses of the ^{1}H–^{1}H COSY and HMBC spectra established that there were four prenyl groups within the molecule ((a) δ_H 3.15, 5.22, 1.66, 1.71; (b) δ_H 3.18, 5.26, 1.65, 1.68; (c) δ_H 3.43, 5.27, 1.75, 1.64; and (d) δ_H 3.27, 5.21, 1.80, 1.72).

The oxygenated methylene at H-2 (δ_H 4.63/4.05) was observed to possess HMBC correlations to C-3 (δ_C 51.7), C-4 (δ_C 196.8), and C-9 (δ_C 162.8), giving the first indications that compound **1** contained an isoflavanone system. HMBC resonances from H-5 (δ_H 7.55) to C-4 (δ_C 196.8), C-9 (δ_C 162.8), and C-7 (δ_C 164.7), and from H-8 (δ_H 6.21) to C-6 (δ_C 125.3) and C-10 (δ_C 112.2) confirmed the isoflavanone moiety. A HMBC correlation from H-5 (δ_H 7.55) to C-7 (δ_C 164.7) placed a hydroxyl group at C-7, given its downfield chemical shift. Shared HMBC correlations between H-8 (δ_H 6.21), H-11 (δ_H 3.15), and H-12 (δ_H 5.22) to C-6 (δ_C 125.3) allowed for the positioning of one of the prenyl groups at C-6. H-2 (δ_H 4.63/4.05) and H-5″ (δ_H 6.14) both possessed HMBC signals to C-1″ (δ_C 113.1), allowing for the beginnings of an aromatic system to be attached to C-3. H-6″ (δ_H 6.79) possessed HMBC correlations to C-2″ (δ_C 156.4) and C-4″ (δ_C 156.7), placing hydroxyl groups at both these positions. H-5″ (δ_H 6.14), H-7″ (δ_H 3.27), and H-8″ (δ_H 5.21) shared HMBC resonances to C-3″ (δ_C 118.0), positioning a second prenyl moiety at this site. The elucidation of this isoflavanone portion of molecule **1** was supported by similarities to NMR data provided for a comparable isoflavanone isolated by McKee et al.

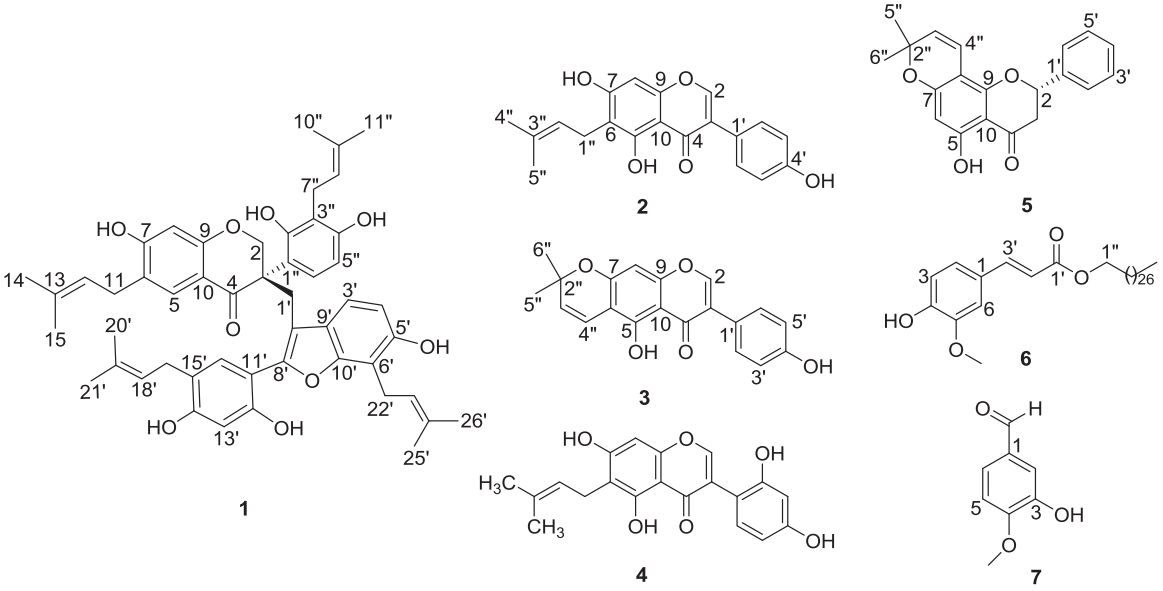

Fig. 1. Structures of compounds **1**–**7** isolated from the stem bark of *E. stricta*.

Table 3
NMR data for erynone (**1**)[a].

Position	δ_H, mult. (*J* in Hz)	δ_C, mult.	COSY	HMBC	ROESY
1					
2	α 4.63, d (12.6)	73.9, CH_2		1′, 1′′, 3, 4, 9	1a′[c], 1b′[c], 2β, 6′′, 16′[c]
	β 4.05, d (12.6)			1′, 1′′, 4, 9	1b′[c], 2α, 3′, 6′′[c], 16′
3		51.7, C			
4		196.8, C			
5	7.55, s	129.7, CH		4, 7, 9, 11	11, 12
6		125.3, C			
7		164.7, C			
8	6.21, s	102.5, CH		4, 9, 6, 7, 10, 11[c]	11[c]
9		162.8, C			
10		112.2, C			
11	3.15, d (7.2)	28.5, CH_2	12	5, 6, 7, 12, 13	5, 12, 14, 15
12	5.22, m	122.7, CH	11	6, 11, 14, 15	5, 11, 14, 15
13		134.1, C			
14	1.66, s	17.8[b]		12, 13, 15	11, 12
15	1.71, s	25.9[b]		12, 13, 14	11, 12
1′	a 3.90, d (14.4)	25.8, CH_2		1′′, 2, 2′, 3, 4, 8′, 9′	1b′, 2α, 3′, 6′′[c], 16′
	b 3.11, d (14.4)			1′′, 2, 2′, 3, 4, 8′, 9′	1a′, 2α, 2β, 3′, 16′
2′		112.3, C			
3′	6.69, d (8.4)	118.7, CH	4′	2′, 5′, 10′	4′, 1a′, 1b′, 2β
4′	6.47, d (8.4)	112.1, CH	3′	5′, 6′, 9′, 22′[c]	3′
5′		152.5, C			
6′		111.5, C			
7′					
8′		151.5, C			
9′		124.0, C			
10′		154.6, C			
11′		110.6, C			
12′		154.8, C			
13′	6.42, s	103.9, CH		8′[c], 11′, 12′, 14′, 15′, 17′[c]	
14′		157.5, C			
15′		121.2, C			
16′	6.96, s	132.6, CH	17′[c]	8′, 12′, 14′, 17′	1a′, 1b′, 2α[c], 2β, 17′, 18′
17′	3.18, dd (7.2, 3.6)	28.3, CH_2	16′[c], 18′	14′, 15′, 16′, 18′, 19′	16′, 18′, 20′, 21′
18′	5.26, m	123.8, CH	17′	15′, 17′, 20′, 21′	16′, 17′, 20′, 21′
19′		133.2, C			
20′	1.65, s	17.9[b]		18′, 19′, 21′	17′, 18′
21′	1.68, s	25.9[b]		18′, 19′, 20′	17′, 18′
22′	3.43, dd (7.2, 4.2)	23.3, CH_2	23′	5′, 6′, 10′, 23′, 24′	23′, 25′, 26′
23′	5.27, m	122.8, CH	22′	6′, 22′, 25′, 26′	22′, 25′, 26′
24′		132.9, C			
25′	1.75, s	18.0[b]		23′, 24′, 26′	22′, 23′
26′	1.64, s	26.0[b]		23′, 24′, 25′	22′, 23′
1′′		113.1, C			
2′′		156.4, C			
2′′-OH	9.05, s			1′′, 2′′, 3′′	
3′′		118.0, C			
4′′		156.7, C			
5′′	6.14, d (8.4)	108.1, CH	6′′	1′′, 3′′, 4′′, 7′′[c]	6′′
6′′	6.79, d (8.4)	126.4, CH	5′′	2′′, 3, 4′′	1a′′[c], 2α, 5′′
7′′	3.27, m	23.4, CH_2	8′′	2′′, 3′′, 4′′, 8′′, 9′′	8′′, 10′′, 11′′
8′′	5.21, m	123.9, CH	7′′	3′′, 7′′, 10′′, 11′′	7′′, 10′′, 11′′
9′′		132.0, C			
10′′	1.80, s	18.1[b]		8′′, 9′′, 11′′	7′′, 8′′
11′′	1.72, s	26.1[b]		8′′, 9′′, 10′′	7′′, 8′′

[a] Spectra were recorded in acetonitrile-d3 at 30 °C.
[b] Signals interchangeable.
[c] Weak correlation.

from *Erythrina lysistemon,* for which no bioactivity has been reported (McKee et al., 1997).

H-1′ (δ_H 3.90/3.11) possessed HMBC correlations to C-2 (δ_C 73.9), C-3 (δ_C 51.7), and C-4 (δ_C 196.8), placing it off C-3, and additionally to C-2′ (δ_C 112.3), C-8′ (δ_C 151.5), and C-9′ (δ_C 124.0). The *ortho*-coupled protons at H-3′ (δ_H 6.69) and H-4′ (δ_H 6.47) demonstrated HMBC resonances to C-2′ (δ_C 112.3), C-5′ (δ_C 152.5), and C-10′ (δ_C 154.6), and C-6′ (δ_C 111.5) and C-9′ (δ_C 124.0), respectively, allowing for a pterocarpan type scaffold to be constructed. H-22′ (δ_H 3.43) and H-23′ (δ_H 5.27) showed HMBC signals to C-6′ (δ_C 111.5), allowing for a third prenyl group to be placed at C-6′.

The two singlet aromatic protons at H-16′ (δ_H 6.96) and H-13′ (δ_H 6.42) possessed HMBC correlations to C-12′ (δ_C 154.8) and C-14′ (δ_C 157.5) and C-11′ (δ_C 110.6) and C-15′ (δ_C 121.2), respectively, allowing for the construction of an aromatic ring with quaternary carbons at C-11′ (δ_C 110.6) and C-15′ (δ_C 121.2) and hydroxylated carbons at C-12′ (δ_C 154.8) and C-14′ (δ_C 157.5). H-17′ (δ_H 3.18) and H-18′ (δ_H 5.26) also demonstrated HMBC signals to C-15′ (δ_C 121.2), allowing the last prenyl group to be placed at C-15′. H-1′ (δ_H 3.90/3.11) and H-16′ (δ_H 6.96) both showed HMBC resonances to C-8′ (δ_C 151.5), positioning this final aromatic system at C-8′ (δ_C 151.5) of the pterocarpan moiety. Thus the planar structure of compound **1**, for which we have assigned the trivial name erynone, was deduced. A strong correlation in the ROESY spectrum between H-4α (δ_H 4.63) and H-6′′ (δ_H 6.79) suggested that the aromatic moiety starting at C-1′′ is on the same face of the molecule as H-4α, thus the relative configuration of position C-3 was assigned. This is the first report of isoflavanone and pterocarpan type sub-structures being linked at position C-3 of the isoflavanone moiety.

Wighteone (**2**) has previously been isolated from the stems of *Glycine wightii* (Lingham et al., 1977), the roots of *Cudrania fruticosa* (Wang et al., 2005), *Ficus ticoua* (Wei et al., 2012), *Lupinus angustifolius* (Lane and Newman, 1986), and *Bolusanthus speciosus* (Erasto et al., 2004), the seeds of *Psoralea corylifolia* (Limper et al., 2013), the leaves of *E. lysistemon* (Pillay et al., 2001) and the whole plant of *Lupinus texensis* (Zhang et al., 2011). Wighteone possesses antibacterial activity against *E. coli*, *B. subtilis, S. aureus* and *C. micoderma* (Erasto et al., 2004), antifungal activity against *C. albicans* (Wang et al., 2005) and species from the *Cryptococcus* and *Aspergillus* genera (Fukai et al., 2003), as well as antioxidant (Zhang et al., 2011) and cytotoxic activity (Kumar et al., 2013; Limper et al., 2013). This is the first report of the isolation of wighteone (**2**) from *E. stricta*.

Alpinum isoflavone has been reported to possess antimicrobial activity against *Mycobacterium smegmatis* and *M. tuberculosis*, antifungal activity against *C. albicans* and *C. glabrata* (Kuete et al., 2008), cytotoxic activity against human KB cells (Nkengfack et al., 2001), as well as antioxidant (Rahman et al., 2010) and anti-inflammatory (Liu et al., 2014) activities. Alpinum isoflavone (**3**) has previously been isolated from the stem bark of *E. stricta* (Hussain et al., 2011), *E. indica* (Nkengfack et al., 2001), *E. variegate* (Rahman et al., 2010), *E. caffra* (Desta and Majinda, 2014), *Ficus nymphaefolia* (Darbour et al., 2007) and *E. lysistemon* (El-Masry et al., 2002), the root bark of *F. chlamydocarpa* and *F. cordata* (Kuete et al., 2008), the seeds of *Milletia thonningii* (Olivares et al., 1982), the fruit of *Maclura tinctoria* (Oyama et al., 2013) and the roots of *Calopogonium mucunoides* (Ndemangou et al., 2013).

Luteone (**4**) has previously been isolated from the fruit of *Lupinus luteus* (Fukui et al., 1973), the roots of *L. albus* (Tahara et al., 1984) and *L. angustifolius* (Lane et al., 1987), the seedpods of *Laburnum anagyroids* (Sato et al., 1995) and the whole grass of *Crotalari ferruginea* (Liu et al., 2014). This compound reportedly possesses antimicrobial activity against *Aspergillus oryzae*, *Cochliobolus miyabeanus*, *Fusarium oxysporum*, *Neurospora crassa*, *Rhizoctonia solani*, *Trametes sanguineae*, *Saccharomyces cerevisiae*, *B. subtilis*, *E. coli*, *S. aureus*, *Chlorella ellipsoidea*, *Trichophyton rubrum*, *T. mentagrophytes,* and *C. albicans* (Fukui et al., 1973),

Helminthosporium carbonum and *Cladosporium herbarum* (Harborne et al., 1976; Tahara et al., 1984). This is the first report of the isolation of luteone from the genus *Erythrina*.

Obovatin (**5**) has previously been isolated from *Tephrosia obovate* (Chen et al., 1978), the seedpods of *T. elata* (Muiva et al., 2009), the roots of *T. tunicate* (Andrei et al., 2000), *Lonchocarpus obtusus* (Cavalcante et al., 2012) and *Dalea boliviana* (Peralta et al., 2011), the stems of *T. toxicaria* (Jang et al., 2003), the fruit of *Derris indica* (Decharchoochart et al., 2014), the aerial parts of *T. major* (Gomez-Garibay et al., 2002) and the bark of *T. purpurea* (Zhong-Hual and Han-Hong, 2011). Obovatin is reported to exhibit insecticidal, antioxidant (Vasconcelos et al., 2009) and anti-HIV activity (Li et al., 2013). This is the first report of the isolation of obovatin from the *Erythrina* genus.

Erythrinassinate B (**6**) possesses cytotoxicity against human KB cells (Nkengfack et al., 2001). This compound has been found in the root bark of *E. sigmoidea* (Nkengfack et al., 1994) and the stem bark of *E. indica* (Nkengfack et al., 2001). This is the first report of its isolation from *E. stricta*.

Isovanillin (**7**) has previously been isolated from many plants including the roots of *Valeriana officinalis* (Wang et al., 2013a), the stems of *Saprosma merrillii* (Zhang et al., 2013), the aerial parts of *Pycnocycla spinosa* (Sadraei et al., 2014) and the bark of *Lannea coromandelica* (Yun et al., 2014). This compound has not previously been isolated from the genus *Erythrina*. Isovanillin has demonstrated antidiarrheal and antispasmodic activities (Sadraei et al., 2014).

3.2.3. GC-MS analysis of n-hexane extract

The *n*-hexane extract of *E. stricta* was analysed by GC-MS (Table 4 and Fig. S17). The major constituents were identified as carophyllene oxide (16.31%), β-caryophyllene (9.06%), β-selinene (7.06%), α-selinene (6.86%), selin-11-en-4-α-ol (6.80%), α-eudesmol (4.60%) and α-copaene (3.31%). β-Caryophyllene, caryophyllene oxide, α-selinene, β-selinene, selin-11-en-4-α-ol, α-copaene, δ-cadenine, and α-eudesmol have been reported to have antibacterial and antifungal activities (Cimanga et al., 2002; Costa et al., 2000; Costantin et al., 2001; Yu et al., 2004). α-Copaene, β-caryophyllene, caryophyllene oxide and δ-cadinene have been reported to have anti-inflammatory activity (Tung et al., 2008). α-Copaene, α-selinene, β-caryophyllene, caryophyllene oxide, and δ-cadinene have also been reported to have antioedematogenic activity (Veiga et al., 2001). Therefore, the antibacterial activities of the *n*-hexane extract against sensitive and resistant strains of *S. aureus* are likely to be associated with the high content of these bioactive compounds.

Table 4
GC-MS analysis of *n*-hexane extract from *E. stricta* on BP-20 and BP-5 columns.

Compounds	Peak position (BP-20 column)	LRI values (BP-20 column)	LRI values (BP-5 column)	Method of identification	% Identified compounds (BP-20 column)
α-Copaene	12	1488	1382	A,B,C	3.31
β-Copaene	13	1589	1437	A,B,C	0.34
β-Caryophyllene	14	1596	1410	A,B,C	9.06
β-Selinene	20	1718	1493	A,B,C	7.06
α-Selinene	21	1723	1504	A,B,C	6.86
7-epi-α-Selinene	22	1756	1527	A,B,C	0.81
δ-Cadinene	23	1762	1531	A,B,C	0.64
Caryophyllene oxide	28	1986	1582	A,B,C	16.31
α-Eudesmol	41	2234	1652	A,B,C	4.60
Selin-11-en-4-α-ol	42	2240	1658	A,B,C	6.80
Methyl hexacosonate			2937	B,C	
Methyl octacosonate			3143	B,C	

A=Published literature data*, B=LRI index, C=mass spectrum, LRI: linear retention indices
*(Adams, 2007; Babushok et al., 2011; Flavornet, 2015; Joulain and König, 1998; Pherobase, 2015; Stenhagen et al., 1974)

Table 5
Minimum inhibitory quantity (MIQ) of the pure compounds.

Compound	*S. aureus* MSSA MIQ (μg)	*S. aureus* MRSA MIQ (μg)	*S. aureus* MDRSA MIQ (μg)
1	3.75	15	15
2	3.75	3.75	7.5
3	15	30	30
4	1.88	1.88	3.75
6	na	na	na
7	na	na	na
Vancomycin	0.09	0.38	0.38

na: Not active.

3.3. Antimicrobial activity of isolated compounds

Due to the limited amount of each pure compound, the antibacterial activities of compounds **1**–**4**, **6** and **7** were determined by overlay TLC bioautography (Table 5). There was insufficient material of compound **5** for testing. Compounds **2**–**4** have already been reported to have antifungal activity and therefore were not re-tested. The antifungal activities of compounds **1**, **6** and **7** against *C. albicans* were determined, also using overlay TLC bioautography, and found to be inactive. Compound **2** has previously been tested against *S. aureus* and demonstrated a minimum inhibitory quantity of 0.01 μg (Erasto et al., 2004).

The data obtained from this study demonstrated that isoflavones and isoflavanones are amongst the chemical classes responsible for the antimicrobial activity of *E. stricta* stem bark, with the isolated compounds demonstrating good to moderate activities (1.88–30 μg) against the tested bacterial strains. In particular, luteone (**4**) showed good activity against all three *S. aureus* strains with MIQ values between 1.88 and 3.75 μg. It was generally observed that prenylated isoflavones and isoflavanones showed greater activity than non-prenylated ones. This is in agreement with a report by Mukne et al. which suggests that there is a significant co-relationship between the presence of prenyl functional groups at particular positions and the antibacterial activity of the compounds tested (Mukne et al., 2011). This is the first report of antibacterial activity of compounds **1**–**4** against the resistant strains of *S. aureus*.

3.4. Antioxidant activity of extracts and isolated compounds

The DPPH free radical scavenging activity and ferric reducing antioxidant power (FRAP) of the crude extracts were evaluated (Fig. 2). All the extracts showed a positive response towards DPPH scavenging and were significantly different from the standard, ascorbic acid ($p < 0.05$). The IC_{50} values for the crude extracts were in the order ethyl acetate < *n*-hexane < dichloromethane < water < methanol (Table 6). The FRAP assay evaluates the antioxidant properties of extracts based on their reducing ability. All the extracts showed dose dependent reducing power and the reducing ability of the extracts was in the range of 476–574 μm Trolox/g (Table 6). The values obtained from the extracts were significantly different from the standard Trolox ($p < 0.05$) and the order was consistent with the DPPH assays.

The antioxidant activities of the isolated compounds were evaluated using dot-blot and DPPH staining methods. The antioxidant activity of compound **5** was not evaluated because of

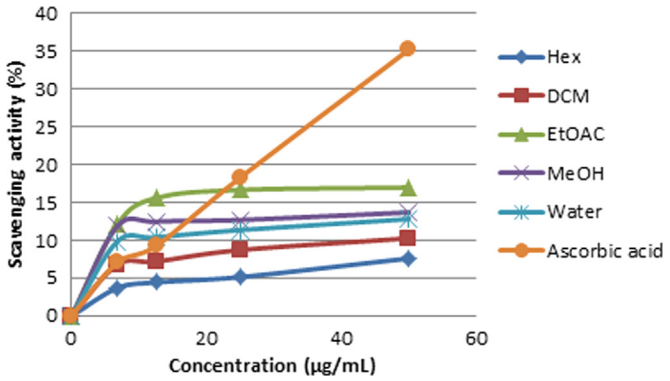

Fig. 2. DPPH scavenging activity (%) at various concentrations (µg/ mL) of crude extracts.

Table 6
Antioxidant activities of *E. stricta* extracts.

Extracts	DPPH IC_{50} (µg/mL)	FRAP (µmol Trolox/g)
n-Hexane	527.75 ± 4.24	484.02 ± 4.46
Dichloromethane	534.69 ± 3.42	481.93 ± 5.46
Ethyl acetate	414.68 ± 3.78	552.47 ± 8.30
Methanol	990.10 ± 1.20	476.88 ± 3.89
Water	599.92 ± 4.23	562.89 ± 5.1
Ascorbic acid	78.13 ± 0.56	
Trolox		3994.23 ± 5.74

Values represented in Table are means ± SD (n=3).

insufficient material. Three of the six tested compounds (**1**, **6** and **7**) showed strong positive responses to free radical scavenging activity, while luteone (**4**) possessed moderate antioxidant activity. Erynone (**1**) demonstrated the greatest activity. In general, it is believed that the radical scavenging activities of polyphenols such as flavonoids can be ascribed to the phenolic hydroxyl groups bonded to the ring structure (Kondo et al., 1999). An increase in the number of phenolic hydroxyl groups increases the radical scavenging activity, as more hydrogen atoms can be donated to stabilise the free radicals (Loo et al., 2008). Erynone (**1**) possesses the highest number (six) of free phenolic hydroxyl groups and was found to be more active than the related structures **2**–**4**. The strong activity of erynone (**1**) is suggested to be due to this compound's ability to readily donate phenolic hydrogens to the DPPH radicals. Additionally, erynone (**1**) possesses a pterocarpan moiety with a double bond at C-2′-C-8′ that extends its conjugation and may act to stabilise the radical formed after loss of hydrogen to DPPH (Yenesew et al., 2009). Luteone (**4**), with three free phenolic groups, showed moderate antioxidant activity. This is the first report of the antioxidant activity of compounds **1**, **6** and **7** (Fig. S18).

4. Conclusions

The present study demonstrated that *E. stricta* stem bark possesses antimicrobial and antioxidant activity. This research resulted in the isolation of a new antibacterial and antioxidant compound, erynone (**1**), three known antimicrobial compounds, wighteone (**2**), alpinum isoflavone (**3**), and luteone (**4**), together with the antioxidant compounds erythrinassinate B (**6**) and isovanillin (**7**). This is the first report of the isolation of luteone, obovatin and isovanillin from the genus *Erythrina* and the first report of the isolation of wighteone and erythrinassinate B from *E. stricta*. GC-MS analysis of the *n*-hexane extract also identified known antimicrobial compounds (*β*-caryophyllene, caryophyllene oxide, *α*-selinene, *β*-selinene, selin-11-en-4-*α*-ol, *α*-copaene, and *δ*-cadenine), which are possibly associated with the antimicrobial activity of the *n*-hexane extract. Together, these results support the customary uses of the stem bark of *E. stricta* by the Chungtia villagers of Nagaland for the treatment of skin and wound infections.

Acknowledgement

The authors thank the Elders from Chungtia Village, Nagaland, India for sharing their knowledge and Dr R. Velmurugan for processing the plant materials. The authors also want to acknowledge Dr John Merlino for providing the microbial strains. This project would not have been possible without the financial support provided by Macquarie University in the form of a Ph.D. scholarship for K. Akter and funding from the National Health and Medical Research Council (NH&MRC, #488504 and #1028092).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2016.03.011.

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3.6 Material and Methods

All relevant procedures are described in the preceding publication. This includes for the preparation of crude extracts and fractions and their antimicrobial and antioxidant activities and the bioassay guided chromatographic isolation of active compounds and their structure elucidation.

3.7 Results and discussions

3.7.1 Summary of isolation of bioactive compounds

As described in the publication, the dried powdered stem bark of *E. stricta* was sequentially extracted with *n*-hexane, dichloromethane, ethyl acetate, methanol and water and screened for antimicrobial activity against methicillin sensitive *S*. *aureus*, methicillin resistant S. *aureus*, multidrug resistant *S*. *aureus*, *E*. *coli*, *P*. *aeruginosa* and *C*. *albicans*. Antimicrobial screening of the extracts was conducted using the disc diffusion[31] and MTT assays.[32] The assay results of the active extracts are shown in Figure 3.9 and Figure 3.10. The methanol and water extracts were found to be inactive against all the tested microbial strains. The dichloromethane extract was found to be the most active extract, with good activity against methicillin sensitive, methicillin resistant and multidrug resistant) strains of *S. aureus* at MIC of 7.81 µg/mL, 31.25 µg/mL and 31.25 µg/mL, respectively and *C*. albicans at MIC of 125 µg/mL (see publication for details). In the disc diffusion assay, the zones of inhibition given by the dichloromethane and ethyl acetate extracts were 17 mm and 13 mm respectively against methicillin sensitive *S. aureus*. Other extracts showed no antibacterial activity in the disc diffusion assay. Antioxidant activity measured using the DPPH and FRAP assay (as detailed in the publication) was moderate.

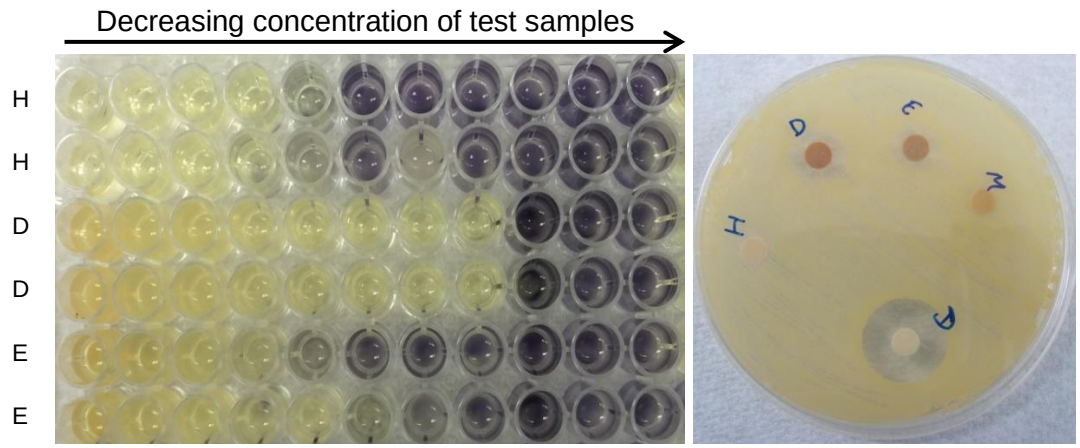


Figure 3.9: MTT microdilution and disc diffusion assay results of *n*-hexane (H), dichloromethane (D), ethyl acetate (E), methanol (M) extracts and antibiotic kanamycin (A) against methicillin sensitive *S. aureus.* Disc: 6 mm; Extracts: 1 mg/disc; Zone of inhibition for A: 23 mm; D: 17 mm and E: 13 mm

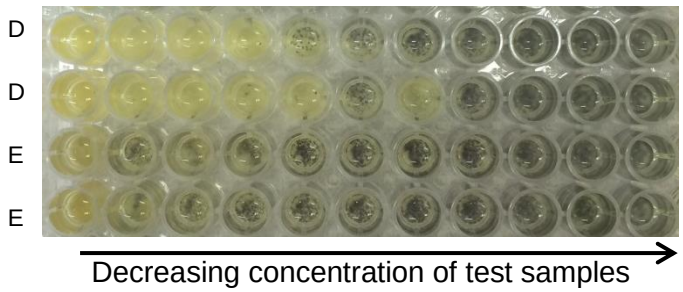


Figure 3.10: MTT assay results of dichloromethane (D) and ethyl acetate (E) extract against *C. albicans*

As a result of the good *S. aureus* and *C. albicans* activity possessed by the dichloromethane extract, it was chosen for bioassay guided chromatographic separation to isolate bioactive compounds (see publication for details). Initial fractionation was undertaken with normal phase silica gel column chromatography, eluting with increasing polarity mixtures of dichloromethane:methanol (100:0 to 80:20). Twelve fractions (F1-F12) were collected (Figure 3.13). As the column fractions F2, F5, F7 and F8 showed the best separation of UV active compounds at 254 nm by TLC, they were chosen for further purification. Chromatographic fractionation of F2, F5, F7 and F8, using a combination of normal phase, size exclusion and preparative thin layer chromatography and bioassays to guide, led to the isolation of seven compounds, including a novel compound, erynone (**1**), and six known compounds, wighteone (**2**), alpinum isoflavone (**3**), luteone (**4**), obovatin (**5**), erythrinassinate B (**6**) and isovanillin (**7**). Examples of the detection of methicillin sensitive *S. aureus* antibacterially active compounds by the TLC bioautography assay are shown in Figure 3.11. Antioxidant activity of the isolated

compounds (except for obovatin due to limited quanitity) was measured using the DPPH dot blot assay (Figure 3.12).[58] A summary of the process of isolation of the bioactive compounds from the dichloromethane extract of *E. stricta* is presented in Figure 3.13, along with assay data of the pure compounds.

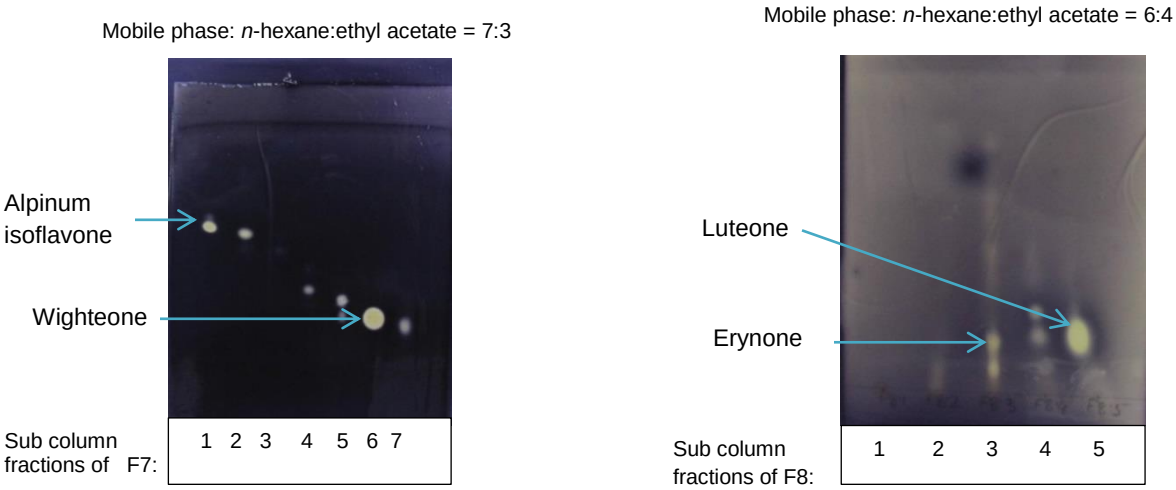


Figure 3.11: examples of TLC bioautography of dichloromethane column fractions showing active compounds against methicillin sensitive *S. aureus*

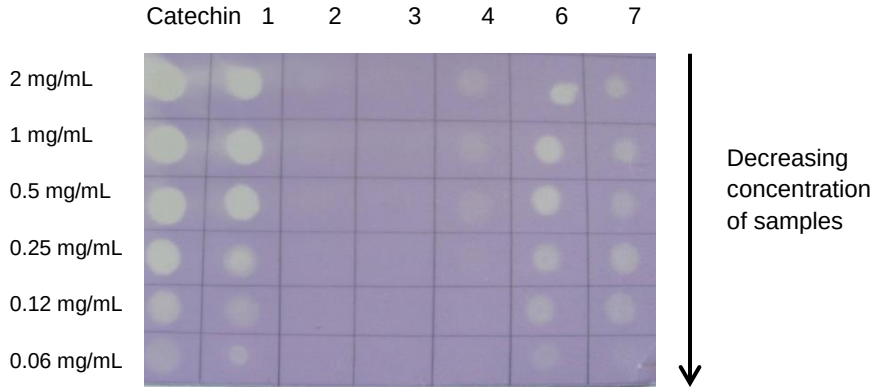
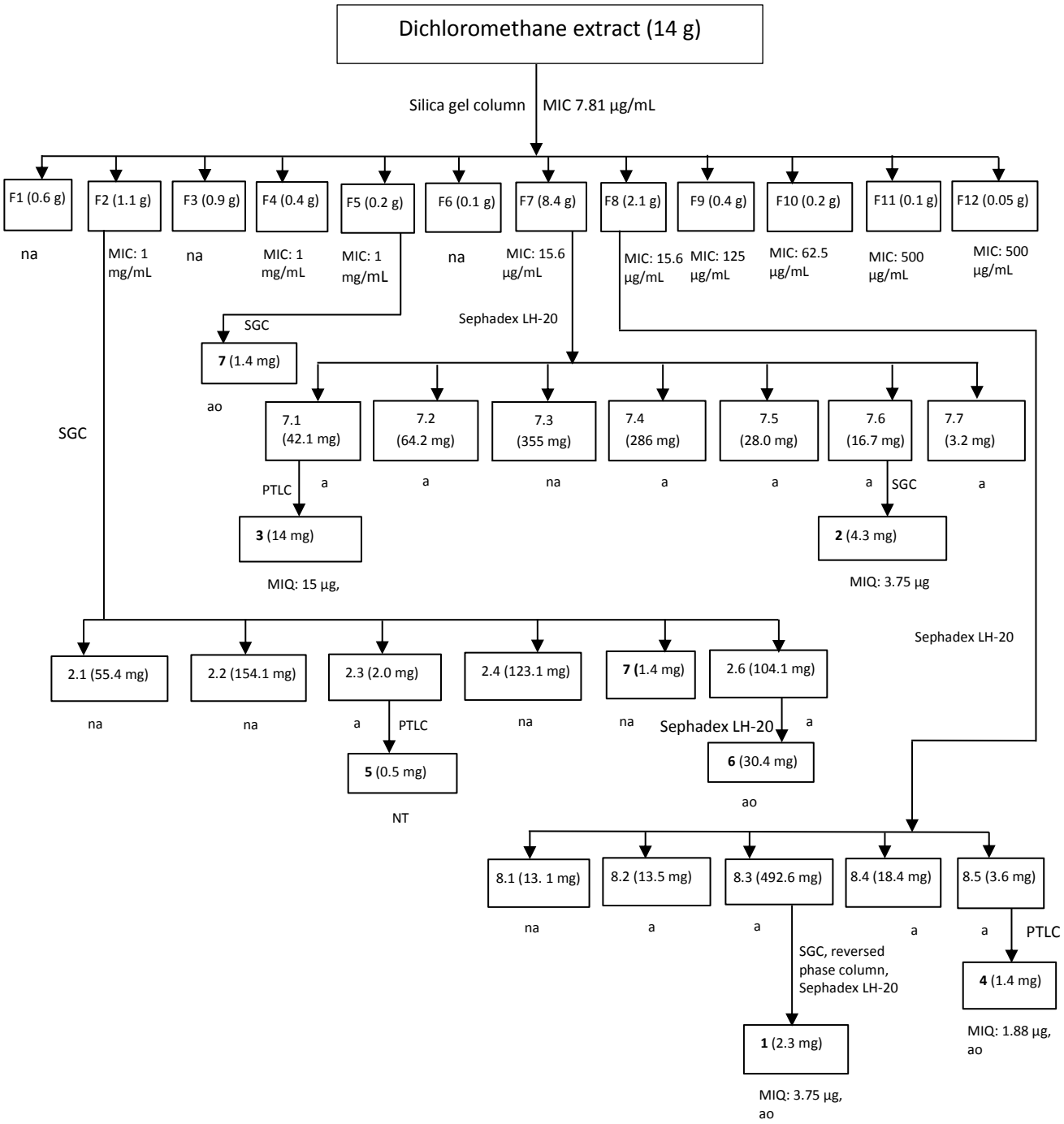


Figure 3.12: DPPH dot blot assay of free radical scavenging activity of pure compounds From left to right samples are catechin (positive control), erynone (**1**), wighteone (**2**), alpinum isoflavone (**3**), luteone (**4**), erythrinassinate B (**6**) and isovanillin (**7**)



MIC: minimum inhibitory concentration; MIQ: minimum inhibitory quantity; a: antibacterially active against methicillin sensitve *S*. aureus; ao: antioxidant active; na: not active; NT: not tested; SGC: normal phase silica gel column; PTLC: normal phase preparative column chromatography

- Because of the low amount of the semipure fractions, antibacterial activity was determined by TLC bioautography

Figure 3.13: Isolation of bioactive compounds from *E. stricta* stem bark

While fractions F2, F5, F7 and F8 were the focus of the isolation studies from the normal phase silica gel column chromatography of the dichloromethane extract, the MTT microdilution assay identified that fractions F9 and F10 possessed good antibacterial activity. Due to complexity of the samples and time constraints, these fractions were not further investigated for isolation of bioactive compounds. TLC bioautography of the subfractions F8-2 and F8-4 from F8 and F7-2, F7-4, F7-5 and F7-7 from F7 also showed the presence of bioactive compounds. These were not further investigated due to limited time. Future investigations of all of these fractions could lead to the isolation of more bioactive compounds.

3.7.2 Characterisation of bioactive compounds

The structures of the isolated compounds **1-7** were elucidated by 1D, 2D NMR and mass spectrometry and also by comparison with reported spectral data. The literature review of the compounds regarding biological studies and isolation is provided in the preceding publication and the structural elucidation is described below.

3.7.2.1 Erynone (1)

Compound **1** (Figure 3.14) was obtained as a brown solid (2.3 mg). From the HRESIMS (high resolution electrospray ionisation mass spectrum) data, the molecular formula of $C_{50}H_{54}O_9$ was inferred for compound **1**. This compound was identified as a novel chromanone and given the trivial name erynone (Figure 3.14). Its structure elucidation is described in detail in the publication and the 1D and 2D NMR spectra are provided in Appendix 2.

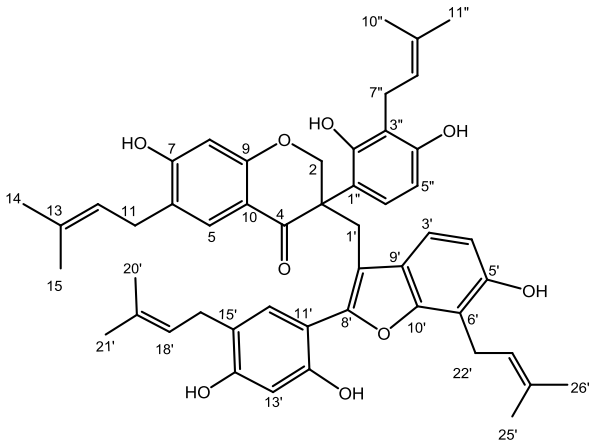


Figure 3.14: Erynone (**1**)

3.7.2.2 Wighteone (2)

Compound **2** was obtained as pale yellow needles. The negative-ion ESIMS (electrospray ionisation mass spectrum) showed an $[M–H]^-$ peak at *m/z* 337.5, consistent with the molecular formula $C_{20}H_{17}O_5$, and the positive-ion ESIMS showed an $[M+H]^+$ peak at 339.5, consistent with the molecular formula $C_{20}H_{19}O_5$. The ^1H and HSQC (heteronuclear single quantum coherence) spectra of compound **2** showed 11 resonances (Table 3.1) including three aromatic signals (δ_H 6.40, 6.79, 7.34). The proton signals were assigned to the respective carbon by analysing of the HSQC spectrum. Twenty resonances were observed in the ^{13}C NMR spectrum, ten of which were identified to be due to quarternary carbons when analysed together with the HSQC spectrum.

Analysis of the ^1H-^1H COSY (correlation spectroscopy) and HMBC (heteronuclear multiple bond correlation) spectra revealed the presence of one prenyl group within the molecule (δ_H 3.20, 5.15, 1.60, 1.70) and its assignment at C-6 was confirmed by the HMBC correlation of H-1'' to C-5 (δ_C 159.4), C-7 (δ_C 163.5) and C-6 (δ_C 111.7).[59] The ^1H NMR signal at δ_H 8.29 (H-2) and the ^{13}C NMR signal at δ_C 154.2 (C-2) are typical of the isoflavone skeleton.[2] The HMBC resonances from H-8 (δ_H 6.40) to C-6 (δ_C 111.7) and C-10 (δ_C 104.6) also confirmed the isoflavone moiety. The signal at δ_C 180.8 indicated the presence of a hydrogen bonded ketone moiety[60] and a downfield signal in the ^1H NMR spectrum at δ_H 13.19 correspondingly indicated the presence of an intramolecular hydrogen bonded phenolic group at the C-5 position.[61]

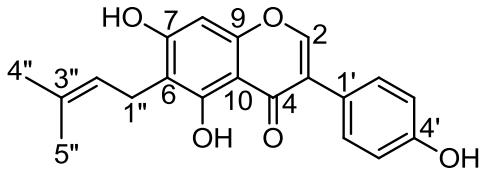


Figure 3.15: Wighteone (**2**)

This compound was identified as wighteone (**2**) and had ^1H NMR and ^{13}C NMR data identical to those in the literature.[62]

3.7.2.3 Alpinum isoflavone (3)

Compound **3** was obtained as yellow needles. The negative-ion HRESIMS showed an $[M–H]^-$ peak at *m/z* 335.0941, consistent with the molecular formula $C_{20}H_{15}O_5$. The ^1H and HSQC spectra showed eleven resonances including three aromatic signals (δ_H 6.36, 6.87, 7.38) and the ^{13}C NMR spectra showed nineteen resonances (Table 3.1). The proton peaks were assigned to the respective carbons by analysing the HSQC spectrum. Analysis of the ^{13}C NMR data along with the HSQC spectrum revealed that ten of the

nineteen carbon signals were due to quarternary carbons.

A one proton singlet at δ_H 8.00, which correlated to the ^{13}C NMR signal at δ_C 154.7 in the HSQC spectrum, is characteristic of an isoflavone skeleton and is assignable to H-2.[63] The ^1H NMR spectrum showed a pair of doublets at δ_H 5.73 (H-3'', J = 10.5 Hz) and at δ_H 6.67 (H-4'', J = 10.5 Hz) and one sharp singlet of six protons at δ_H 1.43 (H-5'' and H-6''), suggesting the presence of a dimethyl pyran ring.[64] The methyl protons at δ_H 1.43 showed HMBC correlation to C-2'' (δ_C 79.2), C-3'' (δ_C 129.9) and C-4'' (δ_C 115.8) respectively and the olefinic protons H-3'' (δ_H 5.73) and H-4'' (δ_H 6.67) showed HMBC correlation to C-5'' and C-6'' at δ_C 28.5 and C-2'' at 79.2 respectively. These HMBC correlations confirm the presence of dimethyl pyran ring originated by cyclisation of isoprenyl moiety.[65] The olefinic protons H-3'' and H-4'' also showed HMBC correlations to C-6 (δ_C 106.3) and C-7 (δ_C 160.4) respectively, which is consistent with that the 2,2-dimethyl pyran ring was fused to C-6 and C-7.[66] A signal at δ_C 182.3 (C-4) in the ^{13}C NMR spectrum is consistent with a hydrogen bonded ketone moiety in the molecule.[60] A downfield signal in the ^1H NMR spectrum at δ_H 13.32 confirmed the presence of an intramolecular hydrogen bonded phenolic group at C-5 as it showed HMBC correlations to C-5 δ_C 157.8.[61]

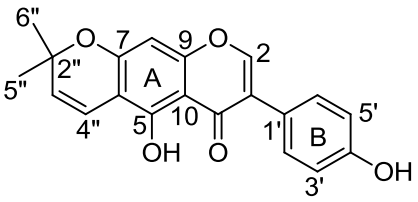


Figure 3.16: Alpinum isoflavone (**3**)

The aromatic proton H-8 (δ_H 6.36) showed HMBC correlations with C-7 (δ_C 160.4) and C-9 (δ_C 158.4), which confirmed its presence in ring A. By analyses of the HSQC and HMBC spectra, the aromatic proton signals at δ_H 6.87 and 7.38 were assigned to C-3' and C-5' and C-4' and C-6' of the B ring, respectively.

This compound was identified as alpinum isoflavone (**3**) and its ^1H NMR spectral data were identical to those in the literature.[67,68]

Table 3.1: ^{1}H (600 MHz) and ^{13}C NMR (150.0 MHz) spectroscopic data for wighteone (**2**), alpinum isoflavone (**3**), luteone (**4**), and obovatin (**5**).

Position	**2**[a]		**3**[b]		**4**[b]		**5**[c]	
	δ_C[d]	δ_H	δ_C[d]	δ_H	δ_C[d]	δ_H	δ_C[d]	δ_H
2	154.2, CH	8.29 (s)	154.7, CH	8.00 (s)	156.5, CH	7.98 (s)	79.3, CH	β 5.45 (dd, 13.1, 3.1)
3	122.7, C		124.2, C		122.3, C		43.5, CH_2	α 3.04 (dd, 16.8, 13.1) β 2.82 (dd,16.8, 3.1)
4	180.8, C		182.3, C		182.5, C		195.8, C	
5	159.4, C		157.8, C		160.2, C		164.0, C	
6	111.7, C		106.3, C		113.1, C		97.9, CH	5.98 (s)
7	163.5, C		160.4, C		163.7, C		162.5, C	
8	93.6, CH	6.40 (s)	95.7, CH	6.36 (s)	94.3, CH	6.50 (s)	102.2, C	
9	156.1, C		158.4, C		157.1, C		156.9, C	
10	104.6, C		107.0, C		105.8, C		103.1, C	
1'	122.1, C		123.3, C		111.8, C		138.7, C	
2'	130.8, CH	7.34 (d, 8.7)	131.5, CH	7.38 (d, 8.3)	132.9, CH	7.04 (d, 8.6)	126.0, CH	7.45 (d, 7.8)
3'	115.7, CH	6.79 (d, 8.7)	116.2, CH	6.87 (d, 8.3)	108.7, CH	6.42 (d, 8.6)	128.9, CH	7.42 (dd, 7.8, 7.2)
4'	157.9, C		158.3, C		160.0, C		128.8, CH	7.37 (t, 7.2)
5'	115.7, CH	6.79 (d, 8.7)	116.2, CH	6.87 (d, 8.3)	105.0, CH	6.41 (s)	128.9, CH	7.42 (dd, 7.8, 7.2)
6'	130.8, CH	7.34 (d, 8.7)	131.5, CH	7.38 (d, 8.3)	157.8, C		126.0, CH	7.45 (d, 7.8)
1''	21.8, CH_2	3.20 (d, 6.7)			22.2, CH_2	3.30 (d, 7.2)		
2''	122.9, CH	5.15 (br t, 6.7)	79.2, C		123.0, CH	5.19 (t, 7.2)	78.4, C	
3''	131.2, C		129.9, CH	5.73 (d, 10.5)	132.8, C		126.7, CH	5.45 (d, 10.2)
4''	18.3, CH_3	1.70 (s)	115.8, CH	6.67 (d, 10.5)	25.8, CH_3	1.75 (s)	115.8, CH	6.53 (d, 10.2)
5''	26.1, CH_3	1.60 (s)	28.5, CH_3	1.43 (s)	18.0, CH_3	1.65 (s)	29.7, CH_3	1.41 (s)
6''			28.5, CH_3	1.43 (s)			29.7, CH_3	1.43 (s)
5-OH		13.19 (s)		13.32 (s)		12.83 (s)		12.07 (s)

[a]Spectra recorded in DMSO-d_6. [b]Spectra recorded in acetonitrile-d_3. [c]Spectra recorded in chloroform-d. [d]Multiplicity. Coupling constants *J* in Hz.

3.7.2.4 Luteone (4)

Compound **4** was obtained as pale yellow needles. The negative-ion HRESIMS showed an $[M–H]^-$ peak at *m/z* 352.1021, consistent with the molecular formula $C_{20}H_{17}O_6$.

The ^{1}H NMR and HSQC spectra showed ten resonances including four aromatic signals (δ_H 6.50, 7.04, 6.42, 6.41). By analysing the ^{13}C NMR and HSQC spectra, the presence of twenty carbons was determined, eleven of which were due to quarternary carbons (Table 3.1).

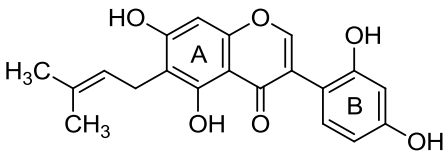


Figure 3.17: Luteone (**4**)

The spectral data of compound **4** closely resembled that of wighteone. The only difference was that C-6' in the B ring is further substituted in **4**. The compound was identified as luteone and its spectral data were in agreement with those reported.[69]

3.7.2.5 Obovatin (5)

Compound **5** was obtained as a white amorphous solid. The positive-ion HRESIMS showed an $[M+H]^+$ peak at 323.1285, consistent with the molecular formula $C_{20}H_{19}O_4$.

The ^{1}H NMR spectrum showed twelve and the ^{13}C NMR spectrum twenty resonances (Table 3.1). Analyses of the ^{13}C NMR and HSQC spectra suggested that eight signals were due to quaternary carbons. Analyses of ^{1}H NMR spectra revealed the presence of three characteristic signals for H-2, H-3 at δ_H 5.45 (1H, dd, J = 13.1, 3.1 Hz), 3.04 (1H, dd, J = 16.8, 13.1 Hz) and 2.82 (1H, dd, J = 16.8, 3.1 Hz), respectively, indicating that the compound had a flavanone skeleton.[70] and were assigned to three protons of a chromanone ring by analysing the HSQC and HMBC spectra. A signal in the ^{13}C NMR spectrum at δ_C 195.8 (C-4) indicated the presence of a ketone moiety in the molecule. The downfield signal in the ^{1}H NMR spectrum at δ_H 12.07 indicated the presence of an intramolecular hydrogen bonded phenol group, which was assignable to C-5 (δ_C 164.0) as the hydroxy proton showed HMBC correlations with C-6 (δ_C 97.9) and C-10 (δ_C 103.1). The ^{1}H NMR spectrum showed a pair of doublets at δ_H 5.45 (H-3'', J = 10.2 Hz) and δ_H 6.53 (H-4'', J = 10.2 Hz) and a pair of sharp three proton singlets at δ_H 1.41 (H-5'') and δ_H 1.43 (H-6''), characteristic of a 2,2-dimethyl pyran ring.[71] The olefinic protons H-3'' and H-4'' at δ_H 5.45 and δ_H 6.53 and the H-6 proton (δ_H 5.98) also showed HMBC correlations to C-7 (δ_C 162.5) and C-8 (δ_C 102.2), suggesting the 2,2-dimethyl pyran ring was fused to C-7 and C-8.[72] By the analysis of the HSQC and HMBC spectra, the aromatic proton signals at δ_H 7.37 (C-4'), 7.42 (C-3', C-5') and 7.45 (C-2', C-6') were assigned to being on the B ring. This compound was identified as obovatin (**5**) and the spectral data were identical to those in the literature.[73]

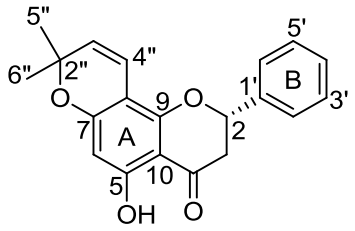


Figure 3.18: Obovatin (**5**)

3.7.2.6 Erythrinassinate B (6)

Compound **6** was obtained as a white powder. The negative-ion APCIMS (atmospheric pressure chemical ionisation mass spectrum) showed an $[M–H]^–$ peak at *m/z* 585.80, consistent with the molecular formula $C_{38}H_{65}O_4$.

The ^{1}H NMR spectrum showed a pair of doublets at δ_H 6.30 (J = 15.9 Hz) and δ_H 7.60 (J = 15.9 Hz) and the large ^{1}H-^{1}H coupling constant suggested they were in a *trans*

configuration. The ^{1}H NMR spectrum also showed three aromatic proton signals at δ_H 6.92 (*d*, *J* = 8.3 Hz), 7.08 (*dd*, *J* = 8.3 and 1.7 Hz) and 7.03 (*d*, *J* = 1.7 Hz) (Table 3.2). The sharp one proton singlet at δ_H 5.86 (4'-OH) indicated the presence of a hydroxy group and the three proton triplet at δ_H 4.19 (H-1'') confirmed the presence of a methylene-oxy group within the molecule. The –OH group was assigned at C-4 (δ_C 147.9) and the methoxyl group at C-5 (δ_C 146.9) by analysis of the HMBC spectrum. Furthermore, the two proton triplet at δ_H 4.19 (H- 1'', *J* = 6.8 Hz), a pair of two proton multiplets at δ_H 1.70 (H-2'') and 1.40 (H-3''), 48 proton broad signal at δ_H 1.23-1.26 (H-4''-H-27'') and a three proton triplet at δ_H 0.88 (H-28'', *J* = 6.8 Hz) is consistent with the presence of a long chain moiety. The ^{13}C NMR spectrum showed a signal at δ_C 167.5 (C-1') typical of a carbonyl group and signals at δ_C 115.7 (C-2') and 144.7 (C-3') due to the side chain C-C double bond.

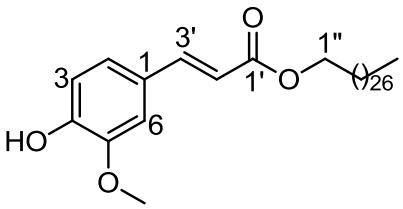


Figure 3.19: Erythrinassinate B (**6**)

Compound **6** was identified as the long chain cinnamate erythrinassinate B and the spectral data were in agreement with the published literature.[74]

3.7.2.7 Isovanillin (7)

Compound **7** was obtained as a white crystalline solid. The negative-ion ESIMS showed an $[M–H]^-$ peak at *m/z* 151.0, consistent with the molecular formula $C_8H_7O_3$. The ^{1}H NMR spectrum showed five and the ^{13}C NMR spectrum eight resonances (Table 3.2). The ^{13}C NMR spectrum showed a signal at δ_C 190.5 (C-7) that indicated the presence of a ketone moiety. The ^{1}H NMR spectrum showed a typical aldehydic signal at δ_H 9.84 (H-7), a C-4 methoxy proton signal at δ_H 4.02, a hydroxy proton signal at δ_H 6.20 (H-3) and three aromatic proton signals at δ_H 7.29 (H-2), 7.05 (H-5, d, *J* = 8.7*)* and 7.43 (H-6, d, *J* = 8.7), suggesting a trisubstituted aromatic ring.[75]

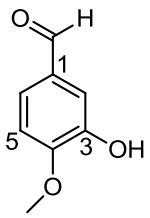


Figure 3.20: Isovanillin (**7**)

The compound was identified as 3-hydroxy-4-methoxybenzaldehyde (isovanillin) and the spectral data were identical to those in the literature.[75, 76]

Table 3.2: ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectroscopic data for erythrinassinate B (**6**) and isovanillin (**7**).

Position	**6**[a]		**7**[a]	
	δ_C[b]	δ_H	δ_C[b]	δ_H
1	127.1, C		129.5, C	
2	123.1, CH	7.08 (dd, 8.3, 1.7)	108.4, CH	7.29 (s)
3	114.7, CH	6.92 (d, 8.3)	146.7, C	
4	147.9, C		151.2, C	
5	146.9, C		114.2, CH	7.05 (d, 8.7)
6	109.3, CH	7.03 (d, 1.7)	128.9, CH	7.43 (d, 8.7)
1'	167.5, C			
2'	115.7, CH	6.30 (d, 15.9)		
3'	144.7, CH	7.60 (d, 15.9)		
1''	64.7, CH_2	4.19 (t, 6.8)		
2''	28.8, CH_2	1.70 (m)		
3''	26.0, CH_2	1.40 (m)		
4''-27''	22.7-32.0, $(CH_2)_{24}$	1.23-1.26 (br m)		
28''	14.1, CH_3	0.88 (t, 6.8)		
4'-OH		5.86 (s)		
3-OH				6.20 (s)
4-OCH_3			55.8, CH_3	4.02
5'-OCH_3	55.9, CH_3	3.94 (s)		
CHO			190.5, C	9.84 (s)

[a]Spectra recorded in chloroform-d. [b]Multiplicity. Coupling constants *J* in Hz.

3.8 Conclusions

Erythrina stricta Roxb. (Fabaceae) is an important medicinal plant for the Chungtia villagers. Stem bark paste of this plant is topically used for the treatment of contact dermatitis, eczema and skin infections. The bark powder of this plant was sequentially extracted to yield *n*-hexane, dichloromethane, ethyl acetate, methanol and water extracts. The extracts were screened for antimicrobial and antioxidant activity. The minimum inhibitory and minimum bactericidal and fungicidal concentrations of the extracts were evalauted by the disc diffusion and MTT microdilution assays. The *n*-hexane, dichloromethane and ethyl acetate extracts showed good antibacterial activity against methicillin sensitive (MSSA) and methicillin resistant (MRSA) as well as multidrug resistant (MDRSA) strains of *S. aureus*. The dichloromethane and ethyl acetate extract also showed antifungal activity against *C. albicans.* All the extracts showed mild antioxidant activity. The *n*-hexane and dichloromethane extracts showed the greatest antimicrobial activity and were chosen for phytochemical studies.

GC-MS analysis of the *n*-hexane extract identified twelve phytochemicals, including carophyllene oxide (16.31%), β-caryophyllene (9.06%), β-selinene (7.06%), α-selinene (6.86%), selin-11-en-4-α-ol (6.80%), α-eudesmol (4.60%) and α-copaene (3.31%). These compounds are well documented in the literature for antimicrobial and anti-inflammatory activities. As the dichloromethane extract showed the greatest antimicrobial activity, it was chosen for isolation of bioactive compounds. Chromatographic techniques including size exclusion, normal and reversed phase silica gel column and preparative thin layer chromatography were used. The seven bioactive compounds erynone, wighteone, alpinum isoflavone, luteone, obovatin, erythrinassinate B and isovanillin, were isolated from normal phase column chromatography fractions of the dichloromethane extract. Only alpinum isoflavone has been previously isolated from this plant. Erynone was identified as a novel compound and this is the first report of the isolation of luteone, obovatin and isovanillin from the *Erythrina* genus and wighteone and erythrinassinate B from this species. Due to the limited amount of the isolated compounds, the antimicrobial activities were evaluated as the minimum inhibitory quantity (MIQ) by TLC bioautography.

Erynone, wighteone, alpinum isoflavone and luteone showed antibacterial activity against MSSA, MRSA and MDRSA. In particular, luteone showed the highest activity and this is the first report of antibacterial activity of these four compounds against methicillin resistant strains and multidrug resistant strains of *S. aureus*. The antioxidant activity of the pure compounds were determined by the DPPH-dot blot assay. Erynone, erythrinassinate B and isovanillin showed good DPPH free radical scavenging activity and erynone demonstrated the greatest activity. A number of further column fractions also showed good antibacterial activity. These were not investigated because of time constraints. However, further studies of these fractions may lead to the isolation of more bioactive compounds.

The identification of bioactive compounds in the *n*-hexane extract and isolation of bioactive compounds from the dichloromethane extract support the traditional uses of the stem bark of *E. stricta* by the Chungtia villagers of Nagaland for the treatment of skin and wound infections.

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CHAPTER FOUR

NSW Medicinal Plants – Literature Review and Chemical and Biological Studies

This chapter provides a literature review on the ethnomedicinal, pharmacological and phytochemical reports of a selection of medicinal plants used by New South Wales Aboriginal people for skin related ailments, followed by the antimicrobial and antioxidant screening and phytochemical profiling studies carried out on these plants within this PhD project.

4.1 Introduction

As described in Chapter one, plants that are traditionally used to treat skin related ailments may be a source of antimicrobial or antioxidant agents. Though a number of plant species have been utilised topically by Australian Aboriginal people for the treatment of skin related conditions, only a limited number of these plants have been investigated for their biological properties and/or chemical studies.[1,2] This is particularly the case in New South Wales (NSW), Australia. For example, a 2012 review on 128 NSW traditional medicinal plants revealed that over half of these had not been investigated for their biological activities and had limited or no chemical investigations.[3]

This chapter presents a review of eight NSW medicinal plants used traditionally for skin related ailments that have had limited studies aligned with their traditional uses and follows this with antimicrobial and antioxidant screening and phytochemical profiling of these plants.

4.2 Literature review of the eight selected plants

The Indigenous Bioresources Research Group has documented 153 medicinal plants used by NSW Aboriginal communities as traditional medicines.[3,4] Among them, fifty-eight plants have been used for the treatment of skin related conditions including sores, wounds and skin infections. An extensive literature review of the fifty-eight plants revealed that only fourteen plants are well documented for their chemical and biological studies aligned with their traditional uses. Out of the fifty-eight plants, eleven had no chemical or biological studies reported, ten had no biological studies reported and had limited reports on chemical studies, and five plants had no reports on chemical studies and had limited biological studies reported. Eighteen plants were found to contain limited reports on both chemical and biological studies (see Appendix 2). Based on these findings, thirty-two plants were identified to be worthy for chemical and biological studies (see Appendix 3). Eight of these medicinal plants, namely *Acacia implexa*, *Acacia falcata*, *Cassytha glabella*, *Eucalyptus haemastoma*, *Hibbertia scandens*, *Smilax glyciphylla*, *Sterculia quadrifida* and *Syncarpia glomulifera* were available in accessible locations and in sustainable quantities for collection, and were thus selected for further studies.

4.2.1 *Acacia falcata*

The genus *Acacia* is one of the important genera of the family Fabaceae (sub family Mimosaceae). It comprises approximately 1350 species.[5,6] These species are widely dispersed in tropical and subtropical regions of Australia, South America, Asia and Africa.[7] Almost 1000 species are currently recognised for Australia.[8] Although the genus is quite large and widespread, little is known about the chemistry of most *Acacia*

species.[6] There are numerous *Acacia* species that have been utilised medicinally across the Australian continent, though the manner of their use varies substantially.[9]

Figure 4.1: *Acacia falcata* (Fabaceae)[10]

Acacia falcata is commonly known as hickory, sally, sickle wattle and *Lignum vitae*. It is native to coastal regions on the eastern slopes of the Great Dividing Range, and is often found in shallow stony soil.[3] It is a shrub that grows up to three metres high with angular branches. Its phyllodes are sickle–shaped and tapered at both ends, 7-18 cm long, with a bluish frosted surface. The flower heads are pale yellow, 2-4 mm in diameter, with about twenty flowers in each head. The fruit is a bluish hued legume, 7-10 cm long and about 5-6 mm broad, containing egg-shaped seeds.[11]

Bark of this plant is reported to be utilised for the treatment of sores and skin complaints[3]. A lotion made from the bark of *A. falcata* is used to treat skin diseases.[11,12] There are no reports for biological or chemical studies on *A. falcata*.

4.2.2 *Acacia implexa*

Acacia implexa is commonly known as lightwood, fish wattle, bastard myall, hickory and hickory wattle.[3] This plant is native to most of the eastern seaboard of Australia where it occurs in well-drained soil in woodlands and open forests. It is a fast growing, erect leguminous tree, reaching about 15 metres.[13] Its phyllodes are thin, sickle-shaped, tapered at both ends, 7-15 cm long or longer and up to 2 cm wide, with sharp pointed tips. Its globular flower heads contain thirty to fifty individual flowers. The legume is bulging, about 8 cm long and 6 mm wide, much curved and twisted and constricted between the seeds. The seed stalk is folded under the seed.[11]

Figure 4.2: *Acacia implexa* Benth. (Fabaceae)[14]

A. implexa bark is reported to be used for the treatment of sores and skin complaints.[3] Australian Aboriginal people make a lotion from the bark for the treatment of skin diseases.[12] The bark and leaves are also reported to be used by Australian Aboriginal people for fish poisoning,[15] and this may denote some toxicity. *A. implexa* has not been reported for any chemical or biological studies.

4.2.3 *Cassytha glabella*

The genus *Cassytha* (Lauraceae) comprises hemi-parasitic twiners without roots and developed leaves. There are 23 species that are distributed in Pacific Rim countries, mainly Australia, Africa, America and Japan.[16,17] The fruits of *Cassytha* species are drupes with the single seed enclosed in a white translucent fleshy pericarp. The mature *Cassytha* vine is usually greenish-orange.[18]

Figure 4.3: *Cassytha glabella* (Lauraceae)[19]

C. glabella is commonly known as devil's twine, dodder laurel, slender devil's twine and slender dodder-laurel and is native to heath, sandstone derived soil and dry sclerophyll forests. This plant is widely distributed all over Australia.[3] Its thread-like stems are hairless with leaves reduced to minute scales. Small white flowers occur in globular heads on a short stalk.[11] The infusion (soaking plant in cold water) of the whole plant is known to be used by the NSW Aboriginal people in cases of high temperature and a decoction of the whole plant is used for bathing of the body to relieve pain.[3,11] Quercetin 3-*O*-rutinoside, cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside have been isolated from fresh aerial parts of *C. glabella* (Figure 4.4).[20] There are no reports of any biological studies on this plant.

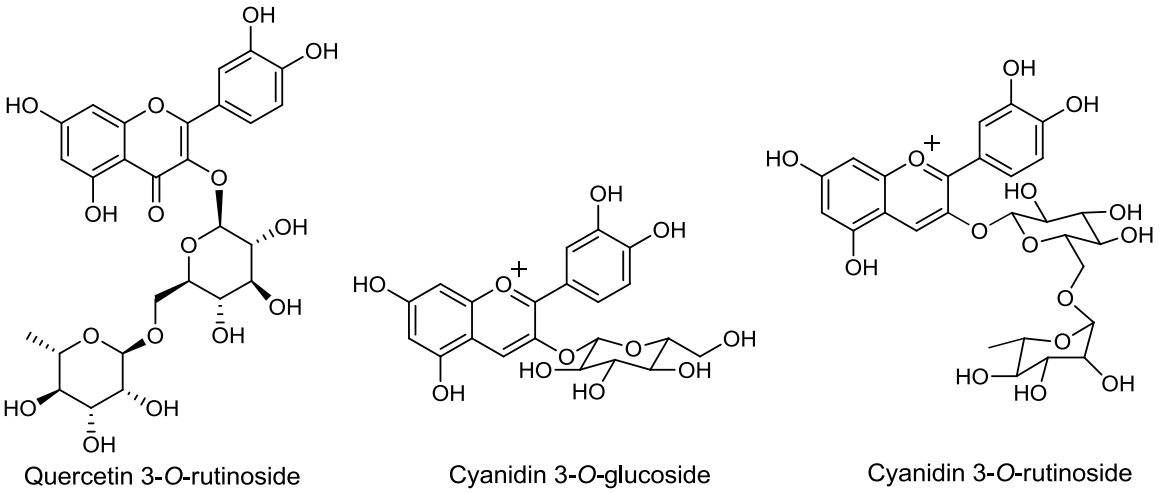

Figure 4.4: Compounds isolated from *C. glabella*

4.2.4 *Eucalyptus haemastoma*

The genus *Eucalyptus* belongs to the family Myrtaceae and is one of the world's most important and most widely planted genera. It comprises 900 species and subspecies.[21] Eucalypts are evergreen aromatic trees, rarely shrubs (mallees), endemic to Australia, Tasmania, New Guinea and the neighboring islands, where they often constitute a large portion of the forest vegetation.[22] The genus *Eucalyptus* is known for its rich source of bioactive compounds.[23] Species of *Eucalyptus* have been reported for various pharmacological actions including antioxidant, antiviral, antimicrobial, anti-inflammatory, antimalarial, antidiabetic and cytotoxic activity.[24] The Aboriginal people of Australia traditionally use the leaves of *Eucalyptus* species to heal wounds and fungal infections.[25]

Figure 4.5: *Eucalyptus haemastoma* (Myrtaceae)[26]

E. haemastoma, commonly known as scribbly gum because of the characteristic insect 'scribbles' that cover their otherwise smooth bark,[27] snappy gum and white gum, is abundant in dry sclerophyll woodlands on shallow infertile sandy soil on sandstone. It is exclusively distributed over NSW.[3] This is a medium tree, growing up to fifteen metres high, with white, grey or yellow scribbly bark, shredding to short ribbons.[28] Adult leaves are alternate, thick and leathery, sickle-shaped and tapered at both ends, 8-12 cm long and 2-3 cm wide. Flowers occur in small bunches either at the end of branches or in leaf forks.[11]

The bright red sap is used topically by the NSW Aboriginal people for the treatment of cuts, wounds and ulcers and is also used internally for the treatment of dysentery.[3,11,29] There are no reports on biological studies of this plant and only one phytochemical report on the identification of abscisic acid (Figure 4.6) in the leaf extract.[30]

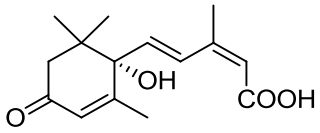

Figure 4.6: Abscisic acid

4.2.5 *Hibbertia scandens*

Hibbertia (Dilleniaceae) includes over 150 species of vines, shrubs and small trees distributed throughout Australasia, Madagascar, Malesia and Fiji.[31] Most of the *Hibbertia* species are endemic to Australia.[32] There are very few reports on this species.

Figure: 4.7: *Hibbertia scandens* (Dilleniaceae)[33]

H. scandens, commonly known as yellow vine, is abundant in sclerophyll forests and is native to NSW.[4] *H. scandens* is a fairly vigorous climber or scrambler, growing to 2 to 5 metres long. It has elliptic to obovate leaves 3-9 cm long by 1-3 cm wide. The large golden yellow flowers, 5-7 cm across, occur throughout the year but are most commonly seen in late spring and summer.[34] This plant (parts used are not known) is reported to be used by the Yaegl Aboriginal community of northern NSW for the treatment of sores and rashes.[3] There is only one biological report on antibacterial studies.[35] This plant has not been reported for any phytochemical studies.

4.2.6 *Smilax glyciphylla*

The genus *Smilax* belongs to the small family Smilacaceae, which is comprised only three genera.[36] *Smilax* is the largest genus of Smilacaceae, consisting of 300 to 350 species, distributed throughout all continents in temperate, subtropical and especially tropical regions.[37,38] Plants of this genus are bramble woody vines with paired tendrils for climbing. Several species of the *Smilax* genus have been traditionally utilised as edible

or pharmaceutical materials in many countries.[38] Species of this genus are well known in folk medicine.[39] The *Smilax* genus is reported to contain flavonoids and steroidal saponins and saponin glycoside of compounds.[40–42] Many species of this genus are also known to exhibit anticancer, antiviral, antioxidant, antimicrobial, anti-inflammatory and antidiabetic activity.[42-47]

Figure 4.8: *Smilax glyciphylla* (Smilacaceae)[48]

Smilax glyciphylla is commonly known as austral sarsaparilla, sarsaparilla, sweet sarsaparilla, sweet tea and wild liquorice.[3] It is distributed across NSW and Queensland (Qld) and native to rainforests, sclerophyll forests, woodlands and heath.[3] It is a climbing plant that grows in moist gullies and rainforest margins in south eastern NSW and Qld.[49] Leaves and small black fruits of this plant have a liquorice-like sweet taste and have been used as a substitute for tea and sugar, to prevent scurvy in colonial times and in treating a range of different conditions including chest ailments, rheumatism, syphilis, pain and cancer.[3,49–51] Syrup made by prolonged boiling of the leaves was marketed in Sydney in the early 1900s as a tonic and a remedy against coughs and catarrh.[49–51] Leaves of this plant have also been used by the Yaegl Elders of northern NSW to clear skin problems.[4]

There are very few reports of chemical and biological studies on this plant. The aqueous extract of the plant is reported to demonstrate antioxidant activity[49] but has been shown to have no antibacterial activity at a concentration of 1 mg/mL.[35] The sweet component of this plant, glycyphyllin (Figure 4.9), has been isolated from the leaves.[52]

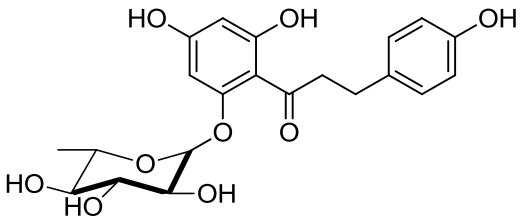

Figure 4.9: Glycyphyllin

4.2.7 *Sterculia quadrifida*

The genus *Sterculia* belongs to the family Malvaceae, which is comprised over 200 genera and is one of the largest genera of this family.[53] This genus consists of about 300 species occurring mostly in tropical regions of the world.[54,55] In various countries *Sterculia* species are reported to be extensively used in traditional medicine for the treatment of cold, fever, boils, diarrhoea, dyspepsia, gonorrhoea, snake bite, syphilis and tapeworm infestation.[56] Many species of this genus have been evaluated for their anti-inflammatory, antimicrobial, antioxidant, antinociceptive, membrane stabilisation and anti-atherothrombosis activities.[54,57–61] The *Sterculia* genus is reported to contain flavonoids, alkaloids, terpenoids and anthocyanins.[62–64]

Figure 4.10: *Sterculia quadrifida* (Malvaceae)[65]

Sterculia quadrifida is commonly known as peanut tree, red fruit kurrajong, kurrajong, kuman and monkey nut tree.[3,11] This species occurs at littoral and riverine rainforests and is distributed in NSW, Northern territory (NT) and Qld.[3] It is a deciduous tree that grows up to 20 metres high with long stalked, alternate, somewhat elongated, heart shaped to oval leaves drawn into a short blunt point. Flowers are in a cluster at the

end of branchlets.[11] The fruit is a leathery pod appearing in clusters of 4 or 5 in the shape of a cross, ripening from May onwards. The ripe red pod splits to reveal 4 or more large shiny and smooth black nuts.[66]

S. quadrifida leaves are known to be used traditionally for the treatment of wounds, sore eyes, stings and bites.[1,3] Although the *Sterculia* genus is well investigated, reports on *S. quadrifida* are very few. There is no report on chemical studies of this species and only one report on antimicrobial screening, which showed that the water partition of the leaves (following partitioning of methanol extract with ethyl acetate and water) is active against *Staphylococcus aureus,* methicillin resistant *S. aureus, Micrococcus luteus* and *Candida albicans* at a concentration of 10 mg/mL.[1]

4.2.8 *Syncarpia glomulifera*

Syncarpia is a small genus in the family Myrtaceae,[67] comprised 120 genera.[68] The genus *Syncarpia* consists of three species (one with two subspecies) and is endemic to eastern Australia.[67] All species are medium to large sized trees that occur in open forests dominated by eucalypts along the eastern coast of Australia.[69] Leaves are clustered at the end of each growth flush and the fruits are united to form a woody mass with seven openings.[70] Plants of this genus contain oil glands within the petioles as well as the leaf lamina.[71]

Figure: 4.11: *Syncarpia glomulifera* (Myrtaceae)[72]

Syncarpia glomulifera is commonly known as turpentine.[73,74] This species typically occurs in wet sclerophyll forest, often on rainforest margins, and has a large latitudinal range along the eastern coast of Australia, from just south of Cooktown in Qld to Bateman's Bay in NSW.[68] *S. glomulifera* grows up to 45 metres.[69] Sap of this species is reported to be used as an antiseptic.[35] The chloroform-ethanol (1:1), chloroform and ethanol extracts of the bark of *S. glomulifera* were reported to exhibit promising

antimicrobial and cytotoxic activity[75] and the water extract of leaves to possess antimicrobial activity.[35] Chemical and biological studies on this plant are very few. Three triterpenoids, betulinic acid, oleanolic acid-3-acetate and ursolic acid-3-acetate were isolated from the bark of this species (Figure 4.12).[68] These compounds have been reported for several biological activities such as antibacterial, anti-inflammatory and cytotoxic activity.[68,76,77] Eucalyptin and 8-desmethyleucalyptin were also reported to be isolated from the leaf wax.[78]

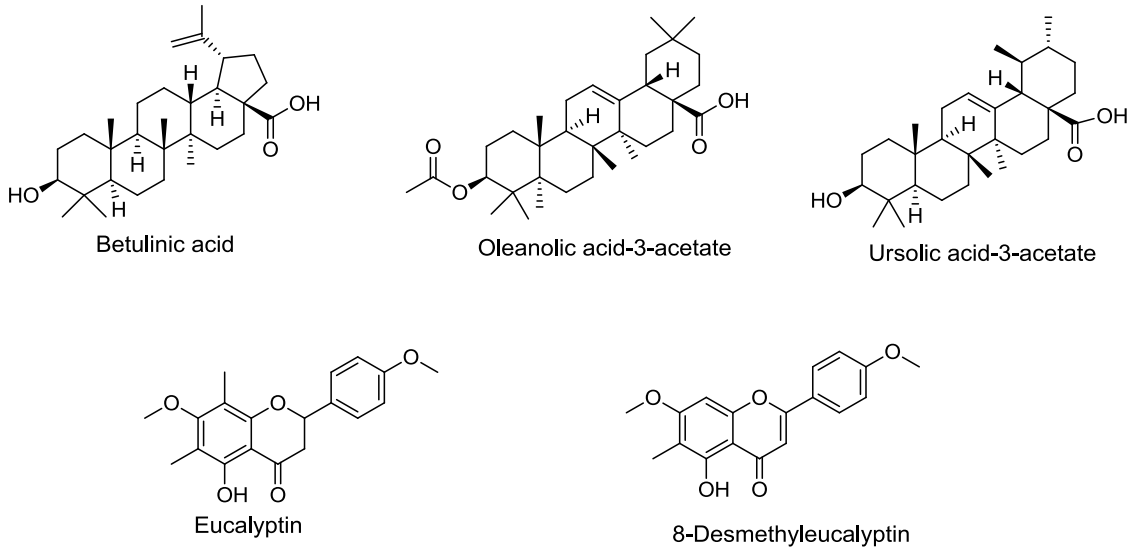

Figure 4.12: Compounds isolated from *S. glomulifera*

4.3 Chemical and biological studies on NSW medicinal plants

4.3.1 *Phytochemical profile and antibacterial and antioxidant activities of medicinal plants used by the Aboriginal people of New South Wales, Australia*

A manuscript entitled “Phytochemical profile and antibacterial and antioxidant activities of medicinal plants used by the Aboriginal people of New South Wales, Australia” was published in the journal of Evidence Based Complementary and Alternative Medicine.[79]

The literature review, chemical and biological studies and the data analysis on the selected eight plants were carried out by the author. The construction of the paper was done predominantly by the author and Dr Emma Barnes. The overall contribution of the author to the paper was 80%.

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2016, Article ID 4683059, 14 pages
http://dx.doi.org/10.1155/2016/4683059

Research Article

Phytochemical Profile and Antibacterial and Antioxidant Activities of Medicinal Plants Used by Aboriginal People of New South Wales, Australia

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Received 29 January 2016; Accepted 29 June 2016

Academic Editor: Laura De Martino

Aboriginal people of Australia possess a rich knowledge on the use of medicinal plants for the treatment of sores, wounds, and skin infections, ailments which impose a high global disease burden and require effective treatments. The antibacterial and antioxidant activities and phytochemical contents of extracts, obtained from eight medicinal plants used by Aboriginal people of New South Wales, Australia, for the treatment of skin related ailments, were assessed to add value to and provide an evidence-base for their traditional uses. Extracts of *Acacia implexa*, *Acacia falcata*, *Cassytha glabella*, *Eucalyptus haemastoma*, *Smilax glyciphylla*, *Sterculia quadrifida*, and *Syncarpia glomulifera* were evaluated. All extracts except that of *S. quadrifida* showed activity against sensitive and multidrug resistant strains of *Staphylococcus aureus* with minimum inhibitory concentration values ranging from 7.81 to 1000 μg/mL. The sap of *E. haemastoma* and bark of *A. implexa* possessed high total phenolic contents (TPC) and strong DPPH radical scavenging abilities. A positive correlation was observed between TPC and free radical scavenging ability. GC-MS analysis of the *n*-hexane extract of *S. glomulifera* identified known antimicrobial compounds. Together, these results support the traditional uses of the examined plants for the treatment of skin related ailments and infections by Aboriginal people of New South Wales, Australia.

1. Introduction

The Aboriginal people of Australia have over 40,000 years of knowledge of flora and fauna as sources of food, healing agents, and other resources [1]. Numerous plant species have been utilised as traditional medicines by Australian Aboriginal people [2], in particular for the topical treatment of sores, wounds, and skin infections, ailments which are especially common in Aboriginal communities [3]. For example, a retrospective review of the medical records of 99 children attending a primary healthcare centre in a remote area of the East Arnhem region in the Northern Territory of Australia found that by one year of age, 68% and 82% of the children had presented with their first case of scabies or streptococcal pyoderma (impetigo), respectively [4]. The use of plants for the treatment of such ailments indicates that they may provide extracts or pure compounds with antimicrobial or wound healing properties. However, to date, only a limited number of these plants have been investigated for their biological activities and/or chemical constituents [2, 3].

Problems associated with skin related infectious diseases and chronic wounds are not limited to Indigenous communities but are serious global threats [5]. It is well known that infection rates have increased and antibiotic resistance has become a growing therapeutic problem [6, 7]. In combination with bacteria being one of the most important

factors responsible for skin infection and delayed wound healing [8], low antioxidant levels are also associated with such diseases [9].

As part of a collaborative research program initiated upon the request of Yaegl Aboriginal people of Northern New South Wales (NSW) to help with conserving, analysing, and developing their medicinal knowledge for ecotourism and healthcare, we have ethnobotanically documented thirty-two Yaegl medicinal plants [10] and conducted preliminary biological and phytochemical studies [11, 12]. To extend this research program, we conducted a literature review in 2012 of 128 plants used as traditional medicines across NSW with regard to their distribution and habitat, documented traditional use, biological activity, and phytochemistry [3]. This review identified significant scope for further biological and chemical investigations of medicinal plants of NSW to add to the growing understanding of this resource. It also highlighted the paucity of community specific details in the published literature.

In recognition of the potential of traditional medicines for topical treatment of skin related ailments, a further literature review of medicinal plants documented in the Yaegl study [10] and NSW review [3] for these applications was conducted. This identified three plants, *Hibbertia scandens* (leaves), *Smilax glyciphylla* (leaves), and *Syncarpia glomulifera* (leaves), used by the Yaegl Aboriginal community, and five NSW plants, namely, *Acacia falcata* (bark), *Acacia implexa* (bark), *Cassytha glabella* (whole plant), *Eucalyptus haemastoma* (sap), and *Sterculia quadrifida* (leaves), for which limited or no biological and/or phytochemical studies had been undertaken. *Acacia implexa* (bark), *Acacia falcata* (bark), *Eucalyptus haemastoma* (sap), and *Sterculia quadrifida* (leaves) are reported to be used for the treatment of sores and skin complaints [3, 13], *Hibbertia scandens* (leaves) is used for the treatment of sores and rashes [10], *Cassytha glabella* (whole plant) is used for bathing (topically) to relieve pain and *Smilax glyciphylla* (leaves) to clear skin problems, aches, and pains [3, 10, 13], and sap and ash from the leaves of *Syncarpia glomulifera* are used as an antiseptic [11] (Table 1). *C. glabella* has been found to be a source of quercetin and anthocyanins [14], but no biological studies have been undertaken on this plant. *S. glyciphylla* possesses antioxidant activity and phenolic compounds have been isolated from its leaves [15]. *S. quadrifida* is reported to have moderate antifungal activity but there were no reports for phytochemical studies [16]. An antibacterial triterpenoid was isolated from the bark of *S. glomulifera* [17], as well as eucalyptin and 8-desmethyleucalyptin from its leaf wax [18]. The identification of essential oils from the leaves of *S. glomulifera* has also been undertaken [19]; however, there are no reports on the biological activity of extracts of its leaves. *A. falcata*, *A. implexa*, *H. scandens*, and *E. haemastoma* have had no reports for either biological or phytochemical studies.

In this study, 70% aqueous ethanolic extracts were prepared from the selected plants. The antibacterial activities of these extracts were determined using the MTT microdilution assay method and antioxidant activity by DPPH free radical scavenging, ABTS radical scavenging activity, and ferric reducing antioxidant power (FRAP) assay methods. Qualitative phytochemical screening and the quantification of the total phenolic, flavonoid, and tannin contents of the extracts were also undertaken. Furthermore, the *n*-hexane extract of *S. glomulifera* was chosen for gas chromatography-mass spectroscopy (GC-MS) analysis, which led to the identification of several known antimicrobial and antioxidant compounds.

2. Materials and Methods

2.1. Ethics. The research with the Yaegl Aboriginal Elders was approved by the Human Research Ethics Committee at Macquarie University (HE27 JUL2007-R05356 and 5201200763). It was conducted under the framework of best ethical practice, working in partnership with Indigenous people [62], and was governed by a cooperative research agreement with the Yaegl Community [63].

2.2. Collection of Plant Material. The leaves of *Syncarpia glomulifera*, *Hibbertia scandens*, and *Smilax glyciphylla*, bark of *Acacia implexa* and *Acacia falcata*, sap of *Eucalyptus haemastoma*, and whole plant of *Cassytha glabella* were collected and identified by plant taxonomist David Harrington. The leaves of *Sterculia quadrifida* were collected by botanist Robert Johnstone and identified by plant taxonomist Alison Downing. The plant samples of *A. implexa* and *A. falcata* were collected from Mulgoa, NSW; the samples of *C. glabella* and *S. glyciphylla* from Macquarie University's NSW Ecology Reserve; the samples of *E. haemastoma*, *H. scandens*, and *S. glomulifera* from Macquarie University's gardens; and *S. quadrifida* from Cudgen Nature Reserve, North Coast NSW. The GPS locations of the collection sites were recorded. Voucher specimens were deposited within the IBRG Herbarium, registered with the Index Herbariorum, New York, except for *S. quadrifida* which was lodged with the Macquarie University Herbarium. The collected plant materials (except the sap of *E. haemastoma*) were thoroughly washed under running tap water and air-dried at room temperature. The dried plant materials were ground into a fine powder using a coffee grinder. The hardened sap of *E. haemastoma* was collected by scraping it from the trunk of the tree; the sap was then directly extracted with solvent.

2.3. Preparation of Extracts. The powdered plant samples of *A. implexa*, *H. scandens*, *S. quadrifida* and *S. glomulifera* (0.7 L × 3, 24 h intervals), *A. falcata* (0.6 L × 3, 24 h intervals), *C. glabella* and *S. glyciphylla* (0.5 L × 3, 24 h intervals), and sap of *E. haemastoma* (0.2 L × 3, 24 h intervals) were each extracted with 70% aqueous ethanol at room temperature with occasional shaking (for plant sample amounts see Table 1). The extracts were filtered under vacuum through Whatman filter paper No. 1; then the solvent removed by evaporation using a Buchi rotary evaporator at 38°C before the crude samples were freeze-dried on a CHRIST alpha 1–4 LD plus (UK) freeze dryer. The quantities of the crude extracts obtained are given in Table 1.

Table 1: Summary of plants, traditional uses, parts used, and quantities of extracts obtained.

Plant name and family	Common names[a,b]	Distribution in Australia	Traditional use	Voucher number	GPS location of plant collection	Part extracted (g)	Extract yield (g/100 g dry wt)
A. falcata, Fabaceae	Hickory, lignum vitae, Sally	NSW, Qld[b]	Bark used for sores and skin complaints[b,c]	IBRG00013	−33.818587, 150.614472	Bark (95.4)	20.6
A. implexa, Fabaceae	Black wattle, lightwood, fish wattle, broad leaf wattle, scrub wattle, hickory, hickory wattle, Sally wattle	ACT, NSW, Qld, Tas, Vic[b]	Bark used for sores and skin complaints[b,c]	IBRG00014	−33.818587, 150.614472	Bark (162.0)	11.2
C. glabella, Lauraceae	Devil's twine, dodder laurel, slender devil's twine, slender dodder-laurel, smooth cassytha	NSW, Qld, SA, Tas, Vic, WA[b]	Whole plant used for bathing of body to relieve pain, rheumatism, and fever[b,c]	IBRG00015	−33.769473, 151.117169	Whole plant (26.2)	11.4
E. haemastoma, Myrtaceae	Scribbly gum, snappy gum, white gum	NSW[b]	Sap used for cuts, sores, wounds, ulcers, and dysentery[b,c]	IBRG00011	−33.771540, 151.119465	Sap (32.6)	65.0
H. scandens, Dilleniaceae	Yellow vine	NSW, Qld[d]	Used to treat sores and rashes (plant part used unknown)[e]	IBRG00017	−33.773865, 151.117391	Leaves (102.0)	9.8
S. glyciphylla, Smilacaceae	Native sarsaparilla, sweet sarsaparilla, smooth sarsaparilla	NSW, Qld[b]	Leaves topically used to clear skin problems[b,e], leaves and black fruits used for aches, pains, rheumatism, blood cleanser/tonic, sickness, cough, colds, congestion, and scurvy[f]	IBRG00012	−33.768539, 151.117406	Leaves (27.6)	17.0
S. glomulifera, Myrtaceae	Luster, red luster, turpentine, red turpentine	NSW, Qld[a]	Leaf ash and sap used as antiseptic[f]	IBRG00018, IBRG00019	−33.781832, 151.114339; −33.776060, 151.117111	Leaves (100.0)	24.0
S. quadrifida, Malvaceae	Kuman, orange fruited kurrajong, red fruited kurrajong, smooth seeded kurrajong, peanut tree, small flowered kurrajong	NSW, NT, Qld[b]	Leaves used to treat wounds, sores, skin complaints, sore eyes, and stings[b,c]	NSW 970302	−28.3526225, 153.564382	Leaves (80.2)	7.0

[a]Retrieved from http://bie.ala.org.au/; [b][3]; [c][13]; [d]retrieved from http://anpsa.org.au/; [e][10]; [f][11].
ACT: Australian Capital Territory; NSW: New South Wales; NT: Northern Territory; Qld: Queensland; SA: South Australia; Tas: Tasmania; Vic: Victoria.

2.4. Chemicals. All chemicals were of the highest purity (≥99.0%). Ferric chloride, Dragendorff's reagent, magnesium metal strips, gallic acid, ascorbic acid, catechin, Folin-Ciocalteu reagent, sodium carbonate, vanillin, aluminium chloride, phosphate buffer, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulfate, and ferric trichloride hexahydrate ($FeCl_3 \cdot 6H_2O$) were all purchased from Sigma Aldrich, USA. Hydrochloric acid (HCl), methanol, chloroform, 98% sulfuric acid, and glacial acetic acid were all analytical grade and purchased from Merck, Germany.

2.5. Phytochemical Analysis. Phytochemical screenings for alkaloids, flavonoids, steroids, terpenoids, tannins, saponins, and anthraquinones were conducted in accordance with published methods [64–66]. For alkaloids, 0.02 g of extract was stirred with 2 mL of 1% HCl on a steam bath and then filtered. A few drops of Dragendorff's reagent was used to treat 1 mL of filtrate. An orange precipitate indicated the presence of alkaloids. For flavonoids, 0.02 g of extract was dissolved in 1 mL of methanol. A chip of magnesium metal was added to the solution followed by the addition of a few drops of 11.6 M HCl. The occurrence of a magenta colour indicated the presence of flavonoids. For steroids, 0.02 g of extract was dissolved in 2 mL of chloroform and filtered (using Whatman No. 1 filter paper). 98% H_2SO_4 was carefully added to the filtrate. A reddish brown colour at the interface indicated the presence of steroids. For terpenoids, 0.02 g of extract was dissolved in 2 mL of methanol and filtered. Acetic anhydride (1 mL) was added to the filtrate and then 2 mL of concentrated H_2SO_4 was added carefully to the side of the tube. Formation of a reddish brown colour at the interface indicated the presence of terpenoids. For saponins, the frothing test was used. About 0.5 g of extract was mixed with 15 mL of Milli-Q water and shaken vigorously for 5 minutes. The formation of a stable froth indicated the presence of saponins. For tannins, 0.02 g of extract was dissolved in 2 mL of Milli-Q water and filtered. A few drops of 1% ferric chloride solution were added to the filtrate. Formation of a blue colour indicated the presence of tannins. Anthraquinone glycosides were detected using the Borntrager's test after hydrolysis of the extract with 10% hydrochloric acid. Chloroform was added to the hydrolysate and the contents were shaken and treated with 10% ammonia solution. The development of a pink colour indicated the presence of anthraquinone glycosides [67].

2.6. Total Phenolic Content of Extracts. The total phenolic content was determined using Folin-Ciocalteu reagent as reported by Muanda et al. [68] with slight modification. The samples were prepared at a concentration of 1.25 mg/mL in methanol. To 250 μL of extract (1.25 mg/mL in methanol), 3.5 mL of distilled water and 250 μL of Folin-Ciocalteu reagent were added and the solution was allowed to stand for 5 min. Next, 1.0 mL of 20% Na_2CO_3 solution was added to the mixture and the solution was left at room temperature for 1 h. Absorbance at 735 nm was read on a spectrophotometer as was that of a blank containing methanol. The phenolic content was calculated as gallic acid equivalent (GAE) by comparison with a calibration curve of gallic acid standard solutions (10–100 μg/mL) and was expressed as mg gallic acid equivalent per gram of dry extract. Data were reported as mean ± SD for three replicates.

2.7. Total Flavonoid Content of Extracts. Total flavonoid content was determined according to the aluminium chloride colorimetric assay with slight modification [68]. The samples were prepared at a concentration of 3.35 mg/mL in methanol. At time of 0 min, 250 μL of standard solution or extract was mixed with 1 mL of Milli-Q water and 75 μL of 5% $NaNO_2$. After 5 min, 75 μL of $AlCl_3$ (10%) was added to the solution and after 1 min, 500 μL of NaOH (1 M) was added to the solution. Then the total solution was made up to 2.5 mL by adding Milli-Q water and mixed thoroughly. Absorbance of the mixture, pink in colour, was determined at 510 nm versus the prepared blank. The total flavonoid content was calculated as catechin equivalent by comparison to a calibration curve of catechin standard solutions (10–100 μg/mL) and was expressed as mg catechin equivalent per gram of dry weight. Samples were analysed in three replications.

2.8. Total Condensed Tannin Content of Extracts. Total condensed tannin content was determined by the method described by Michel et al. [69] with slight modification. The samples were prepared at a concentration of 1.25 mg/mL in methanol. Sample solution (50 μL) was mixed with 3 mL of 4% vanillin in methanol followed by the addition of 1.5 mL of 11.6 M HCl. The well mixed solution was allowed to stand for 15 min and absorbance was measured at 500 nm against a blank. The total condensed tannin content was calculated as catechin equivalent after comparison with a calibration curve of catechin standard solutions (10–100 μg/mL) and was expressed as mg catechin equivalent per gram of dry extracts. Samples were analysed in three replications.

2.9. In Vitro Antioxidant Assays

2.9.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay. The antioxidant activities of the plant extracts were determined using the DPPH radical scavenging protocol described by Liu et al. [70]. The solutions of extracts were prepared at different concentrations (6.75–100 μg/mL) in methanol. DPPH solution (50 μL and 1 mM) in methanol was mixed with 200 μL of sample solution and the solution mixed well by shaking before being left standing at room temperature for 30 min in the dark. The absorbance was measured at 517 nm against the blank (methanol). Ascorbic acid at the same concentrations was used as the standard. All measurements were done in triplicate. The scavenging ability of the extracts was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)\right]}{\text{Abs}_{\text{control}}} \times 100. \quad (1)$$

From a plot of concentration against percentage of inhibition, a linear regression analysis was performed to determine the

IC_{50} value (the extract concentration that could scavenge 50% of the DPPH radicals).

2.9.2. ABTS Radical Cation Scavenging Activity Assay. The ABTS assay method was used as directed by Adedapo et al. [71]. A stock solution was prepared by mixing 7 mM $ABTS^{\bullet+}$ solution in water and 2.4 mM potassium persulfate solution in water in equal volumes and allowing the mixture to react for 12–16 h at room temperature in the dark so that it reached a stable oxidative state. The working solution was then prepared by diluting with methanol to an initial absorbance of 0.700 ± 0.020 ($Abs_{control}$) at 734 nm. The solution was prepared fresh for each analysis. The solutions of extracts were prepared at different concentrations (6.75–100 μg/mL) in methanol. Then 1 mL of sample solution was mixed with 1 mL of $ABTS^{\bullet+}$ solution and the absorbance was measured at 734 nm after 7 min against methanol as the blank. All measurements were done in triplicate. Trolox was used as a positive control. The percentage of scavenging inhibition capacity of $ABTS^{\bullet+}$ of the extract was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)\right]}{\left(\text{Abs}_{\text{control}}\right)} \times 100. \quad (2)$$

IC_{50} values of the plant extracts were also determined for $ABTS^{\bullet+}$.

2.9.3. Ferric Reducing Antioxidant Power (FRAP) Assay. The FRAP assay was carried out by following the method described by Wang et al. [72]. The FRAP reagent included 300 mM acetate buffer (3.1 g of CH_3COONa in 16 mL glacial acetic acid), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution in the ratio of 10 : 1 : 1 (v/v). The solutions of extracts were prepared at a final concentration of 0.2 mg/mL in methanol. Sample solution (400 μL) was mixed with 3 mL of freshly prepared FRAP solution and the solution incubated at 37°C in a water bath for 30 min. The absorbance of the samples was then measured at 593 nm. Trolox was used as a standard solution to draw the calibration curve in a concentration range of 10–100 μg/mL ($Y = 0.0056x + 0.0159$, $R^2 = 0.9993$). The FRAP results were calculated as mg of Trolox equivalent per gram extract. All experiments were done in triplicate.

2.10. In Vitro Antibacterial Activity

2.10.1. Microorganisms. The bacterial strains used included the Gram-positive bacterial strains, methicillin sensitive *Staphylococcus aureus* (MSSA, ATCC 29213), methicillin resistant *Staphylococcus aureus* (MRSA, ATCC BAA1026), and wild multidrug resistant *Staphylococcus aureus* (MDRSA, clinical isolate), and the Gram-negative bacterial strains, *Pseudomonas aeruginosa* (ATCC-27853) and *Escherichia coli* (ATCC 25922). All bacterial strains were kindly provided by Dr. John Merlino (Department of Microbiology, Concord Hospital, Sydney) and the work was approved by the Macquarie University Biosafety Committee (approval reference 08/06/LAB, *KAA110412BHA*).

2.10.2. Culture Media. Müller Hinton II (MH) broth (Bacto Laboratories Pty Ltd., Australia) was used for the growth of all the bacterial strains. All the culture media were prepared according to the manufacturer's instructions.

2.10.3. MTT Microdilution Assay. Minimum inhibitory concentrations (MIC) were determined using the MTT microdilution method as outlined by Appendino et al. with minor modification [73]. A solution of each sample (10 mg/mL) in 20% aqueous DMSO along with that of a suitable antibiotic (1 mg/mL, vancomycin for Gram-positive strains and gentamycin for Gram-negative strains) was prepared and serially diluted to give a final plant sample concentration of 2–1000 μg/mL and antibiotic concentration of 0.05 to 100 μg/mL in 96-well clear bottom microtitre plates. Test samples (20 μL) were inoculated with 175 μL of microbial culture (A_{600} = 0.08 diluted 100-fold in MH broth); a sterile broth control was included. A 20% DMSO control was also included and the plates were incubated at 37°C. After 18 hrs of incubation 5 μL of a methanolic solution (5 mg/mL) of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well and the plates further incubated at 37°C for 1 h to determine the MIC. MTT was used as an indicator of where microbial growth reduced the yellow tetrazolium bromide to a violet formazan. MIC was described as the lowest concentration of the test compounds that inhibited visible growth of the microorganisms (the last well showing no colour change of MTT from yellow to blue).

2.11. GC-MS Analysis of n-Hexane Extract of Syncarpia glomulifera. The 70% aqueous ethanol extract (6.0 g) of *S. glomulifera* leaves was partitioned with *n*-hexane (50 mL × 3), dichloromethane (50 mL × 3), ethyl acetate (50 mL × 3), *n*-butanol (50 mL × 3), and water (50 mL × 3) to give 950 mg, 1.0 g, 540 mg, 1.6 g, and 750 mg of each partition, respectively. The partitions were tested for their antibacterial activity against sensitive and resistant strains of *S. aureus*, *E. coli*, and *P. aeruginosa*. The *n*-hexane extract was selected for GC-MS analysis by gas liquid chromatography (GLC) and gas chromatography-mass spectrometry (GC-MS). GLC was carried out on a BP-20 column (60 m × 0.25 mm × 0.25 μm). The temperature program was 50°C (5 min) to 220°C (15 min) at 3°C/min with helium as the carrier gas. The temperature of the injector and that of detector were both set at 220°C. The BP-20 column (30 m × 0.35 mm × 0.25 μm), programmed from 35°C to 220°C at 3°C/min, was used for GC-MS with helium as the carrier gas and an injector temperature of 220°C for the column. Mass spectra were recorded in electron impact (EI) mode at 70 eV, scanning from 41 to 450 *m/z*. Compounds were identified by their identical GC retention times and retention indices relative to *n*-alkanes and by comparison of their mass spectra with either pure standards or published spectra in the NIST GC-MS library and those in the literature [56–61].

2.12. Statistical Analysis. All results are expressed as means ± standard deviation. Statistical analyses were performed using Microsoft Excel. The IC_{50} values were calculated by regression

Table 2: Qualitative phytochemical screening of plant extracts.

Plant	Alkaloids	Flavonoids	Steroids	Terpenoids	Tannins	Saponins	Anthraquinones
A. implexa	+	+	+	+	+	+	−
A. falcata	−	+	+	+	+	−	+
C. glabella	−	+	+	+	+	−	−
E. haemastoma	+	+	+	+	+	+	+
H. scandens	−	+	+	+	+	−	−
S. glyciphylla	−	+	+	+	+	−	−
S. quadrifida	−	+	+	+	+	−	−
S. glomulifera	−	+	+	+	+	−	−

+ = present; − = not present.

Table 3: Total phenol, flavonoid, and condensed tannin contents of plant extracts.

Plant	Total phenolic content (mg GAE/g plant extract)*	Total flavonoid content (mg CE/g plant extract)*	Total condensed tannin content (mg CE/g plant extract)*
A. falcata	451.67 ± 1.26	183.33 ± 6.04	39.86 ± 2.36
A. implexa	486.71 ± 9.90	133.97 ± 6.12	72.63 ± 5.03
C. glabella	275.52 ± 8.56	168.57 ± 0.35	29.41 ± 2.50
E. haemastoma	656.22 ± 5.07	172.4 ± 3.55	105.97 ± 5.29
H. scandens	174.66 ± 4.09	77.47 ± 3.96	21.97 ± 2.31
S. glyciphylla	243.47 ± 5.90	91.25 ± 4.85	14.67 ± 1.22
S. quadrifida	52.46 ± 0.63	70.5 ± 1.45	9.41 ± 2.04
S. glomulifera	171.41 ± 5.62	58.03 ± 2.15	17.41 ± 2.04

*Results are mean ± SD from three sets of independent experiments, each set in triplicate.

analysis. Values with $p < 0.05$ and $p < 0.01$ were considered statistically significant and very significant, respectively. The experimental results were compared by paired t-test (two sided).

3. Results and Discussion

3.1. Phytochemical Screening. Qualitative phytochemical tests of the 70% aqueous ethanol extracts of the eight plants showed the presence of alkaloids, terpenoids, flavonoids, steroids, saponins, tannins, and anthraquinones (Table 2). These classes of phytochemicals are known to possess a variety of biological activities including antimicrobial, antioxidant, anti-inflammatory, antiplasmodial, and anticancer activities [74–84]. These findings may partially justify the traditional use of the examined plants in the treatment of wound and skin infections and free radical mediated diseases and indicate that they may serve as a source of bioactive compounds against these illnesses.

3.2. Total Phenol, Flavonoid, and Condensed Tannin Contents. Phenolic compounds are effective hydrogen donors, making them good antioxidants [80]. Plant derived polyphenolic flavonoids are also well known to exhibit antioxidant activity. Flavonoids reduce free radicals by quenching, upregulating, or protecting antioxidant defences and chelating radical intermediate compounds [85]. It is also reported that tannins are 15–30 times more effective in quenching peroxyradicals than simple phenolics [86].

The phenolics and polyphenols are one of the largest groups of secondary metabolites to have exhibited antimicrobial activity [87]. The site(s) and number of phenol groups are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity [88]. Naturally occurring plant flavonoids have also been reported to possess antimicrobial activities [79, 89, 90]. The variation in the antibacterial activity of flavonoids is known to be related to their chemical structure, especially in regard to the number and positions of methoxy and phenolic groups within their structures [91–93]. The antimicrobial effects of tannins have also been widely recognised [94–96]. Therefore, the total phenolic, flavonoid, and condensed tannin contents of the eight plant extracts were examined to see if their traditional uses for the treatment of skin related ailments could be linked to the presence of these classes of compounds.

The results showed that the amount of total phenolic, flavonoid, and condensed tannin contents differed significantly ($p < 0.05$) among the extracts of the tested medicinal plants (Table 3, Figure 1). The total phenolic contents were determined as mg GAE/g extract on comparison with a standard gallic acid graph. Three extracts showed very high phenolic contents (>400 mg GAE/g): *E. haemastoma*, *A. implexa*, and *A. falcata* with values of 656.2 ± 5.1, 486.7 ± 9.9, and 451.7 ± 1.3 mg GAE/g of extract, respectively.

Table 4: Antioxidant activities of plant extracts.

Plant	DPPH IC_{50} (μg/mL)	ABTS IC_{50} (μg/mL)	FRAP (μmol Trolox/g)
A. falcata	217.03 ± 3.80	111.47 ± 0.88	1991.46 ± 2.73
A. implexa	130.20 ± 5.37	107.05 ± 1.38	2913.87 ± 6.76
C. glabella	255.23 ± 2.32	203.46 ± 1.25	1796.22 ± 4.58
E. haemastoma	51.99 ± 1.17	61.72 ± 0.53	6189.64 ± 9.45
H. scandens	348.69 ± 2.90	321.03 ± 3.46	1635.51 ± 5.94
S. glyciphylla	439.33 ± 2.05	351.46 ± 1.98	185.80 ± 5.85
S. quadrifida	2190.13 ± 2.16	1824.96 ± 4.26	722.41 ± 6.25
S. glomulifera	235.86 ± 3.50	287.98 ± 1.75	1522.11 ± 4.92
Ascorbic acid	71.58 ± 0.99		
Trolox		231.90 ± 1.76	

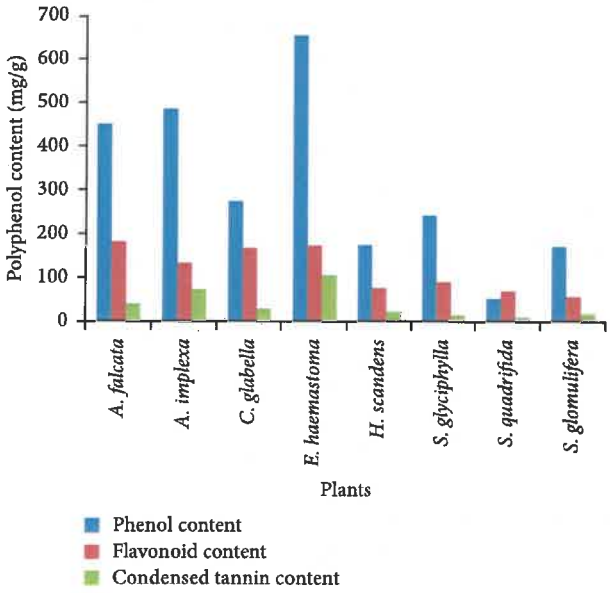

Figure 1: Polyphenolic contents (mg/g) of plant extracts.

C. glabella and *S. glyciphylla* showed reasonable phenolic contents (>200.0 mg GAE/g) of 275.5 ± 8.6 and 243.5 ± 5.9 mg GAE/g of extract. *S. quadrifida* showed the lowest phenolic content at 52.5 ± 0.6 mg GAE/g extract. The total flavonoid content was determined as mg CE/g extract after comparison with a catechin standard graph. The highest total flavonoid content was identified for *A. falcata* at 183.3 ± 6.0 mg CE/g of extract and the lowest for *S. glomulifera* at 58.0 ± 2.2 mg CE/g of extract. The total condensed tannin content was evaluated as mg CE/g of extract after comparison with a catechin standard graph. *E. haemastoma* showed the highest condensed tannin content at 106.0 ± 5.3 mg CE/g of extract and *S. quadrifida* the lowest at 9.4 ± 2.0 mg CE/g of extract.

The results revealed that the level of phenolic compounds and condensed tannins was the highest in the 70% ethanolic extracts from the sap of *E. haemastoma* and bark of *A. falcata* and *A. implexa*. These results were significantly higher than that of the leaves of the other plants investigated.

3.3. *In Vitro* Antioxidant Activity

3.3.1. DPPH Radical Scavenging Activity. The results of the free radical scavenging activity of the extracts are shown in Table 4. The dose-response curves of the DPPH radical scavenging activities of the eight plant extracts were compared with that of ascorbic acid (Figure 2). In the DPPH assay, all extracts examined except for that of *S. quadrifida* showed radical scavenging activity in a concentration dependent manner and were significantly different ($p < 0.01$). This result agreed with an earlier report by Motalleb et al. [97] that showed that the scavenging effects on the DPPH radical increase sharply with increasing concentration of the samples and standards. The highest antioxidant activity was obtained for the extract of *E. haemastoma* (IC_{50} 52.0 ± 1.2 μg/mL and standard ascorbic acid IC_{50} 71.6 ± 1.0 μg/mL).

3.3.2. $ABTS^{\bullet+}$ Scavenging Activity. The antioxidant activities of the plant extracts towards $ABTS^{\bullet+}$ were also determined (Table 4, Figure 2). All extracts showed the ability to neutralise the radical cation $ABTS^{\bullet+}$, with significant differences at $p < 0.01$. The highest activity was obtained for the *E. haemastoma* extract with IC_{50} value of 61.7 ± 0.5 μg/mL, followed by *A. implexa* and *A. falcata* with IC_{50} values of 107.1 ± 1.4 μg/mL and 111.5 ± 0.9 μg/mL, respectively. These extracts could be seen to be rapid and effective scavengers of the $ABTS^{\bullet+}$ radical (Figure 2) and their activities were comparable with that of Trolox.

3.3.3. Ferric Reducing Antioxidant Power (FRAP) Assay. The FRAP assay was used to evaluate the antioxidant properties of the extracts based on their ability to reduce ferric (III) to ferrous (II). The results obtained from the extracts (Table 4) were significantly different ($p < 0.01$). For this assay it was also found that the extract of *E. haemastoma* provided the highest antioxidant activity with a FRAP value of 6189 ± 9.5 μmol Trolox equivalent/g, followed by *A. implexa* and *A. falcata* with FRAP values of 2913 ± 6.8 and 1991 ± 2.7 μmol Trolox equivalent/g, respectively.

Based on the results of all three assays, it can be seen that the sap extract of *E. haemastoma* and bark extracts of *A. implexa* and *A. falcata* possess the strongest free radical scavenging activities and reducing capacities of all the plant

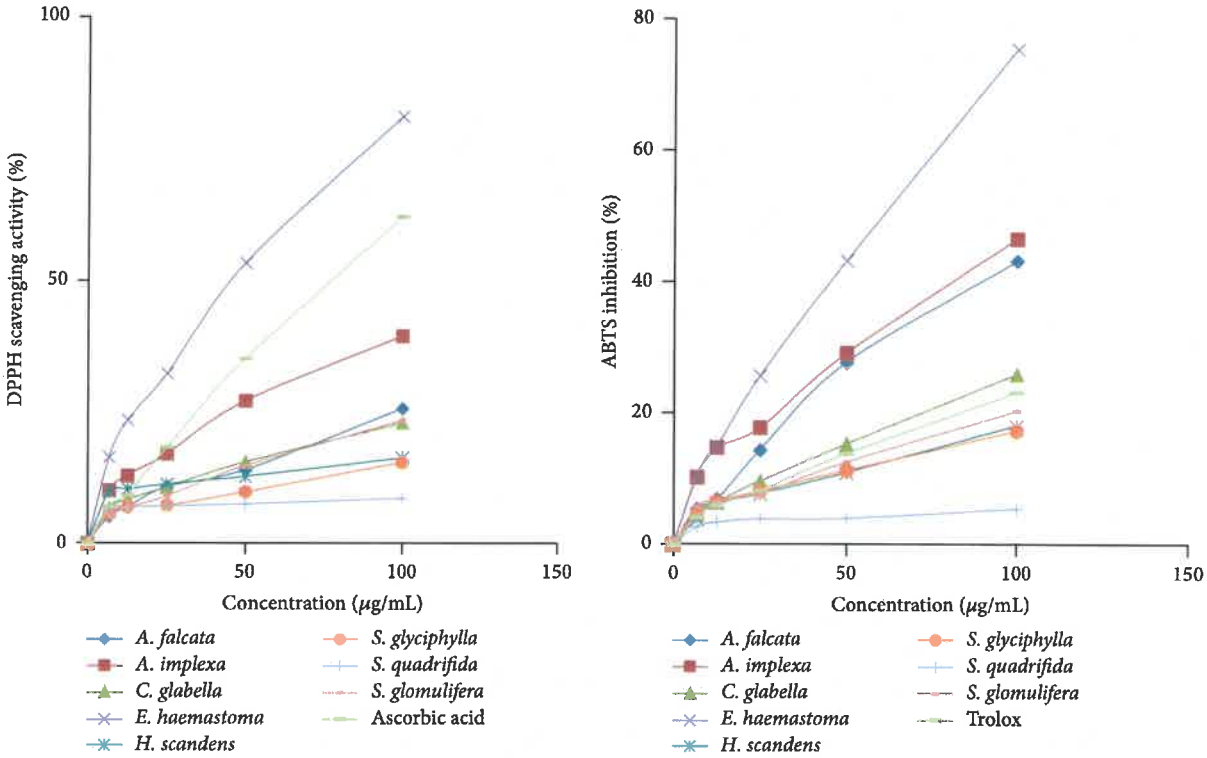

FIGURE 2: DPPH and ABTS scavenging activities of plant extracts.

extracts analysed, indicating that they may be useful for treating free radical-related diseases. The scavenging of the $ABTS^{\bullet+}$ radical by the extracts was found to be higher than that for the DPPH radical. It is well known that the antioxidant activity of a plant extract largely depends on both its composition and the test system [72].

3.4. Correlation between the Total Phenolic and Flavonoid Contents and Antioxidant Activities. Table 5 shows the correlations (linear regression coefficients, R^2) between the total phenolic contents (TPC) and total flavonoid contents (TFC) and the antioxidant assay results for the plant extracts. All the antioxidant assay results showed very good correlation ($R^2 > 0.9$) with the TPC and TFC values except for that of *S. quadrifida*, which showed poor correlation between ABTS and TFC ($R^2 = 0.7953$). The significant correlations between the antioxidant properties and TPCs and TFCs of the extracts may indicate that the phenolic and flavonoid type compounds contained within the plant extracts are the major contributors to their antioxidant properties.

3.5. Antibacterial Activities. The 70% aqueous ethanol extracts of the plants were tested for their antibacterial activity using the MTT microdilution assay method against three Gram-positive (*S. aureus* (MSSA) ATCC 29213, *S. aureus* (MRSA) ATCC BAA 1026, *S. aureus* (MDRSA)) and two Gram-negative (*E. coli* β-lactamase negative ATCC 25922 and *P. aeruginosa* ATCC 27853) bacterial strains. The minimum inhibitory concentration (MIC) values for the extracts are shown in Table 6. None of the extracts showed activity against the Gram-negative bacterial strains, even at a concentration of 1 mg/mL. Seven of the eight extracts showed activity against the sensitive and/or resistant strains of *S. aureus* at MIC values of ≤1 mg/mL, except for *S. quadrifida* which did not show any activity even at a concentration of 1 mg/mL.

According to Ríos and Recio [98] extracts possessing an MIC value equalling or less than 1000 μg/mL are considered to be active and worthy of further investigation. The *S. glomulifera* extract showed the greatest activity against sensitive and resistant strains of *S. aureus* with MIC values of 7.81 μg/mL against all three strains, followed by *E. haemastoma* and *A. implexa* with MIC values of 62.5 μg/mL and 125 μg/mL, respectively, against the sensitive strain of *S. aureus*.

It is well known that phenolic compounds present in plant extracts play an important role in their antimicrobial effects [99]. Phytochemical screening of the extracts in this study showed that *E. haemastoma*, *A. implexa*, and *A. falcata* possess a high content of phenolic compounds. It has also been reported that the active constituents in *A. implexa* and *A. falcata* include tannins [99]. Therefore, it can be inferred that the antibacterial activities of these three plants may be due, at least in part, to their high phenolic and tannin contents. This is the first report of the antibacterial activities of all of these eight medicinal plants against sensitive and resistant strains of *S. aureus*. The promising antibacterial activities of the extracts provides preliminary support for the traditional uses of these plants for the treatment of skin and wound infections.

Table 5: Correlation values (R^2) between the antioxdant activities and total phenolic and total flavonoid contents of the plants extracts.

Plant	R^2 (DPPH)	R^2 (ABTS)	R^2 (FRAP)
A. falcata			
TPC	0.9984	0.9456	0.9844
TFC	0.9881	0.9728	0.9969
A. implexa			
TPC	0.9768	0.9749	0.9832
TFC	0.9157	0.9190	0.9025
C. glabella			
TPC	0.9695	0.9492	0.9675
TFC	0.9431	0.9167	0.9405
E. haemastoma			
TPC	0.9989	0.9969	0.9981
TFC	0.9737	0.9946	0.9926
H. scandens			
TPC	0.9987	0.9999	0.9675
TFC	0.9915	0.9857	0.9806
S. glyciphylla			
TPC	0.9991	0.9967	0.9977
TFC	0.9888	0.9938	0.9922
S. glomulifera			
TPC	0.9962	0.9939	0.9963
TFC	0.9993	0.9981	0.9993
S. quadrifida			
TPC	0.9961	0.9357	0.9988
TFC	0.9261	0.7953	0.9683

TPC: total phenolic content; TFC: total flavonoid content.

Table 6: Antibacterial activities of plant extracts.

Plant	MIC (μg/mL)		
	S. aureus (MSSA)	*S. aureus* (MRSA)	*S. aureus* (MDRSA)
A. falcata	250	1000	1000
A. implexa	125	250	250
C. glabella	500	1000	1000
E. haemastoma	62.5	125	125
H. scandens	500	1000	1000
S. glyciphylla	1000	na	na
S. quadrifida	na	na	na
S. glomulifera	7.81	7.81	7.81
Vancomycin	0.002	0.002	0.002

na: not active at concentration of 1 mg/mL. MIC: minimum inhibitory concentration.

The extract of *S. glomulifera* leaves showed the highest antibacterial activity with MIC values of 7.81 μg/mL against the methicillin sensitive, methicillin resistant, and multidrug resistant strains of *S. aureus*. Therefore, this extract was chosen for further investigation. The crude 70% aqueous ethanol extract was partitioned with *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol, and water and the partitions tested for their antibacterial activity against sensitive and resistant strains of *S. aureus*, *E. coli*, and *P. aeruginosa* (Table 7). Among the five partitions, the *n*-hexane extract showed the greatest antibacterial activities against sensitive and resistant strains of *S. aureus* with MIC values of 7.81 μg/mL against all three strains. None of the extracts showed antibacterial activity against *P. aeruginosa*, and only the *n*-hexane partition showed activity against *E. coli* at a concentration of 1 mg/mL. As the *n*-hexane extract showed the greatest activity, it was chosen for GC-MS analysis to further explore its chemical constituents.

Table 7: Antibacterial activities of *Syncarpia glomulifera* partitions.

Extracts	MIC (μg/mL)			
	S. aureus (MSSA)	*S. aureus* (MRSA)	*S. aureus* (MDRSA)	*E. coli*
n-Hexane	7.81	7.81	7.81	1000
Dichloromethane	31.25	31.25	125	na
Ethyl acetate	na	na	na	na
n-Butanol	1000	1000	1000	na
Water	1000	na	na	na
Vancomycin	0.002	0.002	0.002	NT
Gentamycin	NT	NT	NT	1.69

na: not active at concentration of 1000 μg/mL. NT: not tested. MIC: minimum inhibitory concentration.

3.6. GC-MS Analysis of n-Hexane Extract of S. glomulifera. GC-MS analysis of the *n*-hexane extract of *S. glomulifera* showed that it predominantly contained monoterpene hydrocarbons (α-phellandrene, *p*-cymene, terpinolene), oxygenated monoterpenes (terpinen-4-ol, α-terpineol), sesquiterpene hydrocarbons (α-copaene, β-elemene, aromadendrene, alloaromadendrene, α-selinene, β-selinene, bicyclogermacrene, and viridiflorene), and oxygenated sesquiterpenes (spathulenol, cubenol, epicubenol, cubeban-11-ol, palustrol, epiglobulol, globulol, ledol, and viridiflorol) (Table 8). These phytoconstituents are in accordance with a previous report on the chemical composition of the leaf essential oil of *S. glomulifera*, but they are present in different concentrations [19]. This could be due to seasonal variation, the different collection sites, variances in the extraction processes, or other factors [100].

α-Phellandrene, α-copaene, aromadendrene, terpinen-4-ol, α-terpineol, palustrol, epiglobulol, cubenol, globulol, and spathulenol have been reported to have antibacterial activity against Gram-positive bacteria [20, 28, 31–33, 42, 43, 47, 52] and the presence of these bioactive phytoconstituents could be contributing to the strong antibacterial activity of the *n*-hexane extract. Bicyclogermacrene is reported to be a major component of the antibacterial essential oil from *Zanthoxylum rhoifolium* [39]. In addition, α-phellandrene, *p*-cymene, terpinolene, α-copaene, aromadendrene, terpinen-4-ol, alloaromadendrene, α-terpineol, palustrol, ledol, epicubenol, globulol, viridiflorol, and spathulenol are known to possess other biological activities relevant to skin related ailments including antifungal, antioxidant,

Table 8: GC-MS analysis of *n*-hexane extract of *S. glomulifera* on BP-20 column, phytoconstituents identified and their known biological activities.

Compounds[1]	LRI values	% of identified compounds	Known biological activities
α-Phellandrene	1166	0.93	Antibacterial [20], antifungal [20], antioxidant [21], larvicidal [22]
p-Cymene	1269	0.22	Antifungal [23–26], antioxidant [21]
Terpinolene	1282	0.08	Antioxidant [21], antiviral [27], larvicidal [22]
α-Copaene	1499	0.10	Antibacterial [28], antidermatophytic [29]
β-Elemene	1600	0.10	Anticancer [30]
Aromadendrene	1603	2.33	Antibacterial [31], antioxidant [21]
Terpinen-4-ol	1613	0.18	Antibacterial [32, 33], antifungal [33, 34], antioxidant [21], antiseptic [35], antiviral [27, 36]
Alloaromadendrene	1643	0.64	Antineoplastic [37], antioxidant [38]
Viridiflorene	1681	0.13	None found
Geranial	1685	0.92	None found
α-Terpineol	1696	0.41	Antibacterial [33], antifungal [33, 34], antiviral [27, 36]
β-Selinene	1715	0.23	None found
α-Selinene	1722	0.26	None found
Bicyclogermacrene	1733	0.60	Antibacterial [39]*, antitumor [40]*, cytotoxic [41]
Palustrol	1931	0.26	Antibacterial [42], antifungal [42], antitumor [42]
Cubeban-11-ol (cis)	2012	0.27	None found
Epiglobulol	2018	1.08	Antibacterial [43], uterus relaxant [44]
Ledol	2034	0.95	Antimicrobial [45], anti-inflammatory [45], antineoplastic [37]
Cubenol	2058	0.70	Antibacterial [28]
Cubeban-11-ol (trans)	2064	0.83	None found
Epicubenol	2070	0.36	Antifungal [46]
Globulol	2080	5.31	Antibacterial [43, 47], antifungal [47], antioxidant [48], sedative and anaesthetic [49]
Viridiflorol	2088	1.88	Acetylcholinesterase inhibitory [50], antifungal [51]
Spathulenol	2129	0.96	Antibacterial [52], anticancer [53], anti-inflammatory [54]*, immunomodulatory [55], uterus relaxant [44]

[1]The compounds were identified by their GC retention times and linear retention indices relative to *n*-alkanes and by comparison of their mass spectra with pure standards or published literature data [56–61]. *Major components of essential oils with biological activities.

anti-inflammatory, and antiseptic activities [20, 21, 23–26, 29, 33–35, 38, 42, 45–48, 51, 54].

4. Conclusion

Our study has shown that extracts of *E. haemastoma*, *A. implexa*, *A. falcata*, and *S. glomulifera* contain antioxidant and antibacterial compounds. The highest *in vitro* antioxidant activity of the plant extracts was found for *E. haemastoma*, with results comparable with that of the standard compound, ascorbic acid. *S. glomulifera* and *E. haemastoma* presented the best antibacterial activities against methicillin sensitive, methicillin resistant, and multidrug resistant strains of *S. aureus*, with MIC values between 7.81 and 125 μg/mL. GC-MS analysis of the *n*-hexane extract of *S. glomulifera* revealed the presence of antioxidant and antibacterial compounds. Thus, the results of this study support the use of these plants as traditional medicines for the treatment of skin related ailments including sores, wounds, and skin infections by New South Wales Aboriginal people.

Competing Interests

The authors declare that they have no conflict of interests.

Acknowledgments

The authors thank the Yaegl Community Elders, in particular, Ronald Heron, Jessie Randall, Della Walker, Lillian Williams, Judith Breckenridge, Carmel Charlton, Darren King, Rosemarie Vesper, Muriel Burns, Beatrice Heron, Owen Kapeen, Thelma Kapeen, Eileen McLeay, Glenda McPhail, Lester Mercy, Lenore Parker, Irene Randall, Kevin Randall, and Lenny Waters, for sharing their knowledge on behalf of the Yaegl Community. The authors wish to thank Associate Professor Paul Prenzler and Mr. Daniel Bucio Noble for

providing training on the antioxidant assays. The authors would also like to acknowledge Dr. John Merlino for providing the microbial strains. This project would have not been possible without the financial support provided by Macquarie University in the form of a Ph.D. scholarship for Kaisarun Akter and funding from the National Health and Medical Research Council (NH&MRC, #488504, and #1028092).

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4.3.2 Biological Studies on *Sterculia quadrifida*

As described in Section 4.2.7, leaves of *Sterculia quadrifida* are widely used by the NSW Aboriginal people for the treatment of skin related ailments, including wounds. There are, however, no published reports of chemical studies on this plant and there is only one biological study published. The water partition (following partitioning of methanol extract with ethyl acetate and water) of leaves of *S. quadrifida* has been reported to be active against *Micrococcus luteus, Candida albicans, Staphylococcus aureus* and methicillin resistant strains of *S. aureus* and to be bacteriostatic against *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhimurium* at 10 mg/mL.[1] Due to the use of *S. quadrifida* for treatment of skin related ailments and the limited chemical and biological investigations reported, it was selected for further exploration in this PhD study.

4.3.2.1 Plant collection and extraction

Leaves of *S. quadrifida* were collected in two batches from two different locations to compare the biological assay results. The first collection was carried out in December 2012 from Cumberland State Forest, Sydney and the second collection was in March 2013 from Cudgen Nature Reserve, Northern NSW, Australia. The first collection was done by botanist Mr David Harrington and the second collection was done by Royal Botanical Garden staff of Cudgen Nature Reserve, North Coast, NSW.

The leaves (1st collection from Cumberland State Forest, NSW) were carefully washed and air dried and ground into a coarse powder by using a coffee grinder. The extraction was carried out in a similar manner to that described by Smyth.[1] The prepared plant material (80.3 g) was extracted with methanol (3 x 500 mL, every 24 hr) at room temperature with shaking at 150 rpm, followed by vacuum filtration. The filtrates were then evaporated by using a rotary evaporator at 38°C and freeze dried under vaccum to afford the crude methanol extract (5.5 g, dark green). A fraction of the methanol extract was kept for bioassays and a portion of the extract (2.32 g) was suspended in water (50 mL) and partitioned with ethyl acetate (50 mL × 3). The water partition was freeze dried and the ethyl acetate partition rotary evaporated to afford the water partition (1.81 g, light green) and the ethyl acetate partition (0.41 g, dark green). The leaves (145.1 g) from the 2nd collection (Cudgen Nature Reserve, North Coast, NSW) were washed, air dried, ground to a coarse powder and extracted with methanol (700 mL × 3) to give the methanol extract (10.1 g, dark green). A portion of the methanol extract (5.0 g) was suspendend in 100 mL water, partitioned with ethyl acetate (100 mL × 3), and evaporated/dried as above, to give the water partition (2.9 g, light green) and ethyl acetate partition (1.6 g, dark green). A portion of the methanol extract (3.0 g) from the second collection was also sequentially extracted with *n*-hexane (50 mL × 3), dichloromethane (50 mL × 3), ethyl acetate (50 mL × 3) and water to give after

evaporation the *n*-hexane (680 mg, dark green), dichloromethane (60 mg, green), ethyl acetate (20 mg, light green) and water (1.2 g, brown) partitions. These extracts and partitions were screened for antimicrobial activity.

4.3.2.2 Antimicrobial screening

The MTT microdilution assay method[80] was used to evaluate the antimicrobial activity of the partitions and extracts. This is described in the preceding manuscript. The *S. quadrifida* extracts and partitions were screened against the Gram positive bacterial strains methicillin sensitive *Staphylococcus aureus* and methicillin resistant *S. aureus*, the Gram negative bacterial strain *Escherichia coli*, and the fungal strain *Candida albicans.*

4.3.2.3 Results and discussion

None of the *S*. *quadrifida* extracts or partitions showed activity against any of the tested cultures at a concentration of 1 mg/mL. The first extraction and partitioning of the leaves was carried out in an identical manner to the literature method (*i.e.* methanol extract followed by ethyl acetate-water partition).[1] The literarture reported that the water partition of the leaves possessed antibacterial activity against *S. aureus* and methicillin resistant strains of *S. aureus* and antifungal activity against *C. albicans* at a concentration of 10 mg/mL.[1] We did not test at this concentration as Rios and Reico[81] and Gibbons[82] have stated that extracts displaying antimicrobial activity with MIC values >1 mg/mL have little relevance in terms of clinical application and should be avoided for further investigation. Partitioning of the methanol extract between water and *n*-hexane, dichloromethane and ethyl acetate was done to try to concentrate the active components. However, no antimicrobial activity was observed for any of these partitions at 1 mg/mL.

Although the *S. quadrifida* extracts and partitions did not show any activity against the tested antimicrobial strains at the concentrations tested, this does not discount the plant extracts being active in other biological screening aligned with its traditional uses. As this plant is widely used by the NSW Aboriginal people for the treatment of sores, wounds and skin infections, it would be worthy to explore the plant in more detail in future studies.

4.3.3 Phytochemical and biological studies on *Syncarpia glomulifera n*-hexane partition

As described in the submitted manuscript, *Syncarpia glomulifera* was found to be one of the most active of the species investigated, with the 70% aqueous ethanol extract (MIC 7.81 µg/mL) and *n*-hexane (MIC 7.81 µg/mL), dichloromethane (MIC 31.25 µg/mL) and *n*-butanol (1 mg/mL) partitions active against methicillin sensitive, methicillin resistant

and multidrug resistant strains of *S*. aureus. Its *n*-hexane partition also showed many known biologically active molecules present when examined by GC-MS. Due to these promising features, *S. glomulifera* was further investigated chemically. As detailed in the manuscript, *S. glomulifera* leaves (100.0 g) were extracted with 70% ethanol (500 mL × 3) to obtain 24.0 g of dry crude extract. 6.0 g of the crude extract was suspended in 50 mL of water and was partitioned with *n*-hexane (50 mL × 3), dichloromethane (50 mL × 3), ethyl acetate (50 mL × 3) and *n*-butanol (50 mL × 3) to obtain the *n*-hexane (950 mg), dichloromethane (1.0 g), ethyl acetate (540 m mg), *n*-butanol (1.57 g) and water (746 mg) partitions. The *n*-hexane partition (500 mg) was dry loaded onto a normal phase silica gel column (70 g, 0.040 – 0.063 mm, Merck Germany) and eluted with mixtures of *n*-hexane:ethyl acetate (100:0 to 0:100). Fifty-three fractions were collected and combined to give nine fractions (F1 - F9, in order of elution) according to their R_f values. The fractions were tested against methicillin sensitive strains of *S. aureus* by using the TLC bioautography assay method as described in Chapter three[83,84] to detect the bioactive compounds in the chromatogram. TLC bioautography identified six (F1 and F4–F8) of the eight fractions as antibacterially active against methicillin sensitive *S. aureus* (Figure 4.13).

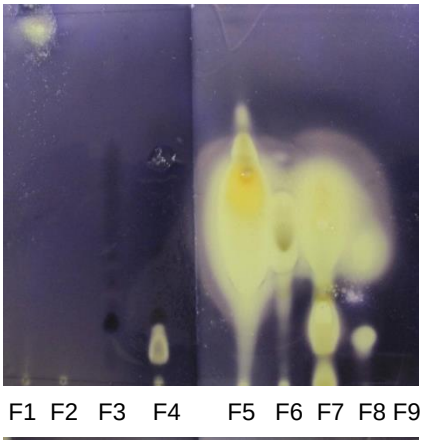
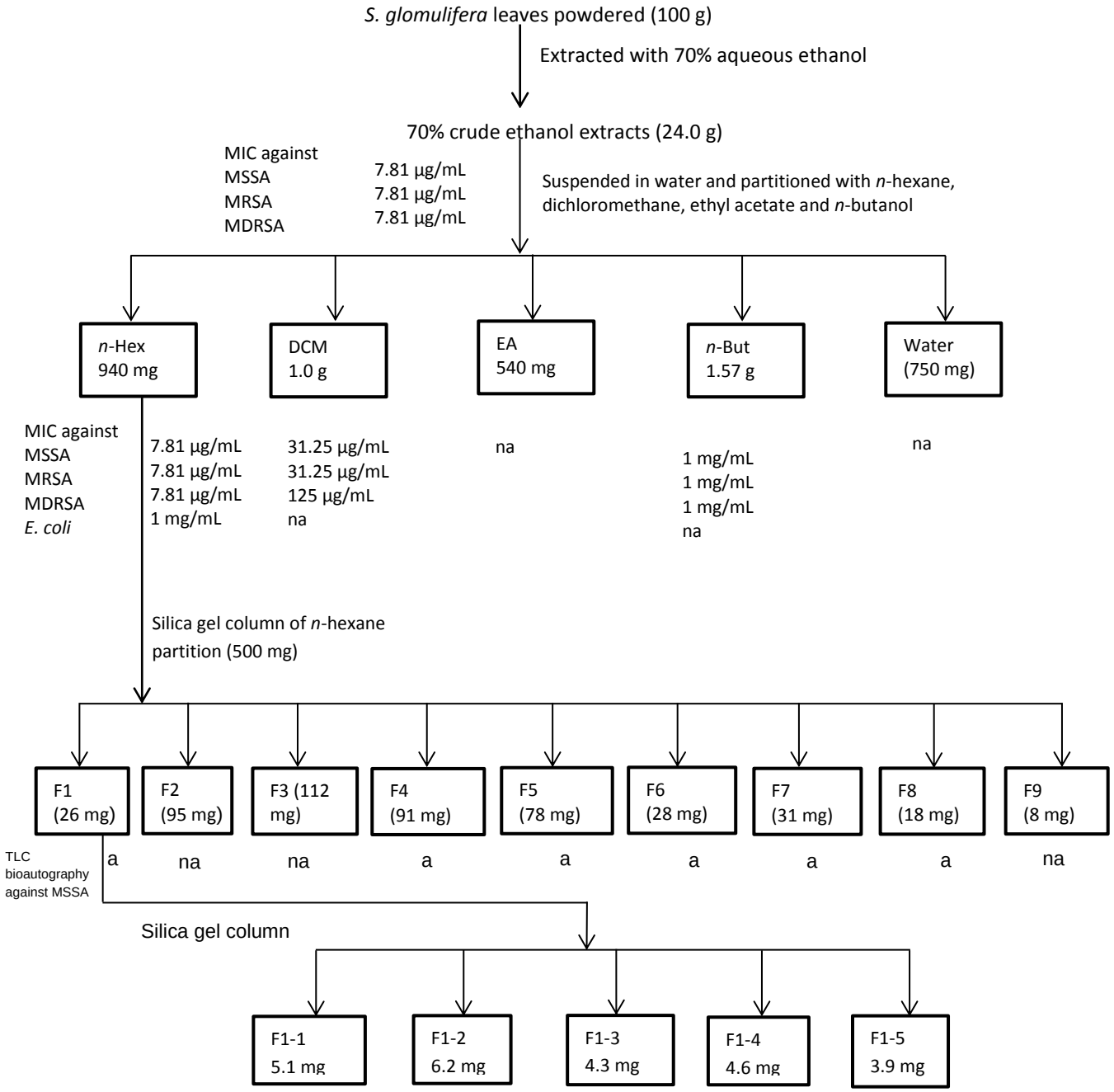


Figure 4.13: TLC bioautography of *n*-hexane column fractions (clear zones indicate active compounds)

As the TLC of fraction F1 showed one moderately clean spot and TLC bioautography showed that the spot was active against methicillin sensitive *S*. *aureus,* this fraction was chosen for further purification. 15 mg of fraction F1 was dry loaded on to a normal phase silica gel column (30 mg, 0.040 – 0.063 mm, Merck Germany) and eluted with *n*-hexane:ethyl acetate (100:0 to 70:30). Twenty fractions were collected and combined to give five fractions (F1-1– F1-5) according to their R_f values. F1-5 was found to be semipure by ^{1}H NMR analysis, but due to time constraints was not further purified.

Other active large scale column fractions were also not examined further due to time constraints. A summary of fractionation of *S*. *glomulifera* is shown in Figure 4.14.



MIC: minimum inhibitory concentration; a: active; na: not active; MSSA: methicillin sensitive *S. aureus;* MRSA: methicillin resistant *S. aureus*, multidrug resistant *S. aureus*; *n*-Hex: *n*-hexane; DCM: dichloromethane; EA: ethyl acetate; *n*-But: *n*-butanol

Figure 4.14: Bioassay guided fractionation of *S. glomulifera* leaves

4.4 Conclusions

A literature review of NSW medicinal plants used for the treatment of skin related ailments by Aboriginal people of NSW was conducted to identify medicinal plants with significant potential for chemical and biological studies. This review resulted in the identification of thirty two plants with none or limited chemical and biological studies aligned with their traditional uses. Out of the thirty two medicinally important NSW plants eight plants *i.e Acacia implexa*, *Acacia falcata*, *Cassytha glabella*, *Eucalyptus haemastoma*, *Hibbertia scandens*, *Smilax glyciphylla*, *Sterculia quadrifida* and *Syncarpia glomulifera* were selected for further investigations. The plant materials were extracted with 70% aqueous ethanol and qualitative and quantitative phytochemical screening of the extracts were conducted. The antimicrobial activity of the extracts against methicillin sensitive, methicillin resistant and multidrug resistant strains of *S. aureus* and antibiotic sensitive strains of *E. coli* and *P. aeruginosa* was evaluated by the MTT microdilution assay and antioxidant activity was evaluated by the DPPH free radical scavenging, ABTS radical scavenging activity and FRAP assay methods.

The qualitative phytochemical screening of the crude extracts revealed the presence of alkaloids, terpenoids, flavonoids, steroids, saponins, tannins and anthraquinone glycoside classes of compounds within the extracts. These groups of compounds are well known for various biological activities including antimicrobial, antioxidant, anti-inflammatory, antiplasmodial and anticancer. The phenolics and polyphenolics are a group of secondary metabolites known to have antimicrobial and antioxidant activities.[85,86] The quantitative phytochemical screening of the extracts showed that *E. haemastoma*, *A. implexa* and *A. falcata* possessed very high phenolic content. The highest flavonoid contents were identified for *A. falcata*, followed by *E. haemastoma.* The highest total condensed tannin contents were possessed by *E. haemastoma*. The lowest amount of total phenolics, total flavonoids and total condensed tannin contents were identified for *S. quadrifida.*

The antioxidant activity evaluated by the three methods showed that the sap extract of *E. haemastoma* and bark extracts of *A. falcata* and *A. implexa* possessed the highest activity. A significant correlation between the antioxidant properties and the total phenolic and flavonoid contents was observed. This indicates that the phenolic and flavonoid type of compounds present in the extracts may be the major contributors to their antioxidant properties. This is the first report of antioxidant activity of *A. falcata*, *A. implexa*, *E. haemastoma*, *H. scandens*, *C. glabella*, *S. quadrifida* and *S. glomulifera.*

The crude extracts of the plants were screened to evaluate their antibacterial activity against methicillin sensitive strains*,* methicillin resistant strains and multidrug resistant strains of *S. aureus,* antibiotic sensitive *E. coli*, *P. aeruginosa* and *Candida albicans*. None of the extracts showed activity against *C. albicans*. Seven of the eight

extracts showed antibacterial activity against sensitive and/or resistant strains of *S. aureus*, except for *S. quadrifida* which did not show any activity at MIC values of ≤ 1 mg/mL. *S. glomulifera* showed the highest antibacterial activity with MIC values of 7.81 µg/mL against all the strains of *S. aureus.* None of the extracts showed activity against the Gram negative bacterial strains. As the phytochemical screening of the extracts showed that *E. haemastoma, A. falcata* and *A. implexa* contained a high content of phenolic compounds, the antibacterial activity of these plants may be due, at least in part, to their high phenolic contents. This is the first report of antibacterial activity of all the extracts against methicillin resistant and multidrug resistant strains of *S. aureus*. Although *S. quadrifida* did not show activity against the tested antimicrobial strains it could be valuable to investigate the plant against different strains and other biological screening in the future, especially given its wide use as a traditional medicine.

As the leaf extract of *S. glomulifera* showed the highest activity (against methicillin sensitive, methicillin resistant and multidrug resistant strains of *S. aureus*) of the plants tested, it was chosen for further investigations. The 70% aqueous ethanolic extract was partitioned with *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol and water. The partitions were tested for their antibacterial activity against the methicillin sensitive and resistant strains of *S. aureus*, *E. coli* and *P. aeruginosa.* Among the five partitions, the *n*-hexane partition showed the greatest activity against methicillin sensitive, methicillin resistant and multidrug resistant strains of *S. aureus,* with MIC values of 7.81 µg/mL. The *n*-hexane extract also showed activity against *E. coli* with MIC values of 1 mg/mL. GC-MS analysis of the *n*-hexane extract of *S. glomulifera* identified 24 phytochemicals, predominantly monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. The identified phytoconstituents are known to possess antimicrobial, antioxidant, anti-inflammatory, antiplasmodial, anticancer and antiviral activities. TLC bioautography of the *n*-hexane large scale column fractions identified six active fractions. These were not further explored due to time constraints, but would be of value to explore in the future. The Dichloromethane partition of leaves of *S. glomulifera* also showed promising antibacterial activity and purification of this partition could also lead to isolation of bioactive compounds.

This study identified thirty two NSW medicinal plants worthy for detailed biological and chemical studies aligned with their traditional use. The present study was involved in the chemical and biological studies of eight of these plants. Detailed chemical and biological studies on not only these but the rest of the plants in future studies would be valuable and may lead to the identification of new healing agents.

In conclusion, the antioxidant and antibacterial activities possessed by the crude extracts of many of the NSW medicinal plants and the identification of biologically active phytoconstituents and active fractions of *S. glomulifera* support the use of these plants

traditionally for the treatment of sores, wounds and skin infections by the Aboriginal people of New South Wales.

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CHAPTER FIVE

Summary, Conclusions and Future Directions

5.1 Summary

This PhD project focused on medicinal plants of Chungtia village, Nagaland, North East India and New South Wales (NSW), Australia that have been used traditionally for the treatment of skin related ailments including sores, wounds and skin infections.

Chapter 1 discussed the significant contribution of traditional medicinal plant knowledge in the discovery of new drug leads and the success of ethnopharmacological research to modern healthcare and drug discovery. This chapter also described the major classes of secondary metabolites and their medicinal properties along with the importance of the search for new antimicrobial and healing agents. The objectives of this PhD study and overview of the thesis were also described.

Chapter 2 introduced Chungtia village as the study area and the best ethical approach adopted in working with Chungtia Elders. This Chapter provided a detailed literature review (up to September 2014) on 135 Chungtia medicinal plants that have been previously ethnobotanically documented. In addition, a subsequent literature review on the documented medicinal plants used for skin related conditions up to March 2016 was presented. The aim of this review was to assist in the selection of Chungtia plants used in the treatment of skin related conditions including sores, wounds and skin infections for further chemical and biological studies.

The first hand information of 135 Chungtia medicinal plants was compared with the reported ethnobotanical and ethnomedicinal uses worldwide as well as chemical and biological studies. The review identified that thirty-five of the thirty-seven plants were reported for either antimicrobial, antioxidant or anti-inflammatory activities that support the traditional uses of these medicinal plants. An extensive literature review of the thirty-seven plants led to the identification of eleven plants with none or limited reports for chemical and biological studies, *i.e. Albizia lucidor*, *Begonia picta*, *Cassia floribunda*, *Dendrocnide sinuata*, *Erythrina stricta*, *Euphorbia royleana, Eurya acuminata*, *Holbolia scandens*, *Myrica esculenta*, *Mussaenda roxburghii* and *Prunus persica.*

Chapter 3 provided a literature review on the important Chungtia medicinal plant *Erythrina stricta* Roxb. (Fabaceae). This review was followed by the description of the chemical and biological studies conducted on the stem bark of this medicinal plant as part of this PhD project. This chapter also provided the methods used throughout the PhD study for antimicrobial and antioxidant screening.

The stem bark paste of *E. stricta* is topically used by the Chungtia villagers for the treatment of skin infections, eczema and contact dermatitis. Other tribal communities have also used the stem bark paste or bark juice for rheumatism, stomach-ache, asthma and dysentery, leucorrhoea and excessive thirst, gout and headache. The literature review revealed that there are limited reports on chemical and biological studies on the

stem bark of *E. stricta*. Therefore, the stem bark of *E. stricta* was selected for antimicrobial and antioxidant studies and isolation of bioactive compounds.

Sequential extraction of the stem bark powder was carried out to give *n*-hexane, dichloromethane, ethyl acetate, methanol and water extracts. The antioxidant activity of the extracts was determined by the DPPH free radical scavenging and ferric reducing antioxidant power (FRAP) assay methods. All the extracts showed antioxidant activity. Antimicrobial screening was performed against the Gram positive bacterial strains methicillin sensitive, methicillin resistant and multidrug resistant *Staphylococcus aureus*; Gram negative bacterial strains *Pseudomonas aeruginosa* and *Escherichia coli*; and the fungal strain *Candida albicans.* The disc diffusion, MTT microdilution and TLC bioautography assay methods were employed. The *n*-hexane, dichloromethane and ethyl acetate extracts showed antibacterial activity against all three strains of *S. aureus*, with minimum inhibitory concentration (MIC) values < 125 µg/mL. The dichloromethane (MIC 125 µg/mL) and ethyl acetate (MIC 1 mg/mL) extracts also showed activity against *C. albicans*. The dichloromethane extract exhibited the greatest activity against the methicillin sensitive strain of *S. aureus*, with MIC and minimum bactericidal concentration (MBC) values of 7.81 µg/mL and 62.5 µg/mL, respectively. This extract also showed the greatest activity of all the extracts against methicillin resistant and multidrug resistant strains of *S. aureus*, with MIC values of 31.25 µg/mL and 62.5 µg/mL, respectively. Therefore, the dichloromethane extract was chosen for isolation of bioactive compounds. Nine bioactive fractions (F2, F4, F5 and F7-F12) obtained from normal phase silica gel column chromatography of the dichloromethane extract showed good activity, with MIC values (15.6-1000 µg/mL) against methicillin sensitive *S. aureus*. Further fractionation and purification of the fractions F2, F5, F7 and F8 led to the isolation of seven bioactive compounds that included the novel compound erynone, together with wighteone, alpinum isoflavone, luteone, obovatin, erythrinassinate B and isovanillin. This is the first report of isolation of luteone, obovatin and isovanillin from the genus *Erythrina* and the first report of isolation of wighteone and erythrinassinate B from the species *E. stricta*. Antimicrobial and antioxidant activity studies of the isolated compounds identified luteone as the most antimicrobial and erynone as the most antioxidant compounds.

Due to time constraints, low amounts and complexity of the samples, some other fractions that also showed good antibacterial activity (MIC < 125 µg/mL) were not further investigated for their bioactive compounds. Thus, there is a potential to isolate more bioactive compounds from the dichloromethane extracts of *E. stricta* bark in future studies.

GC-MS analysis of the *n*-hexane extract of *E. stricta* identified twelve compounds. These included the well-known bioactive compounds caryophyllene oxide (16.31%), *β*-caryophyllene (9.06%), *β*-selinene (7.06%), *α*-selinene (6.86%), selin-11-en-4-*α*-ol

(6.80%), *α*-copaene (3.31%) and *α*-eudesmol (4.60%). These compounds have been reported for antibacterial, antifungal and/or anti-inflammatory activities. Therefore, the significant antibacterial activities of the *n*-hexane extract are likely to be associated with the high content of these bioactive compounds. Thus, the identification of bioactive compounds in the *n*-hexane extract and isolation of antimicrobial and antioxidant compounds from the dichloromethane extract, support the traditional uses of the stem bark of *E*. *stricta* by the Chungtia villagers for the treatment of skin related ailments.

Chapter 4 described the justifications for the selection for chemical and biological studies of eight medicinal plants used by the Aboriginal people of New South Wales for skin related ailments including sores, wounds and skin infections, and provides a literature review on the selected medicinal plants. The literature review included the ethnomedicinal, pharmacological and phytochemical reports of fifty-eight New South Wales medicinal plants. This identified that thirty-two plants had none or limited reports for chemical and biological studies. Out of these thirty-two plants, *Acacia falcata* (bark), *Acacia implexa* (bark), *Cassytha glabella* (whole plant), *Eucalyptus haemastoma* (sap), *Hibbertia scandens* (leaves), *Smilax glyciphylla* (leaves), *Sterculia quadrifida* (leaves) and *Syncarpia glomulifera* (leaves) were chosen for detailed chemical and biological studies due to the limited studies reported on these plants and their availability for collection in sustainable amounts in accessible locations.

The plant materials were extracted with 70% aqueous ethanol and the qualitative and quantitative phytochemical screening of the extracts were conducted. The extracts were also screened for antibacterial and antioxidant activities. The antibacterial activity of the extracts was measured by the MTT microdilution assay and the antioxidant activity was evaluated by the DPPH free radical scavenging, ABTS radical scavenging and FRAP assay methods.

The qualitative phytochemical screening of the extracts identified the presence of terpenoids, flavonoids, steroids, alkaloids, saponins, tannins and anthraquinone glycosides in the extracts. These classes of compounds are widely recognised for various biological activities. Phenolics and the polyphenolics are known to have antimicrobial and antioxidant activities. The quantitative phytochemical screening of the extracts showed that the highest quantities of total phenolic and condensed tannin contents were in *E. haemastoma* followed by *A. implexa*. Similarly, *A. falcata* followed by *E. haemastoma* were identified to contain the highest total flavonoid contents. The antioxidant activity determined for the extracts showed that the extract of *E. haemastoma* had the highest antioxidant activity, with an IC_{50} value of 61.72 ± 0.53 µg/mL. *A. falcata* (111.47 ± 0.88 µg/mL) and *A. implexa* (107.05 ± 1.38 µg/mL) also showed good antioxidant activity. A significant correlation was observed between the antioxidant properties and the total phenolic and flavonoid contents. This correlation suggests that

the phenolic and flavonoid type of compounds present in the extracts are the major contributors to their antioxidant properties. This is the first report of antioxidant activity studies on *A. falcata*, *A. implexa*, *C. glabella*, *E. haemastoma*, *H. scandens*, *S. quadrifida* and *S. glomulifera*.

The crude extracts of the plants were screened for antibacterial activity against methicillin sensitive, methicillin resistant and multidrug resistant strains of *S. aureus, E. coli* and *P. aeruginosa. A. falcata*, *A. implexa*, *C. glabella*, *E. haemastoma*, *H. scandens* and *S. glomulifera* showed activity against methicillin resistant strains of *S. aureus.* None of the plants showed activity against *E. coli* and *P. aeruginosa*. *S. glomulifera* was identified as the most active plant and exhibited antibacterial activity at MIC values of 7.81 µg/mL against all three strains of *S. aureus*. *E. haemastoma* and *A. implexa* also showed good activity with MIC values of < 125 µg/mL. As the phytochemical screening showed that *E. haemastoma* and *A. implexa* extracts contained high phenolic contents, this suggests that the antibacterial activity of these plants may be due to their phenolic contents. This is the first report of antibacterial activity of *A. falcata*, *A. implexa*, *C. glabella*, *E. haemastoma*, *H. scandens* and *S. glomulifera* against methicillin resistant strains of *S. aureus*. The 70% aqueous ethanolic extract of *S. glomulifera* was partitioned to give *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol and water partitions. The *n*-hexane partition showed activity against methicillin sensitive, methicillin resistant and multidrug resistant strains of *S. aureus*, with MIC values of 7.81 µg/mL, and against *E. coli* with an MIC of 1 mg/mL. The dichloromethane partition also showed good activity with an MIC of 31.25 µg/mL against methicillin sensitive and methicillin resistant strains of *S. aureus.* GC-MS analysis of the *n*-hexane extract identified twenty-four phytochemicals, predominantly monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated monoterpenes and oxygenated sesquiterpenes. These phytochemicals are well known for various biological activities including antibacterial, antifungal, antioxidant, anti-inflammatory and cytotoxic activities. Normal phase silica gel column chromatography of the *n*-hexane extract was initiated, but due to limited time, it did not lead to isolation of any compounds. Thus, there is a potential to isolate bioactive compounds from *S. glomulifera* leaves in future studies.

5.2 Conclusions

In conclusion, this PhD study has achieved the primary goal of conducting chemical and biological studies of relatively unexplored medicinal plants used by the Chungtia villagers of Nagaland, North East India, and Aboriginal people of New South Wales, Australia, for the treatment of skin related conditions including sores, wounds and skin infections. This study identified eleven medicinal plants used by the Chungtia villagers and thirty-two plants used by the NSW Aboriginal people to be worthy of detailed biological and

phytochemical studies. This study also identified bioactive fractions and compounds (antimicrobial and antioxidant) for the Nagaland medicinal plant *Erythrina stricta*. The phytochemical, antibacterial and antioxidant studies on eight New South Wales medicinal plants contributed to the understanding of their chemical and biological properties. The findings of this study also supported the traditional usage of these medicinal plants for the treatment of skin related conditions by the Indigenous communities of Nagaland and New South Wales.

5.3 Future directions

As described in Chapter 2, the literature review of the Chungtia medicinal plants identified *Albizia lucidor*, *Begonia picta*, *Cassia floribunda*, *Dendrocnide sinuata*, *Euphorbia royleana, Eurya acuminata*, *Holbolia scandens*, *Myrica esculenta*, *Mussaenda roxburghii* and *Prunus persica* of being worthy for further studies aligned with their traditional uses. Except for *Albizia lucidor* and *Holbolia scandens*, all these plants have been reported for antioxidant and antimicrobial activity but have limited phytochemical studies. Thus, these plants could be a valuable source of new antimicrobial and antioxidant agents. Chapter 3 presented the isolation of compounds from the antibacterially active dichloromethane extract, but also highlighted that there was considerable scope to isolate further bioactive compounds from this extract. As described in Chapter 4, among the NSW traditional medicinal plants screened, *Acacia falcata*, *Acacia implexa, Cassytha glabella*, *Eucalyptus haemastoma* and *Hibbertia scandens* crude extracts showed promising antibacterial and antioxidant activities. These plants are therefore also worthy of further investigations chemically and biologically.

It is recognised that this PhD study employed a representative but small suite of microbial strains and assay methods focusing on the sores, wounds and skin infections. Future investigation of the Chungtia and NSW medicinal plants using a wider range of microorganisms and further biological screens may allow a better understanding of the role these medicinal plants play in treating the various skin related ailments reported that includes eczema, contact dermatitis, scabies, inflammation, burns, boils and abscesses. For example, extending the assays to investigation of the scabies mite *Sarcoptes scabiei* and other fungi including *Trichophyton* sp (associated with ringworm) might align with the traditional use of a number of the medicinal plants for ‘skin infections’, given these conditions are commonly encountered by Indigenous communities. Additionally, complementing the assays used with anti-inflammatory and skin healing functional assays would allow a more holistic approach to understanding the roles of the traditional medicines in the complex process of wound healing.

Appendices

LIST OF APPENDICES

Appendix 1 Literature review of Nagaland medicinal plants up to September 2014 from supplementary materials of Kichu et al., An ethnobotanical study of medicinal plants of Chungtia village, Nagaland, India. *Journal of Ethnopharmacology*, **2015**, *166*, 5-17.

Scientific name (Botanical family) (Herbarium accession number, Assam Acc. No)	Agreement of literature with customary use(s)	Literature with reference to different customary use(s)	Biological activity	Literature with reference to bioactive chemical constituent
Acacia pennata (Linn.) Willd. (Mimosaceae) (69649)	Leaves: to poison fish Neuwinger [1]	Roots, leaves: gynaecological diseases, skin diseases[2]	Leaves: anti-inflammatory; bark: antioxidant[3, 4]	WR [5, 6]
Acorus calamus Linn. (Araceae) (69659)	Roots, leaves: fevers[7]	WR Rhizomes: Stomach ache, epilepsy, toothache, killing head lice, insect repellant[8]	WR Roots, leaves: antibacterial antifungal against; roots: antiviral anticonvulsant, anticancer[7]	WR [7]
Adenia trilobata Engl. (Passifloraceae) (69536)	None found	None found	None found	None found
Adhatoda vasica Nees. (Acanthaceae) (69665)	WR Flowers, fruits: fever, cold[9]	WR Roots: bronchitis, asthma, bilious vomiting, sore eyes[10]	WR Leaves, roots: antibacterial, antipyretic; leaves, roots: antiviral; leaves, flowers: anticholinesterase, abortifacient[9, 10]	WR [9, 10]
Albizia chinensis (Osb) Merr. (Mimosaceae) (69660)	Leaves: to poison fish[11]	WR Bark: gastric ulcer & chronic gastritis, inflammation[12, 13]	WR Bark, leaves: anti-inflammatory; leaves: antioxidant [14, 15]	WR [14, 15]
Albizia lebbeck Linn. Benth. (Mimosaceae) (69677)	None found	WR Leaves: conjunctivitis, ulcer, cold, cough; bark: diabetes; whole plant: bites & stings from venomous animals[16-18]	WR Bark: anti-diabetic, antioxidant, anti-inflammatory[17, 19]	WR [17, 19]
Albizia lucidior (Skud) Hara (Mimosaceae) (69595)	None found	None found	None found	None found
Allium chinense G. Don (Liliaceae) (69679)	Bulbs: cholesterol levels, circulatory system, heart asthma, skin diseases [20]	WR Bulbs: bronchitis, pleurisy, angina pectoris, chest pain, diarrhoea[20]	WR Bulbs: cytotoxic, antioxidant, antimicrobial, cardioprotective[20, 21]	WR [20]
Allium hookeri Thw. (Liliaceae) (69628)	None found	Leaves & rhizomes: skin diseases, veterinary, bone fracture, also used in rituals to protect against evil spirits[22]	None found	None found

Scientific name (Botanical family) (Herbarium accession number, Assam Acc. No)	Agreement of literature with customary use(s)	Literature with reference to different customary use(s)	Biological activity	Literature with reference to bioactive chemical constituent
Allium sativum Linn. (Liliaceae) (69602)	Bulbs: cardiac diseases[23]	WR Leaves & rhizomes: bone fracture; bulbs: tuberculosis[22 24]	WR Bulbs, leaves: cancer; bulbs: hypercholesterolemia, hypertension, peripheral, arterial disease, antibacterial; antioxidant[25, 26]; adverse effects[27]	WR [26, 28]
Alstonia scholaris (Linn.) R. Br. (Apocynaceae) (69541)	Stem: gastrointestinal disorders, diarrhoea [29]	WR Bark: fever, dysentery, astringent, anthelmintic, antiperiodic, dysentery, malarial fever[30, 31]	WR Bark: antimalarial; bark, leaves, stem bark: antibacterial; leaves: anti-inflammatory; aerial parts: antidiarrhoeal[31-33]	WR [31, 33, 34]
Amaranthus gangeticus Linn. (Amaranthaceae) (69699) (N)	None found	WR Whole plant: nutrient, antioxidant [35]	WR Whole plant: anticancer; leaves: menorrhagia, anti-diarrhoea, haemorrhages from the bowels, antipyretic, expectorant [36, 37]	[38]
Aquillaria agallocha Roset. (Thymelaeaceae) (69604)	Roots: dysentery[39]	WR Whole plant: liver complaints[40]	WR Leaves, bark: antibacterial[41]	WR [39, 41]
Artemisia vulgaris Linn. (Asteraceae) (69505)	None found	WR Leaves: cough & common cold[42]	WR Leaves: antibacterial; aerial parts: anti-inflammatory, analgesic[43, 44]	WR [45]
Artocarpus chaplasha Roxb. (Moraceae) (69688)	None found	Stem bark: diarrhoea[46]	Seeds: antioxidant, cytotoxic[47]	None found
Artocarpus heterophyllus Lamk. (Moraceae) (69596)	WR Leaves: wounds; latex: boils[42, 46]	WR Roots: diarrhoea, fever; leaves: ulcers, wound healing; leaves, stem bark: anaemia, asthma, dermatitis, diarrhoea, cough[48]	WR Fruits: cytotoxic; seeds: antibacterial; leaves: wound healing[49-51]	WR [51, 52]
Asclepias curassavica Linn. (Asclepiadaceae) (69617)	Leaves: warts, wounds, healing process [53]	WR Leaves: menstrual problems, piles, gonorrhoea, roundworm infection, abdominal tumours[54, 55]	WR Aerial parts: anti-inflammatory; whole plant: antibacterial; antiviral; latex: antifungal[53]	WR [53]
Averrhoa carambola L. (Averrhoaceae) (69692)	Fruits: diuretic in kidney, bladder complaints[56]	WR Fruits, leaves: vomiting, headache, chicken pox, ringworm[56]	WR Fruits: antioxidant, cardioprotective; roots: antidiabetic; bark: antibacterial, antioxidant, cytotoxic[57-60]	WR [58, 61]
Bambusa tulda Roxb. (Bambusoideae) (69605)	None found	Shoots: tetanus[62, 63]	None found	[64]

Scientific name (Botanical family) (Herbarium accession number, Assam Acc. No)	Agreement of literature with customary use(s)	Literature with reference to different customary use(s)	Biological activity	Literature with reference to bioactive chemical constituent
Basella alba Linn. (Basellaceae) (69633)	Whole plant: demulcent, diuretic, laxative[65]	WR Leaves: dysentery, diarrhoea, anaemia, cancer[66]	WR Leaves: antibacterial, antioxidant, anti-inflammatory, anticancer[66]	WR 66
Bauhinia variegata Linn. (Caesalpiniaceae) (69599)	WR Bark: dysentery, diarrhoea, stomach disorders, ulcers[67]	WR Bark: anthelmintic, skin diseases[67]	WR Leaves: antiulcer, antidiabetic, antibacterial; stem bark: antimicrobial, anthelmintic, hepatoprotective; seeds: antiviral[67, 68]	WR 67, 69
Begonia picta Smith (Begoniaceae) (69642)	Whole plant: cuts & wounds[70]	WR Whole plant: headaches; roots: conjunctivitis;[70, 71]	None found	None found
Brassica oleracea Linn. (Brassicaceae) (69510)	None found	WR Whole plant: laxative, constipation, dyspepsia, hypertension, heat burn[72]	WR Whole plant: anticancer[73]	74
Cajanus cajan (Linn.) (Fabaceae) (69672)	Leaves: yellow fever [75]	WR Roots: cancer; leaves: analgesic, constipation, gingivitis[76, 77]	WR Roots: anticancer; aerial parts: antimicrobial, anthelmintic; leaves: antioxidant; leaves, stem, roots: antiviral[75, 77]	WR 77
Calotropis gigantea Linn. (Asclepiadaceae) (69691)	Stem: latex applied on sprain[78]	WR Latex: dog bite[79]	WR Leaves: antioxidant, antibacterial, antifungal, insecticidal; flowers: analgesic activity; root bark: wound healing[80-83]	WR 84
Cannabis sativa L. (Cannabaceae) (69608)	Leaves, twigs: indigestion & stomach acidity[85]	WR Leaves, seeds: analgesic[86]	WR Flowers, leaves: anticancer, antidepressant; leaves: antibacterial[87-89]	WR 90
Capsicum annum Linn. (Solanaceae) (69658)	Fruits: appetiser[91]	WR Fruits: antiseptic, carminative[92]	WR Fruits: antimicrobial, antiviral, analgesic, cardioprotective, antidiabetic, anti-inflammatory, anti-diarrhoeal[93]	WR 94
Carica papaya Linn. (Caricaceae) (69626)	Fruits: stomach complaints [95]	WR Fruits: diuretic, diarrhoea, dysentery, antibacterial, abortifacient[95]	WR Fruits: antioxidant, wound healing; leaves: antibacterial, anti-inflammatory, anticancer; latex: antifungal; bark: antifertility; roots: diuretic; seeds: anthelmintic[95]	WR 96, 97
Cassia floribunda Cav. (Caesalpiniaceae) (69535)	None found	Young pods are cooked as vegetable[98]	WR Seeds: antioxidant, antidiabetic, antibacterial[99, 100]	WR 100

Scientific name (Botanical family) (Herbarium accession number, Assam Acc. No)	Agreement of literature with customary use(s)	Literature with reference to different customary use(s)	Biological activity	Literature with reference to bioactive chemical constituent
Catharanthus roseus (Linn.) G. Don (Apocynaceae) (69517)	None found	WR Whole plant: dengue fever, diabetes, cancer, diarrhoea[101]	WR Whole plant: anticancer[102]	WR [103]
Celosia cristata L. (Amaranthaceae) (69520)	Whole plant: urinary tract infections; leaves: sores, wounds [104]	WR Whole plant: kidney stone; leaves: dysentery, menstrual bleeding, inflammation & worms[104, 105]	WR Leaves: antiviral, antioxidant, anthelmintic; flowers: antimicrobial[104, 106]	WR [104]
Centella asiatica L. (Apiaceae) (69598)	Leaves: Stomach pain, amoebic dysentery [29]	WR Whole plant: burns[107]	WR Whole plant: antibacterial, wound healing, anti-inflammatory, antioxidant, antinociceptive, collagen stimulant; leaves: antidepressant, antiepileptic, antiulcer, antiviral[108, 109]	WR [108]
Chrysanthemum indicum L. (Asteraceae) (69529)	Flowers: boils, itchiness of skin[110]	WR Flowers, leaves, stem: cough, hepatitis, nerve tonic, rheumatism[72]	WR Leaves: antiplasmodial, hepatoprotective, antifungal; flowers: analgesic, antibacterial, anti-inflammatory[110, 111]	WR [110]
Cissampelos pareira Linn. (Menispermaceae) (69675)	Roots: fever, heart diseases & leprosy, diarrhoea, gastrointestinal disorders[112, 113]	WR Roots: diuretic, febrifuge, for heart trouble, dysentery, sores, snakebite & jaundice[113]	WR Leaves: antidiabetic; roots: antimalarial, antibacterial, antiulcer, cardiovascular activity[113-116]	WR [113, 117]
Cissus repens Lam. (Vitaceae) (69537)	None found	WR Stem, leaves: jaundice, muscle pain, muscle cramp, muscle fatigue, joint pain[118, 119]	WR Whole plant: analgesic, anti-inflammatory; roots: antiulcer, cardioprotective[120-122]	WR [120]
Citrus microcarpa Bunge (Rutaceae) (69618)	None found	WR Infusion of leaves is given during headache & hypertension[123]	WR Fruits: antibacterial, gastroprotective, antiulcer[124, 125]	WR [124, 126-129]
Clerodendron colebrookianum D. Don (Verbenaceae) (69531)	WR Leaves: high blood pressure[8, 130, 131]	WR Leaves: liver pain & viral fever, gastric disorders, dysentery, diarrhoea, abdominal pain, diabetes[130, 132]	WR Leaves: hypertension, diabetes[130, 131, 133]	WR [130, 134]
Coix lacryma-jobi Linn. (Poaceae) (69662)	Seeds: consumed for their nutrients[135]	WR Leaves: urinary complaints, stomach problems, fever, small pox, as tonic; roots: menstrual disorders; seeds: dysentery, diuretic & as diet drink[136, 137]	WR Leaves: anti-trichomonas[138]	WR [135]

Scientific name (Botanical family) (Herbarium accession number, Assam Acc. No)	Agreement of literature with customary use(s)	Literature with reference to different customary use(s)	Biological activity	Literature with reference to bioactive chemical constituent
Costus speciosus (Koenig ex Retz) JE Smith (Costaceae) (69496)	Tubers: applied to decayed tooth to relieve pain [139]	WR Leaves: fever, skin diseases, abortion, diarrhoea, jaundice, arthritis; roots: pneumonia, rheumatism, dropsy, urinary diseases, jaundice[140]	WR Aerial parts: anti-inflammatory, analgesic, antipyretic, antibacterial; roots: antidiabetic, diuretic, estrogenic[140-142]	WR [140]
Crataeva nurvala Buch-Ham. (Capparaceae) (69690)	WR Bark: liver problems, indigestion, flatulence [143]	WR Bark: urinary disorders, kidney & bladder stones; leaves: joint disorders[144]	WR Whole plant: hepatoprotective; stem bark: urinary tract infections, antibacterial anti-inflammatory, antidiabetic, antifertility[144-146]	WR [144, 147]
Croton caudatus Gieseler (Euphorbiaceae) (69664)	Leaf juice: stomach tonic, anticancer [148]	WR Roots & leaves: for the treatment of arthritis & to stop paralysis[149]	WR Leaves: antioxidant, anticancer[150]	WR [151, 152]
Cucurbita pepo L. (Cucurbitaceae) (69682)	None found	WR Seeds: diuretic, urinary system problems, prostate problems[153]	WR Fruits: antioxidant, cytoprotective [154]	WR [155]
*Curculigo capitulata (*Lour.) Kuntze (Hypoxidaceae) (69527)	None found	Leaves: cuts & wounds[156]	Roots: cardioprotective[157]	[157]
Curanga amara Juss. Syn: *Picria fel-terrae* (Scrophulariaceae) (69538)	None found	None found	Leaves: antioxidant [158]	None found
Cyclea peltata Diels. (Menispermaceae) (69650)	Roots: skin disorders [159]	WR Roots: gastric ulcer & allied stomach ailments, jaundice & digestive disorders; leaves: antihypertensive & cardiac depressant activities[160, 161]	WR Whole plant: antibacterial; roots: hepatoprotective[161-163]	WR [160]
Datura stramonium Linn. (Solanaceae) (69528)	Leaves: relieving pain [164]	WR Seeds: purgative, cough, fever, asthma; leaves: wounds, pain[165]	WR Leaves: anti-asthma antiulcer, wounds healing, anti-inflammatory, anti-rheumatic, antimicrobial, cytotoxic; seeds: analgesic effect on both acute & chronic pain[164, 165]	WR [165]
Debregeasia longifolia (Burm. f.) Wedd. (Urticaceae) (69504)	Leaves: diabetes [8]	Bark: bone fracture [166]	Leaves: antibacterial[167]	[168, 169]
Dendrocnide sinuata (Bl.) (Urticaceae) (69508)	Roots: injury, itching skin[170]	Whole plant: elephantiasis[171]	Leaves: antimicrobial, antioxidant[172]	None found

Scientific name (Botanical family) (Herbarium accession number, Assam Acc. No)	Agreement of literature with customary use(s)	Literature with reference to different customary use(s)	Biological activity	Literature with reference to bioactive chemical constituent
Diospyros lanceifolia Roxb. (Ebenaceae) (69544)	Seeds: to poison fish[173]	Fruits: to poison fish; seeds: skin diseases[174]	None found	None found
Dolichos lablab L. (Fabaceae) (69523)	None found	WR Whole plant: boils, pimples[175]	Seeds: antibacterial; whole plant: antidiabetic [176, 177]	None found
Drymaria cordata (Linn.) Willd. (Caryophyllaceae) (69530)	Leaves: sinusitis[8]	Leaves: fever[178]	WR Whole plant: anti-inflammatory, antinociceptive, antipyretic; aerial parts, leaves: antibacterial[178, 179]	WR [179]
Dryopteris filix-mas (L.) syn *Cyclosorus parasiticus* Schott (Dryopteridaceae) (69507)	WR Leaves: gout rheumatism, microscopic insects in chickens[132]	WR Leaves: eye disease[180]	Leaves: antiproliferative[181]	WR [181, 182]
Duabanga grandiflora (Roxb. ex DC.) Walp. (Sonneratiaceae) (69686)	Bark: skin diseases, eczema[183]	Roots: juice to cure upset stomach[184]	Leaves: decreasing skin damage, skin whitening, antibacterial[185, 186]	WR [187]
Elsholtzia blanda Benth. (Lamiaceae) (69524)	Aerial parts: fever, cholera, skin diseases & inflammation; leaves: used to treat inflamed glands[188]	WR Flowers: piles[189]	WR Leaves, roots: antibacterial, antiviral, antioxidant[190-192]	WR [193, 194]
Entada pursaetha DC. (Leguminosae) (69616)	None found	WR Seeds: to reduce pain of inflamed swellings[195]	WR Stem: hepatoprotective, antioxidant[196]	[197]
Equisetum ramosissimum Desf. Subsp. *Debile* (Equisetaceae) (69499)	None found	WR Aerial parts: stomach ache[198]	WR Whole plant: antipyretic, anti-inflammatory[199]	WR [200]
Eryngium foetidum Linn. (Apiaceae) (69668)	Leaves, fruits, roots: indigestion[201]	WR Leaves: abscesses & boils[202]	WR Whole plant: anthelmintic, antibacterial, antimalarial; leaves: anti-inflammatory[203]	WR [204-206]
Erythrina stricta Roxb. (Fabaceae) (69629)	None found	WR Bark: treat disruptions in menstrual cycle[207]	WR Stem: spasmolytic, diuretic, anticonvulsant, analgesic, antiviral, antifungal; bark: antibacterial[208, 209]	WR [210]
Eucalyptus globulus Labill. (Myrtaceae) (69663)	WR Leaves: spasmodic, decongestant, asthma, migraine, congestive headache[211]	WR Leaves, flowers: diabetes, high blood pressure[212]	WR Leaves: antibacterial[213]	WR [213]

Scientific name (Botanical family) (Herbarium accession number, Assam Acc. No)	Agreement of literature with customary use(s)	Literature with reference to different customary use(s)	Biological activity	Literature with reference to bioactive chemical constituent
Eupatorium odoratum Linn. (Asteraceae) (69523)	WR Leaves: cuts & wounds[214]	WR Roots: stomach pains, during gastric ulcer; leaves: cuts & wounds, blisters & skin irritation[132]	WR Leaves: antifungal, antibacterial[215, 216]	WR [215, 216]
Euphorbia pulcherrima Willd. ex Klotzsch (Euphorbiaceae) (69615)	Aerial parts: poisonous for animals[217]	Leaves: rheumatic pain[218]	Flower, leaves, stem, whole plant: antimicrobial; aerial parts: toxic for animals [219, 220]; aerial parts: anticonvulsant, antinociceptive but not toxic[221]	None found
Euphorbia royleana Boiss. (Euphorbiaceae) (69684)	Latex: skin diseases[222]	WR Latex: cuts to stop bleeding; leaves: earache[223]	WR Latex: antibacterial, anti-inflammatory[224, 225]	[226, 227]
Eurya acuminata DC. (Theaceae) (69612)	Leaves: stomach disorders, dysentery, diarrhoea & cholera, skin diseases[228, 229]	Leaves: cough[230]	Leaves, stem: antibacterial [231]	None found
Ficus elastica Roxb. ex Hornem. (Moraceae) (69619)	Leaves: skin infections & skin allergies[232]	Fruits: to stupefy fish & make them float[170]	WR Leaves: antioxidant, anticancer, antibacterial[233-236]	[236]
Garcinia cowa Roxb. (Guttiferae) (69631)	WR Leaves: dysentery & diarrhoea [237]	WR Fruits: coughs, cold [238]	WR Fruits, leaves, twigs: antibacterial, antimalarial, anti-inflammatory; fruits: cytotoxic [239, 240]	WR [239, 240]
Garcinia pedunculata Roxb. Ex Buch.-Ham. (Guttiferae) (69630)	Pericarp: dysentery & diarrhoea[188]	WR Fruits: effective in jaundice[241]	WR Fruits: antioxidant[242]	WR [243]
Girardinia palmata (Forsk.) Gaud. (Urticaceae) (69661)	None found	Roots: to treat allergy caused by food[188]	None found	None found
Glycine max (L.) Merr. (Fabaceae) (69680)	None found	WR Seeds: common cold[244]	WR Whole plant: antioxidant, antibacterial[245, 246]	WR [247]
Gmelina arborea Roxb. (Verbenaceae) (69518)	Roots: skin problems [248]	WR Leaves, bark: constipation, indigestion[249]	WR Stem bark: antibacterial; leaves: anti-inflammatory, antinociceptive[250-252]	WR [252-254]
Gonatanthus pumilus (D. Don) Engler & Krause (Araceae) (69498)	None found	Roots, tubers: burns & wounds[255]	Tubers: antiproliferative[256]	[256]
Gossypium herbaceum Linn. (Malvaceae) (69611)	None found	WR Seeds: cough, asthma, skin disease; roots: abortifacient[257]	WR Whole plant: antidiabetic; leaves: diuretic; flowers: antiulcer; seeds: antifertility, antibacterial[257, 258]	WR [257]

Scientific name (Botanical family) (Herbarium accession number, Assam Acc. No)	Agreement of literature with customary use(s)	Literature with reference to different customary use(s)	Biological activity	Literature with reference to bioactive chemical constituent
Gynocardia odorata R. Br. (Flacourtiaceae) (69501)	None found	Seeds: leprosy, skin disorders[188]	Whole plant: cytotoxic[259]	[259]
Gynura crepidioides Benth. (Asteraceae) (69506)	None found	Leaves: headache, insomnia, constipation, in pregnancy for easy delivery[132]	Leaves: antioxidant[260]	None found
Hedyotis scandens Roxb. (Rubiaceae) (69539)	Leaves: itches, scabies & eczema; roots: gastric problems[261]	WR Roots: sprain; whole plant: peptic ulcer[261]	WR Whole plant: antibacterial[261]	WR [262]
Hodgsonia macrocarpa (Blume) Cogn. (Cucurbitaceae) (69667)	None found	Fruits: skin disease[183]	Leaves: cytotoxic[263]	None found
Holboellia latifolia Wall. (Lardizabalaceae) (69500)	Leaves: burns[159]	Roots: rheumatism[264]	None found	None found
Houttuynia cordata Thunb. (Saururaceae) (69532)	Leaves: dysentery[265]	WR Leaves: cholera, purification of blood, skin diseases[265]	WR Whole plant: anti-inflammatory, antiviral, antibacterial, antidiabetic, antioxidant; leaves: antiobesity[265, 266]	WR [265, 267]
Ipomoea nil (Linn.) Roth. *Pharbitis nil* (Convolvulaceae) (69678)	None found	Seeds: abortifacient[268]	WR Seeds: antimicrobial antitumour[269, 270]	WR [270]
Kalanchoe pinnata (Lam.) Pers. (Crassulaceae) (69515)	WR Leaves: skin diseases, eczema, pruritus[162]	WR Leaves: blood dysentery[271]	WR Roots: antibacterial; leaves: antibacterial, diuretic, hepatoprotective[272, 273]	WR [274]
Lagenaria siceraria (Molina) Standl. (Cucurbitaceae)	WR Roots: wounds; fruits: skin disorders[275, 276]	WR Fruits: cardioprotective, purgative, diuretic; leaves: jaundice; seeds: diuretic, anthelmintic[277]	WR Fruits: cardioprotective, analgesic & anti-inflammatory; seeds: anticancer; leaves: antimicrobial[277, 278]	WR [277, 279]
Lantana camara Linn. (Verbenaceae) (69620)	WR Leaves: fevers, dry cough, jaundice[8]	WR Leaves: cuts, rheumatism, ulcers, vermifuge[280]	WR Flowers, leaves: antibacterial; leaves: anti-inflammatory[281-283]	WR [280, 284]
Lasia spinosa (L.) Thwaites (Araceae) (69655)	Leaves: cuts & injuries[285]	WR Leaves: bone fracture[286]	WR Whole plant: antioxidant; leaves: antioxidant, cytotoxic, antimicrobial[287, 288]	WR [289]

Scientific name (Botanical family) (Herbarium accession number, Assam Acc. No)	Agreement of literature with customary use(s)	Literature with reference to different customary use(s)	Biological activity	Literature with reference to bioactive chemical constituent
Lycopersicon esculentum Linn. (Solanaceae) (69654)	Roots: blood in urine [290]	WR Fruits: feve [291]	WR Fruits: antioxidant, antifungal against[292, 293]	WR [294]
Luffa acutangula Linn. (Cucurbitaceae) (69676)	Whole plant: laxative & purgative[295]	WR Leaves, fruits, seeds: haemorrhoids, diuretic, leprosy, conjunctivitis, demulcent, nutritive[296]	WR Fruits: antioxidant, antidiabetic, anti-inflammatory, antibacterial, hepatoprotective[295, 297]	WR [297]
Macropanax undulatus (Wall. ex G. Don) Seem. (Araliaceae) (69540)	None found	None found	None found	None found
Maesa indica (Roxb.) Wall. (Myrsinaceae) (69514)	None found	Fruits: to expel intestinal parasites[298]	Whole plant: antiviral activity[299]	[300, 301]
Manihot esculenta Crantz. (Euphorbiaceae) (69695)	Tubers: fermentation process[302]	Leaves: viral infections, antitumor[149, 303]	Roots: toxic to humans due to cyanogenic glucosides; leaves: antitumor [149, 302, 304]	[302, 305]
Melastoma malabathricum (Melastomataceae) (69652)	WR Leaves: cuts & wounds[306]	WR Leaves: dysentery, diarrhoea, piles, gastric ulcers[306]	WR Leaves, stems & flowers: antibacterial[306, 307]	WR [281, 306, 308]
Melia composite Willd. (Meliaceae) (69669)	None found	Whole plant: anthelmintic, diuretic[309]	Leaves: antibacterial [309]	None found
Mentha cordifolia Opiz ex Fresen. (Lamiaceae) (69683)	None found	None found	Leaves: antimicrobial[310, 311]	[312]
Mikania cordata (Burm. F.) B. L. Rob. (Asteraceae) (69534)	WR Leaves: cuts & wounds[313]	WR Leaves: blood coagulation, jaundice, snake bite[156, 314]	WR Leaves: antibacterial, antidiarrhoeal[315-317]	WR [314, 318]
Millettia cinerea Benth. (Fabaceae) (69666)	Leaves: to ease back & leg discomfort[319]	None found	None found	None found
Mimosa pudica Linn. (Mimosaceae) (69526)	WR Leaves: dysentery, diarrhoea, ulcer, piles swelling[101, 320]	WR Leaves: cuts & wounds; roots: bilious fevers, leprosy, urinary infections[321, 322]	WR Shoots: wound healing; leaves: antibacterial analgesic, anti-inflammatory, antidiarrhoeal, antiulcer; roots: antifertility[320, 323, 324]	WR [325, 326]
Mirabilis jalapa Linn. (Nyctaginaceae) (69609)	WR Roots: diuretic[327]	WR Roots: purgative, scabies, muscular swelling; leaves: boils, abscesses [327]	WR Flowers: spasmolytic/ spasmodic; leaves: antinociceptive, anti-inflammatory, abortifacient[327]	WR [327]
Mussaenda roxburghii Hk. f. (Rubiaceae) (69502)	WR Leaves: boils, to brew rice beer[328, 329]	WR Bark: diarrhoea[156]	WR Leaves: antimicrobial, cytotoxic[330, 331]	WR [332]

Scientific name (Botanical family) (Herbarium accession number, Assam Acc. No)	Agreement of literature with customary use(s)	Literature with reference to different customary use(s)	Biological activity	Literature with reference to bioactive chemical constituent
Myrica esculenta Buch.-Ham., ex D. Don (Myricaceae) (69522)	Sap: cuts & wounds[333]	Fruits, bark: cough, asthma, dysentery, diarrhoea[334]	Fruits: antioxidant, antipyretic; stem bark: antimicrobial[335, 336]	[335, 336]
Nephrolepis cordifolia (Davalliaceae) (69623)	WR Bulbs & tubers: upset stomach & urinary problems; leaves: wounds[337, 338]	WR Rhizomes: cough, rheumatism, chest congestion, nose blockage & loss of appetite[339]	WR Fronds: antibacterial [340]	WR [341, 342]
Nerium indicum, Mill. (Apocynaceae) (69674)	WR Flowers: insecticidal[343]	WR Roots: snake bite[344]	WR Flowers: larviciadal[345]	WR [346, 347]
Ocimum basilicum Linn. (Lamiaceae) (69543)	None found	WR Leaves: cough & cold[348]	WR Seeds: antibacterial, antioxidant, cytotoxic; leaves: antiviral against influenza[349, 350]	WR: [349]
Oroxylum indicum (Linn.) Vent. (Bignoniaceae) (69637)	Roots: dysentery & rheumatism[351]	WR Roots: biliousness, fevers, bronchitis, intestinal worms, vomiting, asthma; fruits: stomachic, anthelmintic, expectorant[351]	WR Stem bark, root bark: antibacterial, anthelmintic, antiulcer; leaves: gastroprotective, anti-inflammatory, anticancer[351-353]	WR [351]
Oxalis acetosella L. (Oxalidaceae) (69607)	None found	None found	Whole plant: antioxidant, antibacterial[318]	None found
Paederia foetida Linn. (Rubiaceae) (69694)	WR Leaves: intestinal problems, dysentery, diarrhoea [354]	WR Leaves: burns, gout, rheumatism[354]	WR Whole plant: antidiarrhoeal, analgesic; leaves: hepatoprotective, anti-inflammatory, antitussive, antiulcer, anthelmintic[354, 355]	WR [356]
Passiflora edulis Sim. (Passifloraceae) (69700)	WR Fruits: high blood pressure [357, 358]	WR Leaves: cuts, wounds, dysentery, diarrhoea, insomnia[357, 358]	WR Leaves: antioxidant, anti-inflammatory, wound healing; fruits: antihypertensive, anti-hyperglycaemic, anticancer; rind: antihypertensive; leaves, stem, fruits: antibacterial; roots: antiviral[357-359]	WR [360 357, 358]
Phyllanthus emblica, Linn. (Euphorbiaceae) (69671)	WR Fruits: maintain blood pressure, cough, sore throat [91 361]	WR Fruits: loss of appetite[362]	WR Fruits: cardioprotective, anti-hyperglycaemic, antioxidant, kidney-protective, antimicrobial[363, 364 365, 366]	WR [366, 367]
Phyllanthus urinaria Linn. (Euphorbiaceae) (69696)	WR Leaves: dysentery, diarrhoea [368]	WR Whole plant: malaria[361]	WR Leaves & twigs: antiviral, leaves: anti-inflammatory, gastroprotective, antibacterial[369, 370]	WR [370-372]

Scientific name (Botanical family) (Herbarium accession number, Assam Acc. No)	Agreement of literature with customary use(s)	Literature with reference to different customary use(s)	Biological activity	Literature with reference to bioactive chemical constituent
Physalis alkekengi L. (Solanaceae) (69647)	WR Fruits: kidney stones, urinary ailments, diuretic [373, 374]	WR Fruits: jaundice[373]	WR Aerial parts: antifungal; fruits: intestinal microflora balance[375, 376]	WR 376-378
Piper betel (Piperaceae) (69697)	WR Leaves: cuts & wounds[379]	WR Leaves: nerve pain, joint pain, cough & oedema[101]	WR Leaves: analgesic, antimicrobial, anthelmintic[380, 381]	WR 381-384
Plantago erosa Wall. (Plantaginaceae) (69625)	None found	WR Leaves: wounds & boils[385]	WR Leaves: anti-inflammatory[386]	386
Polygonum hydropiper Linn. (Polygonaceae) (69641)	WR Leaves & roots: eczema & scabies[387]	WR Leaves: snake bite[388]	WR Leaves: antibacterial; whole plant: antioxidant & anticholinesterase[389, 390]	WR 390-392
Prunus persica (L.) Stokes (Rosaceae) (69503)	Leaves: killing maggots, skin diseases, ear infections, cough, bronchitis[8]	WR Seeds: external parasites; leaves: laxative[91, 393]	WR Leaves, fruits: cardioprotective, spasmolytic, anti-inflammatory, antioxidant[394, 395]	WR 395, 396
Psidium guajava Linn. (Myrtaceae) (69513)	WR Leaves: vomiting, diarrhoea & dysentery[8]	WR Leaves: cough, pulmonary ailments[397]	WR Leaves: antidiarrhoeal, antibacterial, hepatoprotective, analgesic, anti-inflammatory[398, 399]	WR 398, 399
Punica granatum Linn. (Punicaceae) (69621)	WR Leaves: dysentery, diarrhoea[398]	WR Leaves: epilepsy[400]	WR Leaves: infectious diarrhoea[401]	WR 402-404
Rhus javanica var. *roxburghiana* (Anacardiaceae) (69603)	WR Fruits: dysentery[405]	WR Fruits: swelling & wounds[406]	WR Whole plant: antibacterial against MRSA; fruits: antidiarrhoeal; roots: chronic heart diseases; stem bark: anti-inflammatory[407-411]	WR 411, 412
Rhus roxburghii Hook. f. (Anacardiaceae) (69673)	None found	None found	None found	None found
Ricinus communis Linn. (Euphorbiaceae) (69516)	WR Seeds: laxative[413]	WR Seeds: constipation[414]	WR Leaves: anti-inflammatory, antibacterial, insecticidal, cytotoxic; seeds: analgesic, antiulcer; stem: antioxidant[413]	WR 413
Saccharum officinarum Linn. (Poaceae) (69622)	WR Stem: jaundice[415, 416]	WR Leaves: leucorrhoea[54]	WR Leaves: antibacterial; flowers: antioxidant[417, 418]	WR 416
Scutellaria glandulosa Colebr. (Lamiaceae) (69542)	None found	None found	None found	None found

Scientific name (Botanical family) (Herbarium accession number, Assam Acc. No)	Agreement of literature with customary use(s)	Literature with reference to different customary use(s)	Biological activity	Literature with reference to bioactive chemical constituent
Solanum indicum Linn. (Solanaceae) (69627)	None found	WR Roots: asthma[419]	WR Berries: analgesic, antipyretic, anthelmintic, anti-inflammatory, CNS depressant activity[420]	WR 420, 421
Solanum myriacanthum (Solanaceae) (69597)	WR Seeds, fruits: toothache[422, 423]	WR Fruits: tonsillitis, body worms[424]	Fruits: anthelmintic [425, 426]	426
Sonerila maculata Roxb. (Melastomaceae) (69509)	None found	None found	None found	None found
Spermacoce scaberrima Blume (Rubiaceae) (69643)	None found	None found	None found	None found
Spermacoce poaya Linn. (Rubiaceae) (69698)	None found	None found	None found	None found
Spilanthes acmella Linn. (Asteraceae) (69639)	WR Leaves, flowers: tooth ache[427]	WR Leaves, flowers: rheumatism, fever, diuretic, flu, cough, rabies, tuberculosis, antimalarial[427]	WR Flowers: antipyretic, anti-inflammatory; leaves: analgesic, antibacterial; aerial parts: antibacterial, antioxidant, diuretic; roots: antimalarial[427, 428]	WR 427-429
Spondias pinnata (Linn. F.) Kurz. (Anacardiaceae) (69519)	WR Fruits: bilious dyspepsia[430]	WR Seeds: skin diseases, ringworm, abscess; stem bark: rheumatism, gonorrhoea, anti-tubercular[430, 431]	WR Stem bark: ulcer-protective, antibacterial, anti-diarrhoeal [430]	WR 430-432
Stereospermum chelonoides (Linn. f.) DC (Bignoniaceae) (69610)	WR Flowers: boils caused by diabetes[433]	WR Roots: oedema, blood disorders, bronchial asthma, vomiting, jaundice, rheumatism, paralysis[434]	WR Stem bark: antimicrobial[434, 435]	WR 434, 436, 437
Stixis suaveolens (Roxb.) Pierre (Capparidaceae) (69525)	None found	Leaves: epididymitis and orchitis[2]	None found	None found
Tagetes erecta, Linn. (Asteraceae) (69670)	WR Flowers: skin diseases like sores, burns, wounds, ulcers, eczema[438]	WR Leaves: colic, diuretic, malaria[438]	WR Leaves: antifungal, wound healing; flowers: antinociceptive & anti-inflammatory[439-442]	WR 438, 443-446
Terminalia chebula Retz. (Combretaceae) (69640)	WR Bark, fruits: gastritis, constipation, indigestion, ulcers, vomiting, diarrhoea[368]	WR Fruits: cough[361]	WR Whole plant: antiulcer, gastrointestinal motility[447]	WR 447

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Thunbergia grandiflora Roxb. (Acanthaceae) (69497)	WR Leaves, stem: eye problems[448]	WR Leaves, stem: inflammation, cuts & wounds, astringent[448]	WR Flowers: antibacterial[449]	[450, 451]
Tithonia diversifolia (Hemsl.) A. Gray (Asteraceae) (69687)	WR Leaves: malaria, pain[452]	WR Leaves: hepatitis, diabetes[452]	WR Leaves: antimalarial; aerial parts: anti-hyperglycaemic[453, 454]	WR [452, 454, 455]
Urtica dioica L. (Urticaceae) (69601)	None found	WR Leaves, whole plant: cancer, anti-rheumatic, diabetes, stomachic, cough, colds, throat diseases, oedema, sedative, laxative, asthma, hypertension, kidney stones[456]	WR Leaves: anti-inflammatory, antibacterial, anti-nociceptive[457, 458]	WR [457]
Viola betonicifolia Boj. Ex. baker (Violaceae) (69651)	None found	WR Whole plant: antipyretic, anticancer, febrifuge, purgative, antiepileptic, nervous disorder[459]	WR Whole plant: antipyretic, anti-analgesic, anti-inflammatory[460]	WR [461, 462]
Wedelia chinensis (Osb.) Merrill (Asteraceae) (69693)	WR Leaves: gastrointestinal disorders [463]	WR Leaves: anti-inflammatory, dermatological disorders[464]	WR Leaves: anti-ulcerogenic, anti-inflammatory, antibacterial[465, 466]	WR [467]
Zanthoxylum acanthopodium DC. (Rutaceae) (69600)	WR Leaves: insect repellent; seeds: stomach ache, feve [468]	WR Leaves: fever, dyspepsia, cough, bronchitis; seeds: rheumatism[192]	WR Leaves: antibacterial[192]	WR [469]
Zanthoxylum rhetsa (Roxb.) DC. (Rutaceae) (69689)	Fruits: to poison fish[470]	WR Stem: gastritis & diabetes[471]	WR Stem bark, roots: antibacterial[472, 473]	WR [472, 474]

WR- widely reported

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Appendix 2: Literature review of NSW medicinal plants used for skin related ailments

Scientific name, Distribution	Medicinal uses	Literature with reference to biological study	Literature with reference to chemical study
Acacia falcata, NSW, QLD	Sores, skin complaints	None found	None found
Acacia implexa, ACT, NSW, Qld, Tas, Vic	Sores, skin complaints	None found	None found
Acacia tetragonophylla, NSW, NT, Qld, SA, Vic, WA	Sores, skin complaints, wounds	Leaves: antibacterial[1] Seeds: antioxidant, antidiabetic[2]	None found
Ailanthus triphysa, NSW, Qld, WA	Sores, skin complaints	None found	Leaves: 15-isopimaradiene-2α,3α,19-triol; 6α,7β-dihydroxy-17(20)-*cis*-5α-pregna-16-one; kaempfer-3-*O*-*β*-galactopyranoside; kaempferol-3-*O*-*α*-L-rhamnopyranoside, scopoletin[3]
Ajuga australis, NSW, Qld, SA, Tas, Vic	Sores, skin complaints	Aerial parts: acaricidal and cytotoxic activities[4], anti-inflammatory[5]	Aerial parts: ajugorientin, ajugapitin, 14,15-dihydro-15-hydroxyajugapitin[6]
Alocasia brisbanensis, NSW, Qld, Vic, WA	Cuts, sores, wounds	None found	None found
Alphitonia excelsa, NSW, Qld, NT	Antiseptic	Leaves: anti-inflammatory[5], antimicrobial[7]	Bark: alphitexolide[8]
Alstonia constricta, NSW, Qld	Sores, skin complaints	WR Leaves, roots, bark: antibacterial[9] Bark: antimalarial[10], anticancer[11]	WR Bark: (19,20)-*E*-alstoscholarine, (19,20) *Z*-alstoscholarine[12], manilamine, N4-methylangustilobine B[13]
Amyema maidenii, NSW, NT, SA, Qld, Vic	Inflammation of genital areas	None found	None found
Avicennia marina, NSW, Qld, SA, Vic, WA	Sores, skin complaints, swelling, stings	Roots, leaves stems: antimicrobial, cytotoxic[14]	Roots, leaves, stems: phytochemical constituents[14] Leaves: 2′-cinnamoyl-mussaenoside, 10-*O*-(5-phenyl-2,4-pentadienoyl)-geniposide, 7-*O*-(5-phenyl-2,4-pentadienoyl)-8-epiloganin[15] Twigs: 11-hydroxy-8,11,13-abietatriene 12-*O*-β-xylopyranoside, (7'S,8'R)-4,4',9'-trihydroxy-3,3',5,5'-tetramethoxy-7,8-dehydro-9-al-2,7'-cyclolignan, 6,11,12,16-tetrahydroxy-5,8,11,13-abitetetraen-7-one, lyoniresinol[16]

Scientific name, Distribution	Medicinal uses	Literature with reference to biological study	Literature with reference to chemical study
Canavalia rosea, NSW, Qld, NT, WA	Boils, sores	WR Leaves: antibacterial[17]	WR Aerial parts: canarosine, β-sitosterol, stigmasterol, daucosterol, epi-inositol 6-*O*-methyl ether, rutin[18]
Cassytha glabella, NSW, Qld, SA, Tas, Vic, WA	bathing of body to relieve aches & pains	None found	Aerial parts: quercetin 3-*O*-apiofuranosyl-(1→2)-[rhamnopyranosyl-(1→6)-galactopyranoside], quercetin 3-*O*-apiosylgalactoside, quercetin 3-*O*-apiofuranosyl-(1→2)-arabinopyranoside[19]
Centipeda cunninghamii, NSW, NT, Qld, SA, Vic	Cough, cold,skin infections	Leaves: antioxidant, anti-inflammatory[20]	Leaves: *cis*-chrysanthenyl acetate[21] Flowers: flavonoids[20]
Centaurium spicatum, NSW, NT, Qld, SA, Tas, Vic, WA	Sores, skin complaints	Whole plant: antioxidant[22] Roots: antioxidant[23], antimicrobial, antiprotozoal[24]	WR Whole plant: 3-*O*-[(2,3,4-triacetyl-α-rhamnopyranosyl)-(1→6)]-β-galactopyranoside, quercetin 3-*O*-[(2,3,4-triacetyl-α-rhamnopyranosyl)-(1→6)]-3-acetyl-β-galactopyranoside, quercetin 3-*O*-[(2,3,4- triacetyl-α-rhamnopyranosyl)-(1→6)]-4-acetyl-β-galactopyranoside[22] Roots: 1,5,8-trihydroxy-3,6,7-trimethoxyxanthone, 1,8-dihydroxy-3,6-dimethoxyxanthone, 1-hydroxy-3,5,8-trimethoxyxanthone[24]
Clematis microphylla, NSW, Qld, SA, Tas, Vic, WA	Sores, skin complaints	Stems: anti-inflammatory[25]	Leaves: protoanemonin[26]
Cleome viscosa, NSW, NT, SA, Qld, WA	Sores, skin complaints, wounds	WR Whole plant: antioxidant, anti-inflammatory[27, 28]	WR Seeds: nevirapine[29]
Clerodendrum floribundum, NSW, NT, Qld, WA	Sores, skin complaints, swelling	Aerial parts: cytotoxic[4]	None found
Clerodendrum inerme, NSW, NT, Qld, WA	Sores, skin complaints, wounds	WR Leaves: anti-inflammatory[30], antidiabetic[31]	WR Leaves: hispidulin[32]
Corymbia Dichromophloia, NSW, NT, SA, WA	Wounds	None found	Wood: *cis*- & *trans*-3,5,4'-trihydroxy stilbenes[33]
Corymbia gummifera, NSW, Vic, Qld	Antiseptic, fungal infections	None found	Leaves: GC-MS analysis[34]
Corymbia tessellaris, NSW, Qld, SA, Tas, Vic	Sores, skin complaints	None found	None found

Scientific name, Distribution	Medicinal uses	Literature with reference to biological study	Literature with reference to chemical study
Crinum pedunculatum, NSW, NT, Qld	Stings	None found	None found
Cymbidium canaliculatum, NSW, NT, Qld, Vic, SA	Sores, skin complaints, wounds	None found	None found
Dendrocnide excelsa, NSW, Qld, VIC	Sores, skin complaints	None found	None found
Discorea transversa, NSW, NT, Qld, WA	Sores, skin complaints	None found	Rhizome: hydroxyprotogracillin, protogracillin[35]
Dodonaea viscosa, NSW, NT, Qld, SA, Vic, WA	Wounds	WR Aerial parts: antibacterial[36], antioxidant, anticholinesterase[37]	WR Aerial parts: 6β-hydroxy-15,16-epoxy-5β,8β,9β,10α-cleroda-3,13(16),14-trien-18-oic acid[36], santin, penduletin, viscosine, 6,7-dimethylkaempferol, kaempferol- 3-methyl ether, 3,4′-dimethoxy-5,7-dihydroxyflavone[37]
Eremophila freelingii, NSW, NT, Qld, SA, WA	Sores, skin complaints, wounds	Leaves: antibacterial[1] Leaves & flowers: inhibit platelet aggregation[38]	Whole plants: freelingnite[39], freelingyne[40]
Eremophila gilesii, NSW, NT, Qld, SA, WA	Sores, skin complaints	Aerial parts: inhibit platelet aggregation[41]	Leaves: verbascoside, poliumoside[42]
Eremophila longifolia, NSW, NT, Qld, SA, Vic, WA	Sores, skin complaints	Leaves: antibacterial[43] Aerial parts: antibacterial[44]	Leaves: geniposidic acid[45]
Eremophila sturtii, NSW, NT, SA, Qld, Vic	Sores, skin complaints	Leaves: antimicrobial, anti-inflammatory[46]	Leaves: 3,8-dihydroxyserrulatic acid, serrulatic acid[46]
Ervatamia angustisepala, NSW, Qld	Sores, skin complaints	None found	Aerial parts: alkaloids[47]
Eucalyptus camaldulensis, NSW, NT, Qld, SA, Vic, WA	Sores, skin complaints, wounds	WR Leaves: antimicrobial[48]	WR Leaves: GC-MS analysis[48]
Eucalyptus haemastoma, NSW	Sores, skin complaints	None found	Leaves: abscisic acid[49]

Scientific name, Distribution	Medicinal uses	Literature with reference to biological study	Literature with reference to chemical study
Eucalyptus microtheca, NSW, Qld, NT, SA, WA	Sores, skin complaints	Leaves, bark, roots: allelopathic[50]	Leaves: chemical composition[51]
Exocarpos aphyllus, NSW, Qld	Sores, skin complaints	Aerial parts: antibacterial[52]	None found
Exocaria agallocha, NSW, NT, Qld, WA	Burns	WR Leaves: antimicrobial[53] Bark: cytotoxic[54]	WR Leaves: *n*-hentriacontane, taraxerone, taraxerol[55], chemical composition[56]
Ficus coronata, NSW, NT, Qld, Vic	Wounds	Leaves & barks: antibacterial[57]	Leaves & bark: HPLC analysis[57]
Flagellaria indica, NSW, Qld, NT, WA	Wounds	Leaves: antioxidant[58, 59]	None found
Flindersia maculosa, NSW, Qld	Aches, pains	Leaves: anthelmintic[60]	Bark: alkaloids, 17,19,22,24- tetrahydroxy(l4-p,0-o)cyclophane[61]
Grewia retusifolia, NSW, NT, Qld	Sores, skin complaints	None found	None found
Grevillea striata, NSW, NT, Qld, SA, WA	Wounds	None found	Wood: turrianes[61]
Hibbertia scandens, NSW, Qld, Vic	Sores, rashes	None found	None found
Hibiscus tiliaceus, NSW, NT, Qld	Sores, skin complaints, wounds	WR Flowers: antioxidant, antimutagenic[62], antidepressant[63]	WR Stem: amide[64] Stems & bark: friedelin, pachysandiol, glutinol, lupeol, germanicol, stigmast-4-en-3-one, stigmast-4,22-dien-3-one, ergosta-4,6,8,22-tetraen-3-one, β-sitosterol, stigmasterol[65]
Ipomoea pes-caprae, NSW, NT, Qld, WA	Sores, skin complaints, fungal infections	WR Aerial part: antioxidant, anti-inflammatory[66] Whole plant: antitumor[67]	WR Aerial part: 7-hydroxy-6-methoxycoumarin; 5,7,4'-trimethoxykaempferol; 3,7,8,3'4'-pentahydroxyflavone; *trans*-[3-(4'-hydroxyphenyl)-2-propenoic acid][66]
Lophostemon suaveolens, NSW, Qld, WA, NT	Wounds	Leaves: antibacterial, anti-inflammatory[68]	Leaves: GC-MS analysis[69]
Melaleuca alternifolia, NSW, Qld	Sores, skin complaints, wounds, fungal infections	WR Leaves: antimicrobial[70], antifungal[71]	WR Leaves: terpenoids[72]

Scientific name, Distribution	Medicinal uses	Literature with reference to biological study	Literature with reference to chemical study
Pittosporum angustifolium, NSW, NT, Qld, Vic, SA, WA	Sores, skin complaints	WR Seeds: cytotoxic[73] Leaves: antibacterial[74]	WR Leaves: pittangretosides A-I [75]
Plumbago zeylanica, NSW, NT, Qld, WA	Sores, skin complaints	WR Roots: antibacterial[76]	WR Roots: plumbagin[77], napthaquinone[78]
Pouteria pohlmaniana, NSW, Qld	Wounds	None found	Bark: bayogenin[79]
Pterocaulon sphacelatum, NSW, Qld, Vic, NT,SA, WA	Sores, skin complaints	Aerial parts: antibacterial[43, 80]	Aerial parts: chrysosplenol, 6,7,8-trimethoxycoumarin[80]
Sarcostemma australe, NSW, Qld, SA, WA	Sores, skin complaints, wounds, scurvy	None found	Aerial parts: sarcostin[81]
Smilax glyciphylla, NSW, Qld	Sores	Whole plant: antioxidants[82]	Leaves: glycyphyllin[83]
Sterculia quadrifida, NSW, NT, Qld	Sores , skin complaints, wounds, stings	Leaves: antimicrobial[7]	None found
Syncarpia glomulifera, NSW, Qld	Antiseptic	Bark: antimicrobial, cytotoxic[84] Leaves: antimicrobial[85]	Bark: betulinic acid, oleanolic acid-3-acetate, ursolic acid-3-acetate[86]
Scaevola spinescens, NSW, Qld, Vic, NT,SA, WA	Sores, skin complaints	Whole plant: antibacterial, antiviral, cytotoxicity[87]	None found
Trichodesma zeylanicum, NSW, Qld, NT, SA, WA	Sores, skin complaints	Roots: antioxidant[88]	None found
Trichosanthes palmata, NSW, Qld	Sores, skin complaints	None found	None found
Verbena officinalis, NSW, Qld, Vic, NT, SA, WA	Wounds, sores	WR Aerial parts: anticancer[89]	WR Aerial parts: phenylethanoid glycosides[89] Leaves 4-*epi*-barbinervic acid, 2α,3β-dihydroxyurs-12-en-28-oic acid, 3α,24-dihydroxyurs-12-en-28-oic acid, 3α,24-dihydroxy-olean-12-en-28-oic acid, ursolic acid[90]

Notes: ACT: Australian Capital Territory; NSW: New South Wales; NT: Northern Territory; Qld: Queensland; SA: South Australia; Tas: Tasmania; Vic: Victoria;

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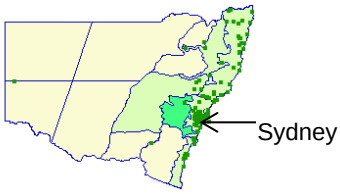
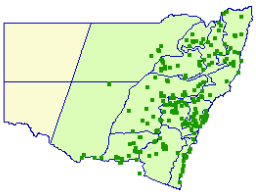
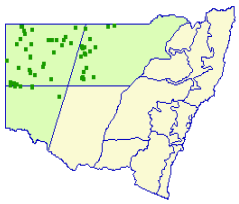
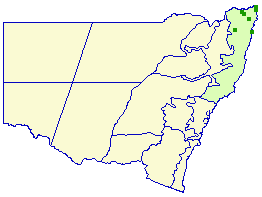
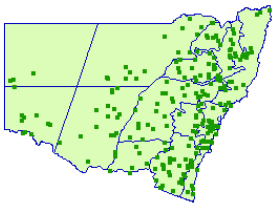
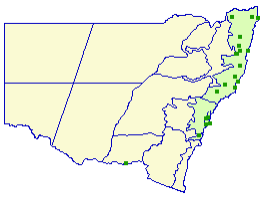
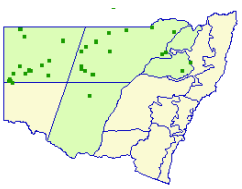
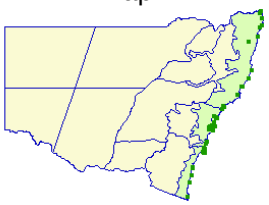
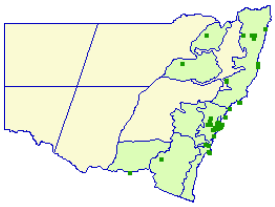
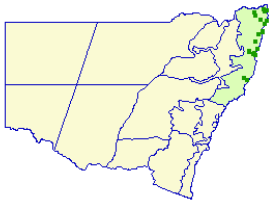
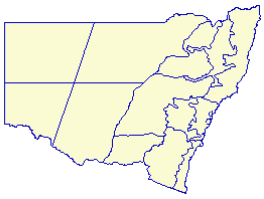
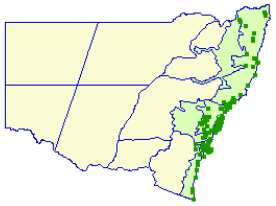
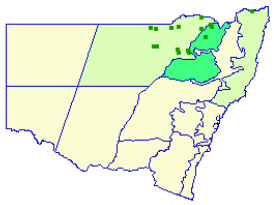
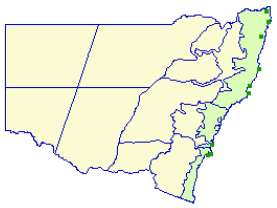
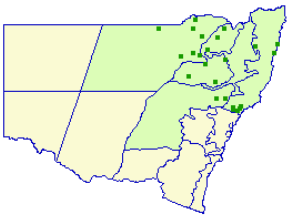
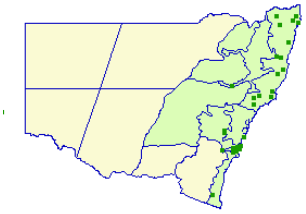
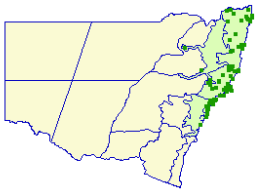
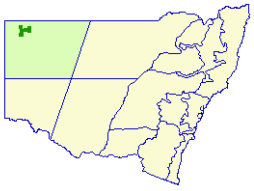
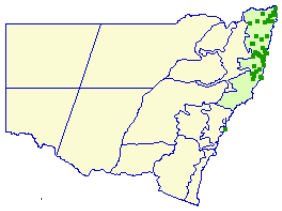
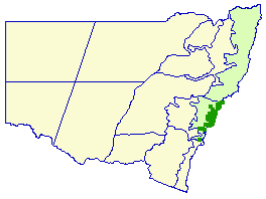
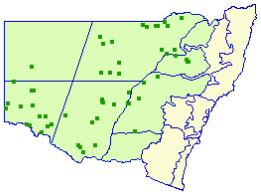
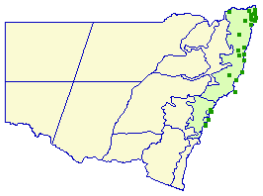
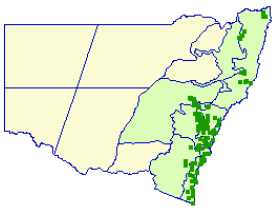
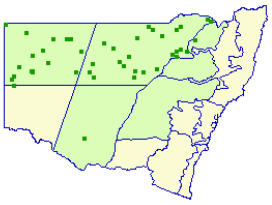
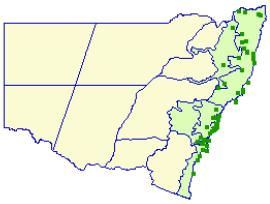
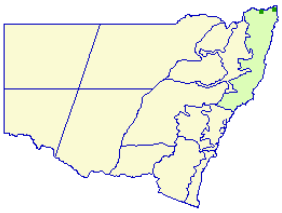
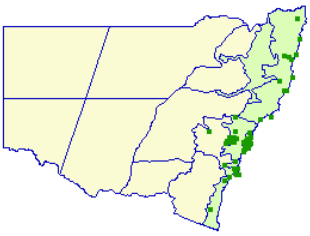
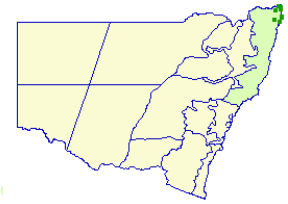
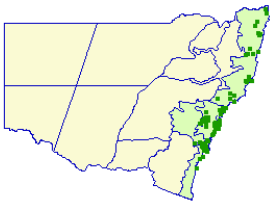
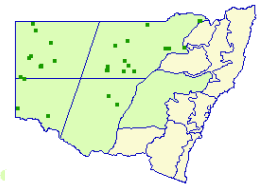
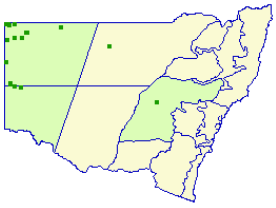
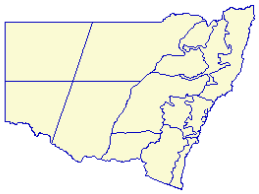
Appendix 3: NSW medicinal plants with none or limited reports on biological and chemical studies and their availability. Retrieved from http://plantnet.rbgsyd.nsw.gov.au/

Plant Name	Notes	Map
Acacia falcata, NSW, Qld	Common, locally available. Was easily obtainable.	
Acacia implexa, ACT, NSW, Qld, Tas, Vic	Common, locally available. Was easily obtainable.	
Acacia tetragonophylla, NSW, NT, Qld, SA, Vic, WA	Not grown in Sydney region. Grown in North Western Plains, North Western Far Plains and South Far Western Plains.	
Ailanthus triphysa, NSW, Qld, WA	Not common in Sydney region. Grown in North Coast.	
Ajuga australis, NSW, Qld, SA, Tas, Vic	Common in Sydney and throughout NSW.	
Alocasia brisbanensis, NSW, Qld, Vic, WA	Common in Sydney. Grown in North Coast and Central Coast.	
Amyema maidenii, NSW, NT, SA, Qld, Vic	Not Common in Sydney region. Grown in North Western Slopes, North Western Plains, South Western Plains and North Far Western Plains.	

Plant Name	Notes	Map
Avicennia marina, NSW, Qld, SA, Vic, WA	Common in Sydney. Grown in North Coast, Central coast and South Coast.	
Cassytha glabella NSW, Qld, SA, Tas, Vic, WA	Common in Sydney. Was easily obtainable.	
Clerodendrum floribundum, NSW, NT, Qld, WA	Not common in Sydney. Grown in North Coast.	
Corymbia Dichromophloia, NSW, NT, SA, WA	Rare in NSW. Available in Northern Territory.	
Corymbia gummifera, NSW, Vic, Qld	Abundant. Grown in North Coast, Central Coast, South Coast, Northern Tablelands and Central Tablelands.	
Corymbia tessellaris, NSW, Qld, SA, Tas, Vic	Not Common in Sydney. Grown in North Western Slopes and North Western Plains.	
Crinum pedunculatum, NSW, NT, Qld	Not common in Sydney. Grown in North Coast, Central coast and South Coast.	

Plant Name	Notes	Map
Cymbidium canaliculatum, NSW, NT, SA, Qld, Vic	A rare and scattered species of orchid. Grown in North Coast, Northern Tablelands, North Western Slopes, Central Western Slopes and North Western Plains.	
Dendrocnide excelsa, NSW, Qld, Vic	Common in Sydney. Grown in North Coast, Northern Tablelands, North Western Slopes, Central Coast, South Coast, Central Western Slopes, Central Tablelands, Central Western Plains and North Western Plains.	
Discorea transversa, NSW, NT, Qld, WA	Abundant in Sydney. Grown in North Coast, Central Coast and Northern Tablelands.	
Eremophila freelingii, NSW, NT, Qld, SA, WA	Does not occur in Sydney. Grown in North Far Western Plains.	
Ervatamia angustisepala, NSW, Qld	Not common in Sydney region. Grown in Central Coast.	
Eucalyptus haemastoma, NSW	Locally available and common. Was easily obtainable.	
Exocarpos aphyllus, NSW, QLD	Not common in Sydney region.	

Plant Name	Notes	MAP
Flagellaria indica, NSW, QLD, NT, WA	Locally available and common. Was easily obtainable.	
Grewia retusifolia, NSW, NT, QLD	Common. Grown in North Coast, Northern Tablelands, North Western Slopes, Central Coast, South Coast, Central Western Slopes, Central Tablelands and Southern Tablelands.	
Grevillea striata, NSW, NT, Qld, SA, WA	Not Common. Grown in Central Western Slopes, Northern Western Slopes, North Western Plains, South Western Plains and North Far Western Plains.	
Hibbertia scandens, NSW, Qld, Vic	Common in Sydney region. Grown in North Coast, Central Coast, South Coast, North Tablelands and Central Tablelands.	
Pouteria pohlmaniana	Rare in NSW. Grown in North Coast.	
Smilax glyciphylla, NSW, Qld	Common in Sydney region. Was easily obtainable.	
Sterculia quadrifida, NSW, NT, Qld	Rare in NSW. Grown in North Coast.	

Plant Name	Notes	Map
Syncarpia glomulifera, NSW, Qld	Abundant. Grown in North Coast, Central coast, South Coast and Central Tablelands. Was easily obtainable.	
Scaevola spinescens, NSW, Qld, Vic, NT,SA, WA	Not common in Sydney region. Grown in Central Western Slopes, North Western Plains, Central Western Plains, North Far Western Plains and South Far Western Plains.	
Trichodesma zeylanicum, NSW, Qld	Not common in Sydney region. Grown in Central Western Slopes, North Far Western Plains, South Western Plains.	
Trichosanthes palmata, NSW, Qld	Rare in NSW. Grown in Byron bay to sunshine Coast.	

Notes: ACT: Australian Capital Territory; NSW: New South Wales; NT: Northern Territory; Qld: Queensland; SA: South Australia; Tas: Tasmania; Vic: Victoria; Retrieved from http://plantnet.rbgsyd.nsw.gov.au/ ;
*Bolded plants were collected for investigation as these were available in accessible locations and in sustainable quantities for collection

APPENDIX 4:

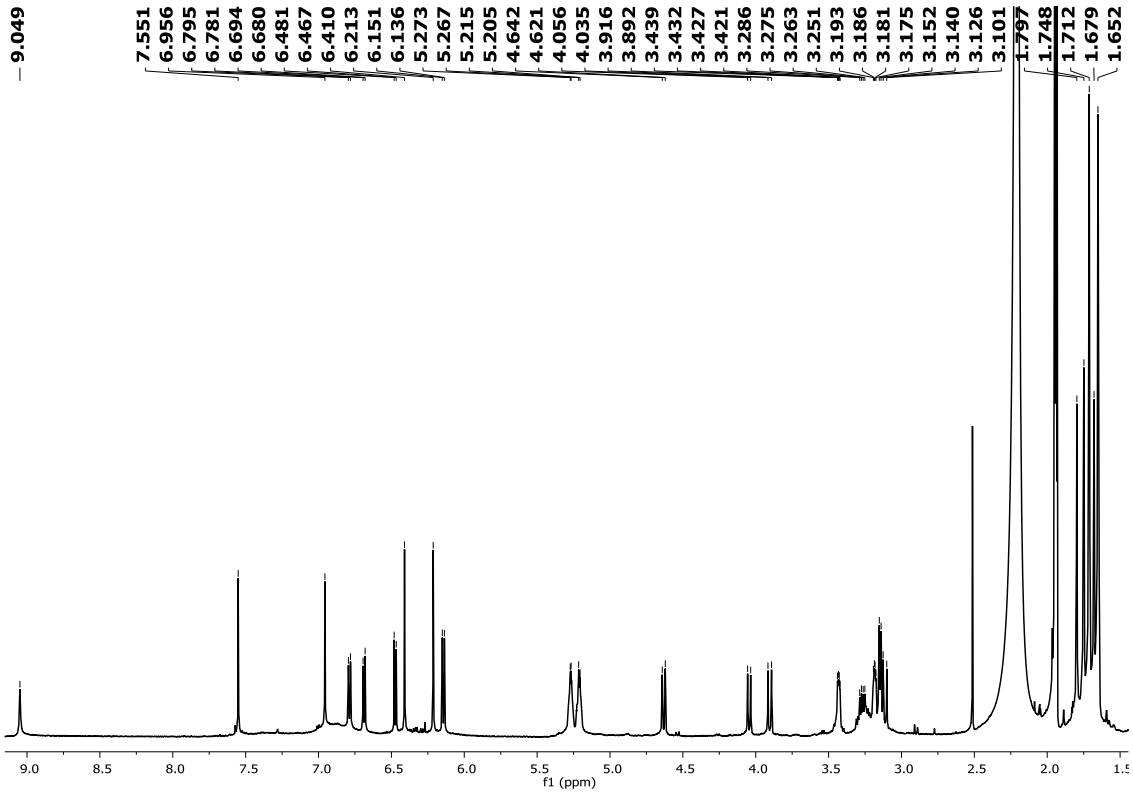


Figure A 1. ^{1}H NMR (acetonitrile-d_3, 600 MHz) spectrum of erynone (**1**)

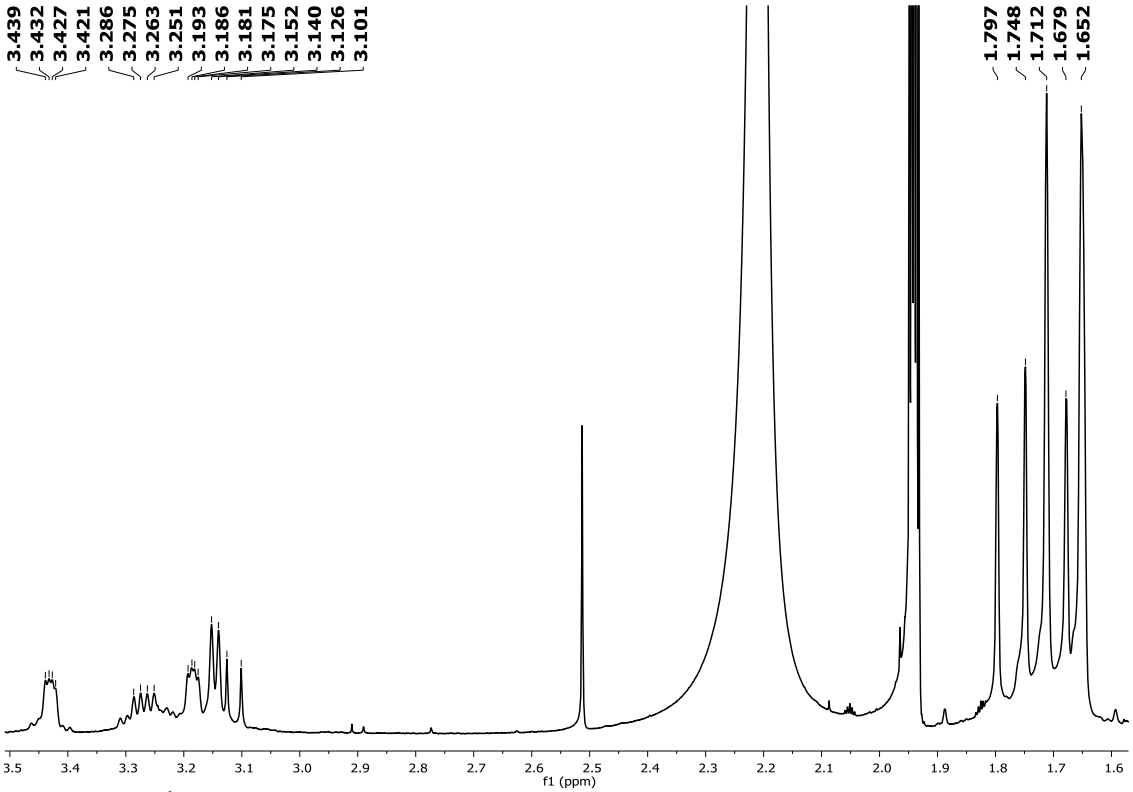


Figure A 2. ^{1}H NMR (acetonitrile-d_3, 600 MHz) spectrum of erynone (**1**) (Insert 1)

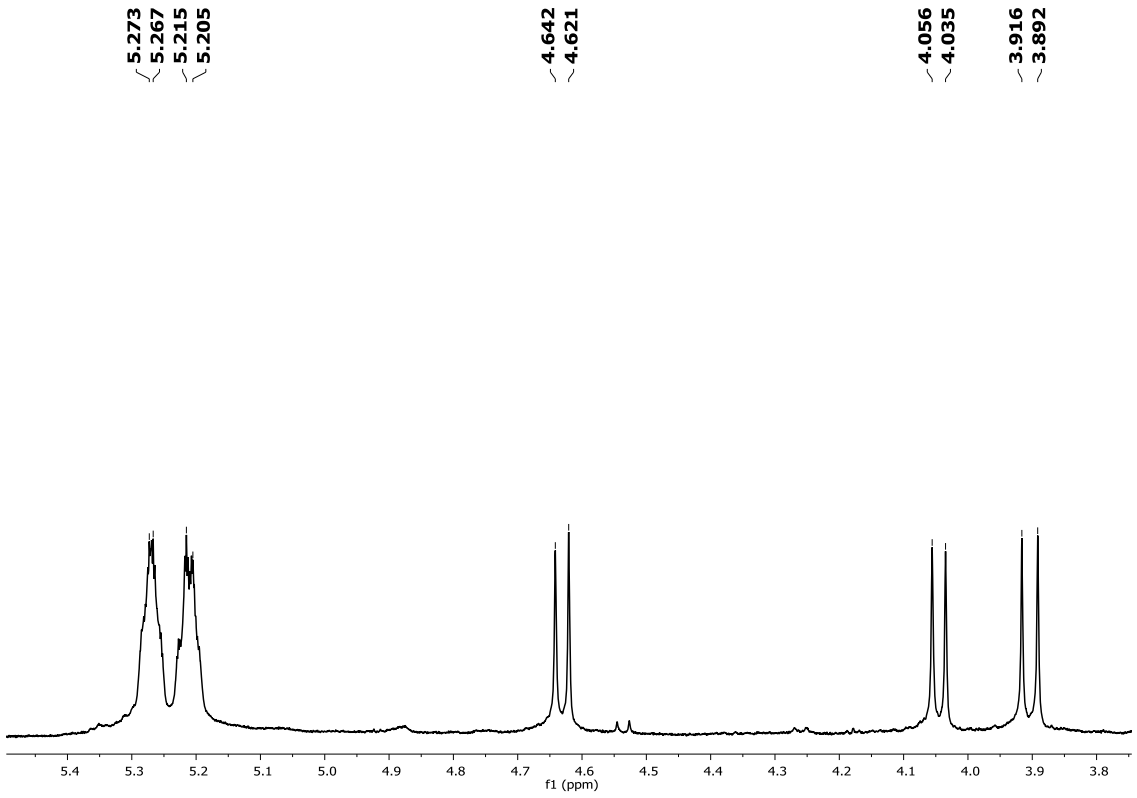


Figure A 3. ^{1}H NMR (acetonitrile-d_3, 600 MHz) spectrum of erynone (**1**) (Insert 2)

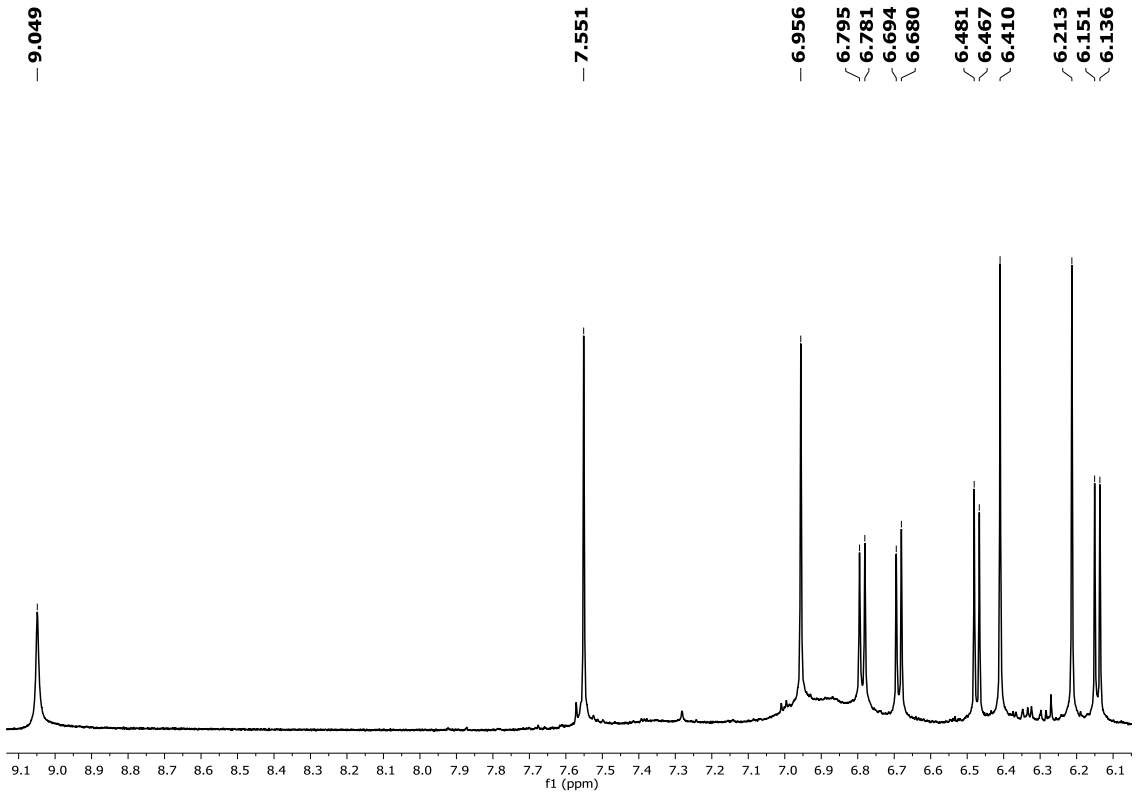


Figure A 4. ^{1}H NMR (acetonitrile-d_3, 600 MHz) spectrum of erynone (**1**) (Insert 3)

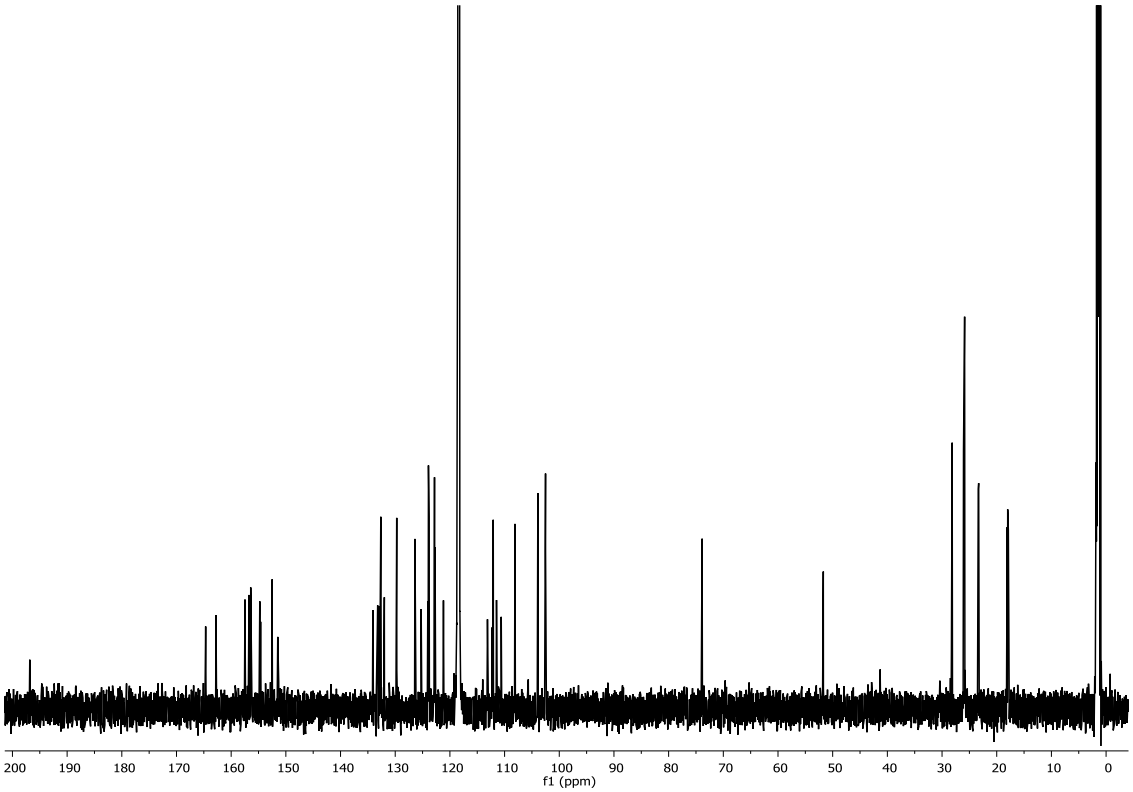


Figure A 5. ^{13}C NMR (acetonitrile-d_3, 150.0 MHz) spectrum of erynone (**1**)

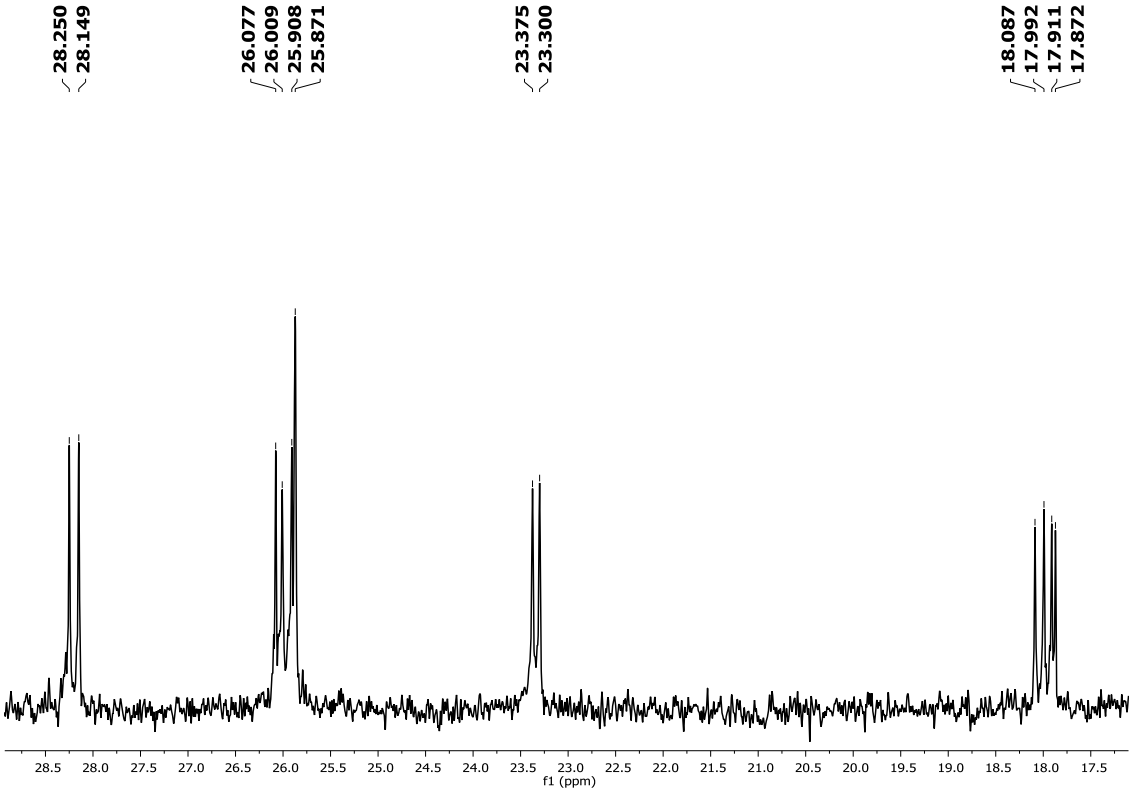


Figure A 6. ^{13}C NMR (acetonitrile-d_3, 150.0 MHz) spectrum of erynone (**1**) (Insert 1)

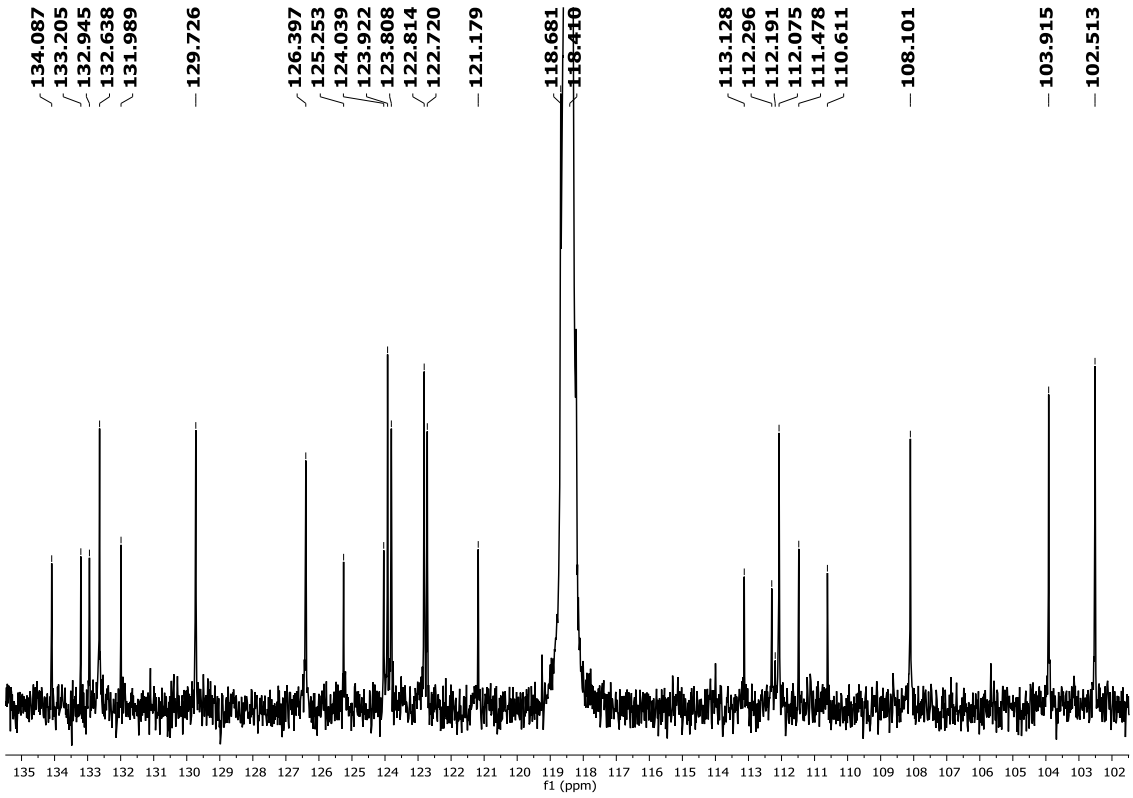


Figure A 7. ^{13}C NMR (acetonitrile-d_3, 150.0 MHz) spectrum of erynone (**1**) (Insert 2)

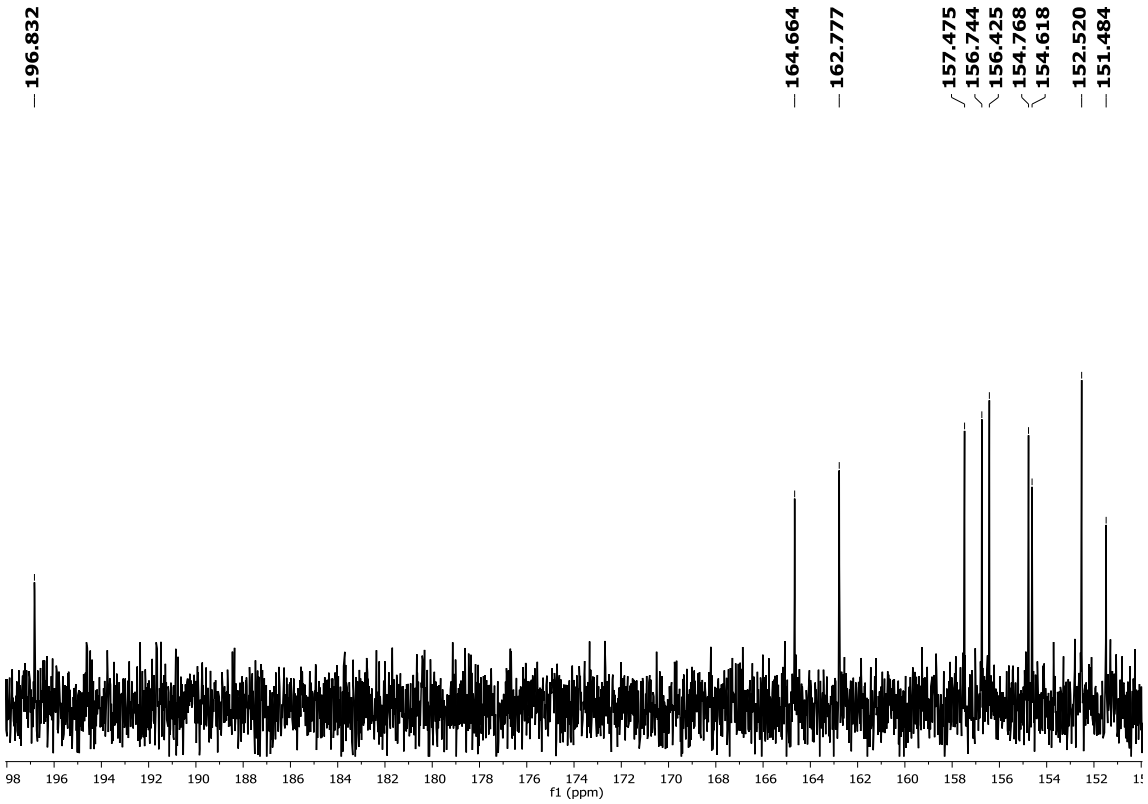


Figure A 8. ^{13}C NMR (acetonitrile-d_3, 150.0 MHz) spectrum of erynone (**1**) (Insert 3)

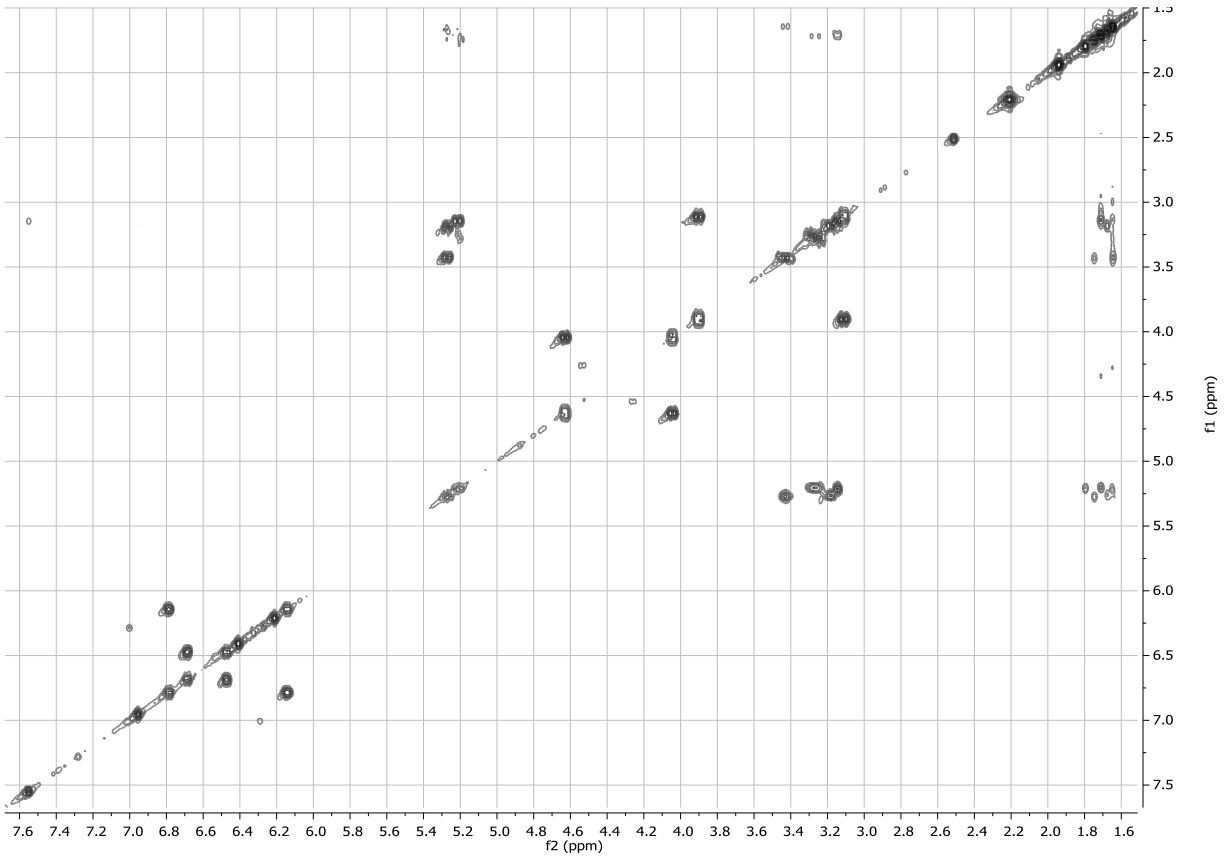


Figure A 9. ^{1}H-^{1}H COSY (acetonitrile-d_3, 600 MHz) spectrum of erynone (**1**)

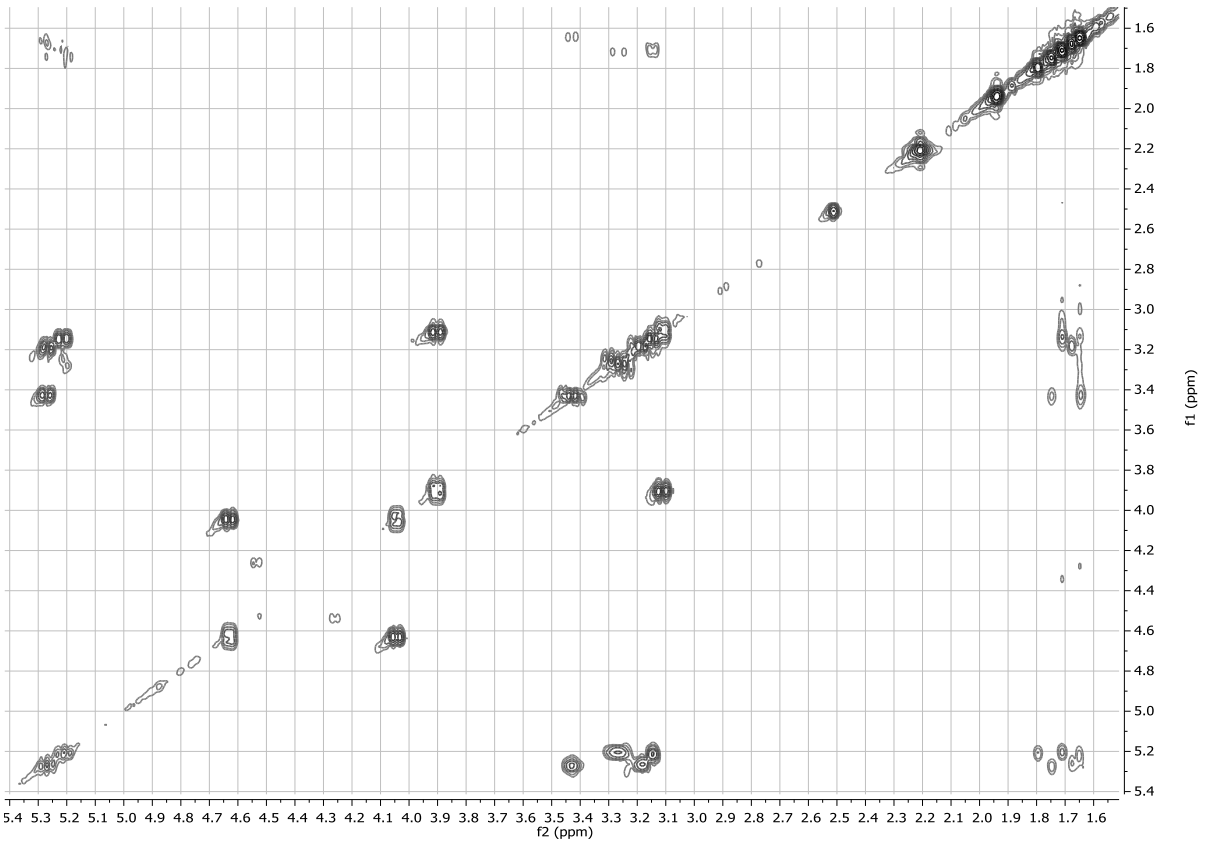


Figure A 10. ^{1}H-^{1}H COSY (acetonitrile-d_3, 600 MHz) spectrum of erynone (**1**) (Insert)

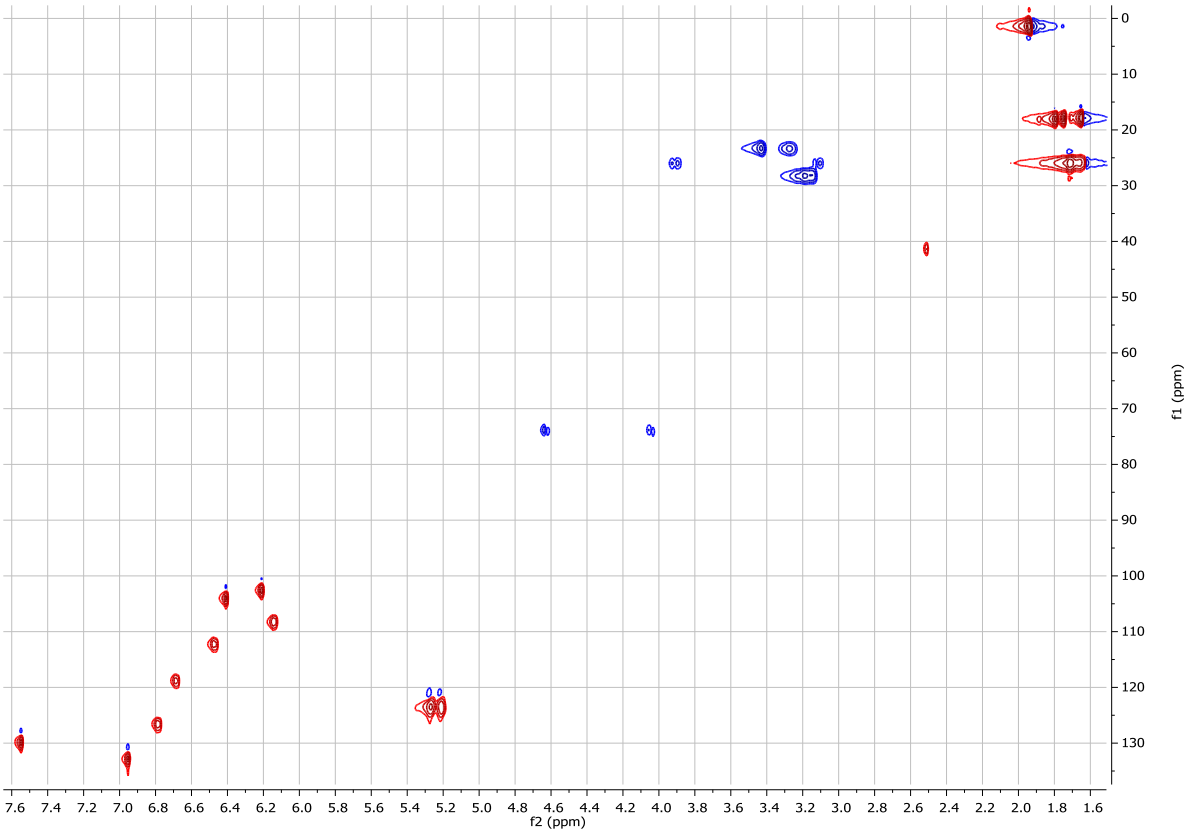


Figure A 11. HSQC (acetonitrile-d_3, 600 MHz) spectrum of erynone (**1**)

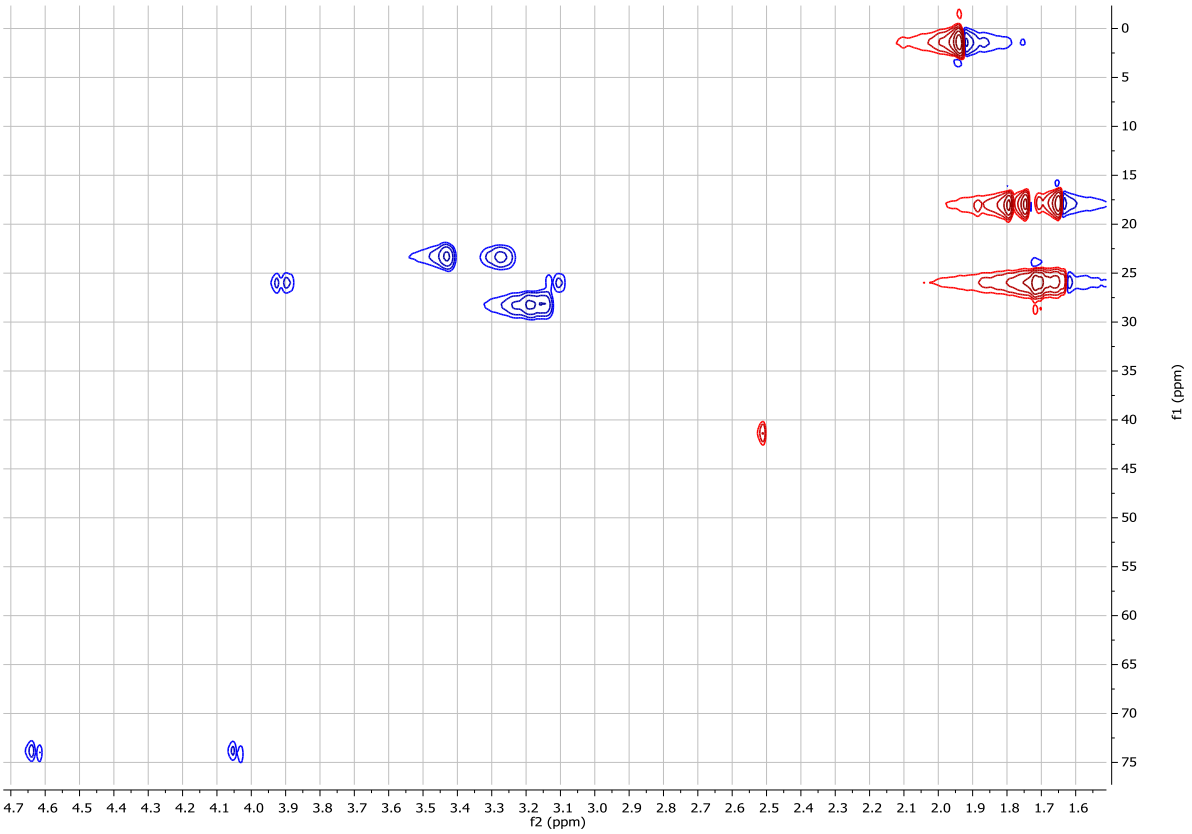


Figure A 12. HSQC (acetonitrile-d_3, 600 MHz) spectrum of erynone (**1**) (Insert)

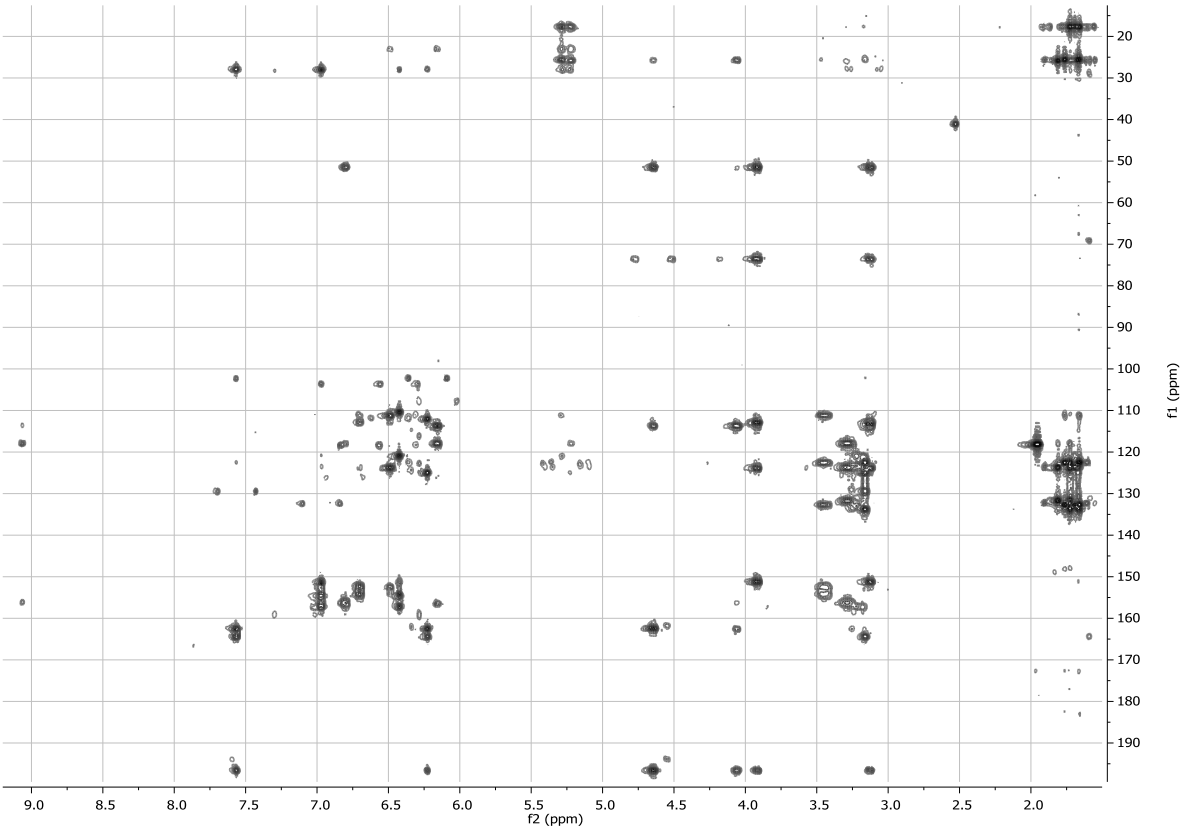


Figure A 13. HMBC (acetonitrile-d_3, 600 MHz) spectrum of erynone (**1**)

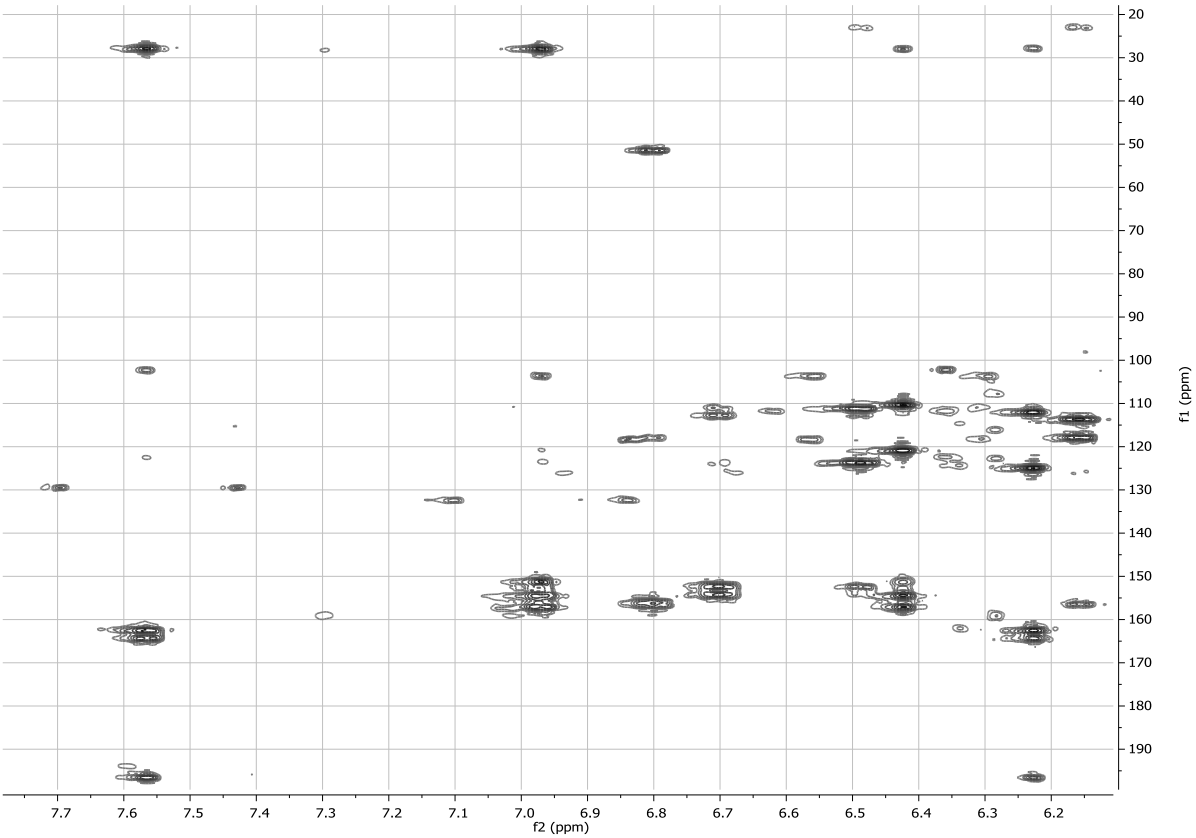


Figure A 14. HMBC (acetonitrile-d_3, 600 MHz) spectrum of erynone (**1**) (Insert)

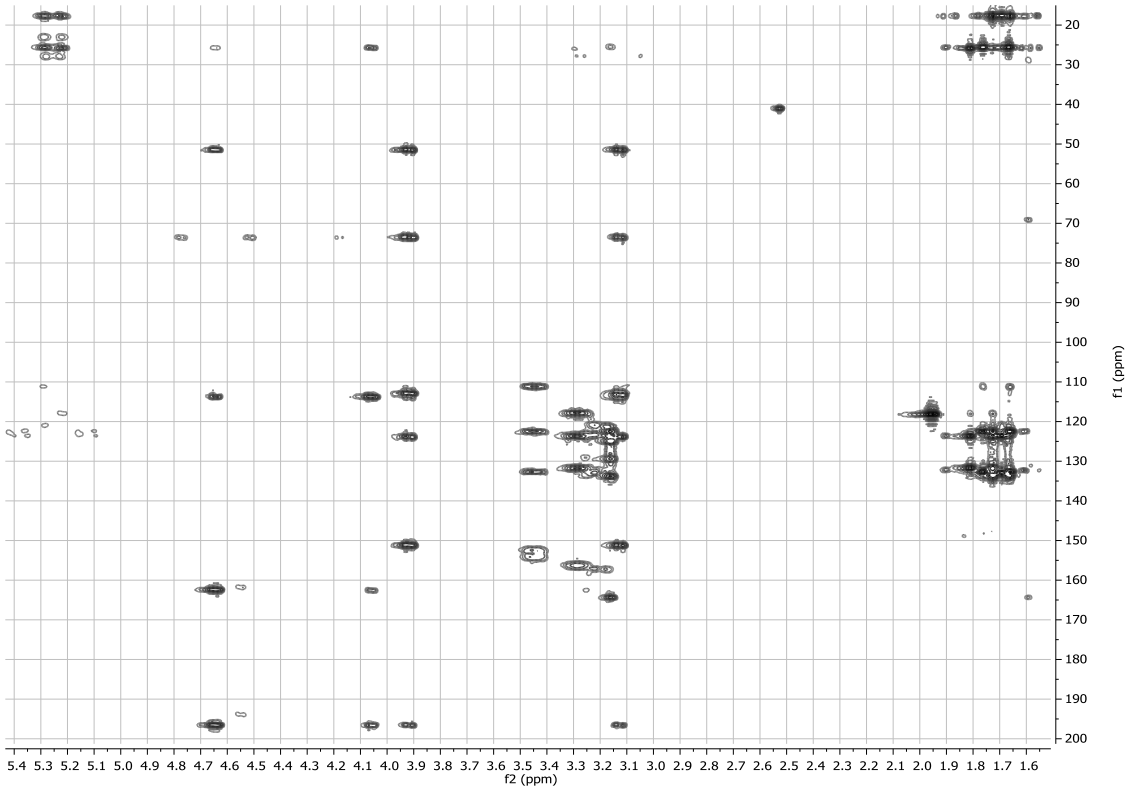


Figure A 15. HMBC (acetonitrile-d_3, 600 MHz) spectrum of erynone (**1**) (Insert)

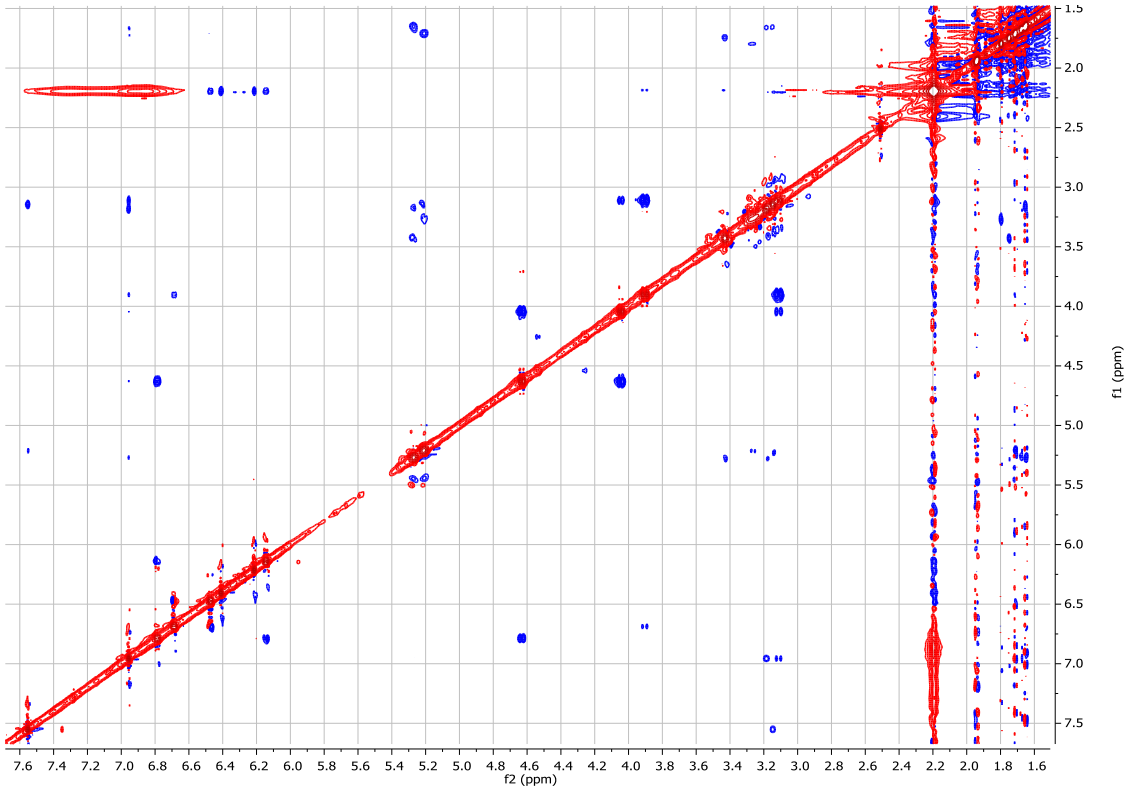


Figure A 16. ROESY (acetonitrile-d_3, 600 MHz) spectrum of erynone (**1**)

APPENDIX 5:

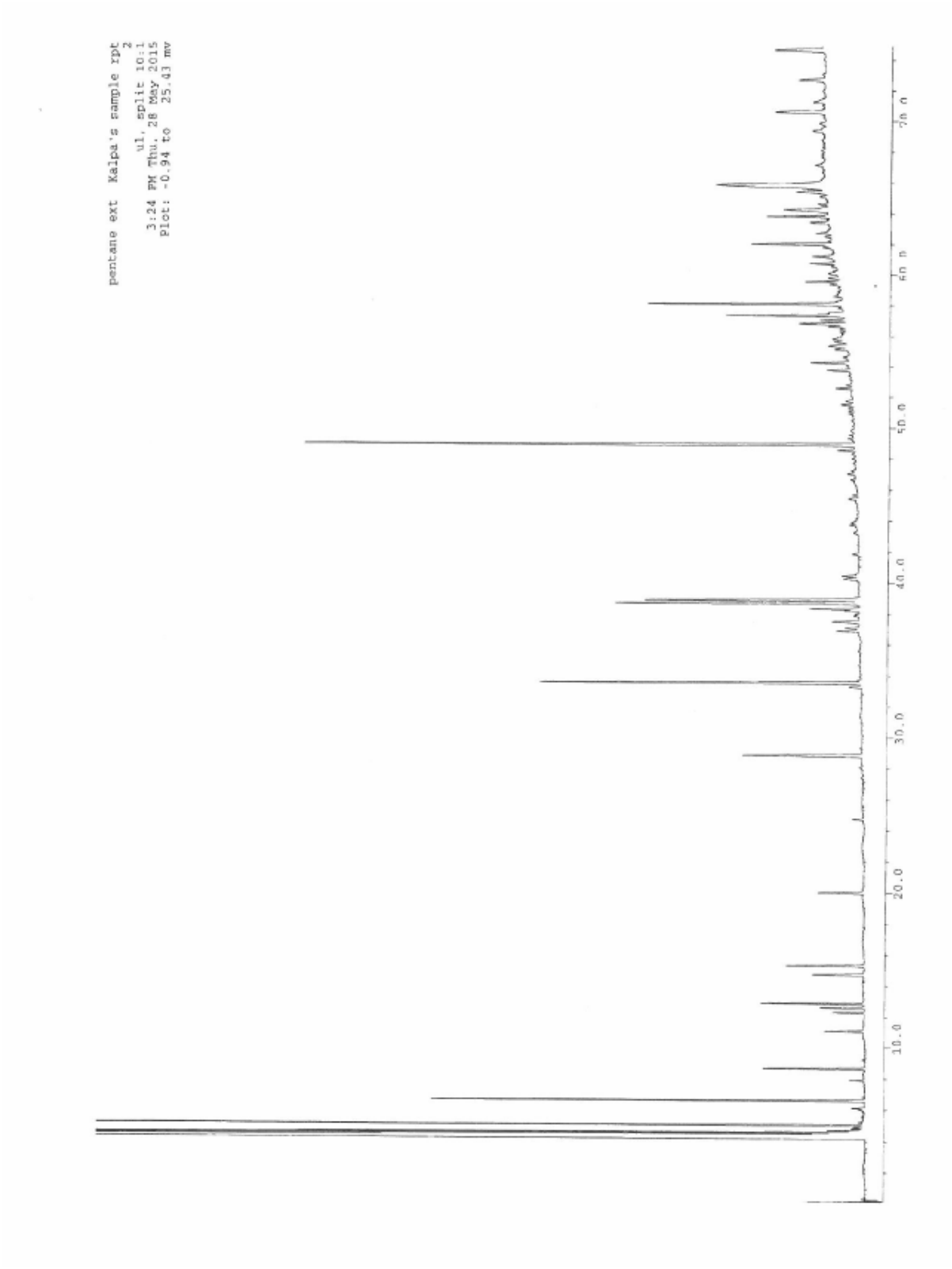


Figure A 17. GLC-FID trace of the *n*-hexane extract of *Erythrina stricta* on a BP-20 column

APPENDIX 6

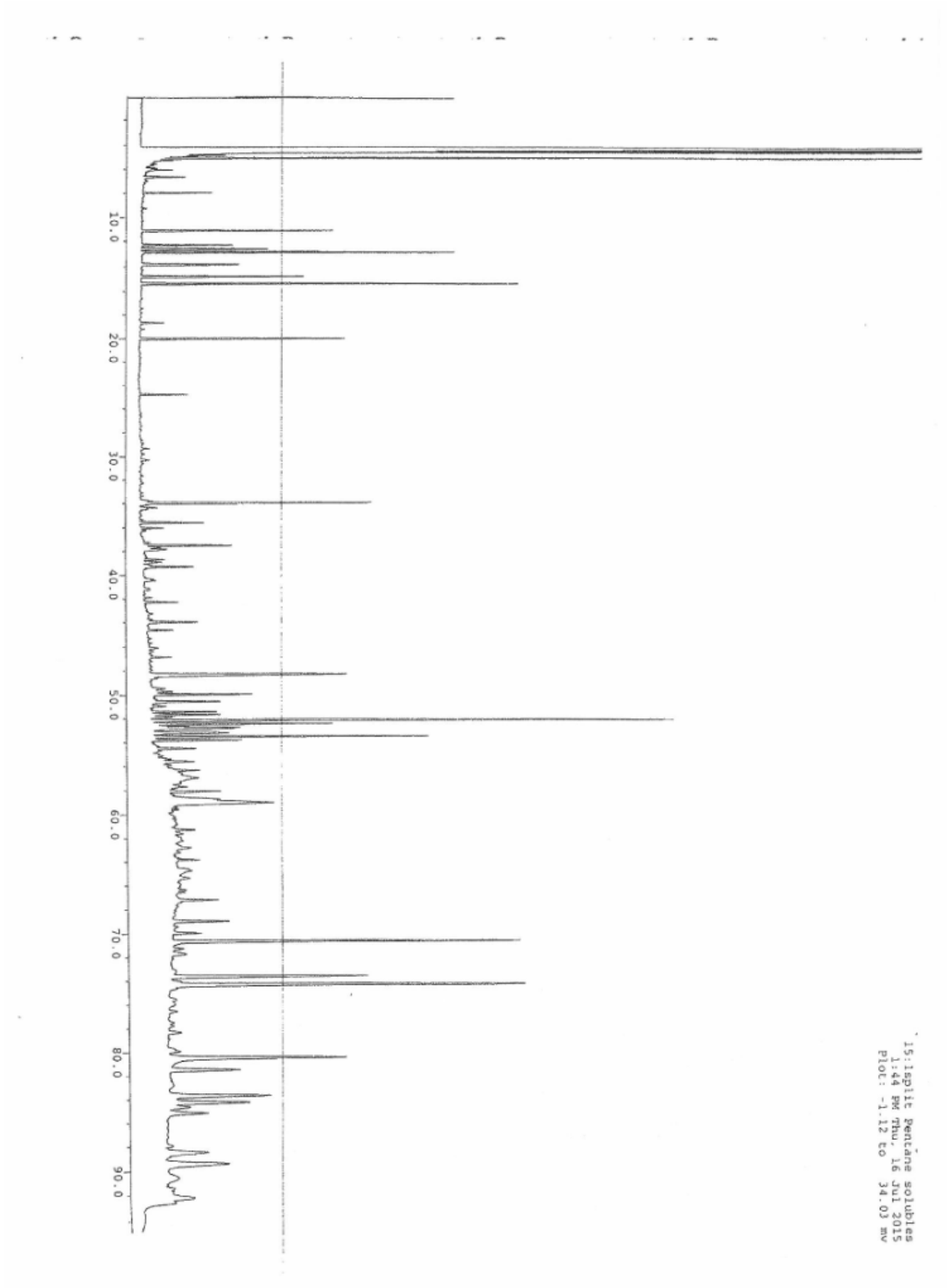


Figure A 18. GLC-FID trace of the *n*-hexane extract of *Syncarpia glomulifera* on a BP-20 column

APPENDIX 7

MACQUARIE UNIVERSITY

Research Office
Research Hub, Building C5C East
MACQUARIE UNIVERSITY NSW 2109

Phone +61 (0)2 9850 8612
Fax +61 (0)2 9850 4465
Email ro@mq.edu.au

Biosafety Committee
Phone +61 (0)2 9850 4194
Email biosafety@mq.edu.au

23 April 2010

Associate Professor Subramanyam Vemulpad
Department of Chiropractic
Faculty of Science
Macquarie University

IBC Reference: 08/06/LAB

Dear Associate Professor Vemulpad

Title of project: Antimicrobial Assays with Medicinal Plant Materials

The above application was considered by the Biosafety Committee at its meeting on 22 October 2008. Final Approval of the above application is granted, effective 22 October 2008.

You must inform the Committee of your willingness to accept and comply with any conditions by signing the Agreement Statement at the end of this letter, and returning one copy of this Agreement to the Committee Secretary, Ms Nicola Myton, in the Research Office at Macquarie University.

STANDARD REQUIREMENTS ATTACHED TO APPROVAL:

1. Approval will be for a period of twelve (12) months. At the end of this period, if the project is continuing then a progress report must be submitted. If, at the end of this period the project has been completed, abandoned, discontinued or not commenced for any reason, you are required to submit a Final Report. If you complete the work earlier than you had planned you must submit a Final Report as soon as the work is completed. These reports are located at the following address:
http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/biosafety_research_ethics/forms

A Progress/Final Report for this study will be due on: 1 October 2009.

2. Please remember the Committee must be notified of any alteration to the project.

3. If you will be applying for or have applied for internal or external funding for the above project **it is your responsibility** to provide Macquarie University's Research Grants Officer with a copy of this letter as soon as possible. The Research Grants Officer will not inform external funding agencies that you have final approval for your project and funds will not be released until the Research Grants Officer has received a copy of this final approval letter.

APPENDIX 8

Ethics Secretariat <ethics.secretariat@mq.edu.au>

to A/Prof, A/Prof, Mr, Prof, Dr, joanne.packer, Mrs, me

Dear A/Prof Jamie

Re: "An Ethnopharmacological study of Australian Medicinal plants" (Ethics Ref: 5201200763)

Thank you for your recent correspondence. Your response has addressed the issues raised by the Human Research Ethics Committee and you may now commence your research.

This research meets the requirements of the National Statement on Ethical Conduct in Human Research (2007). The National Statement is available at the following web site:

http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/e72.pdf.

The following personnel are authorised to conduct this research:

A/Prof Joanne Jamie
A/Prof Subramanyam Vemulpad
Mr David Harrington
Mrs Kaisarun Akter
Mrs Tarannum Naz
Ms Joanne Michelle Packer
Prof Shoba Ranganathan

NB. STUDENTS: IT IS YOUR RESPONSIBILITY TO KEEP A COPY OF THIS APPROVAL
EMAIL TO SUBMIT WITH YOUR THESIS.

Please note the following standard requirements of approval:
1. The approval of this project is conditional upon your continuing compliance with the National Statement on Ethical Conduct in Human Research (2007).
2. Approval will be for a period of five (5) years subject to the provision of annual reports.

Progress Report 1 Due: 24 January 2014
Progress Report 2 Due: 24 January 2015
Progress Report 3 Due: 24 January 2016
Progress Report 4 Due: 24 January 2017
Final Report Due: 24 January 2018
NB. If you complete the work earlier than you had planned you must submit a Final Report as soon as the work is completed. If the project has been discontinued or not commenced for any reason, you are also required to submit a Final Report for the project.

Progress reports and Final Reports are available at the following website:

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/human_research_ethics/forms

3. If the project has run for more than five (5) years you cannot renew approval for the project. You will need to complete and submit a Final Report and submit a new application for the project. (The five year limit on renewal of approvals allows the Committee to fully re-review research in an environment where legislation, guidelines and requirements are continually changing, for example, new child protection and privacy laws).

4. All amendments to the project must be reviewed and approved by the Committee before implementation. Please complete and submit a Request for Amendment Form available at the following website:

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/human_research_ethics/forms

5. Please notify the Committee immediately in the event of any adverse effects on participants or of any unforeseen events that affect the continued ethical acceptability of the project.

6. At all times you are responsible for the ethical conduct of your research in accordance with the guidelines established by the University. This information is available at the following websites:

http://www.mq.edu.au/policy/

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/human_research_ethics/policy

If you will be applying for or have applied for internal or external funding for the above project it is your responsibility to provide the Macquarie University's Research Grants Management Assistant with a copy of this email as soon as possible. Internal and External funding agencies will not be informed that you have final approval for your project and funds will not be released until the Research Grants Management Assistant has received a copy of this email.
Please retain a copy of this email as this is your official notification of final ethics approval.

Yours sincerely
Dr Karolyn White
Director of Research Ethics
Chair, Human Research Ethics Committee