BIOLOGICAL AND PHYTOCHEMICAL ANALYSIS OF CHUNGTIA MEDICINAL PLANTS OF NAGALAND, INDIA

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

MASTER OF PHILOSOPHY

Department of Chemistry and Biomolecular Sciences
MACQUARIE UNIVERSITY
SYDNEY

October 2014

DECLARATION

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

Teresa Malewska

October 2014

ACKNOWLEDGEMENTS

First and utmost, I thank my supervisors, A/Profs Joanne Jamie and Subra Vemulpad for providing me with this MPhil opportunity. I am very grateful to Joanne and Subra for all the help and support I have received. It has been my privilege to work closely with you, I have enjoyed the opportunity to watch and learn from your knowledge and experience. Your insights and patience with me will always be remembered and appreciated. I am very proud of what we have achieved together, thank you both.

I am also indebted to Prof. Zbigniew Sidorczyk and Dr Krystyna Zych who's counsel had steered me through my postgraduate studies at University of Lodz, Poland.

I am thankful to all my Departmental friends, past and present who have made the work place a pleasant environment: Tarranum Naz (Sony), Mikolaj (Nick) Gad, Kaisarun (Kalpa) Akter, Jason Smith, Meyanungsang (Meza) Kichu and Ping Yin. Your wise advice have been very valuable. Thank you Ling Lee for your company and for making me laugh.

My special thanks goes to Wendy Loa for all the NMR, ESI-MS and Chemdraw help. Thank you Alpesh Patel for help with NMR and Mark Tran for help with IR and EI-MS. Thank you Tony for CD. Thank you Dave Harrington for the local specimen of my plant and associated vouchering work.

GC-MS work in this thesis was performed at the University of New South Wales by Dr Joe Brophy. I am really grateful to Dr Brophy for assisting me with this. Thanks also goes to Dr Russel Barrow of the Australian National University, Canberra for organising the HR-MS of the isolated compounds.

I wish to thank my dad Pawel and mum Anna, my sister Alicja and her husband Adam, who have always welcomed me warmly back in Poland. A big thanks to you all for being supportive, loving and understanding. I owe infinitely much to my husband, Boris. I would not have completed this work without your love, support and encouragement. You are the best person I have ever met.

LIST OF ABBREVIATIONS

The following abbreviations are used throughout the text:

ATCC	American Type Culture Collection
br	broad
$CDCl_3$	Deuterated chloroform
CD_3COCD_3	Deuterated aceton
$CDOD_3$	Deuterated methanol
Cfu	Colony Forming Units
$CHCl_3$	Chloroform
COSY	(Proton-Proton) Correlation Spectroscopy
CSMT	Chungtia Senso Mokokchung Town
CVC	Chungtia Village Council
d	doublet (MNR)
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
EtOAc	Ethyl acetate
EI	Electronic impact
ESI	Electrospray Ionization
EtOH	Ethanol
GS-MS	Analytical gas chromatography mass spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
HR-MS	High Resolution Mass Spectroscopy
HSQC	Heteronuclear Single Quantum Correlation
IBRG	Indigenous Bioresources Research group
ISEP	Indigenous Science Education Program
J	Coupling constant
LC-MS	Liquid Chromatography-Mass Spectroscopy
m	Multiplet (NMR)
mg	Milligram
m/z	Mass to charge ratio

MDRSA	Multi drug resistant *Staphylococcus aureus*
MeOH	Methanol
MH II	Muller Hinton II
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser effect spectroscopy
R_f	Retention factor
s	singled (NMR)
SEC	Size exclusion chromatography
t	triplet (NMR)
TLC	Thin layer chromatography
UV	Ultraviolet
2D NMR	Two-Dimensional Nuclear Magnetic Resonance Spectroscopy
µg	Microgram

TABLE OF CONTENTS

Abstract

The MPhil study presented in this thesis was an extension of a collaborative research partnership between the Indigenous Bioresources Research Group (IBRG) of Macquarie University and Chungtia village (Chungtia Senso Mokokchung Town, CSMT), Nagaland, for documentation of ethnobotanical knowledge of Chungtia village Elders and healers as well as phytochemical and biological activity investigation and isolation of bioactive constituents from Nagaland medicinal plants.

The project was initiated by Meyanungsang Kichu, a Nagaland person, who conducted an ethnobotanical study of medicinal plants used by Chungtia villagers and documented 135 plants for their various ethnomedicinal and ethnobotanical applications. This MPhil study completed an up to date literature review of the 135 medicinal plants, then investigated the antimicrobial potential of those plants used by Chungtia villagers for skin conditions, conducted antimicrobial screening of a selection of these, and finally investigated in detail one plant for its antimicrobial activity and bioactive constituents.

A comprehensive literature review covering traditional usages of all 135 plants by other Indigenous traditional healers' worldwide and phytochemical and biological properties of these plants was conducted. This revealed that the traditional usages by the Chungtia community of 93 of their medicinal plants are in agreement with the uses of other Indigenous communities. Thirteen species were found to have no reports on their traditional uses, other than our first-hand accounts of the Chungtia community. Out of 93 species that were found to be used in a similar way by other communities, 80 had traditional uses that were consistent with pharmacological studies that have been reported in the literature and 55 of these plants had also had phytochemical studies conducted that showed bioactive compounds that aligned with their traditional uses by the Chungtia villagers.

A detailed literature review was conducted on the antimicrobial properties and relevant phytoconstituents of 35 plants used by the Chungtia villagers for skin related conditions of a possible microbial origin. This highlighted twelve species with either no antimicrobial properties reported and/or no antimicrobial compounds identified. Out of these, seven species (*Dendrocnide sinuata, Duabanga grandiflora, Erythrina stricta, Eurya acuminata, Holboellia latifolia, Maesa indica* and *Prunus persica*) that were available for collection were selected for antimicrobial screening.

The antimicrobial screening of the 70% aqueous ethanolic extracts of the plants (*D. sinuata* stem, *D. grandiflora* stem bark, *E. stricta* stem, *E. acuminata* leaves, *H. latifolia* leaves, *M. indica* leaves and *P. persica* roots) was performed using disc diffusion and MTT microdilution assays against the human pathogenic microorganisms *Staphylococcus aureus* (susceptible *S. aureus*), methicillin resistant *S. aureus* (MRSA) and multi drug resistant *S. aureus* (MDRSA), susceptible beta-lactamase negative *Escherichia coli* (β- *E. coli*), β-lactamase positive (antibiotic resistant) *E. coli* (β+ *E. coli*), *Pseudomonas aeruginosa, Streptococcus pyogenes, Salmonella typhimurium* and *Candida albicans*. The highest inhibitory activities were exhibited by the *P. persica* root extract, with MIC values of 156 µg/mL for all tested *S. aureus* strains. Based on the antibacterial screening results, *P. persica* was selected for further biological and chemical investigations for its antibacterial constituents.

The 70% aqueous ethanolic *P. persica* roots extract was subjected to partitioning with different polarity solvents (*n*-hexane, dichloromethane, ethyl acetate). The most potent inhibitory activity was observed for the *n*-hexane and ethyl acetate partitions against susceptible and resistant strains of *S. aureus*. The GS-MS analysis of the *n*-hexane partition revealed the presence of eight constituents, out of which three were reported in the literature as antibacterial against *S. aureus*.

TLC bioautographic methods reported in the literature were trialled with the aim to develop the most appropriate technique for the bioautography guided isolation process. The overlay method was found to be the most effective for the purpose of this study. TLC bioautography guided isolation by normal phase chromatography, size exclusion chromatography and preparative TLC led to the isolation of β-sitosterol (5.1) from the *n*-hexane partition and afzelechin (5.2) and *ent*-epiafzelechin-(2α→O→7',4α→8')-(-)-*ent*-afzelechin (5.3) from the ethyl acetate partition. The structures of these three compounds were determined based on various spectroscopic methods, including mass spectrometry, nuclear magnetic resonance spectroscopy, Infrared spectroscopy and circular dichroism.

β-Sitosterol was found to be moderately active (MIC 1250 µg/mL) against *P. aeruginosa* as well as weakly active (MIC 2500 µg/mL) against susceptible strains of *S. aureus*, *E. coli* and *S. typhimurium. ent*-Epiafzelechin-(2α→O→7',4α→8')-(-)-*ent*-afzelechin showed good antibacterial activity against all the tested strains of *S. aureus* (MIC 156 µg/mL for susceptible and 312 µg/mL for resistant) as well as weak activity against the susceptible

strains of *E. coli*, *P. aeruginosa* and *S. typhimurium* (MIC 2500 µg/mL, for all bacteria). This is the first report of this compound possessing antibacterial activity. The antimicrobial properties of afzelechin were not tested due to the small quantity of sample.

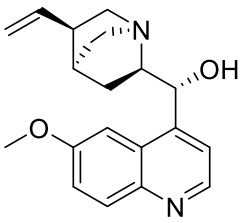
Chapter 1

This chapter describes the importance of medicinal plant research in the process of ethnomedicinally guided drug discovery and provides an overview of major groups of antimicrobial compounds that can be derived from plants. The outline of the thesis aims is also provided.

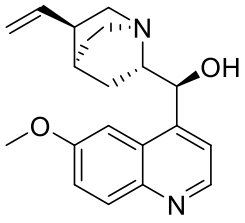
1.1 Introduction

With the escalating occurrence of multidrug resistant microorganisms, much research effort is being focussed on identifying new antimicrobial compounds, including those isolated from nature (Kirst 2013). Since the introduction of conventional antibiotics in the 1950's, there has been little use of plant derivatives as antimicrobials. However, interest in using phytochemicals (products from secondary plant metabolism) for the treatment of microbial infections has increased from the late 1990's following the poor efficacy of conventional antibiotics, due in part to their often excessive and inappropriate use in mammalian infections (Cowan 1999).

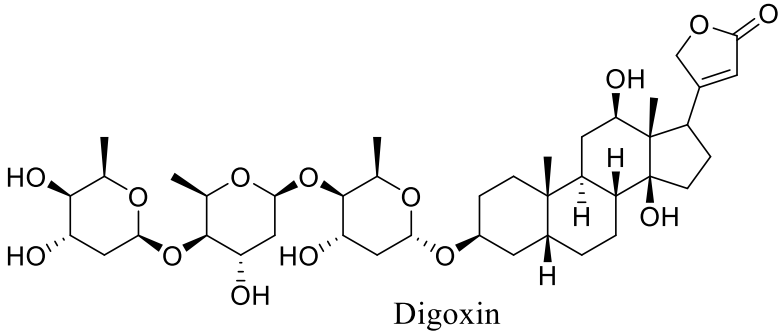
Nowadays about 25% of the drugs prescribed worldwide come from plants. Of the 252 drugs considered as basic and essential by the World Health Organisation (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural plant precursors (Zhang *et al.* 2013). Examples of important drugs obtained from plants are digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum* (Zhang *et al.* 2013) (Figure 1.1).



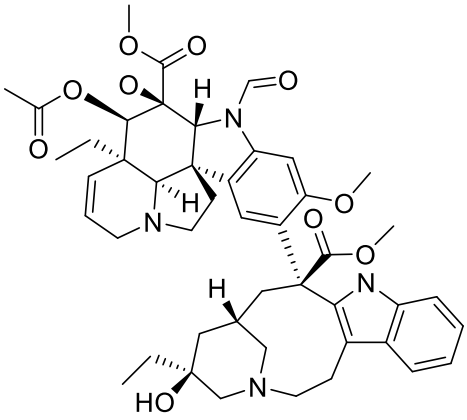
Quinine



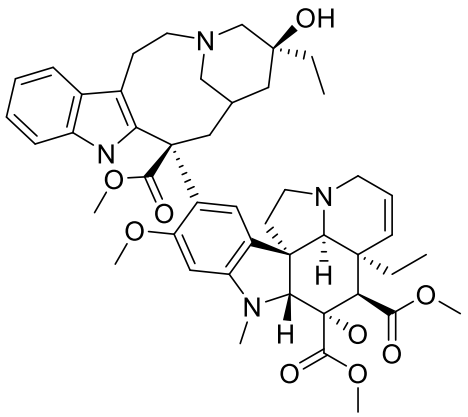
Quinidine



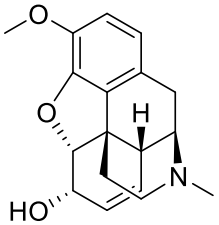
Digoxin



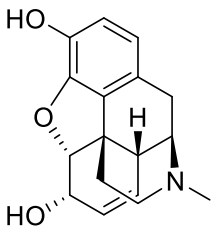
Vincristine



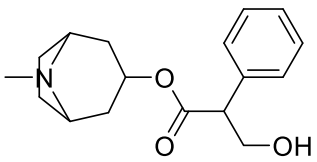
Vinblastine



Codeine



Morphine



Atropine

Figure 1.1 Examples of drugs derived from plants.

1.2 How microbial drug resistance develops

Antimicrobial resistance is driven by evolutionary processes and is therefore inevitable (Wright 2003). Most antibiotics used to treat human diseases originate from natural templates produced by particular species of bacteria or fungi as a mechanism of competition to ensure their own survival (for example, to gain a larger share of environmental food supplies by inhibiting competitors). As the ability to produce poisonous chemicals was developed by microorganisms to combat competitors, so was the counter-measure, namely antibiotic resistance. In natural environments such as soil, microbes can develop resistance through mutations, or can exchange genetic information (including resistance genes), thus permitting the transmission of resistance to other microbes with great ease (Hancock 2005).

Microbial resistance to antibiotics emerged soon after the first use of these agents in the treatment of infectious diseases, and continues to pose a significant challenge for the healthcare sector. The emergence of bacterial strains that exhibit resistance to a variety of antibiotics, *ie* strains that are multidrug resistant, is becoming the major cause of treatment failure of infectious diseases worldwide (Eells *et al.* 2013). This is attested by the spread, with associated deaths, of infections by methicillin-resistant *Staphylococcus aureus* (MRSA) (Eells *et al.* 2013). This bacterium has achieved the status of a "superbug", in that there are few antibiotics available for therapy against this pathogen (Eells *et al.* 2013). The widespread use of antibiotics, both for human consumption and animal feed, has also fostered the development of resistance in a variety of Gram-negative and Gram-positive pathogenic bacteria (Eells *et al.* 2013).

1.3 Development of medicines from natural products

1.3.1 A short history of herbal medicine

The use of natural products for medicinal purposes is as ancient as human civilisation, but the history of plant remedies being used as anti-infectious agents is much chequered with plants falling in and out of favour.

The Bible offers descriptions of approximately 30 healing plants (Cowan 1999). Hippocrates (in the late fifth century B.C.) mentioned 300 to 400 medicinal plants (Thomson and Schultes 1978). In the first century A.D., Dioscorides wrote *De Materia Medica*, a medicinal plant catalogue, which became the prototype for modern pharmacopoeias (Cowan 1999). The fall of ancient civilisations forestalled Western

advances in the understanding of medicinal plants, with much of the documentation of plant pharmaceuticals being destroyed or lost (Cowan 1999). During the Dark Ages, Arab as well as Asian cultures were excavating their own older works to build upon them. Also in the West, the Renaissance years saw a revival of ancient medicine, which was built largely on the use of plants as medicines (Cowan 1999).

Among Europeans living in the New World, the use of botanicals was a reaction against invasive or toxic mainstream medicinal practices of the day. Oliver Wendell Holmes noted that medical treatments in the 1800s could be dangerous and ineffective. Examples include the use of mercury baths in London "barber shops" to treat syphilis and dangerous hallucinogens as a tuberculosis "cure." In 1861 Holmes wrote, "If the whole *materia medica* as now used could be sunk to the bottom of the sea, it would be all the better for mankind - and all the worse for the fishes" (Holmes 1861, Cowan 1999).

The Industrial Revolution and the development of organic chemistry resulted in the preference for synthetic products for pharmacological treatments. The reasons for this were that pure compounds were easily obtained, structural modifications to produce potentially more active and safer drugs could be easily performed and the economic power of the pharmaceutical companies was increasing. Throughout the development of human culture, the use of natural products has had magical-religious significance and different points of view regarding the concepts of health and disease that existed within each culture. Obviously this approach was against that of the industrialised Western societies, in which drugs from natural resources were considered either an option for poorly educated or low income people or simply as religious superstition with no pharmacological value (Rates 2001). However, when traditional antibiotics (products of microorganisms or their synthesised derivatives) became ineffective, mainstream medicine again acknowledged the usefulness of antimicrobial and other drugs derived from plants (Cowan 1999).

Most recently, the ascendancy of the human immunodeficiency virus (HIV) as well as the rapid emergence of multidrug resistant microbial strains have spurred intensive investigation into plant derivatives that may be effective, especially for use in underdeveloped nations with little access to expensive Western medicines (Magiorakos *et al.* 2012).

1.4 Major groups of antimicrobial compounds from plants

Plants produce an enormous array of phytochemicals that can be divided into two major groups, namely basic and secondary metabolites. Basic metabolites are the substances that aid anabolic and catabolic processes required for respiration, nutrient assimilation, and growth/development of the plants. These processes are required for cell maintenance and proliferation. In contrast, secondary metabolites are compounds present in cells that are not necessary for cell survival but are thought to be required for the plants' survival in the environment. These compounds are responsible for preventing attacks from insect and pathogen's whilst aiding plant reproduction through, for example, providing pollinator attraction as either floral scent or colouration. This requirement for secondary metabolites to have highly diverse biological activities has led plants to accumulate a vast catalogue of compounds (Kliebenstein 2004). Many secondary metabolites are aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Cowan 1999, Waterhouse *et al.* 2001, Simoes *et al.* 2009). Some other phytochemicals such as terpenoids, give plants their odour, and others, like quinones and tannins are responsible for plant pigments or flavours (Cowan 1999). Some examples of plant phytochemicals possessing antimicrobial activity are described below.

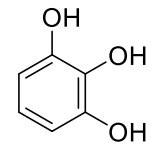
Phenols and polyphenols

Phenols or polyphenols constitute one of the most numerous and widely distributed groups of substances in the plant kingdom (Harborne 1993). Natural phenols can range from simple molecules, such as phenolic acids, to highly polymerised compounds, such as tannins. Polyphenols mainly exist in conjugated form, with one or more sugar residues linked to phenolic groups, although the sugar units may also be directly linked to an aromatic carbon. The associated sugars can be present as monosaccharides, disaccharides, or as oligosaccharides. The most common sugar residue is β-D-glucopyranose, although galactose, rhamnose, xylose, arabinose, glucuronic and galacturonic acids and many others are also commonly found. Associations with other compounds, such as carboxylic and organic acids, amines, and lipids, and linkages with other phenols are also common (Bravo 1998).

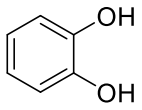
Many phenolic and polyphenolic compounds have antimicrobial properties. Some examples are described below, including their antimicrobial significance.

Simple phenols and phenolic acids

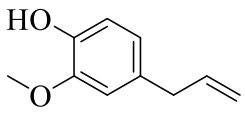
Phenols and phenolic acids (containing a phenolic ring and an organic carboxylic acid function) are commonly found in nature (Cowan 1999). The simple phenols catechol and pyrogallol are toxic to microorganisms. Substituted derivatives of hydroxybenzoic and hydroxycinnamic acids are the predominant phenolic acids in plants, with hydroxycinnamic acid being the most common. The hydroxycinnamic acid and is found in common herbs such as rosemary, sage and thyme, and it is effective against viruses (Chiang *et al.* 2002, Wang *et al.* 2004, Wang *et al.* 2009), bacteria (Fernández *et al.* 1996) and fungi (Wahdan 1998). The site(s) and number of hydroxyl groups on the phenolic ring are thought to be related to the level of toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Geissman 1963, Cowan 1999, Sher 2004). The mechanisms thought to be responsible for phenolic antimicrobial activity include enzyme inhibition by the oxidised compounds, possibly through reaction with sulfhydryl groups or through more non-specific interactions with the proteins (Mason and Bruce P 1987, Cowan 1999), leading to inactivation of the protein and loss of its function. Probable targets in the microbial cell are surface exposed adhesions, cell wall polypeptides, and membrane-bound enzymes (Naz *et al.* 2006). Phenolic compounds possessing a C3 side chain containing no oxygen are classified as essential oils and are often cited as antimicrobials. Eugenol is a well known representative found in clove oil (Sher 2004). Eugenol possesses bacteriostatic and antifungal properties (Sher 2004).



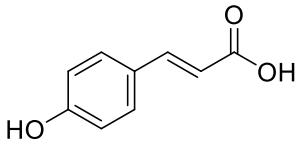
Pyrogallol



Catechol



Eugenol



p-Hydroxycinnamic acid

Figure 1.2 Examples of simple phenols and phenolic acids.

Flavonoids - flavones, flavonols and anthocyanins

Flavonoids (Figure 1.3) represent the most common and widely distributed group of plant phenolics. Their common structure is that of diphenylpropanes, with two aromatic rings linked through three carbons that usually form an oxygenated heterocycle. Flavonoids occasionally occur in plants as aglycones, although they are most commonly found as glycoside derivatives (Bravo 1998). Among the flavonoids, flavones (*e.g.*, apigenin, luteolin, diosmetin), flavonols (*e.g.*, quercetin, myricetin, kaempferol) and their glycosides are the most common classes. Flavonoids are widespread in the plant kingdom, with the exception of algae and fungi. Flavonols occur as flavone *O*-glycosides and *C*-glycosides (Herrmann 1988), with the latter characterised by possessing a carbon-carbon linkage between the anomeric carbon of a sugar molecule and the C-6 or C-8 carbon of the flavone nucleus. Unlike *O*-glycosides, sugars in *C*-glycosides are not cleaved by acid hydrolysis. Flavanones (*e.g.*, naringenin, hesperidin) can also occur as *O*- or *C*-glycosides and are especially abundant in citrus fruits and prunes (Bravo 1998). Isoflavones (*e.g.*, genistein, daidzein), with ring B of the flavone molecule attached to the carbon 3 of the heterocycle, especially occur in legumes. Flavonoids (*e.g.*, catechin, epicatechin, gallocatechin) are the monomeric constituents of the condensed tannins, although they are also very common as free monomers (Bravo 1998). Anthocyanins are the most important group of water-soluble plant pigments and are responsible for the colour of flowers and fruits of higher plants. The term anthocyanin refers to the glycosides of anthocyanidin (*e.g.*, pelargonidin, malvidin, cyanidin). In addition to glycosylation, common linkages with aromatic and aliphatic acids, as well as methyl ester derivatives, also occur. Anthocyanins and polymeric pigments formed from anthocyanins by condensation with other flavonoids are responsible for the colour of red wine (Bravo 1998). Examples of flavonoids are presented in Figure 1.3.

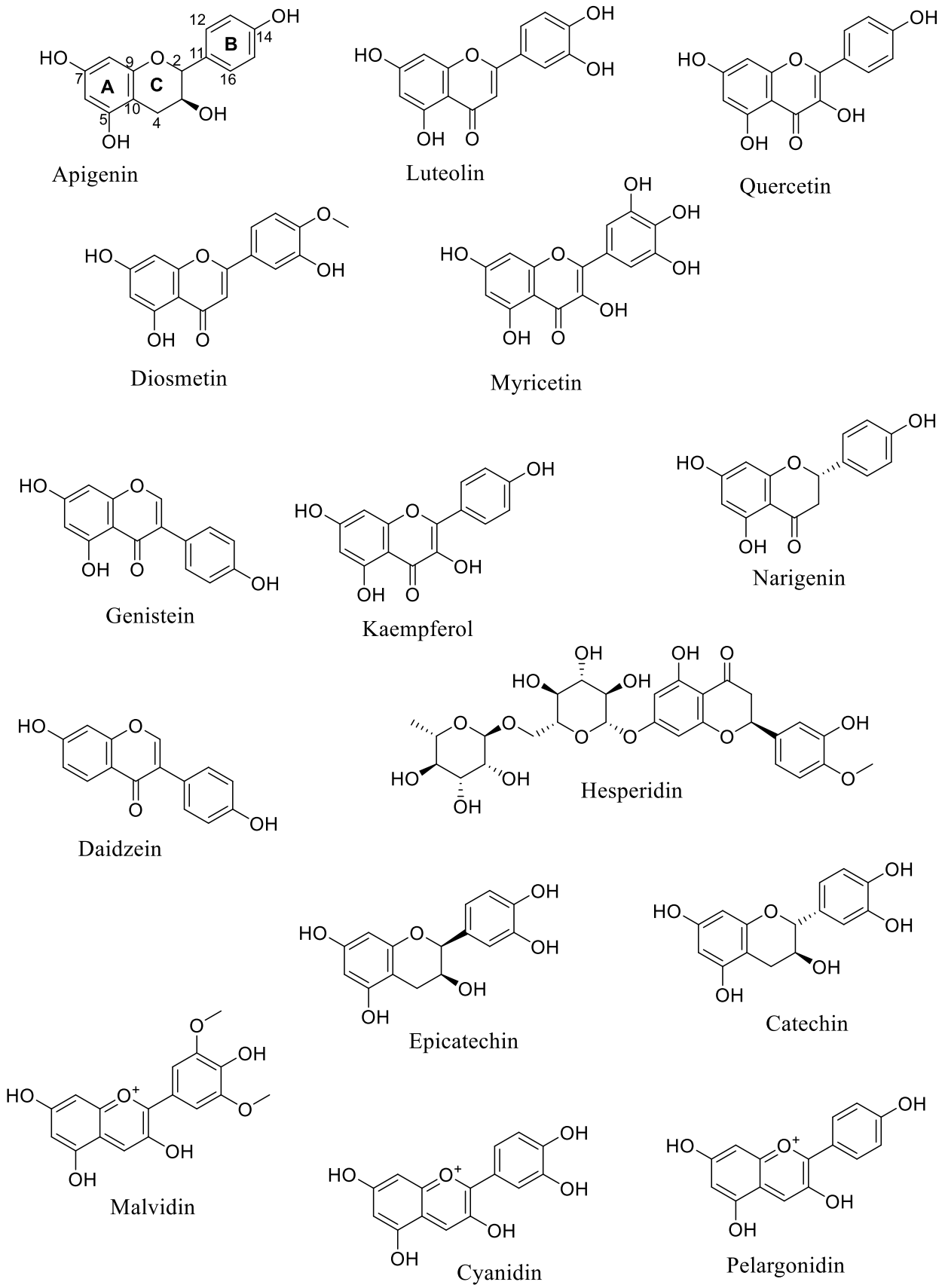


Figure 1.3 Examples of flavonoids.

Flavonoids are synthesised by plants in response to microbial infection (Dixon *et al.* 1983), thus it is no surprise that they have been found *in vitro* to be effective antimicrobial agents against a wide array of microorganisms. The efficacy of flavonoids against such a variety of pathogens can be attributed to the cell-wall permeability of the porins in the outer membrane of microorganisms – it seems likely that the compounds may block the charges of the amino acids in porins (Cowan 1999). The activity of flavonoids may also be due to their ability to complex with extracellular and soluble proteins and then with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes (Saleem *et al.* 2010). Flavonoids having free hydroxyl groups at C-5 and C-7 in ring A have been shown to have the greatest antimicrobial activity (Cowan 1999). Catechins, which contain the most reduced form of the C3 unit in flavonoid compounds, deserve special mention. These flavonoids have been extensively researched due to their occurrence in oolong green teas. It was proven that these teas have antimicrobial activity (Toda *et al.* 1989) and that they contain a mixture of catechin compounds. These compounds inhibit *in vitro Vibrio cholerae* O1 (Toda *et al.* 1992), and have been proven active against methicillin resistant *Staphylococcus aureus* (Zhao *et al.* 2001), *Bacillus cereus* (Friedman *et al.* 2006) and *Candida albicans* (Hirasawa and Takada 2004). Anthocyanidins pelargonidin, delphinidin and cyanidin, as well as cyanidin-3-glucoside, have been found to be active against *Escherichia coli* (Puupponen-Pimiä *et al.* 2001).

Tannins

"Tannin" is a general descriptive name for a group of polymeric phenolic substances capable of transforming animal hides into leather by forming stable tannin-protein complexes with skin collagen and precipitating gelatin from solution - a property known as astringency (Bravo 1998, Cowan 1999). They are found in almost every plant part: bark, wood, leaves, fruits and roots (Scalbert 1991). Tannins are divided into two groups, hydrolysable and condensed tannins (Bravo 1998). Hydrolysable tannins are based on gallic acid, usually as multiple esters with β-D-glucose, while the more numerous condensed tannins (often called proanthocyanidins) are derived from flavonoid monomers (Cowan 1999). Tannins are also formed by condensations of flavan derivatives that have been transported to woody tissues of plants. Alternatively, tannins may be formed by polymerisation of quinone units (Geissman 1963). Tannins have an ability to complex with proteins through non-specific interactions such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation (Haslam 1996, Cowan 1999). Thus, their

mode of antimicrobial action, as described in the section on quinones, may be related to their ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins. They also complex with polysaccharides (Cowan 1999). The toxicity of tannins towards microorganisms is well documented (Scalbert 1991). Tannins have been reported to be bacteriostatic or bactericidal against *S. aureus* (Akiyama *et al.* 2001), *Shigella boydii*, *Shigella flexneri*, *E. coli* and *Pseudomonas aeruginosa* (Banso and Adeyemo 2007). Proanthocyanidins isolated from *Cinnamomum burmannii* have been shown to possess antimicrobial activity against common foodborne pathogenic bacteria such as *Bacillus cereus*, *Listeria monocytogenes*, *S. aureus*, *E. coli* and *Salmonella anatum* (Shan *et al.* 2007).

Coumarins

Coumarins are phenolic substances made of fused benzene and α-pyrone rings (O'Kennedy and Thornes 1997). Coumarins give hay its characteristic odour (Cowan 1999). They are a large family of compounds of natural or synthetic origin, associated with various pharmacological activities, although reports concerning antimicrobial activity of naturally occurring coumarins are very scarce (Matos *et al.* 2013). Umbelliferone and scopoletin isolated from *Petroselinum crispum* and *Ruta graveolens* have been reported for their antibacterial activity, albeit weak, against *S. aureus*, *E. coli*, *B. subtilis* and *Streptococcus epidermidis* (Ojala *et al.* 2000, Sardari *et al.* 2000, Lee *et al.* 2003). Decursinol angelate, isolated from *Angelica gigas* roots, has been found to be inhibitory toward *B. subtilis* (Lee *et al.* 2003). The fused heterocyclic framework of coumarins has been used as a prototype scaffold for the synthesis of a wide variety of analogues in order to study and improve their antimicrobial properties (Matos *et al.* 2013). A study of coumarin derivatives substituted on the pyrone ring indicated that 3-carboxyl derivatives show significant antibacterial activity against *S. aureus* (Matos *et al.* 2013). Different 4-substituted coumarins, such as 4-chlorocoumarin, also exhibit an interesting antimicrobial profile (Matos *et al.* 2013). Figure 1.4 shows examples of coumarins.

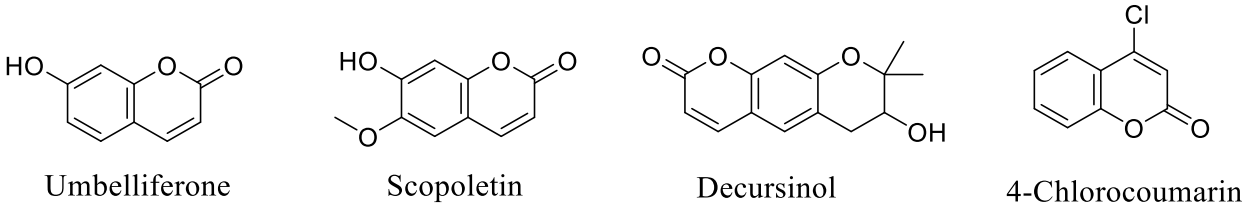


Figure 1.4 Examples of coumarins.

Quinones

Quinones are aromatic rings with two ketone substitutions (Cowan 1999). They are characteristically highly reactive and ubiquitous in nature. These compounds are responsible for the browning of cut or injured fruits and vegetables and are intermediate products in the melanin synthesis pathway in human skin (Schmidt 1988). The change from diphenol (or hydroquinone) and diketone (or quinone) occurs easily through oxidation and reduction reactions. The individual redox potential of the particular quinone-hydroquinone pair is very important in many biological systems, for example the function of ubiquinone (coenzyme Q) in mammalian electron transport systems (Cowan 1999). In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins (Stern *et al.* 1996), often leading to inactivation of the protein and loss of function. For that reason, the potential range of quinone antimicrobial effects is great. Probable targets in the microbial cell are surface-exposed adhesins, cell wall polypeptides, and membrane-bound enzymes. Quinones may also render substrates unavailable to the microorganism (Cowan 1999).

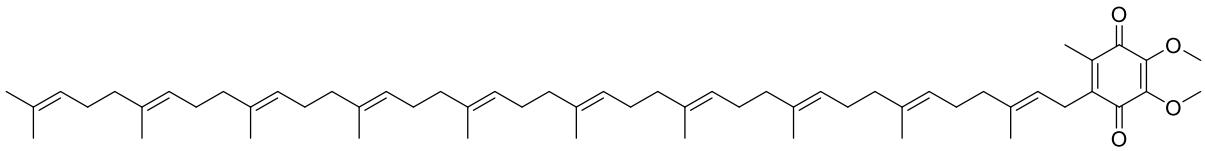


Figure 1.5 Ubiquinone.

Terpenoids and essential oils

Essential oils are plant volatile compounds responsible for their fragrance. These oils, based on an isoprene structure, are called terpenes; their general chemical structure being $C_{10}H_{16}$. They may occur as diterpenes, triterpenes, and tetraterpenes (C20, C30 and C40, respectively), as well as hemiterpenes (C5) and sesquiterpenes (C15) (Cowan 1999). When the compounds contain additional elements, usually oxygen, they are termed terpenoids. Terpenoids are biosynthesised from acetate units, and as such they share their origins with fatty acids. They differ from fatty acids in that they contain extensive branching and are cyclised (Cowan 1999). Examples of common terpenoids are menthol and camphor (monoterpenes) and farnesol and artemisin (sesquiterpenoids) (Cowan 1999). Terpenes or terpenoids have been found to be active against bacteria, fungi, viruses and protozoa (Cowan 1999). Investigation of three monoterpenes, namely linalyl acetate, (+)-menthol

and thymol, against the Gram positive bacterium *S. aureus* and the Gram negative bacterium *E. coli* suggest that the antimicrobial effect of various monoterpenes may result from a perturbation of the lipid fraction of microorganism plasma membranes, resulting in alterations of membrane permeability and in leakage of intracellular materials (Trombetta *et al.* 2005). The study of the antibacterial effects of the terpene alcohols farnesol, nerolidol and plaunotol on *S. aureus*, focusing on the leakage of K+ ions, indicated that they act on cell membranes as well (Inoue *et al.* 2004). Additionally, increasing the hydrophobicity of kaurene diterpenoids by the addition of a methyl group has been shown to drastically reduce their antimicrobial activity (Mendoza *et al.* 1997). The pentacyclic triterpenoids, oleanolic and ursolic acids have been found to be active against *L. monocytogenes* (Kurek *et al.* 2010). The diterpene aframodial, isolated from the seeds of *Aframonum longilolius*, was shown to be active against both antibiotic sensitive and methicillin-resistant strains of *S. aureus* (Sandjo and Kueteb 2013)*;* the same diterpene was proven to be a broad-spectrum antifungal (Cowan 1999). Examples of terpenoids are provided in Figure 1.6.

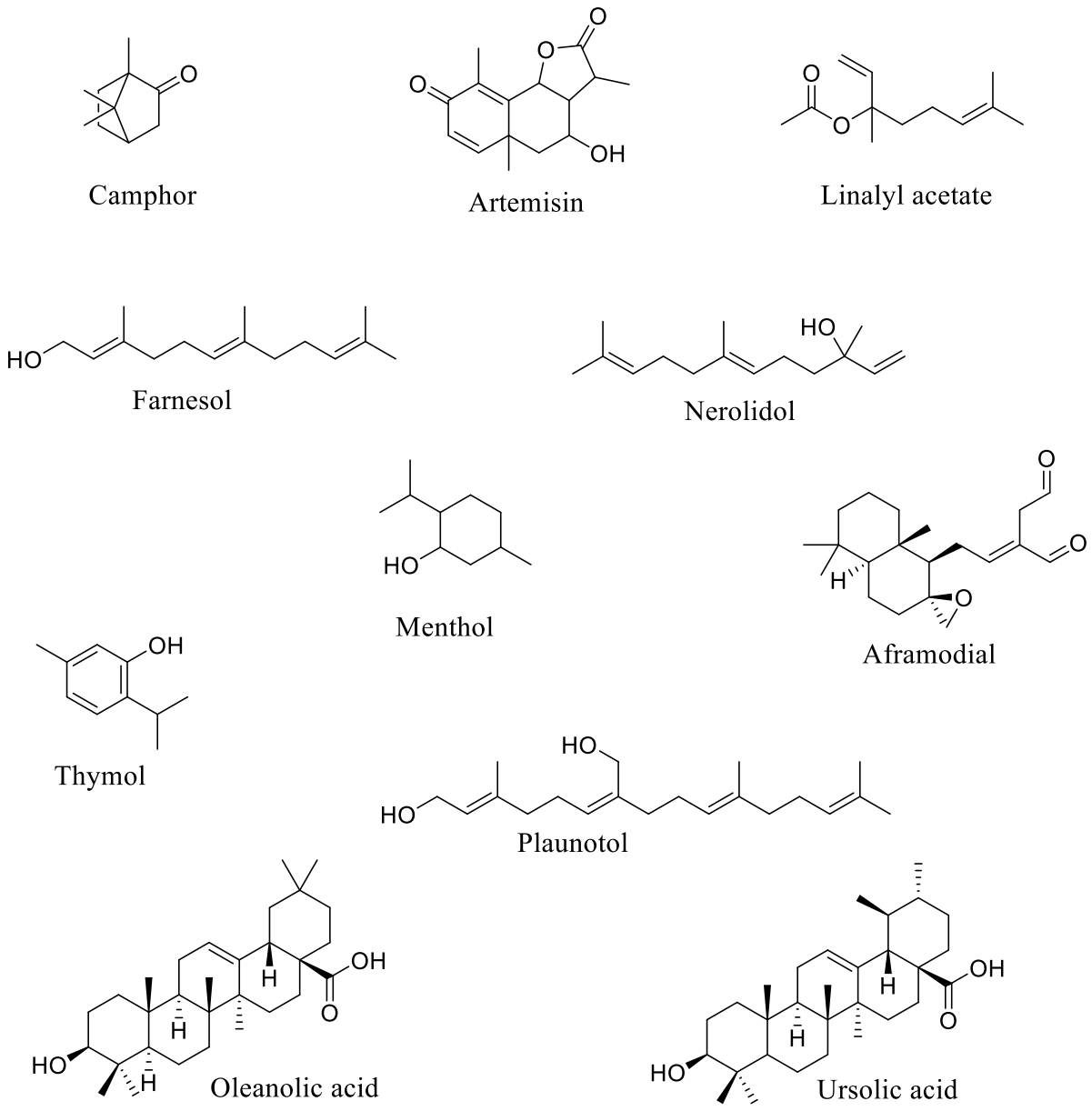
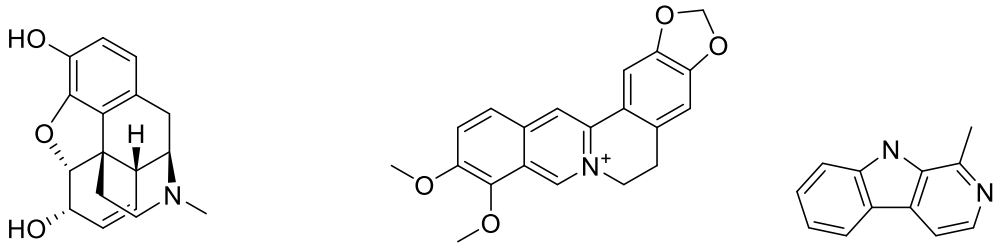


Figure 1.6 Examples of terpenoids.

Alkaloids

Heterocyclic nitrogen containing compounds are called alkaloids (Cowan 1999). The first medically important alkaloid, morphine, was isolated in 1805 from the opium poppy *Papaver somniferum* by German pharmacist Friedrich Serturner (Wright 2011). Relatives of many plant families are known to produce antimicrobial alkaloids (Saleem *et al.* 2010). Alkaloids may be useful against HIV infection as well as intestinal infections associated with AIDS. Berberine and harmane are important representatives of the alkaloid group (Al-Bayati and Al-Mola 2008). Berberine has been shown to be active against methicillin-resistant *S. aureus* (Yu *et al.* 2005), *E. coli*, *P. aeruginosa* and *B. subtilis* (Čerňáková and

Košťálová 2002), effective against trypanosomes and plasmodia (Čerňáková and Košťálová 2002), and active against *C. albicans* (Freile *et al.* 2003). Harmane has been shown to be active against *B. subtilis*, *S. aureus*, *E. coli* and *Proteus vulgaris* and two fungal strains, one filamentous, *Aspergillus niger*, and one yeast, *C. albicans* (Nenaah 2010). The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmane is attributed to their ability to intercalate with DNA (Cowan 1999). Example of alkaloids are depicted in Figure 1.7.



Morphine Berberine Harmane

Figure 1.7 Examples of alkaloids.

1.5 Approaches for drug development

The drug development process is a long and arduous one designed to ensure that therapies released to market are effective as well as safe. It takes many years for a substance to become commercially available as a drug (Cox and Balick 1994, Schwikkard and Mulholland 2014) and the process is very costly. It was estimated in the United States that for every 10,000 pure compounds that are biologically active in screening tests, 20 would be tested in animal models, and 10 of these would be clinically evaluated but only one would reach U.S. Food and Drug Administration approval for marketing (Schwikkard and Mulholland 2014). The time required for this process was estimated as 10 years at a cost of $231 million (U.S.) (Fabricant and Farnsworth 2001, Schwikkard and Mulholland 2014).

The efficient and effective selection of appropriate plants for investigative purposes in a drug discovery program is of crucial importance for a successful outcome. A variety of approaches have been used by researchers with varying levels of success (Schwikkard and Mulholland 2014). The major selection approaches that are relevant in the search for bioactive molecules are random screening, the chemotaxonomic approach and the ethnobotanical approach (Cox 2008).

1.5.1 Random and targeted screening

Random screening

Random screening relies on collection and screening of available plants from a biodiverse region that provides chemical diversity. This random collection and broad screening method has successfully led to the discovery of anticancer drugs, including taxol (from *Taxus brevifolia*) and camptothecin (from *Campotheca acuminata*) performed by the National Cancer Institute (NCI) USA. The process was, however, tedious and very costly (Farnsworth 1988, Schwikkard and Mulholland 2014). The NCI screened around 200000 extracts between 1955 and 1980 with limited success. This led to a reduction in focus on random screenings until 1986, when, with the improvement of screening methods, the NCI started screening again. By 1995, 40000 extracts had been prepared and 18000 were screened for activity. The success rate was about 1% (Schwikkard and Mulholland 2014).

Chemotaxonomic approach

The chemotaxonomic approach is based on the fact that botanically related plants very often produce chemically related secondary metabolites. If a promising agent of a rare structure is found in a species belonging to a certain genus, other species belonging to the same genus offer a good chance of yielding related compounds (Schwikkard and Mulholland 2014). As an example, quinine and closely related alkaloids, have until now only been found in the bark of Cinchona or Remijia species, both belonging to Rubiaceae (Tringali 2003).

The Ethnobotanical approach

Over the past several decades, ethnobotany has reemerged as an interdisciplinary approach to drug discovery, involving collaboration between Western trained scientists and indigenous healers (Oubre *et al.* 1997). Bruhn and Holmstedt defined the ethnobotanical approach as "*the interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by man*" (Schwikkard and Mulholland 2014). This approach has proven to be very effective, with many of the current pharmaceutical drugs derived from higher plants having been discovered in an ethnobotanical context (Cox 2008). Fabricant and Farnsworth reported in 1991 that out of 156 bioactive compounds identified to date, 122 were discovered following ethnobotanical leads. Furthermore, 80% of these compounds were used by native communities for the same (or related) medicinal purposes (Fabricant and Farnsworth 2001). For example, galegine, an antihyperglycemic

agent, was isolated from the plant *Galega officinalis* L. This plant has been used traditionally for the treatment of diabetes. Also, the very strong painkiller affinin was isolated from the plant *Heliopsis longipes*, which is traditionally applied by Mexican native healers for the treatment of tooth pain (Schwikkard and Mulholland 2014). The history of drug discovery and development seems to confirm that the ethnobotanical approach is a valuable avenue for new medicines. Furthermore, drugs derived from plants with documented ethnobotanical use are likely to be safer than active compounds isolated from these with no records of human use (Fabricant and Farnsworth 2001, Cox 2008, Schwikkard and Mulholland 2014).

The ethnobotanical approach has been applied in the study presented in this thesis as a method of choice for the discovery of antimicrobial fractions and compounds extracted from medicinal plants used by the Chungtia tribe of Nagaland, India.

1.6 Objectives of this study

The overall objective of this MPhil project was to undertake chemical and biological investigations, in partnership with Chungtia villagers, guided by their traditional knowledge on the medicinal plants they have used for the treatment of skin related ailments such as wounds, cuts and sores.

This project involved:
Conduct of a comprehensive literature review on plants documented first-hand from Chungtia villagers for various ethnomedicinal and ethnobotanical uses.
Conduct of a literature review on the plants used by Chungtia elders for the treatment of skin ailments and subsequent antimicrobial screening of the most promising plants.
Bioassay guided phytochemical investigations and isolation of active compounds from the one plant determined as having significant potential from the literature reviews and antimicrobial screening.

Akiyama, H., K. Fujii, O. Yamasaki, T. Oono and K. Iwatsuki (2001). "Antibacterial Action of Several Tannins against *Staphylococcus aureus.*" *Journal of Antimicrobial Chemotherapy* **48**(4): 487-491.

Al-Bayati, F. A. and H. F. Al-Mola (2008). "Antibacterial and Antifungal Activities of Different Parts of *Tribulus terrestris* L. Growing in Iraq." *Journal of Zhejiang University Science B* **9**(2): 154-159.

Banso, A. and S. Adeyemo (2007). "Evaluation of Antibacterial Properties of Tannins Isolated from *Dichrostachys cinerea.*" *African Journal of Biotechnology* **6**(15).

Bravo, L. (1998). "Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance." *Nutrition reviews* **56**(11): 317-333.

Čerňáková, M. and D. Košťálová (2002). "Antimicrobial Activity of Berberine — a Constituent of *Mahonia aquifolium.*" *Folia Microbiologica* **47**(4): 375-378.

Chiang, L. C., W. Chiang, M. Y. Chang, L. T. Ng and C. C. Lin (2002). "Antiviral Activity of *Plantago major* Extracts and Related Compounds *in vitro.*" *Antiviral Research* **55**(1): 53-62.

Cowan, M. M. (1999). "Plant Products as Antimicrobial Agents." *Clinical Microbiology Reviews* **12**(4): 564-582.

Cox, P. A. (2008). "Etnnopharrnacology and the Search for New Drugs." *Bioactive Compounds From Plants* **735**(9): 40-68.

Cox, P. A. and M. J. Balick (1994). "The Ethnobotanical Approach to Drug Discovery." *Scientific American* **270**(6): 60-65.

Dixon, R., P. Dey and C. Lamb (1983). "Phytoalexins: Enzymology and Molecular Biology." *Advances in Enzymology and Related Areas of Molecular Biology* **55**(5): 1-136.

Eells, S., J. McKinnell, A. Wang, N. Green, D. Whang, P. O'hara, M. Brown and L. Miller (2013). "A Comparison of Clinical Outcomes between Healthcare-Associated Infections Due to Community-Associated Methicillin-Resistant *Staphylococcus aureus* Strains and Healthcare-Associated Methicillin-Resistant *S. aureus* Strains." *Epidemiology and Infection* **141**(10): 2140-2148.

Fabricant, D. S. and N. R. Farnsworth (2001). "The Value of Plants Used in Traditional Medicine for Drug Discovery." *Environmental Health Perspectives* **109**(1): 69-75.

Farnsworth, N. R. (1988). "Screening Plants for New Medicines." *Biodiversity* **5**(Part II): 83-97.

Fernández, M. A., M. D. García and M. T. Sáenz (1996). "Antibacterial Activity of the Phenolic Acids Fractions of *Scrophularia frutescens* and *Scrophularia sambucifolia.*" *Journal of Ethnopharmacology* **53**(1): 11-14.

Freile, M. L., F. Giannini, G. Pucci, A. Sturniolo, L. Rodero, O. Pucci, V. Balzareti and R. D. Enriz (2003). "Antimicrobial Activity of Aqueous Extracts and of Berberine Isolated from *Berberis heterophylla.*" *Fitoterapia* **74**(7–8): 702-705.

Friedman, M., P. R. Henika, C. E. Levin, R. E. Mandrell and N. Kozukue (2006). "Antimicrobial Activities of Tea Catechins and Tea Flavins and Tea Extracts against *Bacillus cereus.*" *Journal of Food Protection* **69**(2): 354-361.

Geissman, T. (1963). "Flavonoid Compounds, Tannins, Lignins and Related Compounds." *Pyrrole Pigments, Isoprenoid Compounds and Phenolic Plant Constituents* **9**(2): 265-272.

Hancock, R. E. W. (2005). "Mechanisms of Action of Newer Antibiotics for Gram-Positive Pathogens." *The Lancet Infectious Diseases* **5**(4): 209-218.

Harborne, J. B. (1993). The Flavonoids Advances in Research since 1986, CRC Press.

Haslam, E. (1996). "Natural Polyphenols (Vegetable Tannins) as Drugs: Possible Modes of Action." *Journal of Natural Products* **59**(2): 205-215.

Herrmann, K. (1988). "On the Occurrence of Flavonol and Flavone Glycosides in Vegetables." *Zeitschrift für Lebensmittel-Untersuchung und Forschung* **186**(1): 1-5.

Hirasawa, M. and K. Takada (2004). "Multiple Effects of Green Tea Catechin on the Antifungal Activity of Antimycotics against *Candida albicans.*" *Journal of Antimicrobial Chemotherapy* **53**(2): 225-229.

Holmes, O. W. (1861). Currents and Counter-Currents in Medical Science: With Other Addresses and Essays, Ticknor and Fields.

Inoue, Y., A. Shiraishi, T. Hada, K. Hirose, H. Hamashima and J. Shimada (2004). "The Antibacterial Effects of Terpene Alcohols on *Staphylococcus aureus* and Their Mode of Action." *FEMS Microbiology Letters* **237**(2): 325-331.

Kirst, H. A. (2013). "Developing New Antibacterials through Natural Product Research." *Expert Opinion on Drug Discovery* **8**(5): 479-493.

Kliebenstein, D. (2004). "Secondary Metabolites and Plant/Environment Interactions: A View through *Arabidopsis thaliana* Tinged Glasses." *Plant, Cell & Environment* **27**(6): 675-684.

Kurek, A., A. M. Grudniak, M. Szwed, A. Klicka, L. Samluk, K. I. Wolska, W. Janiszowska and M. Popowska (2010). "Oleanolic Acid and Ursolic Acid Affect Peptidoglycan Metabolism in *Listeria monocytogenes.*" *Antonie van Leeuwenhoek* **97**(1): 61-68.

Lee, S., D.-S. Shin, J. S. Kim, K.-B. Oh and S. S. Kang (2003). "Antibacterial Coumarins from *Angelica gigas* Roots." *Archives of Pharmacalological Research* **26**(6): 449-452.

Magiorakos, A. P., A. Srinivasan, R. Carey, Y. Carmeli, M. Falagas, C. Giske, S. Harbarth, J. Hindler, G. Kahlmeter and B. Olsson-Liljequist (2012). "Multidrug-Resistant, Extensively Drug-Resistant and Pandrug-Resistant Bacteria: An International Expert

Proposal for Interim Standard Definitions for Acquired Resistance." *Clinical Microbiology and Infection* **18**(3): 268-281.

Mason, T. L. and W. Bruce P (1987). "Inactivation of Red Beet β-Glucan Synthase by Native and Oxidized Phenolic Compounds." *Phytochemistry* **26**(8): 2197-2202.

Matos, M. J., S. Vazquez-Rodriguez, L. Santana, E. Uriarte, C. Fuentes-Edfuf, Y. Santos and A. Muñoz-Crego (2013). "Synthesis and Structure-Activity Relationships of Novel Amino/Nitro Substituted 3-Arylcoumarins as Antibacterial Agents." *Molecules* **18**(2): 1394-1404.

Mendoza, L., M. Wilkens and A. Urzúa (1997). "Antimicrobial Study of the Resinous Exudates and of Diterpenoids and Flavonoids Isolated from Some Chilean *Pseudognaphalium* (*Asteraceae*)." *Journal of Ethnopharmacology* **58**(2): 85-88.

Naz, S., S. Ahmad, S. Ajaz Rasool, S. Asad Sayeed and R. Siddiqi (2006). "Antibacterial Activity Directed Isolation of Compounds from *Onosma hispidum*." *Microbiological Research* **161**(1): 43-48.

Nenaah, G. (2010). "Antibacterial and Antifungal Activities of β-Carboline Alkaloids of *Peganum harmala* (L) Seeds and Their Combination Effects." *Fitoterapia* **81**(7): 779-782.

O'Kennedy, R. and R. D. Thornes (1997). Coumarins: Biology, Applications, and Mode of Action, John Wiley & Sons.

Ojala, T., S. Remes, P. Haansuu, H. Vuorela, R. Hiltunen, K. Haahtela and P. Vuorela (2000). "Antimicrobial Activity of Some Coumarin Containing Herbal Plants Growing in Finland." *Journal of Ethnopharmacology* **73**(1–2): 299-305.

Oubre, A., T. Carlson, S. King and G. Reaven (1997). "From Plant to Patient: An Ethnomedical Approach to the Identification of New Drugs for the Treatment of Niddm." *Diabetologia* **40**(5): 614-617.

Puupponen-Pimiä, R., L. Nohynek, C. Meier, M. Kähkönen, M. Heinonen, A. Hopia and K. M. Oksman-Caldentey (2001). "Antimicrobial Properties of Phenolic Compounds from Berries." *Journal of Applied Microbiology* **90**(4): 494-507.

Rates, S. M. K. (2001). "Plants as Source of Drugs." *Toxicon* **39**(5): 603-613.

Saleem, M., M. Nazir, M. S. Ali, H. Hussain, Y. S. Lee, N. Riaz and A. Jabbar (2010). "Antimicrobial Natural Products: An Update on Future Antibiotic Drug Candidates." *Natural Product Reports* **27**(2): 238-254.

Sandjo, L. P. and V. Kueteb (2013). "Three Diterpenoids from the Medicinal Plants of Africa." *Medicinal Plant Research in Africa: Pharmacology and Chemistry* **15**(3): 105.

Sardari, S., S. Nishibe and M. Daneshtalab (2000). Coumarins, the Bioactive Structures with Antifungal Property. *Studies in Natural Products Chemistry*. **23:** 335-393.

Scalbert, A. (1991). "Antimicrobial Properties of Tannins." *Phytochemistry* **30**(12): 3875-3883.

Schmidt, H. (1988). "Phenol Oxidase (Ec 1.14. 18.1) a Marker Enzyme for Defense Cells." *Progress in Histochemistry and Cytochemistry* **17**(3): III-186.

Schwikkard, S. L. and D. A. Mulholland (2014). "Useful Methods for Targeted Plant Selection in the Discovery of Potential New Drug Candidates." *Planta Medica* **2**: 12-16

Shan, B., Y.-Z. Cai, J. D. Brooks and H. Corke (2007). "Antibacterial Properties and Major Bioactive Components of Cinnamon Stick (*Cinnamomum burmannii*): Activity against Foodborne Pathogenic Bacteria." *Journal of Agricultural and Food Chemistry* **55**(14): 5484-5490.

Sher, A. (2004). "Antimicrobial Activity of Natural Products from Medicinal Plants." *Gomal Journal of Medical Sciences* **7**(1).

Simoes, M., R. N. Bennett and E. A. Rosa (2009). "Understanding Antimicrobial Activities of Phytochemicals against Multidrug Resistant Bacteria and Biofilms." *Natural Product Reports* **26**(6): 746-757.

Stern, J. L., A. E. Hagerman, P. D. Steinberg and P. K. Mason (1996). "Phlorotannin-Protein Interactions." *Journal of Chemical Ecology* **22**(10): 1877-1899.

Thomson, W. A. and R. E. Schultes (1978). Medicines from the Earth, McGraw-Hill.

Toda, M., S. Okubo, H. Ikigai, T. Suzuki, Y. Suzuki, Y. Hara and T. Shimamura (1992). "The Protective Activity of Tea Catechins against Experimental Infection by *Vibrio cholerae* O1." *Microbiology and Immunology* **36**(9): 999-1001.

Toda, M., S. Okubo, R. Ohnishi and T. Shimamura (1989). "Antibacterial and Bacteriocidal Activities of Japanese Green Tea." *Nihon Saikingaku Zasshi. Japanese Journal of Bacteriology* **44**(4): 669-672.

Tringali, C. (2003). Bioactive Compounds from Natural Sources: Isolation, Characterization and Biological Properties, CRC Press.

Trombetta, D., F. Castelli, M. G. Sarpietro, V. Venuti, M. Cristani, C. Daniele, A. Saija, G. Mazzanti and G. Bisignano (2005). "Mechanisms of Antibacterial Action of Three Monoterpenes." *Antimicrobial Agents and Chemotherapy* **49**(6): 2474-2478.

Wahdan, H. (1998). "Causes of the Antimicrobial Activity of Honey." *Infection* **26**(1): 26-31.

Wang, G.-F., L.-P. Shi, Y.-D. Ren, Q.-F. Liu, H.-F. Liu, R.-J. Zhang, Z. Li, F.-H. Zhu, P.-L. He, W. Tang, P.-Z. Tao, C. Li, W.-M. Zhao and J.-P. Zuo (2009). "Anti-Hepatitis B Virus Activity of Chlorogenic Acid, Quinic Acid and Caffeic Acid i*n vivo* and *in vitro*." *Antiviral Research* **83**(2): 186-190.

Wang, H., G. J. Provan and K. Helliwell (2004). "Determination of Rosmarinic Acid and Caffeic Acid in Aromatic Herbs by Hplc." *Food Chemistry* **87**(2): 307-311.

Waterhouse, P. M., M.-B. Wang and T. Lough (2001). "Gene Silencing as an Adaptive Defence against Viruses." *Nature* **411**(6839): 834-842.

Wright, G. D. (2003). "Mechanisms of Resistance to Antibiotics." *Current Opinion in Chemical Biology* **7**(5): 563-569.

Wright, J. (2011). "A History of Opium, from Medical to Recreational Use." *British Journal of School Nursing* **6**(10): 512.

Yu, H.-H., K.-J. Kim, J.-D. Cha, H.-K. Kim, Y.-E. Lee, N.-Y. Choi and Y.-O. You (2005). "Antimicrobial Activity of Berberine Alone and in Combination with Ampicillin or Oxacillin against Methicillin-Resistant *Staphylococcus aureus.*" *Journal of Medicinal Food* **8**(4): 454-461.

Zhang, A., H. Sun and X. Wang (2013). "Recent Advances in Natural Products from Plants for Treatment of Liver Diseases." *European Journal of Medicinal Chemistry* **63**(4): 570-577.

Zhao, W.-H., Z.-Q. Hu, S. Okubo, Y. Hara and T. Shimamura (2001). "Mechanism of Synergy between Epigallocatechin Gallate and B-Lactams against Methicillin-Resistant *Staphylococcus aureus.*" *Antimicrobial Agents and Chemotherapy* **45**(6): 1737-1742.

Chapter 2

This chapter introduces Chungtia village as the study area and provides a comprehensive literature review of the plants that had first-hand information reported for their various ethnomedicinal and ethnobotanical applications by Chungtia community members.

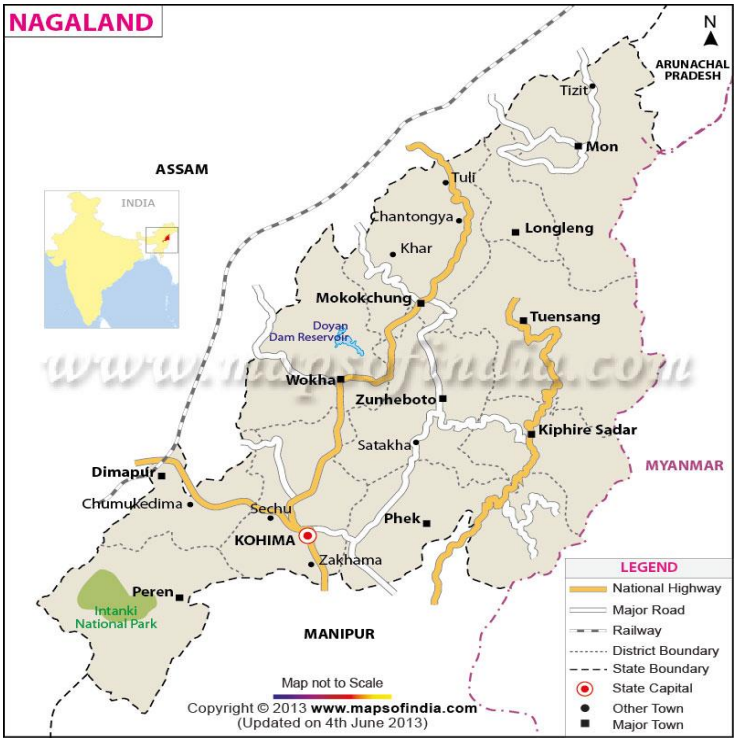


Figure 2.1 Map of the study area

2.1 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 (Kichu 2010).

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 (Kichu 2010).

Due to globalisation and westernisation of native cultures, Indigenous medicinal plant knowledge is being lost worldwide as well as in Nagaland (Cordell 2002, Mawere 2014). Elderly people that possess this knowledge are dying and communities are being dislocated. The number of studies that have been undertaken to document and preserve the medicinal plant knowledge in Nagaland is very scarce (Kichu 2010). There is still much to be documented in Nagaland given the nature of the biodiversity of the country and the cultures and traditions of its people (Kichu 2010). Moreover, the Chungtia village Ao tribe, with which this study concerns, is the only Nagaland tribal community that has not had their medicinal knowledge formally documented to date (Pfoze *et al.* 2014).

This project was originally established by former PhD student and Nagaland native Meyanungsang Kichu, who followed 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 (Kichu 2010). He undertook a systematic documentation of 135 medicinal plants following first-hand interviews with Elders of Chungtia Village. The motivation of these villagers to document their customary medicinal plant 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 (Kichu 2010).

2.2 Establishment of the collaborative research partnership and the authorising body

2.2.1 Chungtia village

Chungtia inhabitants belong to the Ao tribe, and their village is located in the Mokokchung district, Nagaland, India. Topographically it is a hilly area, with vegetation predominantly of a semi-evergreen forest type. January and February are the coldest months, when the temperature can drop down to 2 ºC, and the summer months' average temperatures are mild and oscillate between 27 to 32 ºC. 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 2,500 mm. July and August receive the most rainfall (Kichu 2010).

2.2.2 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) (Kichu 2010).

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 in which the executive members are represented by one member each from the 14 clans of Chungtia village. 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 (Kichu 2010).

2.2.3 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 (Kichu 2010). 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. 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.
- For any commercial interest, a process of further negotiation would be undertaken for appropriate benefit sharing with the village community.

2.3 First-hand medicinal plants documentation

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 considered to be the key custodians of traditional knowledge to participate, upon their consent (Kichu 2010). Kichu made two field trips over a period of two years to Chungtia village for the interviews and collection of voucher specimens of plants.

The interviews identified that the villagers had a strong preference for using medicinal plants for treatment of common ailments such as fever, cold, cuts and wounds and gastrointestinal problems, even though conventional treatments are available to them (Kichu 2010).

Overall, the interviews conducted by Kichu resulted in the collection of first-hand information on ethnomedicinal and ethnobotanical applications of 135 plants. This chapter documents the recorded uses, preparation and mode of administration by the Chungtia villagers of the 135 medicinal plants and provides a review of literature up to September 2014 on each plant including customary (traditional and contemporary) medicinal usages worldwide and available biological and chemical data. The results are presented in Table 2.1.

Table 2.1 Ethnobotanical plants documented in Chungtia village and their reported ethnobotanical usages worldwide as well as phytochemical and pharmacological properties

Scientific name (Botanical family) (CD = cultivated/WD = wild) (Herbarium accession number, (ASSAM Acc. No) Himalayan Endemic (E) Native (N) Cosmopolitan (C) Introduced (I)	**Local name**	**Part Used[a]**	**Recorded uses, preparation and mode of administration by Chungtia villagers**	**N I[b]**	**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) (WD) (69649) (N)	Zanghi	Stem (F)	Stem is crushed into river or creek water to poison fish	3	Leaves: to poison fish Neuwinger (2004)	Roots, leaves: gynaecological diseases, skin diseases (Zheng and Xing 2009)	Leaves: anti-inflammatory; bark: antioxidant (Dongmo *et al.* 2007) (Sowndhararajan *et al.* 2013)	WR[c] (Daduang *et al.* 2011, Sowndharar ajan *et al.* 2013)
Acorus calamus Linn. (Araceae) (WD) (69659) (C)	Mukupen	Leaves (F)	Leaf decoction is used in bath for the treatment of influenza	2	Roots, leaves: fevers (Rajput *et al.* 2014)	WR Rhizomes: Stomach ache, epilepsy, toothache, killing head lice, insect repellant (Pfoze *et al.* 2012)	WR Roots, leaves: antibacterial against *S. aureus, P. aeruginosa, E. coli, B. subtilis, B. cereus, S. dysenteriae, S. flexneri, V. cholerae, S. paratyphi, S. pyogenes, K. pneumoniae*, antifungal against *C. albicans*; roots: antiviral against HSV-1, HSV-2, anticonvulsant, anticancer (Rajput *et al.* 2014)	WR (Rajput *et al.* 2014)

[a] Plants parts used. F – fresh, D – dry, B – both. [b]N I – Number of informants. [c]WR – Widely referenced.

Adenia trilobata Engl. (Passifloraceae) (WD) (69536) (E)	Tenik tepang	Leaves (F)	Leaf poultice is bandaged onto the knee to relieve pain	3	None found	None found	None found	None found
Adhatoda vasica Nees. (Acanthaceae) (CD) (69665) (N)	Sungjem wa	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	WR Flowers, fruits: fever, cold (Roy *et al.* 2013)	WR Roots: bronchitis, asthma, bilious vomiting, sore eyes (Lone *et al.* 2013)	WR Leaves, roots: antibacterial against *S. aureus, B. subtilis, P. aeruginosa, S. typhimurium*, antipyretic; leaves, roots: antiviral against HIV-1, HIV-2, H1N1; leaves, flowers: anticholinesterase, abortifacient (Lone *et al.* 2013, Roy *et al.* 2013)	WR (Lone *et al.* 2013, Roy *et al.* 2013)
Albizia chinensis (Osb) Merr. (Mimosaceae) (WD) (69660) (N)	Mokokwa	Leaves & 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	Leaves: To poison fish (Leaman *et al.* 1995)	WR Bark: gastric ulcer & chronic gastritis, inflammation (Perumal *et al.* 2010, Yu *et al.* 2013)	WR Bark, leaves: anti-inflammatory; leaves: antioxidant (Kumari *et al.* 2011, Kokila *et al.* 2013)	WR (Kumari *et al.* 2011, Kokila *et al.* 2013)
Albizia lebbeck Linn. Benth. (Mimosaceae) (WD) (69677) (N)	Moang	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 & burned until red hot. It is then	7	None found	WR Leaves: conjunctivitis, ulcer, cold, cough; bark: diabetes; whole plant: bites & stings from venomous animals (Zia-Ul-	WR Bark: anti-diabetic, antioxidant, anti-inflammatory (Ahmed *et al.* 2014, Bajpai *et al.* 2014)	WR (Ahmed *et al.* 2014, Bajpai *et al.* 2014)

			immersed in water for 1 second. This results in a hardening of the metal			Haqi *et al.* 2013, Ahmed *et al.* 2014, Meena *et al.* 2014)		
Albizia lucidior (Skud) Hara (Mimosaceae) (WD) (69595) (N)	Sunemtong	Roots (F)	Root infusion is applied topically to abscesses & boils	3	None found	None found	Bark: anticancer (Cai *et al.* 2002)	None found
Allium chinense G. Don (Liliaceae) (CD) (69679) (N)	Alolasung	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 & snake bites & skin diseases	10	Bulbs: cholesterol levels, circulatory system, heart asthma, skin diseases (Bah *et al.* 2012)	WR Bulbs: bronchitis, pleurisy, angina pectoris, chest pain, diarrhoea (Bah *et al.* 2012)	WR Bulbs: cytotoxic, antioxidant, antimicrobial against *P. aeruginosa*, *S. aureus*, cardioprotective (Bah *et al.* 2012, Lanzotti *et al.* 2014)	WR (Bah *et al.* 2012)
Allium hookeri Thw. (Liliaceae) (CD) (69628) (N)	Repchalasung	Leaves (F)	Leaves are eaten raw for vermifuge	10	None found	Leaves & rhizomes: skin diseases, veterinary, bone fracture, also used in rituals to protect against evil spirits (Namsa *et al.* 2011)	None found	None found
Allium sativum Linn. (Liliaceae) (CD) (69602) (C)	Lasung	Bulbs (B)	During high blood pressure, bulbs are kept in the mouth without chewing for half an hour each day. Bulb paste is	10	Bulbs: cardiac diseases (Jouad *et al.* 2001)	WR Leaves & rhizomes: bone fracture; bulbs: tuberculosis	WR Bulbs, leaves: cancer; bulbs: hypercholesterolemia, hypertension, peripheral,	WR (Bhandari *et al.* 2014, Gulfraz *et al.* 2014)

			applied externally for spider & snake bites			(Namsa *et al.* 2011) (Bunalema *et al.* 2014)	arterial disease, antibacterial against *S. aureus*, *E. coli*, *P. aeruginosa*, *S. epidermidis*, *K. pneumoniae*, antioxidant (Pittler and Ernst 2007, Gulfraz *et al.* 2014); adverse effects: (Borrelli *et al.* 2007)	
Alstonia scholaris (Linn.) R. Br. (Apocynaceae) (CD) (69541) (N)	Loomi	Leaves, roots & stem (B)	Decoction of stem, leaves & roots is taken orally to treat gastritis, jaundice & also drunk as a liver tonic	4	Stem: gastrointestinal disorders, diarrhoea (Dey and De 2012)	WR Bark: fever, dysentery, astringent, anthelmintic, antiperiodic, dysentery, malarial fever (Meena *et al.* 2011, Khyade *et al.* 2014)	WR Bark: antimalarial; bark, leaves, stem bark: antibacterial against *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. typhi*, *S. dysentery*; leaves: anti-inflammatory; aerial parts: antidiarrhoeal (Arulmozhi *et al.* 2007, Mukherjee *et al.* 2012, Khyade *et al.* 2014)	WR (Mukherjee *et al.* 2012, Khyade *et al.* 2014, Zhu *et al.* 2014)
Amaranthus gangeticus Linn. (Amaranthaceae) (CD) (69699) (N)	Tsumarlua	Leaves (F)	Boiled leaves are consumed to treat indigestion & also taken as a laxative	2	None found	WR Whole plant: nutrient, antioxidant (Njume *et al.* 2014)	WR Whole plant: anticancer; leaves: menorrhagia, anti-diarrhoea, haemorrhages from the bowels, antipyretic, expectorant (Tripathi *et al.* 1996, Escobedo-López *et al.* 2014)	(Sani *et al.* 2004)
Aquillaria agallocha Roset. (Thymelaeaceae) (CD) (WD) (69604) (N)	Sungza	Roots & stem (F)	Either an infusion or decoction of the roots or stem is	10	Roots: dysentery	WR Whole plant: liver complaints	WR Leaves, bark: antibacterial against *E.*	WR (Dash *et al.* 2008,

			taken orally thrice a day to treat dysentery & malaria		(Talukdar 2014)	(Shamsi-Baghbanan *et al.* 2014)	*coli, B. subtilis,* (Dash *et al.* 2008)	Talukdar 2014)
Artemisia vulgaris Linn. (Asteraceae) (WD) (69505) (C)	Chinangchibaza	Roots (F)	Roots infusion is taken orally to treat dysentery	5	None found	WR Leaves: cough & common cold (Partha 2014)	WR Leaves: antibacterial against *S. aureus, E. coli, P. aeruginosa, B. cereus*; aerial parts: anti-inflammatory, analgesic (Ashok and Upadhyaya 2013, Ahmadizadeh *et al.* 2014)	WR (Melguizo-Melguizo *et al.* 2014)
Artocarpus chaplasha Roxb. (Moraceae) (WD) (69688) (N)	Unem	Ripe fruits (B)	Eaten either raw or as an infusion taken orally twice a day for liver, kidney & gall bladder problems	4	None found	Stem bark: diarrhoea (Sharma *et al.* 2001)	Seeds: antioxidant, cytotoxic (Ahmed *et al.* 2013)	None found
Artocarpus heterophyllus Lamk. (Moraceae) (CD) (69596) (N)	Polong	Sap	Sap is applied topically to treat skin disease	10	WR Leaves: wounds; latex: boils (Sharma *et al.* 2001, Partha 2014)	WR Roots: diarrhoea, fever; leaves: ulcers, wound healing; leaves, stem bark: anaemia, asthma, dermatitis, diarrhoea, cough (Jagtap and Bapat 2010)	WR Fruits: cytotoxic; seeds: antibacterial against *S. aureus, E. coli, P. aeruginosa, B. subtilis*; leaves: wound healing (Baliga *et al.* 2011, Nair *et al.* 2013, Zheng *et al.* 2014)	WR (Baliga *et al.* 2011, Madruga *et al.* 2014)
Asclepias curassavica Linn. (Asclepiadaceae) (CD) (69617) (I)	Noklangchang	Fruits (F) & leaves (D)	Leaf paste is applied topically for cuts & wounds	4	Leaves: warts, wounds, healing process	WR Leaves: menstrual problems, piles, gonorrhoea,	WR Aerial parts: anti-inflammatory; whole plant: antibacterial	WR (Sundararaj an and

					(Sundararajan and Koduru 2014)	roundworm infection, abdominal tumours (Baskar *et al.* 2012, Ghildiyal *et al.* 2014)	against *S. aureus, E. coli, P. aeruginosa, B. subtilis*, antiviral against adeno virus, coxsackie B2, herpes type-1, measles, poliovirus-1; latex: antifungal against *C. albicans*; (Sundararajan and Koduru 2014)	Koduru 2014)
Averrhoa carambola L. (Averrhoaceae) (CD) (69692) (N)	Jarkona	Fruits (F) & leaves (D)	Either fresh fruit or dried leaves or dried fruits made into a powder are consumed during high blood pressure, bladder & intestinal problems	8	Fruits: diuretic in kidney, bladder complaints (Kumar 2014)	WR Fruits, leaves: vomiting, headache, chicken pox, ringworm (Kumar 2014)	WR Fruits: antioxidant, cardioprotective; roots: antidiabetic; bark: antibacterial against *S. typhi, P. aeruginosa, E. coli*, antioxidant, cytotoxic (Scholz *et al.* 2010, Das *et al.* 2013, Pantaleón-Velasco *et al.* 2014, Xu *et al.* 2014)	WR (Scholz *et al.* 2010, Zainudin *et al.* 2014)
Bambusa tulda Roxb. (Bambusoideae) (WD) (CD) (69605) (N)	Longme	Ash, leaves (B) & roots (F)	Ash is used as dye, leaf decoction is used in bath during common cold & fresh roots juice is taken orally as vermifuge	6	None found	Shoots: tetanus (Sharma and Borthakur 2008, Singh *et al.* 2010)	None found	(Nirmala *et al.* 2014)
Basella alba Linn. (Basellaceae) (WD) (69633) (C)	Latsungen	Leaves (F)	Leaves eaten either raw or boiled to treat gastritis & as a laxative	3	Whole plant: demulcent, diuretic, laxative (Rahman *et al.* 2013)	WR Leaves: dysentery, diarrhoea, anaemia, cancer (Adhikari *et al.* 2012)	WR Leaves: antibacterial against *S. typhimurium*, antioxidant, anti-inflammatory, anticancer (Adhikari *et al.* 2012)	WR (Adhikari *et al.* 2012)

Bauhinia variegata Linn. (Caesalpiniaceae) (CD) (69599) (N)	Owepanghef	Leaves (F)	Boiled immature leaves are consumed during gastrointestinal problems	1	WR Bark: dysentery, diarrhoea, stomach disorders, ulcers (Lim 2014)	WR Bark: anthelmintic, skin diseases (Lim 2014)	WR Leaves: antiulcer, antidiabetic, antibacterial against *S. aureus, E. coli, P. aeruginosa, B. subtilis, K. pneumoniae, V. cholerae, S. typhimurium, S. dysenteriae*; stem bark: antimicrobial against *S. aureus, E. coli, P. aeruginosa, C. albicans, A. niger*, anthelmintic, hepatoprotective; seeds: antiviral (Mali *et al.* 2007, Lim 2014)	WR (Jash *et al.* 2014, Lim 2014)
Begonia picta Smith (Begoniaceae) (WD) (69642) (E)	Tesenlawa	Leaves (F)	Leaves are used to cleanse hands by crushing between palms. Leaves are also used in cooking for their sour taste	2	Whole plant: cuts & wounds (Sapkota 2014)	WR Whole plant: headaches; roots: conjunctivitis; (Singh and Hamal 2013, Sapkota 2014)	None found	None found
Brassica oleracea Linn. (Brassicaceae) (CD) (69510) (I)	Pandacobi	Foliage (F)	The fresh juice of the foliage is consumed to treat jaundice	1	None found	WR Whole plant: laxative, constipation, dyspepsia, hypertension, heat burn (Ahmed *et al.* 2014)	WR Whole plant: anticancer (Chaudhary *et al.* 2014)	(Stoewsand 1995)

Cajanus cajan (Linn.) (Fabaceae) (CD) (69672) (N)	Mahajang	Leaves (F)	Leaf decoction is consumed to provide relief from fever	2	Leaves: yellow fever (Nwodo *et al.* 2011)	WR Roots: cancer; leaves: analgesic, constipation, gingivitis (Pal *et al.* 2011, Amri 2014)	WR Roots: anticancer; aerial parts: antimicrobial against *S. aureus, B. subtilis, S. epidermidis*, anthelmintic; leaves: antioxidant; leaves, stem, roots: antiviral against measles (Nwodo *et al.* 2011, Pal *et al.* 2011)	WR (Pal *et al.* 2011)
Calotropis gigantea Linn. (Asclepiadaceae) (WD) (69691) (N)	Kutjak moli	Leaves (F)	Leaf poultice is used topically to treat bone dislocation, body pain, sprain & burns	10	Stem: latex applied on sprain (Uprety *et al.* 2011)	WR Latex: dog bite (Elkington *et al.* 2014)	WR Leaves: antioxidant, antibacterial against *S. aureus, E. coli, P. aeruginosa, S. flexneri, S. dysenteriae, S. sonnei, B. cereus, B. subtilis*, antifungal against *C. albicans*, insecticidal; flowers: analgesic activity; root bark: wound healing (Pathak and Argal 2007, Deshmukh *et al.* 2009, Kuldeep *et al.* 2014, Parvin *et al.* 2014)	WR (Dhalendra *et al.* 2014)
Cannabis sativa L. (Cannabaceae) (CD) (69608) (N)	Ganja	Leaves (F)	Leaf decoction is taken orally for stomach ache	3	Leaves, twigs: indigestion & stomach acidity (Ghosh and Das 2011)	WR Leaves, seeds: analgesic (Adnan *et al.* 2014)	WR Flowers, leaves: anticancer, antidepressant; leaves: antibacterial against *S. aureus, E. coli, P. aeruginosa, E. faecalis, S. typhimurium* (de Mello *et al.* 2014,	WR (Kusari *et al.* 2014)

							Naveed *et al.* 2014, Romano *et al.* 2014)	
Capsicum annum Linn. (Solanaceae) (CD) (69658) (I)	Metsu	Fruits (F)	Fruits are eaten during loss of appetite, indigestion & to purify blood	2	Fruits: appetiser (Bhatia *et al.* 2014)	WR Fruits: antiseptic, carminative (Iwu 2014)	WR Fruits: antimicrobial against *S. aureus, E. coli, P. aeruginosa, P. mirabilis, V. cholerae, C. albicans,* antiviral against HSV-1, analgesic, cardioprotective, antidiabetic, anti-inflammatory, anti-diarrhoeal (Khan *et al.* 2014)	WR (Materska 2014)
Carica papaya Linn. (Caricaceae) (CD) (69626) (I)	Kumita	Fruits & sap (F)	Decoction of unripe fruit is consumed as liver tonic & to treat gastritis. Boiled unripe fruit is consumed as a laxative. The sap is used as preservative for citrus juices & extracts of other herbs	6	Fruits: stomach complaints (Vij and Prashar 2014)	WR Fruits: diuretic, diarrhoea, dysentery, antibacterial, abortifacent (Vij and Prashar 2014)	WR Fruits: antioxidant, wound healing; leaves: antibacterial against *S. aureus, E. coli, P. aeruginosa, K. pneumonia, P. mirabilis*, anti-inflammatory, anticancer; latex: antifungal against *C. albicans*; bark: antifertility; roots: diuretic; seeds: anthelmintic (Vij and Prashar 2014)	WR (Eke *et al.* 2014, Nguyen *et al.* 2014)
Cassia floribunda Cav. (Caesalpiniaceae) (WD) (69535) (I)	Napongchami	Leaves (F)	Warmed leaves are made into a paste & applied externally for fungal infection, eczema, contact	8	None found	Young pods are cooked as vegetable (Chandra *et al.* 2013)	WR Seeds: antioxidant, antidiabetic, antibacterial against *E. coli, S. pyogenes, S. typhi, S.*	WR (Vadivel *et al.* 2011)

			dermatitis, allergic reaction, prickly heat & burns. Caution - only for external use				*paratyphi* (Vadivel *et al.* 2011, Singh *et al.* 2013)	
Catharanthus roseus (Linn.) G. Don (Apocynaceae) (CD) (69517) (I)	Supienaro	Leaves (F)	Leaf decoction is taken orally for gastroenteritis problems & as a laxative	6	None found	WR Whole plant: dengue fever, diabetes, cancer, diarrhoea (Islam *et al.* 2014)	WR Whole plant: anticancer (Inoue and Craker 2014)	WR (Rai *et al.* 2014)
Celosia cristata L. (Amaranthaceae) (WD) (69520) (N)	Alonaro	Flowers & leaves (F)	Decoction of flowers is taken orally for urinary tract infection. Leaf paste is applied topically for cuts & wounds	3	Whole plant: urinary tract infections; leaves: sores, wounds (Surse Sunita and City 2014)	WR Whole plant: kidney stone; leaves: dysentery, menstrual bleeding, inflammation & worms (Desale *et al.* 2013, Surse Sunita and City 2014)	WR Leaves: antiviral, antioxidant, anthelmintic; flowers: antimicrobial against *S. aureus, E. coli, P. aeruginosa, B. subtilis, S. typhimurium, C. albicans* (Yun *et al.* 2008, Surse Sunita and City 2014)	WR (Surse Sunita and City 2014)
Centella asiatica L. (Apiaceae) (WD) (69598) (N)	Longtsukolok	Whole plant (F)	Plant is boiled & consumed for gastrointestinal problems	7	Leaves: Stomach pain, amoebic dysentery (Dey and De 2012)	WR Whole plant: burns (Bahramsoltani *et al.* 2014)	WR Whole plant: antibacterial against *S. aureus, E. coli, P. aeruginosa, B. subtilis, S. sonnei*, wound healing, anti-inflammatory, antioxidant, antinociceptive, collagen stimulant; leaves: antidepressant, antiepileptic, antiulcer, antiviral against HSV-1,	WR (Gohil *et al.* 2010)

							HSV-2 (Jagtap *et al.* 2009, Gohil *et al.* 2010)	
Chrysanthemum indicum L. (Asteraceae) (CD) (69529) (I)	Asurongmang	Leaves (F)	Leaf paste is applied topically to treat lip scab (angular cheilitis) & scabies	3	Flowers: boils, itchiness of skin (Lim 2014)	WR Flowers, leaves, stem: cough, hepatitis, nerve tonic, rheumatism (Ahmed *et al.* 2014)	WR Leaves: antiplasmodial, hepatoprotective, antifungal against *C. albicans*; flowers: analgesic, antibacterial against *S. aureus, E. coli, P. aeruginosa, S. pyogenes, S. mutans*, anti-inflammatory (Wang *et al.* 2009, Lim 2014)	WR (Lim 2014)
Cissampelos pareira Linn. (Menispermaceae) (WD) (69675) (I)	Likhazung	Roots (B)	Roots are eaten raw or infusion is taken orally for high blood pressure, malaria, dysentery, piles, gastrointestinal problems & diabetes. Powdered dried roots are used for long term storage	6	Roots: fever, heart diseases & leprosy, diarrhoea, gastrointestinal disorders (Rudrapal *et al.* 2012, Semwal *et al.* 2014)	WR Roots: diuretic, febrifuge, for heart trouble, dysentery, sores, snakebite & jaundice (Semwal *et al.* 2014)	WR Leaves: antidiabetic; roots: antimalarial: active against *P. falciparum*, antibacterial against *S. aureus, E. coli, S. typhimurium, K. pneumoniae*, antiulcer, cardiovascular activity (Basumata *et al.* 2012, Singh *et al.* 2013, Ngoci *et al.* 2014, Semwal *et al.* 2014)	WR (Patel *et al.* 2014, Semwal *et al.* 2014)
Cissus repens Lam. (Vitaceae) (WD) (69537) (N)	Zerebliwa	Leaves (B)	Leaf decoction is taken orally for high blood pressure, urinary, spleen & kidney problems	2	None found	WR Stem, leaves: jaundice, muscle pain; muscle cramp; muscle fatigue; joint pain (de Boer *et al.*	WR The whole plant: analgesic, anti-inflammatory; roots: antiulcer, cardioprotective (Umbare *et al.* 2011, Chang *et al.* 2012,	WR (Fernandes and Banu 2012)

						2012, Sarker *et al.* 2012)	Fernandes and Banu 2012)	
Citrus microcarpa Bunge (Rutaceae) (CD) (69618) (C)	Nimbutinga	Fruits (F)	Fresh fruit juice is taken judiciously for stomach ache & gas formation (purgative)	10	None found	WR Infusion of leaves is given during headache & hypertension (Olowa *et al.* 2012)	WR Fruits: antibacterial against *Streptococcus* spp, *E. coli, V. cholerae, Salmonella* spp, *E. tarda*, gastroprotective, antiulcer (Sharma 2011, Lim 2012)	WR (Dharmawan *et al.* 2008, Ghafar *et al.* 2010, Cheong *et al.* 2012, Cheong *et al.* 2012, Lim 2012)
Clerodendron colebrookianum D. Don (Verbenaceae) (WD) (CD) (69531) (N)	Oremwa	Leaves (F)	Boiled leaves are consumed to treat high blood pressure & also eaten as a delicacy. Caution - ingestion of drupe may induce body swelling & vomiting	10	WR Leaves: high blood pressure (Nath and Bordoloi 1991, Kalita *et al.* 2012, Pfoze *et al.* 2012)	WR Leaves: liver pain & viral fever, gastric disorders, dysentery, diarrhoea, abdominal pain, diabetes (Kalita *et al.* 2012, Singh *et al.* 2013)	WR Leaves: hypertension, diabetes (Nath and Bordoloi 1991, Kotoky *et al.* 2005, Kalita *et al.* 2012)	WR (Majaw and Moirangthem 2009, Kalita *et al.* 2012)
Coix lacryma-jobi Linn. (Poaceae) (WD) (69662) (N)	Jemur	Fruits & leaves (F)	Fruits & leaves are cooked & consumed as vitamin source	7	Seeds: consumed for their nutrients (Bhandari *et al.* 2012)	WR Leaves: urinary complaints, stomach problems, fever, small pox, as tonic; roots: menstrual disorders; seeds: dysentery, diuretic & as diet drink (Katewa *et*	WR Leaves: anti-trichomonas (Brandelli *et al.* 2013)	WR (Bhandari *et al.* 2012)

						al. 2001, Sangtam *et al.* 2012)		
Costus speciosus (Koenig ex Retz) JE Smith (Costaceae) (WD) (69496) (N)	Aokmejang	Stem (F)	Inner stem is chewed for tooth ache & as vermifuge	10	Tubers: applied to decayed tooth to relieve pain (Renuga and Bai 2013)	WR Leaves: fever, skin diseases, abortion, diarrhoea, jaundice, arthritis; roots: pneumonia, rheumatism, dropsy, urinary diseases, jaundice (Pawar and Pawar 2014)	WR Aerial parts: anti-inflammatory, analgesic, antipyretic, antibacterial against *S. aureus*; roots: antidiabetic, diuretic, estrogenic (Saraf 2010, Srivastava *et al.* 2013, Pawar and Pawar 2014)	WR (Pawar and Pawar 2014)
Crataeva nurvala Buch-Ham. (Capparaceae) (WD) (69690) (N)	Kongkawa	Leaves (F)	Warmed leaves are applied externally to relieve pain & body swelling. Boiled leaves are consumed as liver tonic	8	WR Bark: liver problems, indigestion, flatulence (Sharma *et al.* 2012)	WR Bark: urinary disorders, kidney & bladder stones; leaves: joint disorders (Bhattacharjee and Shashidhara 2012)	WR Whole plant: hepatoprotective; stem bark: urinary tract infections, antibacterial against *B. cereus, E. coli*, anti-inflammatory, antidiabetic, antifertility (Bopana and Saxena 2008, Bhattacharjee and Shashidhara 2012, Prasobh 2014)	WR (Kalidhar 2006, Bhattacharjee and Shashidhara 2012)
Croton caudatus Gieseler (Euphorbiaceae) (WD) (69664) (N)	Khemetsu koila	Leaves & roots (F)	Fresh leaves or roots are chopped into fine pieces & soaked in water overnight & the extract is drunk twice a day for cancer, sinusitis &	1	Leaf juice: stomach tonic, anticancer (Medhi and Borthakur 2012)	WR Roots & leaves: for the treatment of arthritis & to stop paralysis (Yusuf *et al.* 2007)	WR Leaves: antioxidant, anticancer (Nath *et al.* 2013)	WR (Zou *et al.* 2010, Lokendrajit *et al.* 2012)

			gastrointestinal problems					
Cucurbita pepo L. (Cucurbitaceae) (CD) (69682) (I)	Moyamatsu	Fruits & leaves (F)	Leaves & fruits are cooked & consumed as vitamin source	10	None found	WR Seeds: diuretic, urinary system problems, prostate problems (Khan *et al.* 2013)	WR Fruits: antioxidant, cytoprotective (Song *et al.* 2013)	WR (Emmanuel and Ganiyu 2013)
*Curculigo capitulata (*Lour.) Kuntze (Hypoxidaceae) (WD) (69527) (N)	Kurivu	Rhizomes (F)	Outer skin of rhizomes is peeled off & soaked in water until it turns slimy & then consumed for gastritis & squeezed into eyes for treating eye infection (dirt, conjunctivitis)	9	None found	Leaves: cuts & wounds (Shil *et al.* 2014)	Roots: cardioprotective (Chang *et al.* 1997)	(Chang *et al.* 1997)
Curanga amara Juss. Syn: *Picria fel-terrae* (Scrophulariaceae) (WD) (CD) (69538) (N)	Longri	Leaves (F)	Fresh leaves are chewed or infusion taken orally to treat dysentery, high blood pressure, food poisoning, gastroenteritis & loss of appetite. Caution - extremely bitter	10	None found	None found	Leaves: antioxidant (Choi and Hwang 2005)	None found
Cyclea peltata Diels. (Menispermaceae) (WD) (69650) (N)	Tsungrempangmoli	Leaves (B)	Leaf decoction is applied topically to abscesses & boils. Leaves also added to bath to ward off evil spirits	6	Roots: skin disorders (Begum and Nath 2000)	WR Roots: gastric ulcer & allied stomach ailments, jaundice & digestive	WR Whole plant: antibacterial against *S. aureus, S. haemolyticus, B. cereus*; roots: hepatoprotective	WR (Shine *et al.* 2014)

						disorders; leaves: antihypertensive & cardiac depressant activities (Abraham and Thomas 2012, Shine *et al.* 2014)	(Abraham and Thomas 2012, Bhat *et al.* 2014, Shine *et al.* 2014)	
Datura stramonium Linn. (Solanaceae) (WD) (69528) (C)	Kohima sangjem	Leaves (F)	Warmed leaves are applied externally to relieve back pain	5	Leaves: relieving pain (Das *et al.* 2012)	WR Seeds: purgative, cough, fever, asthma; leaves: wounds, pain (Sayyed and Shah 2014)	WR Leaves: anti-asthma antiulcer, wounds healing, anti-inflammatory, anti-rheumatic, antimicrobial against *S. aureus, E. coli, P. aeruginosa, P. vulgaris, A. niger*, cytotoxic; seeds: analgesic effect on both acute & chronic pain (Das *et al.* 2012, Sayyed and Shah 2014)	WR (Sayyed and Shah 2014)
Debregeasia longifolia (Burm. f.) Wedd. (Urticaceae) (WD) (69504) (N)	Natsulawa	Leaves (F)	Leaf decoction is taken orally to treat diabetes, fever & high blood pressure. Boiled leaves are eaten as a delicacy	3	Leaves: diabetes (Pfoze *et al.* 2012)	Bark: bone fracture (Dangwal *et al.* 2010)	Leaves: antibacterial against *S. aureus* (Mariani *et al.* 2014)	(Jian *et al.* 2010, Zhao *et al.* 2013)
Dendrocnide sinuata (Bl.) (Urticaceae) (WD) (69508) (N)	Zaklojawa	Stem (F)	Outer fresh stem is scraped off & the mucilage secreted is applied on fresh cuts & wounds (haemostatic). Caution - produces	10	Roots: injury, itching skin (Srivastava 2010)	Whole plant: elephantiasis (Rahman *et al.* 2013)	Leaves: antimicrobial against *E. coli, P. aeruginosa, E. aerogenes*, antioxidant (Tanti *et al.* 2011)	None found

			extremely painful sting					
Diospyros lanceifolia Roxb. (Ebenaceae) (WD) (69544)	Urcha	Fruits & roots (F)	Fruits or roots are crushed in stream to poison fish	10	Seeds: to poison fish (Wiart 2006)	Fruits: to poison fish; seeds: skin diseases (Mallavadhani *et al.* 1998)	None found	None found
Dolichos lablab L. (Fabaceae) (CD) (69523) (N)	Napakauv	Leaves & pods (B)	Cooked pods are consumed to treat diarrhoea, nausea, vomiting & poor appetite. For insect & poisonous spider bites & bee stings, leaf paste is applied topically. Caution - fatal when consumed after a dog bite	8	None found	WR Whole plant: boils, pimples (Juyal and Ghildiyal 2013)	Seeds: antibacterial against *S. aureus, E. coli, P. aeruginosa*; whole plant: antidiabetic (Jain 2014, Singhal *et al.* 2014)	None found
Drymaria cordata (Linn.) Willd. (Caryophyllaceae) (WD) (69530) (C)	Pipivula	Whole plant (F)	Plants warmed in fire are crushed into a paste & applied topically to treat fungal infection (ringworm), contact dermatitis & lip scab (angular cheilitis). For sinusitis, leaf paste is inserted into the nostril. To deodorise armpits, plant is wrapped in banana leaves & toasted for 5-10 minutes & then	6	Leaves: sinusitis (Pfoze *et al.* 2012)	Leaves: fever (Kalyan and Pallwabee 2014)	WR Whole plant: anti-inflammatory, antinociceptive, antipyretic; aerial parts, leaves: antibacterial against *S. aureus, E. coli, P. aeruginosa, B. subtilis* (Kalyan and Pallwabee 2014, Nono *et al.* 2014)	WR (Nono *et al.* 2014)

			applied to armpits. For ear pain & infection (otitis media), leaves, mustard oil & spider exuvia are pounded, filtered & a few drops instilled into the ear					
Dryopteris filix-mas (L.) syn *Cyclosorus parasiticus* Schott (Dryopteridaceae) (WD) (69507) (C)	Nachav	Whole plant (F)	Whole plant is crushed into the stream to poison fish. Infusion is sprayed as pesticide & insecticide. Leaves are laid down in chicken coop for killing chicken ticks/bugs. Leaf paste is used to treat skin irritation & snake & insect bites	3	WR Leaves: gout rheumatism, microscopic insects in chickens (Singh *et al.* 2013)	WR Leaves: eye disease (Zheng *et al.* 2013)	Leaves: antiproliferative (Wei *et al.* 2013)	WR (Wei *et al.* 2013, Hunyadi *et al.* 2014)
Duabanga grandiflora (Roxb. ex DC.) Walp. (Sonneratiaceae) (CD) (WD) (69686) (N)	Kisati	Stem bark (F)	Fresh bark is scraped off & applied topically to treat skin diseases, cuts & wounds	3	Bark: skin diseases, eczema (Shankar and Devalla 2012)	Roots: juice to cure upset stomach (Biswas *et al.* 2010)	Leaves: decreasing skin damage, skin whitening, antibacterial against *S. aureus, E. coli* (Tsukiyama *et al.* 2010, Othman *et al.* 2011)	WR (Auamcharoen *et al.* 2009)
Elsholtzia blanda Benth. (Lamiaceae) (WD) (69524) (N)	Changjang	Leaves (B)	Leaf paste is applied to fresh cuts & decoction of leaves is added to bath during cold or fever. Leaf paste is also inserted into	4	Aerial parts: fever, cholera, skin diseases & inflammation; leaves: used to treat	WR Flowers: piles (Singh *et al.* 2014)	WR Leaves, roots: antibacterial against *S. aureus*, antiviral against influenza virus, antioxidant (Liu *et al.*	WR (Lu *et al.* 2000, Chen *et al.* 2005)

			the nostril to treat sinusitis		inflamed glands (Rai and Lalramnghinglova 2010)		2007, Guo *et al.* 2012, Ishwori *et al.* 2014)	
Entada pursaetha DC. (Leguminosae) (WD) (69616) (N)	Keling	Seeds (B)	Seeds extract is used for head wash to treat head lice & dandruff	10	None found	WR Seeds: to reduce pain of inflamed swellings (Sutha *et al.* 2010)	WR Stem: hepatoprotective, antioxidant (Gupta *et al.* 2011)	(Tapondjou *et al.* 2005)
Equisetum ramosissimum Desf. Subsp. *Debile* (Equisetaceae) (WD) (69499) (C)	Avpenba	Stem (F)	Stem decoction is taken orally for kidney problems	6	None found	WR Aerial parts: stomach ache (Bulut and Tuzlaci 2013)	WR Whole plant: antipyretic, anti-inflammatory (Amoroso *et al.* 2014)	WR (Wang and Jia 2004)
Eryngium foetidum Linn. (Apiaceae) (WD) (69668) (I)	Aong thonia	Leaves (F)	Leaves are consumed either raw or cooked for indigestion	3	Leaves, fruits, roots: indigestion (Rahmatullah *et al.* 2009)	WR Leaves: abscesses & boils (Fongod *et al.* 2014)	WR Whole plant: anthelmintic, antibacterial against *Erwinia* spp, *S. choleraesuis*, antimalarial; leaves: anti-inflammatory (Paul *et al.* 2011)	WR (Paul *et al.* 2011, Bhavana *et al.* 2013, Mohammad hosseini *et al.* 2013)
Erythrina stricta Roxb. (Fabaceae) (WD) (69629) (N)	Lochet	Stem bark (F)	Bark paste is applied topically to treat contact dermatitis, eczema & skin infections	10	None found	WR Bark: treat disruptions in menstrual cycle (Kumar and Bharati 2014)	WR Stem: spasmolytic, diuretic, anticonvulsant, analgesic, antiviral, antifungal against *C. albicans, A. niger*; bark: antibacterial against *B. cereus, B. subtilis, S. aureus, S. lutea, E. coli, P. aeruginosa, S. typhi,*	WR (Rukachaisirikul *et al.* 2007)

							S. paratyphi, S. boydii, S. dysenteriae, V. mimicus, V. parahaemolyticus (Hussain *et al.* 2010, de Araújo-Júnior *et al.* 2012)	
Eucalyptus globulus Labill. (Myrtaceae) (CD) (69663) (I)	Eucalyptus	Leaves (B)	Steam from boiling leaves is inhaled for nasal decongestion	3	WR Leaves: spasmodic, decongestant, asthma, migraine, congestive headache (Sivasankari *et al.* 2014)	WR Leaves, flowers: diabetes, high blood pressure (Amel 2013)	WR Leaves: antibacterial against *P. aeruginosa* (Pereira *et al.* 2014)	WR (Pereira *et al.* 2014)
Eupatorium odoratum Linn. (Asteraceae) (WD) (69523) (I)	Zasen	Leaves (F)	Leaf paste is applied topically to fresh cuts & wounds	10	WR Leaves: cuts & wounds (Hasan *et al.* 2013)	WR Roots: stomach pains, during gastric ulcer; leaves: cuts & wounds, blisters & skin irritation (Singh *et al.* 2013)	WR Leaves: antifungal against *C. albicans*, antibacterial against *S. aureus, E. coli, P. aeruginosa, B. subtilis, B. cereus, P. mirabilis* (Sharma *et al.* 2013, Unnithan *et al.* 2014)	WR (Sharma *et al.* 2013, Unnithan *et al.* 2014)
Euphorbia pulcherrima Willd. ex Klotzsch (Euphorbiaceae) (CD) (69615) (I)	Muluchangnaro	Flowers & leaves (F)	Death may result when flowers or leaves are fed accidentally to pigs	2	Aerial parts: poisonous for animals (Cortinovis and Caloni 2013)	Leaves: rheumatic pain (Adsul *et al.* 2013)	Flower, leaves, stem, whole plant: antimicrobial against *S. typhi, S. paratyphi, E. coli, C. albicans, A. niger*; aerial parts: toxic for animals (Yakubu and Mukhtar 2011, Caloni *et al.* 2013); aerial parts: anticonvulsant,	None found

							antinociceptive but not toxic (Singh *et al.* 2012)	
Euphorbia royleana Boiss. (Euphorbiaceae) (CD) (69684) (N)	Takterak	Milky sap (F)	Milky sap is applied topically to treat skin diseases & body pain	3	Latex: skin diseases (Yadav and Bhandoria 2013)	WR Latex: cuts to stop bleeding; leaves: earache (Joshi *et al.* 2011)	WR Latex: antibacterial against *B. subtilis, E. coli,* anti-inflammatory (Bani *et al.* 2000, Sharma 2013)	(Singh and Singh 2012, Rai *et al.* 2014)
Eurya acuminata DC. (Theaceae) (WD) (69612) (C)	Mesetwa	Fruits & leaves (F)	Leaf infusion is taken orally to treat dysentery/diarrhoea . Leaf paste is applied topically to cuts & wounds. Fresh fruits are crushed & mixed with water & drunk 3 to 4 times to treat gas formation	4	Leaves: stomach disorders, dysentery, diarrhoea & cholera, skin diseases (Adhikari *et al.* 2007, Joshi and Joshi 2007)	Leaves: cough (Holdsworth and Sakulas 1986)	Leaves, stem: antibacterial against *S. aureus* (Grosvenor *et al.* 1995)	None found
Ficus elastica Roxb. ex Hornem. (Moraceae) (CD) (69619) (N)	Ngisa	Roots & sap (F)	Root juice or sap is applied topically to snake bite & cuts & wounds	3	Leaves: skin infections & skin allergies (Van Kiem *et al.* 2012)	Fruits: to stupefy fish & make them float (Srivastava 2010)	WR Leaves: antioxidant, anticancer, antibacterial against *P. aeruginosa, B. cereus, K. pneumoniae, P. mirabilis, E. aerogenes* (Almahyl *et al.* 2003, Parekh and Chanda 2007, Sirisha *et al.* 2010, Kaur and Arora 2013)	(Almahyl *et al.* 2003)
Garcinia cowa Roxb. (Guttiferae) (WD) (69631) (N)	Songtula	Stem bark & seeds (D)	Decoction of dried bark or seed covers are taken orally to treat	10	WR Leaves: dysentery & diarrhoea	WR Fruits: coughs, cold (Kabir *et al.* 2014)	WR Fruits, leaves, twigs: antibacterial against *S. aureus, E. coli, P.*	WR (Ritthiwigrom *et al.* 2013,

			dysentery/diarrhoea . Seeds are edible		(Hazarika and Nautiyal 2012)		*aeruginosa, B. subtilis, B. cereus, S. typhimurium, S. epidermidis,* antimalarial, anti-inflammatory; fruits: cytotoxic (Ritthiwigrom *et al.* 2013, Auranwiwat *et al.* 2014)	Auranwiwat *et al.* 2014)
Garcinia pedunculata Roxb. Ex Buch.-Ham. (Guttiferae) (WD) (69630) (N)	Asong	Stem bark & seeds (D)	Decoction of dried bark or seed covers are taken orally to treat dysentery/diarrhoea . Seeds are edible	10	Pericarp: dysentery & diarrhoea (Rai and Lalramnghinglova 2010)	WR Fruits: effective in jaundice (Barukial and Sarmah 2011)	WR Fruits: antioxidant (Sharma *et al.* 2014)	WR (Chowdhury 2014)
Girardinia palmata (Forsk.) Gaud. (Urticaceae) (WD) (69661) (N)	Ongpangzakl	Leaves (F)	Leaf paste is applied to dog bites. Dogs stung with this plant may die	10	None found	Roots: to treat allergy caused by food (Rai and Lalramnghinglova 2010)	None found	None found
Glycine max (L.) Merr. (Fabaceae) (CD) (69680) (I)	Alichami	Seeds (B)	Roasted seeds are made into powder & taken with tea to treat dysentery	3	None found	WR Seeds: common cold (Negi and Maikhuri 2013)	WR Whole plant: antioxidant, antibacterial against *S. aureus, E. coli, P. aeruginosa, B. subtilis, S. pyogenes, S. viridans, S. faecalis, K. pneumonia, P. vulgaris* (Ponnusha *et al.* 2011, Sharma 2011)	WR (Medic *et al.* 2014)
Gmelina arborea Roxb. (Verbenaceae) (WD) (69518) (N)	Ekong	Drupe (F)	Mesocarp of the drupe is applied topically to treat skin diseases	6	Roots: skin problems (Acharya *et al.* 2012)	WR Leaves, bark: constipation, indigestion	WR Stem bark: antibacterial against *E. coli, S. typhi, K. pneumoniae, P.*	WR (Chothani *et al.* 2011, Kulkarni *et*

						(Doley *et al.* 2014)	*mirabilis, S. dysentriae*, leaves: anti-inflammatory, antinociceptive (El-Mahmood *et al.* 2010, Kulkarni *et al.* 2013, Panda 2014)	*al.* 2013, Chothani and Patel 2014)
Gonatanthus pumilus (D. Don) Engler & Krause (Araceae) (WD) (69498) (N)	Longtong	Leaves & stem (F)	Small quantity of the leaves or stem is mixed with food & given as vermifuge to pigs. Caution - extremely poisonous & death may result if ingested by humans. Rhizomes also causes extreme itching when contacted	8	None found	Roots, tubers: burns & wounds (Bhat *et al.* 2013)	Tubers: antiproliferative (Dhuna *et al.* 2007)	(Dhuna *et al.* 2007)
Gossypium herbaceum Linn. (Malvaceae) (CD) (69611) (I)	Khumpa	Roots (F)	Root decoction is taken orally as diuretic	2	None found	WR Seeds: cough, asthma, skin disease; roots: to cause abortion (Khaleequr *et al.* 2012)	WR Whole plant: antidiabetic; leaves: diuretic; flowers: antiulcer; seeds: antifertility, antibacterial against *B. cereus, S. typhimurium, S. epidermidis*, (Khaleequr *et al.* 2012, Velmurugan and Bhargava 2014)	WR (Khaleequr *et al.* 2012)
Gynocardia odorata R. Br. (Flacourtiaceae) (WD) (69501) (N)	Lamen	Leaves (F)	Leaf paste is applied to bee sting & leaves are used to protect from bee sting during honey	6	None found	Seeds: leprosy, skin disorders (Rai and	Whole plant: cytotoxic (Asif *et al.* 2014)	(Asif *et al.* 2014)

			harvest. Caution - plant growing near drinking water source or channel can contaminate the drinking water & if consumed results in abnormal enlargement of neck			Lalramnghinglova 2010)		
Gynura crepidioides Benth. (Asteraceae) (WD) (69506) (I)	Monglibaza	Leaves (F)	Leaf decoction is taken orally to treat diabetes	4	None found	Leaves: headache, insomnia, constipation, in pregnancy for easy delivery (Singh *et al.* 2013)	Leaves: antioxidant (Zhang *et al.* 2012)	None found
Hedyotis scandens Roxb. (Rubiaceae) (WD) (69539) (E)	Termoli	Leaves & roots (B)	Leaf paste is applied topically to cuts & wounds & infusion or decoction is taken orally to treat urinary tract infection, piles, & gastrointestinal problems & also taken as a laxative. Roots are chewed & applied topically to bee sting	8	Leaves: itches, scabies & eczema; roots: gastric problems (Subba and Basnet 2014)	WR Roots: sprain; whole plant: peptic ulcer (Subba and Basnet 2014)	WR Whole plant: antibacterial against *S. aureus, E. coli, P. vulgaris, K. pneumoniae* (Subba and Basnet 2014)	WR (Ke-qun 2013)
Hodgsonia macrocarpa (Blume) Cogn. (Cucurbitaceae) (WD) (69667) (E)	Assa	Leaves & seeds (F)	Leaf paste is used for massaging body pain & roasted seed	6	None found	Fruits: skin disease (Shankar and Devalla 2012)	Leaves: cytotoxic (Rizwana *et al.* 2010)	None found

			is consumed as a laxative					
Holboellia latifolia Wall. (Lardizabalaceae) (WD) (69500) (E)	Mezetsuk	Leaves (F)	Foam from crushed leaves is applied topically to burns	5	Leaves: burns (Begum and Nath 2000)	Roots: rheumatism (Lepcha and Das 2011)	None found	None found
Houttuynia cordata Thunb. (Saururaceae) (CD) (69532) (N)	Nokna	Whole plant (F)	Eaten raw to treat dysentery/diarrhoea, gas formation & as vermifuge	10	Leaves: dysentery (Kumar *et al.* 2014)	WR Leaves: cholera, purification of blood, skin diseases (Kumar *et al.* 2014)	WR Whole plant: anti-inflammatory, antiviral against SARS, influenza, HSV-1, HSV-2, dengue virus, antibacterial against *S. typhimurium*, antidiabetic, antioxidant; leaves: antiobesity (Wei 2001, Kumar *et al.* 2014)	WR (Chen *et al.* 2014, Kumar *et al.* 2014)
Ipomoea nil (Linn.) Roth. *Pharbitis nil* (Convolvulaceae) (CD) (69678) (C)	Makenchangnaro	Flower & leaves (F)	Leaf & flower paste is applied topically to burns	2	None found	Seeds: abortifacient (Bhatt *et al.* 2013)	WR Seeds: antimicrobial against *S. aureus, S. pneumonia, E. coli, C. freundii, K. pneumoniae, C. albicans, A. niger*, antitumour (Hussain *et al.* 2014, Lee *et al.* 2014)	WR (Lee *et al.* 2014)
Kalanchoe pinnata (Lam.) Pers. (Crassulaceae) (CD) (69515) (I)	Nokchamoli	Leaves (F)	Warmed leaf paste is applied topically to treat ringworms, skin diseases & burns	6	WR Leaves: skin diseases, eczema, pruritus (Bhat *et al.* 2014)	WR Leaves: blood dysentery (Anisuzzaman *et al.* 2007)	WR Roots: antibacterial against *S. aureus, E. coli, P. aeruginosa*; leaves: antibacterial against *B. cereus, B. subtilis*, diuretic, hepatoprotective (Wiart *et al.* 2004, Quazi Majaz *et al.* 2011)	WR (Milad *et al.* 2014)

Lagenaria siceraria (Molina) Standl. (CD) (Cucurbitaceae) (I)	Aakuf	Leaves (F)	Juice extract of leaves is applied topically to treat skin diseases & inflammation	6	WR Roots: wounds; fruits: skin disorders (Goji *et al.* 2006, Giday *et al.* 2007)	WR Fruits: cardioprotective, purgative, diuretic; leaves: jaundice; seeds: diuretic, anthelmintic (Kubde *et al.* 2010)	WR Fruits: cardioprotective, analgesic & anti-inflammatory; seeds: anticancer; leaves: antimicrobial against *S. aureus, E. coli, P. aeruginosa, C. albicans, A. niger* (Badmanaban 2010, Kubde *et al.* 2010)	WR (Gangwal *et al.* 2010, Kubde *et al.* 2010)
Lantana camara Linn. (Verbenaceae) (WD) (69620) (I)	Aiangketba naro	Whole plant (F)	Plant decoction is taken orally to treat jaundice, cold & fever	1	WR Leaves: fevers, dry cough, jaundice (Pfoze *et al.* 2012)	WR Leaves: cuts, rheumatism, ulcers, vermifuge (Ghisalberti 2000)	WR Flowers, leaves: antibacterial against *E. coli, P. aeruginosa, B. subtilis, E. faecalis*; leaves: anti-inflammatory (Kumar *et al.* 2013, Pradeep *et al.* 2013, Arya *et al.* 2014)	WR (Ghisalberti 2000, Ingawale and Goswami-Giri 2014)
Lasia spinosa (L.) Thwaites (Araceae) (WD) (69655) (N)	Turang	Leaves & 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	Leaves: cuts & injuries (Rao and Jamir 1982)	WR Leaves: bone fracture (Uddin *et al.* 2014)	WR Whole plant: antioxidant; leaves: antioxidant, cytotoxic, antimicrobial against *B. cereus, B. subtilis, S. aureus, S. lutea, E. coli, P. aeruginosa, S. typhi, S. paratyphi, S. boydii, S. dysenteriae, V. mimicus, C. albicans, A. niger* (Goshwami *et al.* 2013, Wong *et al.* 2014)	WR (Brahma *et al.* 2014)

Lycopersicon esculentum Linn. (Solanaceae) (CD) (69654) (I)	Benganatasula	Fruits (F)	Juice from unripe fruits is taken orally twice a day to treat urinary problems & kidney pain	3	Roots: blood in urine (Maroyi 2012)	WR Fruits: fever (Rahmatullah *et al.* 2013)	WR Fruits: antioxidant, antifungal against *C. albicans* (Alam *et al.* 2014, Nour *et al.* 2014)	WR (Gautam 2013)
Luffa acutangula Linn. (Cucurbitaceae) (CD) (69676) (N)	Pokka	Flowers, fruits & leaves (F)	Flowers, fruits & leaves are boiled & consumed as a laxative & to aid digestion	6	Whole plant: laxative & purgative (Abid 2005)	WR Leaves, fruits, seeds: haemorrhoids, diuretic, leprosy, conjunctivitis, demulcent, nutritive (Rahman 2013)	WR Fruits: antioxidant, antidiabetic, anti-inflammatory, antibacterial against *B. subtilis, S. aureus, E. coli, H. pylori, K. pneumoniae, S. typhi*, hepatoprotective (Abid 2005, Mohan G and Sanjey J 2014)	WR (Mohan G and Sanjey J 2014)
Macropanax undulatus (Wall. ex G. Don) Seem. (Araliaceae) (WD) (69540) (N)	Semza	Leaves (F)	During common cold & high fever leaves are laid down & slept on. Leaves can be seen in many bird nests, particularly flamingo & bulbul	4	None found	None found	None found	None found
Maesa indica (Roxb.) Wall. (Myrsinaceae) (WD) (69514) (N)	Kensametong	Leaves (F)	Leaf paste is applied topically to cuts & wounds (haemostatic)	3	None found	Fruits: to expel intestinal parasites (Jamir and Takatemjen 2010)	Whole plant: antiviral activity (Sindambiwe *et al.* 1998)	(Ahmad and Zaman 1973, Kuruvilla *et al.* 2010)
Manihot esculenta Crantz. (Euphorbiaceae) (CD) (69695) (I)	Alicha	Tubers (B)	Tubers are used with some rice & herbs to produce fermented beer & this is taken for	10	Tubers: fermentation process (Jansz and	Leaves: viral infections, antitumor (Yusuf *et al.* 2007,	Roots: toxic to humans due to cyanogenic glucosides; leaves: antitumor (Aregheore and Agunbiade 1991,	(Jansz and Uluwaduge 2012, Nuwamany

			gastrointestinal problems		Uluwaduge 2012)	Ohemu *et al.* 2014)	Yusuf *et al.* 2007, Jansz and Uluwaduge 2012)	a *et al.* 2014)
Melastoma malabathricum (Melastomataceae) (WD) (69652) (N)	Nemna	Fruits & leaves (F)	Fruits are edible & leaf paste is applied to cuts & wounds	3	WR Leaves: cuts & wounds (Joffry *et al.* 2011)	WR Leaves: dysentery, diarrhoea, piles, gastric ulcers (Joffry *et al.* 2011)	WR Leaves, stems & flowers: antibacterial against *S. aureus* (Joffry *et al.* 2011, Dej-adisai *et al.* 2014)	WR (Malek *et al.* 2003, Joffry *et al.* 2011, Arya *et al.* 2014)
Melia composite Willd. (Meliaceae) (WD) (69669) (N)	Aiet	Fruits & leaves (F)	Fruits/leaves are consumed to expel gas from stomach	2	None found	Whole plant: anthelmintic, diuretic (Sutha *et al.* 2014)	Leaves: antibacterial against *E. aerogenes, S. flexneri* (Sutha *et al.* 2014)	None found
Mentha cordifolia Opiz ex Fresen. (Lamiaceae) (CD) (69683) (I)	Pudina	Leaves (F)	Leaf paste is applied topically to fresh cuts & skin diseases. Crushed leaves are inhaled for nostril decongestion	10	None found	None found	Leaves: antimicrobial against *B. subtilis, P. aeruginosa, C. albicans, A. niger* (Ragasa *et al.* 2001, Saranraj and Sivasakthi 2014)	(Ragasa *et al.* 2001)
Mikania cordata (Burm. F.) B. L. Rob. (Asteraceae) (WD) (69534) (I)	Indialeelang	Leaves & stem (B)	Leaf/stem paste is inserted into the rectum for about 5 minutes to treat dysentery/diarrhoea & piles. Leaf/stem paste is applied topically to treat skin diseases & cuts. Dried powdered leaves are also used with other plants	10	WR Leaves: cuts & wounds (Uddin and Hassan 2014)	WR Leaves: blood coagulation, jaundice, snake bite (Nayeem *et al.* 2011, Shil *et al.* 2014)	WR Leaves: antibacterial against *S. aureus, S. typhi, S. sonnei, S. pyogenes, P. aeruginosa,* antidiarrhoeal (Hamill *et al.* 2003, Salgado *et al.* 2005, Nayeem *et al.* 2011)	WR (Nayeem *et al.* 2011, Chetia *et al.* 2014)

Millettia cinerea Benth. (Fabaceae) (WD) (69666) (E)	Suli	Roots & vines (F)	Roots are crushed into stream or creek to poison fish. Vine extract is used in massages to relieve body pain	10	Leaves: To ease back & leg discomfort (Tao 2001)	None found	None found	None found
Mimosa pudica Linn. (Mimosaceae) (WD) (69526) (I)	Amidangku-ayaklawa	Leaves (F)	Leaf paste is applied topically to treat inflammation & decoction is taken orally for gastrointestinal problems	8	WR Leaves: dysentery, diarrhoea, ulcer, piles swelling (Joseph *et al.* 2013, Islam *et al.* 2014)	WR Leaves: cuts & wounds; roots: bilious fevers, leprosy, urinary infections (Morvin *et al.* 2014, Pazyar *et al.* 2014)	WR Shoots: wound healing; leaves: antibacterial against *K. pneumoniae*, analgesic, anti-inflammatory, antidiarrhoeal, antiulcer; roots: antifertility (Gandhiraja *et al.* 2009, Joseph *et al.* 2013, Mishra *et al.* 2013)	WR (Zhang *et al.* 2011, Ahmad *et al.* 2012)
Mirabilis jalapa Linn. (Nyctaginaceae) (CD) (69609) (I)	Chumdangnaro	Leaves & roots (F)	Decoction of leaves/roots is taken orally as a diuretic & to treat ear ache	4	WR Roots: diuretic (Lim 2014)	WR Roots: purgative, scabies, muscular swelling; leaves: boils, abscesses (Lim 2014)	WR Flowers: spasmolytic/ spasmodic; leaves: antinociceptive, anti-inflammatory, abortifacient (Lim 2014)	WR (Lim 2014)
Mussaenda roxburghii Hk. f. (Rubiaceae) (WD) (69502) (E)	Andipeernaro	Leaves (B)	Fresh leaf paste is applied topically to cuts & wounds. Leaves are also used in combination with other herbs to brew rice beer	10	WR Leaves: boils, to brew rice beer (Deori *et al.* 2007, Rahman 2010)	WR Bark: diarrhoea (Shil *et al.* 2014)	WR Leaves: antimicrobial against *B. cereus, B. subtilis, S. aureus, S. lutea, E. coli, P. aeruginosa, S. typhi, S. paratyphi, S. boydii, S. dysenteriae, V. mimicus, V. parahaemolyticus*, cytotoxic (Roy and Saraf 2006, Vidyalakshmi *et*	WR (Roy and Saraf 2006, De Utpal *et al.* 2012)

							al. 2008, Hossain *et al.* 2013)	
Myrica esculenta Buch.-Ham., ex D. Don (Myricaceae) (CD) (69522) (N)	Mediong	Fruits & leaves (F)	Fruits are edible & leaf paste is applied to cuts & wounds	10	Sap: cuts & wounds (Manandhar 1995)	Fruits, bark: cough, asthma, dysentery, diarrhoea (Singh *et al.* 2014)	Fruits: antioxidant, antipyretic; stem bark: antimicrobial against *B. subtilis, S. aureus , S. epidermidis, E. coli, P. aeruginosa, A. niger* (Agnihotri *et al.* 2012, Pant *et al.* 2014)	(Agnihotri *et al.* 2012, Pant *et al.* 2014)
Nephrolepis cordifolia (Davalliaceae) (WD) (69623) (C)	Seraenjen	Tubers (F)	Tubers are crushed & taken orally with water to treat hiccups & 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 & applied topically or a slice of tuber is rubbed into the affected areas for the treatment of skin infections	4	WR Bulbs & tubers: upset stomach & urinary problems; leaves: wounds (Samuel and Andrews 2010, Balendra and Ravi 2012)	WR Rhizomes: cough, rheumatism, chest congestion, nose blockage & loss of appetite (Kumari *et al.* 2011)	WR Fronds: antibacterial against *P. mirabilis, E. aerogenes, P. aeruginosa, S. typhimurium, E. coli, K. pneumoniae, B. subtilis, B. cereus, S. faecalis* (Rani *et al.* 2010)	WR (Chakrabort y and Verma 2005, Chan *et al.* 2006)
Nerium indicum, Mill. (Apocynaceae) (CD) (69674) (C)	Sharonnaro	Flowers (F)	Flower decoction is used to kill lice & insects	1	WR Flowers: insecticidal: (Hiremath *et al.* 1997)	WR Roots: snake bite (Khan *et al.* 2014)	WR Flowers: larviciadal (Raveen *et al.* 2014)	WR (Pandya *et al.* 2013, Dey and Chaudhuri 2014)

Ocimum basilicum Linn. (Lamiaceae) (CD) (69543) (N)	Nangperra	Whole plant (B)	Infusion is taken orally to treat stomach upset & gas formation. It is also added in bath to treat influenza	10	Aerial parts: stomach ache (Alanis *et al.* 2005)	WR Leaves: cough & cold (Kadirvelmurugan *et al.* 2014)	WR Seeds: antibacterial against *S. aureus, E. coli, S. typhi, B. subtilis*, antioxidant, cytotoxic; leaves: antiviral against influenza (Jadhav *et al.* 2014, Shirazi *et al.* 2014)	WR: (Shirazi *et al.* 2014)
Oroxylum indicum (Linn.) Vent. (Bignoniaceae) (WD) (69637) (N)	Ochamiliau	Bark (F)	Decoction is taken orally to treat dysentery & rheumatism	2	Roots: dysentery & rheumatism (Deka *et al.* 2013)	WR Roots: biliousness, fevers, bronchitis, intestinal worms, vomiting, asthma; fruits: stomachic, anthelmintic, expectorant, (Deka *et al.* 2013)	WR Stem bark, root bark: antibacterial against *S. aureus, E. coli, B. subtilis, S. dysenteriae*, anthelmintic, antiulcer; leaves: gastroprotective, anti-inflammatory, anticancer (Dev *et al.* 2010, Deka *et al.* 2013, Raghu *et al.* 2013)	WR (Deka *et al.* 2013)
Oxalis acetosella L. (Oxalidaceae) (WD) (69607) (C)	Waroetsu	Leaves (F)	Eaten raw as diuretic, vermifuge & to treat gas formation & dysentery	2	None found	None found	Whole plant: antioxidant, antibacterial against *B. subtilis, B. cereus, P. vulgaris, E. coli* (Chetia *et al.* 2014)	None found
Paederia foetida Linn. (Rubiaceae) (WD) (69694) (N)	Atsulelang	Stem (F)	Decoction is taken orally to treat intestinal problems	6	WR Leaves: intestinal problems, dysentery, diarrhoea: (Chanda *et al.* 2013)	WR Leaves: burns, gout, rheumatism (Chanda *et al.* 2013)	WR Whole plant: antidiarrhoeal, analgesic; leaves: hepatoprotective, anti-inflammatory, antitussive, antiulcer, anthelmintic (Chanda *et al.* 2013, Soni *et al.* 2013)	WR (Kumar *et al.* 2014)

Passiflora edulis Sim. (Passifloraceae) (CD) (69700) (I)	Entsulashi	Leaves (F)	Leaf decoction is taken orally once a day after meal to treat high blood pressure	8	WR Fruits: high blood pressure (Lim 2012, Lim 2012)	WR Leaves: cuts, wounds, dysentery, diarrhoea, insomnia (Lim 2012, Lim 2012)	WR Leaves: antioxidant, anti-inflammatory, wound healing; fruits: antihypertensive, anti-hyperglycaemic anticancer; rind: antihypertensive; leaves, stem, fruits: antibacterial against *S. aureus, B. subtilis, E. coli, P. aeruginosa, S. paratyphi, K. pneumoniae*; roots: antiviral against HSV-1 (Lim 2012, Lim 2012, Konta *et al.* 2014)	WR (Ingale and Hivrale 2010) (Lim 2012, Lim 2012)
Phyllanthus emblica, Linn. (Euphorbiaceae) (WD) (CD) (69671) (N)	Lher	Fruits (B)	Fresh or dried fruits are eaten raw or infusion or decoction is taken orally to treat cough, high blood pressure, bladder & kidney problems & also taken as a laxative	10	WR Fruits: maintains blood pressure, cough, sore throat (Bhatia *et al.* 2014) (Khuankaew *et al.* 2014)	WR Fruits: loss of appetite (Badhan Biswas *et al.* 2014)	WR Fruits: cardioprotective, anti-hyperglycaemic, antioxidant, kidney-protective, antimicrobial against *S. aureus, B. subtilis, B. cereus, E. coli, P. aeruginosa, S. typhi, C. albicans, A. niger* (Fatima *et al.* 2014, Seraj *et al.* 2014) (Liu *et al.* 2009, Tasanarong *et al.* 2014)	WR (Liu *et al.* 2009, Yang and Liu 2014)
Phyllanthus urinaria Linn. (Euphorbiaceae) (WD) (69696) (N)	Asularlir	Fruits & leaves (F)	Fruits or leaves are eaten raw to treat fever, kidney pain, jaundice, dysentery & gastrointestinal problems. They are	10	WR Leaves: dysentery, diarrhoea (Thapa *et al.* 2014)	WR Whole plant: malaria (Khuankaew *et al.* 2014)	WR Leaves & twigs: antiviral against enterovirus 71, coxsackie virus A16, leaves: anti-inflammatory,	WR (Lai *et al.* 2008, Hu *et al.* 2014, Sarin *et al.* 2014)

			also taken as a laxative				gastroprotective, antibacterial against *H. pylori* (Lai *et al.* 2008, Yeo *et al.* 2014)	
Physalis alkekengi L. (Solanaceae) (WD) (69647) (N)	Entsupilvu	Fruits (F)	Eaten raw or infusion is taken orally for kidney & bladder problems	5	WR Fruits: kidney stones, urinary ailments diuretic (Jarić *et al.* 2007, Joharchi 2014)	WR Fruits: jaundice (Joharchi 2014)	WR Aerial parts: antifungal against *C. albicans*; fruits: intestinal microflora balance (Torabzadeh and Panahi 2013, Li *et al.* 2014)	WR (Zhou *et al.* 2012, Fu and Jian-qin 2013, Li *et al.* 2014)
Piper betel (Piperaceae) (CD) (69697) (N)	Patiwa	Leaves (F)	Leaf paste is applied topically to cuts & wounds. Fresh leaves are chewed with lime, areca nut & tobacco to treat dental caries	10	WR Leaves: cuts & wounds (Latheef *et al.* 2014)	WR Leaves: nerve pain, joint pain, cough & oedema (Islam *et al.* 2014)	WR Leaves: analgesic, antimicrobial against *S. aureus, E. coli, S. typhi, S. dysenteriae*, anthelmintic (Akter *et al.* 2014, Venkateswarlu 2014)	WR (Nagori *et al.* 2011, Akter *et al.* 2014, Saxena *et al.* 2014, Venkateswarlu *et al.* 2014)
Plantago erosa Wall. (Plantaginaceae) (WD) (69625)	Sangnem	Leaves (F)	Boiled leaves are consumed as a laxative	4	None found	WR Leaves: wounds & boils (Sanglakpam *et al.* 2012)	WR Leaves: anti-inflammatory (Barua *et al.* 2011)	(Barua *et al.* 2011)
Polygonum hydropiper Linn. (Polygonaceae) (WD) (69641) (C)	Nikmeremlawa	Leaves (F)	Leaf paste is applied topically to treat fungal	4	WR Leaves & roots: eczema &	WR	WR Leaves: antibacterial against *S. aureus, B. subtilis, S. epidermidis,*	WR (Duraipandiyan *et al.* 2010,

			infections & skin infections		scabies (Rahmatullah *et al.* 2009)	Leaves: snake bite (Van Minh *et al.* 2014)	*E. coli, E. faecalis, P. aeruginosa, K. pneumoniae, P. vulgaris, Erwinia* sp; whole plant: antioxidant & anticholinesterase (Duraipandiyan *et al.* 2010, Ayaz *et al.* 2014)	Akhter *et al.* 2014, Ayaz *et al.* 2014)
Prunus persica (L.) Stokes (Rosaceae) (CD) (69503) (I)	Mokori	Leaves, roots & seeds (F)	Fresh roots are soaked in water overnight & taken orally to treat typhoid. Also used to treat skin related infections. Seed endosperms are consumed to treat dysentery/diarrhoea, & leaf extract is applied topically to treat skin diseases (acne)	10	Leaves: killing maggots, skin diseases, ear infections, cough, bronchitis (Pfoze *et al.* 2012)	WR Seeds: external parasites; leaves: laxative (Gilani *et al.* 2000, Bhatia *et al.* 2014)	WR Leaves, fruits: cardioprotective, spasmolytic, anti-inflammatory, antioxidant (Kono *et al.* 2013, Kruger *et al.* 2014)	WR (Kim *et al.* 2014, Kruger *et al.* 2014)
Psidium guajava Linn. (Myrtaceae) (CD) (69513) (I)	Monaim	Leaves (F)	Leaves are chewed & swallowed to treat constipation & dysentery/diarrhoea	10	WR Leaves: vomiting, diarrhea & dysentery (Pfoze *et al.* 2012)	WR Leaves: cough, pulmonary ailments (Alsarhan *et al.* 2014)	WR Leaves: antidiarrhoeal, antibacterial against *S. enteritidis*, *B. cereus*, hepatoprotective, analgesic, anti-inflammatory (Joseph and Priya 2011, Ravi and Divyashree 2014)	WR (Joseph and Priya 2011, Ravi and Divyashree 2014)
Punica granatum Linn. (Punicaceae) (CD) (69621) (N)	Jarem	Fruits (D) & leaves (B)	Leaf decoction is taken twice a day before meals to treat dysentery/diarrhoea	10	WR Leaves: dysentery, diarrhoea	WR Leaves: epilepsy (Sharma *et al.* 2013)	WR Leaves: infectious diarrhoea (Birdi *et al.* 2010)	WR (Prakash and Prakash 2011,

			. Dried fruit cover is also used for the same purpose		(Joseph and Priya 2011)			Rummun *et al.* 2013, Rathi *et al.* 2014)
Rhus javanica var. *roxburghiana* (Anacardiaceae) (WD) (69603) (N)	Tangma	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 & allergies (rash)	10	WR Fruits: dysentery (Mahato and Chaudhary 2005)	WR Fruits: swelling & wounds (Malla *et al.* 2014)	WR Whole plant: antibacterial against MRSA; fruits: antidiarrhoeal; roots: chronic heart diseases; stem bark: anti-inflammatory (Kumar *et al.* 2010, Su and Kuo 2012, Cho *et al.* 2013, You *et al.* 2013, Yang *et al.* 2014)	WR (Rayne and Mazza 2007, Cho *et al.* 2013)
Rhus roxburghii Hook. f. (Anacardiaceae) (WD) (69673) (N)	Jarak	Whole plant (F)	Causes contact dermatitis	10	None found	None found	None found	None found
Ricinus communis Linn. (Euphorbiaceae) (WD) (CD) (69516) (N)	Pakawa	Leaves & seeds (F)	A quarter of a roasted seed is chewed & swallowed as a laxative. Caution - high dose causes extreme diarrhoea (not advisable for children). Leaves are used for rearing silk worms	10	WR Seeds: laxative (Preeti and Verma 2014)	WR Seeds: constipation (Belayneh and Bussa 2014)	WR Leaves: anti-inflammatory, antibacterial against *S. aureus, P. aeruginosa, K. pneumoniae, B. subtilis,* insecticidal, cytotoxic; seeds: analgesic, antiulcer; stem: antioxidant (Preeti and Verma 2014)	WR (Preeti and Verma 2014)
Saccharum officinarum Linn. (Poaceae) (CD) (69622) (N)	Mostutong	Culms (F)	Juice is taken orally twice a day to treat jaundice	10	WR Stem: jaundice (Nath and	WR Leaves: women ailments: leucorrhoea	WR Leaves: antibacterial against *S. aureus, B. subtilis, P. aeruginosa,*	WR (Abbas *et al.* 2014)

					Choudhury 2010, Abbas *et al.* 2014)	(Ghildiyal *et al.* 2014)	*P. mirabilis*; flowers: antioxidant (Palaksha *et al.* 2013, Thangavelu *et al.* 2014)	
Scutellaria glandulosa Colebr. (Lamiaceae) (WD) (69542) (N)	Yimramoli	Whole plant (B)	Plant infusion is taken orally to treat stomach upset & gas formation. Infusion is also used in bath to treat influenza	10	None found	None found	None found	None found
Solanum indicum Linn. (Solanaceae) (CD) (69627) (N)	Ao longkok	Fruits & leaves (F)	Unripe fruits are roasted & consumed once or twice a day as anthelminthic (pinworm). Fresh leaves, rice & water are ground, dried, powdered & used as brewing cake for rice beer	10	None found	WR Roots: asthma (Savithramma *et al.* 2007)	WR Berries: analgesic, antipyretic, anthelmintic, anti-inflammatory, CNS depressant activity (Deb *et al.* 2014)	WR (Deb *et al.* 2014, Yin *et al.* 2014)
Solanum myriacanthum (Solanaceae) (WD) (69597) (C)	Atsu longkok	Seeds (F)	Fumes from the burning seeds are channelled into the affected area to treat tooth ache	10	WR Seeds, fruits: tooth ache (Kala 2005, Rajkumari *et al.* 2013)	WR Fruits: tonsillitis, body worms (Nanda *et al.* 2013)	Fruits: anthelmintic (Tandon *et al.* 2011, Yadav and Tangpu 2012)	(Yadav and Tangpu 2012)
Sonerila maculata Roxb. (Melastomaceae) (WD) (69509) (N)	Alichang	Leaves (F)	Leaf paste is applied topically to treat insect bites & inflammation	4	None found	None found	None found	None found

Spermacoce scaberrima Blume (Rubiaceae) (WD) (69643) (N)	Ongpangentilawa	Leaves (F)	Leaf paste is applied immediately to snake bite	4	None found	None found	None found	None found
Spermacoce poaya Linn. (Rubiaceae) (WD) (69698) (N)	Intifada	Leaves (B)	Fresh leaves are chewed & swallowed as a laxative. Dried leaf paste pounded with rice grains is used for brewing rice beer	4	None found	None found	None found	None found
Spilanthes acmella Linn. (Asteraceae) (WD) (69639) (I)	Okensencha	Flowers (F)	Flowers are chewed 2 to 4 times a day to treat tooth ache	6	WR Leaves, flowers: tooth ache (Prachayasittikul *et al.* 2013)	WR Leaves, flowers: rheumatism, fever, diuretic, flu, cough, rabies, tuberculosis, antimalarial (Prachayasittikul *et al.* 2013)	WR Flowers: antipyretic, anti-inflammatory; leaves: analgesic, antibacterial against *K. pneumoniae*; aerial parts: antibacterial against *S. pyogenes, S. mutans, P. gingivalis*, antioxidant, diuretic; roots: antimalarial (Prachayasittikul *et al.* 2013, Pavunraj *et al.* 2014)	WR (Prachayasittikul *et al.* 2013, Das 2014, Pavunraj *et al.* 2014)
Spondias pinnata (Linn. F.) Kurz. (Anacardiaceae) (WD) (69519) (N)	Pakho	Drupes & 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	10	WR Fruits: bilious dyspepsia (Bora *et al.* 2014)	WR Seeds: skin diseases, ringworm, abscess; stem bark: rheumatism, gonorrhoea, anti-tubercular (Akhter	WR Stem bark: ulcer-protective, antibacterial against *S. aureus, E. coli, B. subtilis, P. mirabilis*, anti-diarrhoeal (Bora *et al.* 2014)	WR (Akhter 2014, Bora *et al.* 2014, Khan 2014)

			liver tonic & appetiser			2014, Bora *et al.* 2014)		
Stereospermum chelonoides (Linn. f.) DC (Bignoniaceae) (WD) (69610) (N)	Sengpet	Stem (F)	Stem bark paste is applied to treat cuts & wounds & skin diseases. Stem bark paste is also used as an antiseptic & a decoction of stem bark is taken orally to treat allergies	7	WR Flowers: diabetic boils (Gunasekaran and Balasubramanian 2012)	WR Roots: oedema, blood disorders, bronchial asthma, vomiting, jaundice, rheumatism, paralysis (Sumanth *et al.* 2013)	WR Stem bark: antimicrobial against *S. aureus, B. subtilis, B. cereus, E. coli, P. aeruginosa, S. typhi, S. paratyphi, S. boydii, S. dysenteriae, V. mimicus, V. parahaemolyticus, C. albicans, A. niger* (Haque *et al.* 2007, Sumanth *et al.* 2013)	WR (Haque *et al.* 2006, Choudhury *et al.* 2011, Sumanth *et al.* 2013)
Stixis suaveolens (Roxb.) Pierre (Capparidaceae) (WD) (69525) (N)	Aiemaluv	Fruits, roots & seeds (F)	Root infusion is taken orally to treat spleen enlargement. Fruits & seeds are edible	10	None found	Leaves: epididymitis and orchitis (Zheng and Xing 2009)	None found	None found
Tagetes erecta, Linn. (Asteraceae) (CD) (69670) (I)	Pesunaro	Whole plant (F)	Plant infusion is taken orally to treat intestinal problems, rheumatic pain, boils, skin infection & sinusitis	1	WR Flowers: skin diseases like sores, burns, wounds, ulcers, eczema (Lim 2014)	WR Leaves: colic, diuretic, malaria (Lim 2014)	WR Leaves: antifungal against *C. albicans*, *S. aureus*, MRSA, *S. epidermidis*, wound healing; flowers: antinociceptive & anti-inflammatory activities (Chakraborthy 2009, Chomnawang *et al.* 2009, Chatterjee and Ali 2010, Kiranmai *et al.* 2011)	WR (Piccaglia *et al.* 1996, Gupta and Vasudeva 2010, Rhama and Madhavan 2011, Dasgupta *et al.* 2012, Lim 2014)

Terminalia chebula Retz. (Combretaceae) (WD) (CD) (69640) (N)	Ningkha	Drupes (B)	Drupes are eaten as a good source of vitamins & to treat stomach ache	10	WR Bark, fruits: gastritis, constipation, indigestion, ulcer, vomiting, diarrhoea (Thapa *et al.* 2014)	WR Fruits: cough (Khuankaew *et al.* 2014)	WR Whole plant: antiulcer, gastrointestinal motility (Rathinamoorthy and Thilagavathi 2014)	WR (Rathinamoorthy and Thilagavathi 2014)
Thunbergia grandiflora Roxb. (Acanthaceae) (WD) (69497) (N)	Koktsuli	Stem (F)	Fluid from stem is added to the eye to treat eye infection	3	WR Leaves, stem: eye problems (Hossan *et al.* 2014)	WR Leaves, stem: inflammation, cuts & wounds, astringent (Hossan *et al.* 2014)	WR Flowers: antibacterial against *S. aureus, E. coli, P. aeruginosa, K. pneumoniae, B. cereus, S. typhi, P. mirabilis, S. pyogenes* (Jeeva *et al.* 2011)	(Subramanian and Nair 1971, Ismail *et al.* 1996)
Tithonia diversifolia (Hemsl.) A. Gray (Asteraceae) (CD) (69687) (C)	Zoninaro	Leaves (F)	Infusion is taken orally to treat high blood pressure, malaria & leaf paste is applied topically to treat abscesses & body pain	1	WR Leaves: malaria, pain (Lifongo *et al.* 2014)	WR Leaves: hepatitis, diabetes (Lifongo *et al.* 2014)	WR Leaves: antimalarial: active against *P. falciparum*; aerial parts: anti-hyperglycaemic (Adebayo and Krettli 2011, Zhao *et al.* 2012)	WR (Zhao *et al.* 2012, Gu 2014, Lifongo *et al.* 2014)
Urtica dioica L. (Urticaceae) (WD) (69601) (N)	Zaklutasula	Leaves (F)	Leaf paste is applied topically to treat dog & snake bites	10	None found	WR Leaves, whole plant: cancer, anti-rheumatic, diabetes, stomachic, cough, colds, throat diseases, oedema, sedative, laxative,	WR Leaves: anti-inflammatory, antibacterial against *S. aureus, E. coli, P. aeruginosa, S. typhi, S. flexneri, E. faecalis, K. pneumoniae*, anti-nociceptive (Sabzar *et al.*	WR (Sabzar *et al.* 2012)

						asthma, hypertension, kidney stones (Altundag and Ozturk 2011)	2012, Hajhashemi and Klooshani 2013)	
Viola betonicifolia Boj. Ex. baker (Violaceae) (WD) (69651) (N)	Hingpangmoli	Leaves (F)	Leaf paste is applied to draw out stings or thorns	4	None found	WR Whole plant: antipyretic, anticancer, febrifuge, purgative, epilepsy, nervous disorder (Alamgeer *et al.* 2013)	WR Whole plant: antipyretic, anti-analgesic, anti-inflammatory (Muhammad *et al.* 2012)	WR (Muhammad *et al.* 2012, Muhammad *et al.* 2013)
Wedelia chinensis (Osb.) Merrill (Asteraceae) (WD) (CD) (69693) (N)	Enze	Leaves (F)	Fresh leaves are eaten raw in the form of a salad to treat gastrointestinal problems	10	WR Leaves: gastrointestinal disorders (Mollik *et al.* 2010)	WR Leaves: anti-inflammatory, dermatological disorders (Mundepi *et al.* 2014)	WR Leaves: anti-ulcerogenic, anti-inflammatory, antibacterial against *S. aureus, E. coli, P. aeruginosa, S. pneumoniae* (Manjamalai *et al.* 2012, Nomani *et al.* 2013)	WR (Banu and Nagarajan 2013)
Zanthoxylum acanthopodium DC. (Rutaceae) (WD) (CD) (69600) (N)	Changpet	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 influenza. Leaf infusion is sprayed	8	WR Leaves: insecticides, insect repellent; seeds: stomach ache, fever (Gupta and Mandi 2013)	WR Leaves: fever, dyspepsia, cough, bronchitis; seeds: rheumatism (Ishwori *et al.* 2014)	WR Leaves: antibacterial against *S. aureus, P. aeruginosa, B. cereus, C. perfringens* (Ishwori *et al.* 2014)	WR (Negi *et al.* 2012)

			on pest infected plants as pesticide					
Zanthoxylum rhetsa (Roxb.) DC. (Rutaceae) (WD) (69689) (N)	Ongret	Seeds (D)	Seeds are crushed in the stream to poison fish. Leaf infusion is sprayed on pest infected plants as pesticide	10	Fruits: to poison fish (Yumnam and Tripathi 2013)	WR Stem: gastritis & diabetes (Tangjang *et al.* 2011)	WR Stem bark, roots: antibacterial against *S. aureus, E. coli, P. aeruginosa, S. typhimurium* (Nagaraja 2011, Tantapakul *et al.* 2012)	WR (Krohn *et al.* 2011, Tantapakul *et al.* 2012)

CD – Cultivated. WD – Wild. E - Himalayan endemic. N – Native. C – Cosmopolitan. I – Introduced. WR - widely reported. F – Fresh. D – Dry. B – Both. N I - number of informants. Bacteria: *B. cereus - Bacillus cereus, B. subtilis - Bacillus subtilis, C. perfringens - Clostridium perfringens, C. freundii – Citrobacter freundii, E. aerogenes - Enterobacter aerogenes, E. coli - Escherichia coli, E. tarda - Edwardsiella tarda, H. pyroli - Helicobacter pylori, K. pneumoniae - Klebsiella pneumoniae, Porphyromonas gingivalis – P. gingivalis, P. aeruginosa - Pseudomonas aeruginosa, P. mirabilis - Proteus mirabilis, P. vulgaris - Proteus vulgaris, S. choleraesuis - Salmonella choleraesuis, S. enteritidis - Salmonella enteritidis, S. paratyphi - Salmonella paratyphi, S. typhi - Salmonella typhi, S. typhimurium - Salmonella typhimurium, S. lutea - Sarcinia lutea, S. boydii - Shigella boydii, S. dysenteriae - Shigella dysenteriae, S. flexneri - Shigella flexneri, S. sonnei - Shigella sonnei, S. aureus - Staphylococcus aureus,* MRSA – methicillin resistant *S. aureus*, MDRSA – multi drug resistant *S. aureus, S. epidermidis - Staphylococcus epidermidis, S. haemolyticus - Staphylococcus haemolyticus, S. mutans - Streptococcus mutans, S. pneumoniae – Streptacoccus pneumoniae, S. pyogenes - Streptococcus pyogenes, S. viridans - Streptococcus viridans, V. cholerae - Vibrio cholera, V. mimicus - Vibrio mimicus, V. parahaemolyticus - Vibrio parahaemolyticus*. Parasite: *P. falciparum - Plasmodium falciparum*. Fungi: *A. niger - Aspergillus niger, C. albicans - Candida albicans.*

The literature review presented above on the 135 plants used by Chungtia villagers (Kichu 2010) compared reported traditional usages of the plants by communities worldwide with the Chungtia applications. Ninety three species were found to be utilised for the same ethnomedicinal or ethnobotanical purposes by other communities and 31 were found to be used for different purposes. Thirteen had no ethnomedicinal or ethnobotanical uses reported by any community other than the first-hand reports by the Chungtia villagers. Out of the ninety three 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 thirteen species exclusively reported for their usages by Chungtia villagers, only three have been validated by pharmacological research and no bioactive compounds responsible for the activities have been determined. Detailed analysis of the literature search results are presented below.

2.4 Results and discussion

2.4.1 Informants and data collection

Over a period of two years (2007-2009), Kichu made two trips to Chungtia village to conduct first-hand interviews and for collection of voucher specimens. During the first trip, ten village elders, including two herbal practitioners were interviewed. 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. This could be attributed to the facts that several key custodians of that knowledge have died before passing it on, the younger generation are using more Western medicines and the villager's general wellbeing is improving due to modernisation of their lifestyle. The lack of awareness on the importance of traditional knowledge preservation was noted as a concern by the village Elders (Kichu 2010).

During the second trip, follow up interviews were conducted with two herbal practitioners and one village Elder (Kichu 2010).

2.4.2 Medicinal plants recorded, their habit and habitat

In the study conducted by Kichu, a total of 135 plant species belonging to sixty nine families and 123 genera were identified as having medicinal and household maintenance value. The most often cited were plants belonging to the Asteraceae family with nine

species used, followed by the Euphorbiaceae family with seven species and Solanaceae with six species (Kichu 2010). Of all the plants, sixty eight were woody (tree, shrub, woody vine or liana) and sixty seven were herbs or herbaceous vines. Seventy eight were collected in the wild, forty seven were cultivated and ten were collected as both, wild and cultivated (Kichu 2010).

2.4.3 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, followed by fruit, stem, roots, flowers, sap/latex and bulbs, with the whole plant least used (Kichu 2010). The most preferred modes of preparation were in the form of pastes, decoctions, infusions, juices and poultices. 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 most utilised followed by topical applications. Preparations in most cases were made from single plants, but some plant mixtures were also recorded (Kichu 2010).

2.4.4 Usage categories

Table 2.2 summarises the ailments and classifies them into categories according to Chungtia villagers' system of classification and the number of plants in each category. From the 135 plants recorded first-hand, 53 have been used for one ailment or ailments relative to one category and eighty two have been applied to treat various disorders classified under different categories. The most common uses of the plants are for gastrointestinal complaints, followed by skin infections, cuts, sores and wounds, then musculoskeletal problems.

Table 2.2 Usage grouped by category and number of plants used for the treatment of each (Kichu 2010).

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, 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	35
Musculoskeletal problems	Knee pain, body ache, body pain, back pain, body swelling, bone dislocation, sprain, rheumatism, inflammation	16
Influenza/cold/fever	Influenza, 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 decongestion, eye infection, ear pain and infection (otitis media), ear ache,	11
Others	To ward off evil spirits, tempering machete (hardening metal), food preservative, ripening process, dyes, armpit deodorisation	9
Fish poisoning	Fish poisoning	7
Beer fermentation	Beer fermentation	4
Dental disorders	Tooth ache, dental caries	4
Pesticides/insecticides	pesticide and insecticide, head lice	4
Diabetes	Diabetes	3
Malaria	Malaria	3
Cancer	Cancer	1

2.4.5 Comparing traditional usages

The uses of plant species described by Chungtia community members were compared with several studies related to the traditional ethnomedicinal and ethnobotanical knowledge worldwide.

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, 123 species have been additionally found to have different ethnomedicinal applications reported worldwide and twelve species (*A. trilobata, A. lucidor, C. amara, M. undulatus, M.*

cordifolia, O. acetosella, R. roxburghii, S. glandulosa, S. maculata, S. scabberima, S. poaya and *S. suaveolens*) were found to be exclusively applied by Chungtia community members, not being mentioned in any other customary knowledge study.

Interestingly, *B. alba*, *C. papaya* and *C. roseus*, which are used by the Chungtia community for their laxative effects were reported by other communities as antidiarrhoeal, and *P. persica* used as an antidiarrhoeal by Chungtia villagers, was reported by others as a laxative. *P. persica* leaves contains 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.*

2.4.6 Comparing pharmacological and phytochemical studies with Chungtia community traditional applications

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

The most common pharmacological properties reported with relevance to gastrointestinal complaints was antibacterial activity against bacteria known to cause diarrhoea and stomach cramps (*E. aerogenes*, *S. sonnei, S. flexneri*, *E. coli* and *S. boydii*) (Murray *et al.* 2013); dysentery (*S. dysenteriae)* and cholera (*V. cholerae*) (Murray *et al.* 2013); gastroenteritis (*E. tarda, V. parahaemolyticus, V. mimicus*) (Murray *et al.* 2013); food poisoning (*S. choleraesuis, S. typhimurium, S. enteritidis, B. cereus, B. subtilis, C. perfringens, Erwinia* spp) (Murray *et al.* 2013); enteric fever (*S. typhi, S. paratyphi*); and peptic ulcers (*H. pylori*). Thirty one of the forty four plants were reported as possessing antibacterial activity against at least one of the bacterial species listed above. Moreover, of these plants, pharmacological studies identified antidiarrhoeal properties for *A. scholaris*, *A. gangeticus*, *C. annum, M. cordata, M. pudica, P. foetida, P. guajava* and *P. granatum*; anti-ulcer properties for *C. asiatica*, *C. pareira, C. macrocarpa, M. pudica*, *O. indicum*, *P. foetida, S. pinnata T. chebula* and *W. chinensis*; hepatoprotective properties for *B.*

variegata, *L. acutangula*, *P. foetida* and *P. guajava*; gastroprotective properties for *C. macrocarpa*, *O. indicum* and *P. urinaria*; anthelmintic properties for *B. variegata*, *C. papaya*, *E. foetidum*, *O. indicum* and *P. foetida*; and 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 twenty nine plants were reported for antimicrobial properties against at least one of the dermatologically relevant pathogens such as the bacteria *S. aureus, S. pyogenes, S. epidermidis, S. lutea, S. haemolyticus* and *P. aeruginosa* and the fungi *C. albicans* and *A. niger* (Murray *et al.* 2013). Additionally, *A. scholaris*, *A. curassavica*, *C. indicum*, *D. cordata*, *E. royleana*, *G. arborea*, *L. siceraria*, *M. pudica*, *P. persica* and *T. erecta* have been reported for their anti-inflammatory activity; *A. chinense, C. floribunda, C. cristata, D. sinuata*, *F. elastica*, *L. spinosa*, *M. esculenta*, *P. hydropiper* and *P. persica* have been found to be antioxidant; and *A. heterophyllus*, *D. 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 twenty species.

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

Influenza, colds and fever: For the plants used for influenza, colds and fever, *A. calamus* and *N. cordifilia* were found to be antibacterially active against *S. pyogenes* (causes throat infections) and *K. pneumoniae* (causes lung and lower respiratory tract infections). *A. calamus*, *A. vasica*, *E. blanda*, *O. basilicum* and *C. cajan* were found to be antiviral and *A.*

vasica and *D. cordata* antipyretic. *B. tulda, M. undulatus* and *S. glandulosa* have not been reported for pharmacological or phytochemical studies.

Hypertension: Ten of the plants were reported for possessing relevant biological activities for use in treatment of hypertension. Those included: cardioprotective (*A. chinense, A. carambola, C. annum, C. pareira, C. repens* and *R. javanica*), anti-hyperglycaemic (*A. sativum, P. eludis, P. emblica* and *T. diversifolia*), and anti-hypertensive (*A. sativum, C. colebrookianum, P. eludis* and *P. emblica*). All of these plants have also been shown to have compounds with pharmacological properties aligned with their medicinal uses.

Urinary tract infections and kidneys and bladder problems: Out of the twelve plants used for urinary, kidney or bladder problems, only three were found to be validated by pharmacological and phytochemical research. *G. herbaceum* was found to be diuretic, *H. scandens* was found to be active against *P. 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 *S. scabberima* were not reported for pharmacological or phytochemical studies, while *A. chinense*, *A. sativum*, *D. lablab* and *H. scandens* have been found to be antimicrobial against *S. aureus, P. aeruginosa, E. coli* and *S, epidermidis* and *U. 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, E. globulus, M. cordifolia, N. cordifolia, T. erecta* and *T. grandiflora* have been shown to be antibacterial; and *D. cordata, M. jalapa* and *T. erecta* are anti-nociceptive.

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

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

Four species (*Z. rhetsa, Z. acanthopodium, N. indicum* and *E. 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.

Three plants (*G. crepidioides*, *D. longifolia* and *C. pareira*) are used by the Chungtia community as a treatment for diabetes. *C. pareira* has been found to possess antidiabetic properties in pharmacological research; however, the compounds responsible for the antidiabetic properties have not been isolated.

Of the three plants (*T. diversifolia*, *C. pareira* and *A. 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 *P. 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.

2.4.7 Toxicity

Chungtia village healers indicated five plants that might cause toxic or adverse effects such as body swelling and vomiting, contact dermatitis, animal and even human death. The literature search of all the 135 plants highlighted a total of fifteen plants that could have adverse effects, but literature reports confirmed toxic effects have been reported for only two of the Chungtia reported plants, namely *Ricinus communis* and *Euphorbia pulcherrima*. In the case of *R. communis* the plant have been reported to cause diarrhoea. It was confirmed in the literature that poisoning might occur when broken seeds are ingested. Unbroken seeds will pass through the digestive system without expelling its toxin (ricin) and thus will not cause unwanted effects. *E. pulcherrima,* which has been reported by Chungtia Elders as poisonous when fed to pigs, has also been reported for its toxicity to ruminants. For the thirteen remaining plants, excluding these two confirmed cases, the

toxicity reports concerned plant parts different to these used by Chungtians. It might be that the plant parts applied by Chungtia villagers have no toxic effects, but caution with the usage of them is warranted. Toxicity reported by Chungtia community members' and that in the literature is summarised in Table 2.3.

Table 2.3 Literature study of the plants surveyed having toxic/adverse effects

Scientific name	Plant part	Toxic/adverse effects reported by Chungtia community (Kichu 2010)	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/oxytocic	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 & 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 & vomiting	No reports	No reports	No reports	No reports
Costus speciosus	Fruits & 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 & leaves	Death may result when fed accidently to pigs	Poisonous for animals	Aerial parts	Eaten fresh	(Cortinovis and Caloni 2013)
Gonatanthus pumilus	Leaves & stem	Extremely poisonous & death may result if ingested by humans. Rhizome also causes extreme itching when contacted	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 & lotaustralin	(Rivadeneyra-Domínguez *et al.* 2013)
Mimosa pudica	Leaves	No reports	Anti-fertility/gonadotropin & estradiol secretion	roots	Aqueous roots extract	(Joseph *et al.* 2013)
Mirabilis jalapa	Leaves & 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 & leaves	High doses causes extreme diarrhoea	Bloody diarrhoea	Seeds	Ricin	(Fahim Kadir *et al.* 2014)

2.5 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 and on their reported biological properties and bioactive constituents. Sixty eight different applications by the Chungtia villagers were identified, which were classified into sixteen categories. Ninety thee plants' traditional usages by the Chungtia community were found to be in agreement with other community reports. Additionally, 31 plants were reported to be used differently by other communities. Thirteen plants 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 have been investigated for their biological properties. Out of the ninety three 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.

Traditional healers and Elders of Chungtia village possess significant knowledge of medicinal plants and their application for the treatments of many ailments. It has been proven in pharmacological studies that many traditionally used medicinal plants of the villagers possess important therapeutic value. These findings validate their traditional medicinal usage by Chungtia villagers and highlight the significance of the traditional knowledge of the Chungtia Elders.

Abbas, S. R., S. M. Sabir, S. D. Ahmad, A. A. Boligon and M. L. Athayde (2014). "Phenolic Profile, Antioxidant Potential and DNA Damage Protecting Activity of Sugarcane (*Saccharum officinarum*)." *Food Chemistry* **147**: 10-16.

Abid, M. (2005). Phytochemical and Pharmacological Evaluation of Fruit Extracts of *Luffa acutangula* Roxb. Var. Amara. (*Cucurbitaceae*), Rajiv Gandhi University of Health Sciences.

Abraham, J. and T. D. Thomas (2012). "Antibacterial Activity of Medicinal Plant *Cyclea peltata* (Lam) Hooks & Thoms." *Asian Pacific Journal of Tropical Disease* **2**(2): 280-284.

Acharya, N. S., S. R. Acharya, M. B. Shah and D. D. Santani (2012). "Development of Pharmacognostical Parameters and Estimation of β-Sitosterol Using Hptlc in Roots of *Gmelina arborea* Roxb." *Pharmacognosy Journal* **4**(30): 1-9.

Adebayo, J. and A. Krettli (2011). "Potential Antimalarials from Nigerian Plants: A Review." *Journal of Ethnopharmacology* **133**(2): 289-302.

Adhikari, B. S., M. M. Babu, P. L. Saklani and G. S. Rawat (2007). "Distribution, Use Pattern and Prospects for Conservation of Medicinal Shrubs in Uttaranchal State, India." *Journal of Mountain Science* **4**(2): 155-180.

Adhikari, R., H. Naveen Kumar and S. Shruthi (2012). "A Review on Medicinal Importance of *Basella alba* L." *International Journal of Pharmaceutical Sciences and Drug Research* **4**(2): 110-114.

Adnan, M., I. Ullah, A. Tariq, W. Murad, A. Azizullah, A. Khan and N. Ali (2014). "Ethnomedicine Use in the War Affected Region of North-West Pakistan." *Journal of Ethnobiology and Ethnomedicine* **10**: 16.

Adsul, Y. D., R. T. Mahajan and S. B. Badgujar (2013). "Ethnobotanical Euphorbian Plants of North Maharashtra Region." *Journal of Pharmacy and Biological Sciences* **7**(1): 29-35.

Agnihotri, S., S. Wakode and M. Ali (2012). "Essential Oil of *Myrica esculenta* Buch. Ham.: Composition, Antimicrobial and Topical Anti-Inflammatory Activities." *Natural Product Research* **26**(23): 2266-2269.

Ahmad, H., S. Sehgal, A. Mishra and R. Gupta (2012). "*Mimosa pudica* L. (Laajvanti): An Overview." *Pharmacognosy Reviews* **6**(12): 115-124.

Ahmad, S. A. and A. Zaman (1973). "Chemical Constituents of *Glochidion venulatum*, *Maesa indica* and *Rhamnus triquetra*." *Phytochemistry* **12**(7): 1826.

Ahmadizadeh, C., A. Monadi, A. Rezaie, M. Pashazadeh and B. Jafari (2014). "Antibacterial Activity of Methanolic Extract and Essence of Sagebrush (*Artemisia vulgaris*) against Pathogenic Bacteria." *Bulletin of Environment, Pharmacology and Life Sciences* **3**(2): 121-125.

Ahmed, D., V. Kumar, M. Sharma and A. Verma (2014). "Target Guided Isolation, *in vitro* Antidiabetic, Antioxidant Activity and Molecular Docking Studies of Some Flavonoids from *Albizzia lebbeck* Benth. Bark." *BMC Complementary and Alternative Medicine* **14**: 155.

Ahmed, N., A. Mahmood, A. Mahmood, S. S. Tahir, A. Bano, R. N. Malik, S. Hassan and M. Ishtiaq (2014). "Relative Importance of Indigenous Medicinal Plants from Layyah District, Punjab Province, Pakistan." *Journal of Ethnopharmacology* **155**(1): 509–523.

Ahmed, T., M. N. Uddin, M. K. Hossain, N. Hasan and M. S. Rana (2013). "Evaluation of Antioxidant and Cytotoxic Potential of *Artocarpus chama* Buch. Seeds Using *in vitro* Models." *International Journal of Pharmacy and Pharmaceutical Sciences* **5**(1): 283-289.

Akhter, R. (2014). Chemical and Biological Investigations on Chloroform Extract of *Spondias pinnata* Bark, Diss. East West University.

Akhter, R., M. A. Haque, M. A. Bhuiyan and M. Shahriar (2014). "*In vivo* Pharmacological Investigation of Leaf of *Polygonum hydropiper* (L.)." *Dhaka University Journal of Pharmaceutical Sciences* **12**(2): 145-149.

Akter, K. N., P. Karmakar, A. Das, S. N. Anonna, S. A. Shoma and M. M. Sattar (2014). "Evaluation of Antibacterial and Anthelmintic Activities with Total Phenolic Contents of *Piper betel* Leaves." *Avicenna Journal of Phytomedicine* **4**(5): 320-329.

Alam, S. B., N. G. Benyelles, M. E. A. Dib, N. Djabou, L. Tabti, J. Paolini, A. Muselli and J. Costa (2014). "Antifungal Activity of Essential Oils of Three Aromatic Plants from Western Algéria against Five Fungal Pathogens of Tomato (*Lycopersicon esculentum* Mill)." *Journal of Applied Botany and Food Quality* **87**: 56-61.

Alamgeer, T. A., M. Rashid, M. N. H. Malik and M. N. Mushtaq (2013). "Ethnomedicinal Survey of Plants of Valley Alladand Dehri, Tehsil Batkhela, District Malakand, Pakistan." *International Journal of Basic Medical Sciences and Pharmacy* **3**(1): 23-32.

Alanis, A., F. Calzada, J. Cervantes, J. Torres and G. Ceballos (2005). "Antibacterial Properties of Some Plants Used in Mexican Traditional Medicine for the Treatment of Gastrointestinal Disorders." *Journal of Ethnopharmacology* **100**(1): 153-157.

Almahyl, H. A., M. Rahmani, M. A. Sukari and A. M. Ali (2003). "Investigation on the Chemical Constituents of the Leaves of *Ficus elastica* Roxb. And Their Antimicrobial Activity." *Pertanika Journal of Science & Technology* **11**(1): 57-63.

Alsarhan, A., N. Sultana, A. Al-Khatib and M. R. A. Kadir (2014). "Review on Some Malaysian Traditional Medicinal Plants with Therapeutic Properties." *Journal of Basic and Applied Sciences* **10**(0): 149-159.

Altundag, E. and M. Ozturk (2011). "Ethnomedicinal Studies on the Plant Resources of East Anatolia, Turkey." *Procedia-Social and Behavioral Sciences* **19**(0): 756-777.

Amel, B. (2013). "Traditional Treatment of High Blood Pressure and Diabetes in Souk Ahras District." *Journal of Pharmacognosy and Phytotherapy* **5**(1): 12-20.

Amoroso, V. B., D. A. Antesa, D. P. Buenavista and F. P. Coritico (2014). "Antimicrobial, Antipyretic, and Anti-Inflammatory Activities of Selected Philippine Medicinal Pteridophytes." *Asian Journal of Biodiversity* **5**(1).

Amri, E. (2014). "The Role of Selected Plant Families with Dietary Ethnomedicinal Species Used as Anticancer." *Journal of Medicinal Plants Studies* **2**(1): 28-39.

Anisuzzaman, M., A. Rahman, M. Harun-Or-Rashid, A. Naderuzzaman and A. Islam (2007). "An Ethnobotanical Study of Madhupur, Tangail." *Journal of Applied Sciences Research* **3**(7): 519-530.

Aregheore, E. and O. Agunbiade (1991). "The Toxic Effects of Cassava (*Manihot esculenta* Grantz) Diets on Humans: A Review." *Veterinary and Human Toxicology* **33**(3): 274-275.

Arulmozhi, S., P. M. Mazumder, P. Ashok and S. Narayanan (2007). "Pharmacological Activities of *Alstonia scholaris* Linn. (*Apocynaceae*) - A Review." *Pharmacognosy Reviews* **1**(1): 163-170.

Arya, V., D. Kumar and M. Gautam (2014). "Phytopharmacological Review on Flowers: Source of Inspiration for Drug Discovery." *Biomedicine & Preventive Nutrition* **4**(1): 45-51.

Ashok, P. and K. Upadhyaya (2013). "Evaluation of Analgesic and Anti-Inflammatory Activities of Aerial Parts of *Artemisia vulgaris* L. In Experimental Animal Models." *Journal of Biologically Active Products from Nature* **3**(1): 101-105.

Asif, F., A. Z. Boby, N. Alam, M. Taraquzzaman, S. R. Chowdhury and M. A. Rashid (2014). "Canvassing of Thrombolytic, Cytotoxic, and Erythrocyte Membrane-Stabilizing Attributes in *in-vitro* Screening of *Gynocardia odorata*." *Journal of Analytical Science and Technology* **5**(1): 1-7.

Auamcharoen, W., A. Chandrapatya, A. Kijjoa, A. Silva and W. Herz (2009). "Chemical Constituents of *Duabanga grandiflora* (*Lythraceae*)." *Biochemical Systematics and Ecology* **37**(4): 535-537.

Auranwiwat, C., K. Trisuwan, A. Saiai, S. G. Pyne and T. Ritthiwigrom (2014). "Antibacterial Tetraoxygenated Xanthones from the Immature Fruits of *Garcinia cowa*." *Fitoterapia* **98**(0): 178-183.

Ayaz, M., M. Junaid, J. Ahmed, F. Ullah, A. Sadiq, S. Ahmad and M. Imran (2014). "Phenolic Contents, Antioxidant and Anticholinesterase Potentials of Crude Extract, Subsequent Fractions and Crude Saponins from *Polygonum hydropiper* L." *BMC Complementary and Alternative Medicine* **14**(1): 145.

Badhan Biswas, M., Y. Arafat, M. S. Hossain, M. Sakib-uz-Zaman, Z. Khatun and M. Rahmatullah (2014). "Ethnomedicinal Practices of a Village Folk Medicinal Practitioner in Faridpur District, Bangladesh." *American-Eurasian Journal of Sustainable Agriculture* **8**(1): 20-27.

Badmanaban, R. (2010). "Studies on Anthelmintic and Antimicrobial Activity of the Leaf Extracts of *Lagenaria siceraria* Mol." *Journal of Global Pharma Technology* **2**(4): 66-70.

Bah, A. A., F. Wang, Z. Huang, I. H. Shamsi, Q. Zhang, G. Jilani, S. Hussain, N. Hussain and E. Ali (2012). "Phyto-Characteristics, Cultivation and Medicinal Prospects of Chinese Jiaotou (*Allium chinense*)." *Interational Journal of Agriculture and Biology* **14**(4): 650-657.

Bahramsoltani, R., M. H. Farzaei and R. Rahimi (2014). "Medicinal Plants and Their Natural Components as Future Drugs for the Treatment of Burn Wounds: An Integrative Review." *Archives of Dermatological Research* **306**: 601-617.

Bajpai, S., R. Pathak and T. Hussain (2014). "Anti-Inflammatory Activity of Ethno-Botanical Plants Used as Traditional Medicine: A Review." *Research and Reviews: Journal of Pharmacognosy and Phytochemistry* **2**(1): 24-34.

Balendra, P. S. and U. Ravi (2012). "Ethno-Botanical Importance of Pteridophytes Used by the Tribe of Pachmarhi, Central India." *Journal of Medicinal Plants Research* **6**(1): 14-18.

Baliga, M. S., A. R. Shivashankara, R. Haniadka, J. Dsouza and H. P. Bhat (2011). "Phytochemistry, Nutritional and Pharmacological Properties of *Artocarpus heterophyllus* Lam (Jackfruit): A Review." *Food Research International* **44**(7): 1800-1811.

Bani, S., A. Kaul, B. Jaggi, K. Suri, O. Suri and O. Sharma (2000). "Anti-Inflammatory Activity of the Hydrosoluble Fraction of *Euphorbia royleana* Latex." *Fitoterapia* **71**(6): 655-662.

Banu, H. R. and N. Nagarajan (2013). "Gc-Ms Determination of Bioactive Components of *Wedelia chinensis* (Osbeck) Merrill." *Journal of Chemical & Pharmaceutical Research* **5**(4): 279-285.

Barua, C. C., S. K. Pal, J. D. Roy, B. Buragohain, A. Talukdar, A. G. Barua and P. Borah (2011). "Studies on the Anti-Inflammatory Properties of *Plantago erosa* Leaf Extract in Rodents." *Journal of Ethnopharmacology* **134**(1): 62-66.

Barukial, J. and J. Sarmah (2011). "Ethnomedicinal Plants Used by the People of Golaghat District, Assam, India." *International Journal of Medicinal and Aromatic Plants* **1**(3): 203-211.

Baskar, A.-A., M.-A. Alsaif and S. Ignacimuthu (2012). "*In vitro* Antioxidant and Antiproliferative Potential of Medicinal Plants Used in Traditional Indian Medicine to Treat Cancer." *Redox Report* **17**(4): 145-156.

Basumata, C., J. Kalita, P. Mohan and K. Baruah (2012). "Antidiabetic Effect of *Cissampelos pareira* Linn, (*Menispermaceae*) on Fructose-Aloxan Diabetic Rats." *International Journal of Integrative sciences, Innovation and Technology* **1**(2): 27-38.

Begum, D. and S. C. Nath (2000). "Ethnobotanical Review of Medicinal Plants Used for Skin Diseases and Related Problems in Northeastern India." *Journal of Herbs, Spices & Medicinal Plants* **7**(3): 55-93.

Belayneh, A. and N. F. Bussa (2014). "Ethnomedicinal Plants Used to Treat Human Ailments in the Prehistoric Place of Harla and Dengego Valleys, Eastern Ethiopia." *Journal of Ethnobiology and Ethnomedicine* **10**(1): 1-18.

Bhandari, S. R., S.-K. Park, Y.-C. Cho and Y.-S. Lee (2012). "Evaluation of Phytonutrients in Adlay (*Coix lacryma-jobi* L.) Seeds." *African Journal of Biotechnology* **11**(8): 1872-1878.

Bhandari, S. R., M. K. Yoon and J.-H. Kwak (2014). "Contents of Phytochemical Constituents and Antioxidant Activity of 19 Garlic (*Allium sativum* L.) Parental Lines and Cultivars." *Horticulture, Environment, and Biotechnology* **55**(2): 138-147.

Bhaskar, V., K. M. Profulla, B. Balakrishnan, N. Balakrishnan and B. Sangameswaran (2009). "Evaluation of the Anti-Fertility Activity of Stem Bark of *Crataeva nurvala* Buch-Hum." *African Journal of Biotechnology* **8**(22): 6453-6456.

Bhat, J. A., M. Kumar and R. W. Bussmann (2013). "Ecological Status and Traditional Knowledge of Medicinal Plants in Kedarnath Wildlife Sanctuary of Garhwal Himalaya India." *Journal of Ethnobiology and Ethnomedicine* **9**(1): 1-18.

Bhat, P., G. R. Hegde, G. Hegde and G. S. Mulgund (2014). "Ethnomedicinal Plants to Cure Skin Diseases — an Account of the Traditional Knowledge in the Coastal Parts of Central Western Ghats, Karnataka, India." *Journal of Ethnopharmacology* **151**(1): 493-502.

Bhatia, H., Y. P. Sharma, R. K. Manhas and K. Kumar (2014). "Ethnomedicinal Plants Used by the Villagers of District Udhampur, India." *Journal of Ethnopharmacology* **151**(2): 1005-1018.

Bhatt, D., R. Kumar, G. Joshi and L. Tewari (2013). "Indigenous Uses of Medicinal Plants by the Vanraji Tribes of Kumaun Himalaya, India." *Journal of Medicinal Plants Research* **7**(37): 2747-2754.

Bhattacharjee, A. and S. C. Shashidhara (2012). "Phytochemical and Ethno-Pharmacological Profile of *Crataeva nurvala* Buch-Hum (Varuna): A Review." *Asian Pacific Journal of Tropical Biomedicine* **2**(2): 1162-1168.

Bhavana, G., R. Chandrika and K. Saraswathi (2013). "Quantitative Determination of Secondary Compounds in Populations of *Eryngium foetidum* L. From India." *International Journal of Current Science* **9**: 24-28.

Birdi, T., P. Daswani, S. Brijesh, P. Tetali, A. Natu and N. Antia (2010). "Newer Insights into the Mechanism of Action of *Psidium guajava* L. Leaves in Infectious Diarrhoea." *BMC Complementary and Alternative Medicine* **10**(1): 33.

Biswas, A., M. Bari, M. Roy and S. Bhadra (2010). "Inherited Folk Pharmaceutical Knowledge of Tribal People in the Chittagong Hill Tracts, Bangladesh." *Indian Journal of Traditional Knowledge* **9**(1): 77-89.

Bopana, N. and S. Saxena (2008). "*Crataeva nurvala*: A Valuable Medicinal Plant." *Journal of Herbs, Spices & Medicinal Plants* **14**(1-2): 107-127.

Bora, N. S., B. B. Kakoti, B. Gogoi and A. K. Goswami (2014). "Ethno-Medicinal Claims, Phytochemistry and Pharmacology of *Spondias pinnata*: A Review." *International Journal of Pharmaceutical Sciences and Research* **5**(4): 1138-1145.

Borrelli, F., R. Capasso and A. A. Izzo (2007). "Garlic (*Allium sativum* L.): Adverse Effects and Drug Interactions in Humans." *Molecular Nutrition & Food Research* **51**(11): 1386-1397.

Boumba, V. A., A. Mitselou and T. Vougiouklakis (2004). "Fatal Poisoning from Ingestion of *Datura stramonium* Seeds." *Veterinary and Human Toxicology* **46**(2): 81-82.

Brahma, J., S. Chakravarty and P. Rethy (2014). "Qualitative Estimation of the Presence of Bioactive and Nutritional Compound in *Lasia spinosa*: An Important Vegetable Plant Used by the Bodos of Kokrajhar District." *International Journal of ChemTech Research* **6**(2): 1405-1412.

Brandelli, C. L. C., P. d. B. Vieira, A. J. Macedo and T. Tasca (2013). "Remarkable Anti-*Trichomonas vaginalis* Activity of Plants Traditionally Used by the Mbyá-Guarani Indigenous Group in Brazil." *BioMed Research International* **2013**: 7.

Bulut, G. and E. Tuzlaci (2013). "An Ethnobotanical Study of Medicinal Plants in Turgutlu (Manisa — Turkey)." *Journal of Ethnopharmacology* **149**(3): 633-647.

Bunalema, L., S. Obakiro, J. R. S. Tabuti and P. Waako (2014). "Knowledge on Plants Used Traditionally in the Treatment of Tuberculosis in Uganda." *Journal of Ethnopharmacology* **151**(2): 999-1004.

Cai, B., H. Zhang, D. Zhang, C. Cui and W. Li (2002). "Apoptosis-Inducing Activity of Extract from Chinese Herb, *Albizzia lucidior* I. Nielsen." *Chinese Journal of Cancer* **21**(4): 373-378.

Caloni, F., C. Cortinovis, M. Rivolta, S. Alonge and F. Davanzo (2013). "Plant Poisoning in Domestic Animals: Epidemiological Data from an Italian Survey (2000–2011)." *Veterinary Record* **172**(22): 580.

Chakraborthy, G. S. (2009). "Antinociceptive and Anti-Inflammatory Activities of *Tagetes erecta* Flowers in Animal Models." *Archives of Applied Science Research* **1**(1): 92-96.

Chakraborty, R. and P. Verma (2005). Identification and Evaluation of Antibacterial Phytochemicals of Fishbone Fern (*Nephrolepis cordifolia*). *Journal of Biotechnology* **118**: 74-75

Chan, X.-q., Y.-c. Su, X.-j. LI, Y.-q. LIN and D.-q. LU (2006). "Extraction and Purification Antibacterial Polysaccharide from *Nephrolepis cordifolia* [J]." *Journal of Zhangzhou Teachers College (Natural Science)* **4**.

Chanda, S., I. P. Sarethy, B. De and K. Singh (2013). "*Paederia foetida*—a Promising Ethno-Medicinal Tribal Plant of Northeastern India." *Journal of Forestry Research* **24**(4): 801-808.

Chandra, K., B. Nautiyal and M. Nautiyal (2013). "Ethno-Botanical Resources as Supplementary Foods and Less Known Wild Edible Fruits in District Rudraprayag, Uttarakhand, India." *Journal of Human Ecology* **42**(3): 259-271.

Chang, C.-W., W.-T. Chang, J.-C. Liao, Y.-J. Chiu, M.-T. Hsieh, W.-H. Peng and Y.-C. Lin (2012). "Analgesic and Anti-Inflammatory Activities of Methanol Extract of *Cissus repens* in Mice." *Evidence-Based Complementary and Alternative Medicine* **2012**: 10.

Chang, W.-L., M.-J. Su and S.-S. Lee (1997). "Bioactive Norlignan Glucosides from *Curculigo capitulata*." *Journal of Natural Products* **60**(2): 76-80.

Chatterjee, S. and A. S. Ali (2010). "Studies on the Antibacterial Activity of *Tagetes erecta* Linn Leaves." *Archives of Pharmacal Research* **2**(2): 360-361.

Chaudhary, A., U. Sharma, A. P. Vig, B. Singh and S. Arora (2014). "Free Radical Scavenging, Antiproliferative Activities and Profiling of Variations in the Level of Phytochemicals in Different Parts of Broccoli (*Brassica oleracea*)." *Food Chemistry* **148**(0): 373-380.

Chen, H., C. Zhou, Y. Lou, Z. Duan and Y. Zhao (2005). "Chemical Constituents from *Elsholtzia blanda*." *China Journal of Chinese Materia Medica* **30**(20): 1589-1591.

Chen, J., W. Wang, C. Shi, J. Wan, L. Deng and J. Fang (2014). "Determination of Four Volatile Compounds with Anti-Inflammatory Biological Activity in *Houttuynia cordata* Thunb. By Gas Chromatography and Gas Chromatography–Mass Spectrometry." *Analytical Letters* **47**(5): 730-741.

Cheong, M. W., Z. S. Chong, S. Q. Liu, W. Zhou, P. Curran and B. Yu (2012). "Characterisation of Calamansi (*Citrus microcarpa*). Part I: Volatiles, Aromatic Profiles and Phenolic Acids in the Peel." *Food Chemistry* **134**(2): 686-695.

Cheong, M. W., D. Zhu, J. Sng, S. Q. Liu, W. Zhou, P. Curran and B. Yu (2012). "Characterisation of Calamansi (*Citrus microcarpa*). Part Ii: Volatiles, Physicochemical Properties and Non-Volatiles in the Juice." *Food Chemistry* **134**(2): 696-703.

Chetia, J., S. Upadhyaya and D. Bora (2014). "Screening of Phytochemicals, Antioxidant and Antimicrobial Activity of Some Tea Garden Weeds." *International Journal of Pharmaceutical Sciences Review and Research* **26(1)**(33): 193-196.

Cho, J.-Y., K.-D. Lee, S.-Y. Park, W. C. Jeong, J.-H. Moon and K.-S. Ham (2013). "Isolation and Identification of α-Glucosidase Inhibitors from the Stem Bark of the Nutgall Tree (*Rhus javanica* Linné)." *Journal of the Korean Society for Applied Biological Chemistry* **56**(5): 547-552.

Choi, E.-M. and J.-K. Hwang (2005). "Screening of Indonesian Medicinal Plants for Inhibitor Activity on Nitric Oxide Production of Raw 264.7 Cells and Antioxidant Activity." *Fitoterapia* **76**(2): 194-203.

Chomnawang, M. T., S. Surassmo, K. Wongsariya and N. Bunyapraphatsara (2009). "Antibacterial Activity of Thai Medicinal Plants against Methicillin-Resistant *Staphylococcus aureus*." *Fitoterapia* **80**(2): 102-104.

Chothani, D. L. and N. Patel (2014). "Pharmacognostic and Physicochemical Evaluation on Fruits of *Gmelina arborea*." *PharmaTutor* **2**(7): 162-166.

Chothani, D. L., H. Vaghasiya and N. Pate (2011). "A Review on *Gmelia arborea* Roxb. (Gambhari): Traditional Uses, Phytochemical Constituents, Pharmacological Activity, Marketed Formulation." *Inventi Rapid: Ethnopharmacology* **4**(1): 55-61.

Choudhury, S., S. Datta, A. D. Talukdar and M. D. Choudhury (2011). "Phytochemistry of the Family Bignoniaceae-a Review." *Assam University Journal of Science and Technology* **7**(1): 145-150.

Chowdhury, T. (2014). "Virtual Screening of Compounds Derived from *Garcinia pedunculata* as an Inhibitor of Gamma Hemolysin Component a of *Staphylococcus aureus*." *Bangladesh Journal of Pharmacology* **9**(1): 67-71.

Cordell, G. A. (2002). "Natural Products in Drug Discovery – Creating a New Vision." *Phytochemistry Reviews* **1**(3): 261-273.

Cortinovis, C. and F. Caloni (2013). "Epidemiology of Intoxication of Domestic Animals by Plants in Europe." *The Veterinary Journal* **197**(2): 163-168.

Daduang, J., S. Daduang, P. Hongsprabhas and P. Boonsiri (2011). "High Phenolics and Antioxidants of Some Tropical Vegetables Related to Antibacterial and Anticancer Activities." *African Journal of Pharmacy and Pharmacology* **5**(5): 608-615.

Dangwal, L., A. Sharma, N. Kumar, C. Rana and U. Sharma (2010). "Ethnomedico Botany of Some Aquatic Angiospermea from North-West Himalaya." *Researcher* **2**(4): 49-54.

Das, J., Z. Datta, A. Saha, S. M. Nur, P. Barua, M. M. Rahman, K. Ahmed, M. Mostofa, R. Hossain and A. Mannan (2013). "A Comprehensive Study on Antioxidant, Antibacterial, Cytotoxic and Phytochemical Properties of *Averhoa carambola*." *International Journal of Bioassays* **2**(5): 803-807.

Das, M. (2014). Evaluation of the Laxative Effects of Methanolic Extract of *Spilanthes acmella*, East West University.

Das, S., P. Kumar and S. Basu (2012). "Phytoconstituents and Therapeutic Potentials of *Datura stramonium* Linn." *Journal of Drug Delivery and Therapeutics* **2**(3): 4-7.

Dasgupta, N., S. Ranjan, P. Saha, R. Jain, S. Malhotra and M. Mohamed Saleh (2012). "Antibacterial Activity of Leaf Extract of Mexican Marigold (*Tagetes erecta*) against Different Gram Positive and Gram Negative Bacterial Strains." *Journal of Pharmacy Research* **5**(8): 4201-4203.

Dash, M., J. K. Patra and P. P. Panda (2008). "Phytochemical and Antimicrobial Screening of Extracts of *Aquilaria agallocha* Roxb." *African Journal of Biotechnology* **7**(20): 3531-3534.

de Araújo-Júnior, J. X., M. S. de Oliveira, P. G. Aquino, M. S. Alexandre-Moreira and A. E. Sant'Ana (2012). A Phytochemical and Ethnopharmacological Review of the Genus *Erythrina*, InTech**:** 327-352.

de Boer, H. J., V. Lamxay and L. Björk (2012). "Comparing Medicinal Plant Knowledge Using Similarity Indices: A Case of the Brou, Saek and Kry in Lao Pdr." *Journal of Ethnopharmacology* **141**(1): 481-500.

de Mello, S. A., R. N. de Oliveira, D. Coutinho, S. Machado, O. Arias-Carrión, A. Nardi and A. Silva (2014). "Antidepressant-Like and Anxiolytic-Like Effects of Cannabidiol: A Chemical Compound of *Cannabis sativa*." *CNS & Neurological Disorders-Drug Targets* **13**(6): 953-960.

De Utpal, C., R. Ghosh, S. Chowdhury and B. Dinda (2012). "New Iridoid from Aerial Parts of *Mussaenda roxburghii*." *Natural Product Communications* **7**(1): 1-2.

Deb, P. K., R. Ghosh, R. Chakraverty, R. Debnath, L. Das and T. Bhakta (2014). "Phytochemical and Pharmacological Evaluation of Fruits of *Solanum indicum* Linn." *International Journal of Pharmaceutical Sciences Review & Research* **25**(2): 28-32.

Dej-adisai, S., I. Meechai, J. Puripattanavong and S. Kummee (2014). "Antityrosinase and Antimicrobial Activities from Thai Medicinal Plants." *Archives of Pharmacal Research* **37**(4): 473-483.

Deka, D., V. Kumar, C. Prasad, K. Kumar, B. Gogoi, L. Singh and R. Srivastava (2013). "*Oroxylum indicum* – a Medicinal Plant of North East India: An Overview of Its Nutritional, Remedial, and Prophylactic Properties." *Journal of Applied Pharmaceutical Science* **3**(1): 104-112.

Deori, C., S. S. Begum and A. Mao (2007). "Ethnobotany of Sujen, a Local Rice Beer of Deori Tribe of Assam." *Indian Journal of Traditional Knowledge* **6**(1): 121-125.

Desale, M., P. Bhamare, P. Sawant, S. Patil and S. Kamble (2013). "Medicinal Plants Used by the Rural People of Taluka Purandhar, District Pune, Maharashtra." *Indian Journal of Traditional Knowledge* **12**(2): 334-338.

Deshmukh, P. T., J. Fernandes, A. Atul and E. Toppo (2009). "Wound Healing Activity of *Calotropis gigantea* Root Bark in Rats." *Journal of Ethnopharmacology* **125**(1): 178-181.

Dev, L. R., M. Anurag and G. Rajiv (2010). "*Oroxylum indicum*: A Review." *Pharmacognosy Journal* **2**(9): 304-310.

Dey, A. and J. N. De (2012). "Ethnobotanical Survey of Purulia District, West Bengal, India for Medicinal Plants Used against Gastrointestinal Disorders." *Journal of Ethnopharmacology* **143**(1): 68-80.

Dey, P. and T. K. Chaudhuri (2014). "Pharmacological Aspects of *Nerium indicum* Mill: A Comprehensive Review." *Pharmacognosy Reviews* **8**(16): 156-162.

Dhalendra, G., P. Rathore, T. Satapathy and A. Roy (2014). "Pharmacognostical, Phytochemical and Pharmacological Study of *Calotropis procera*: A Review." *Research Journal of Pharmacy and Technology* **7**(3): 346-351.

Dharmawan, J., S. Kasapis, P. Sriramula, M. J. Lear and P. Curran (2008). "Evaluation of Aroma-Active Compounds in Pontianak Orange Peel Oil (*Citrus nobilis* Lour. Var. Microcarpa Hassk.) by Gas Chromatography− Olfactometry, Aroma Reconstitution, and Omission Test." *Journal of Agricultural and Food Chemistry* **57**(1): 239-244.

Dhuna, V., S. Kamboj Sukhdev, A. Kaur, K. Saxena Ajit, V. Bhide Shobhana, Shanmugavel and J. Singh (2007). "Characterization of a Lectin from *Gonatanthus pumilus* D. Don Having Anti-Proliferative Effect against Human Cancer Cell Lines." *Protein and Peptide Letters* **14**(1): 71-78.

Doley, B., P. Gajurel, P. Rethy and R. Buragohain (2014). "Uses of Trees as Medicine by the Ethnic Communities of Arunachal Pradesh, India." *Journal of Medicinal Plants Research* **8**(24): 857-863.

Dongmo, A. B., T. Miyamoto, K. Yoshikawa, S. Arihara and M.-A. Lacaille-Dubois (2007). "Flavonoids from *Acacia pennata* and Their Cyclooxygenase (Cox-1 and Cox-2) Inhibitory Activities." *Planta Medica* **73**: 1202-1207.

Duraipandiyan, V., F. Indwar and S. Ignacimuthu (2010). "Antimicrobial Activity of Confertifolin from *Polygonum hydropiper*." *Pharmaceutical Biology* **48**(2): 187-190.

Eke, O., A. Augustine and H. Ibrahim (2014). "Qualitative Analysis of Phytochemicals and Antibacterial Screening of Extracts of *Carica papaya* Fruits and Seeds." *International Journal of Modern Chemistry* **6**(1): 48-56.

El-Mahmood, A., J. Doughari and H. Kiman (2010). "*In vitro* Antimicrobial Activity of Crude Leaf and Stem Bark Extracts of *Gmelina arborea* (Roxb) against Some Pathogenic Species of *Enterobacteriaceae*." *African Journal of Pharmacy and Pharmacology* **4**(6): 355-361.

Elkington, B. G., P. Phiapalath, K. Sydara, V. Somsamouth, N. I. Goodsmith and D. D. Soejarto (2014). "Assessment of the Importance of Medicinal Plants among Communities around Khiat Ngong of Southern Laos." *Journal of Environmental Biology* **35**(4): 607-615.

Emmanuel, I. A. and O. Ganiyu (2013). "Comparative Chemical Compositions and Phytate/Minerals Molar Ratios of Melon (*Citrullus vulgaris*) and Squash (*Cucurbita pepo*) Seeds." *Research & Reviews: Journal of Food Science & Technology* **2**(1): 1-8.

Escobedo-López, D., C. A. Núñez-Colín and E. Espitia-Rangel (2014). "Adaptation of Cultivated Amaranth (Amaranthus Spp.) and Their Wild Relatives in Mexico." *Journal of Crop Improvement* **28**(2): 203-213.

Fabricant, D. S. and N. R. Farnsworth (2001). "The Value of Plants Used in Traditional Medicine for Drug Discovery." *Environmental Health Perspectives* **109**(1): 69-75.

Fahim Kadir, M., M. Shahdaat Bin Sayeed, N. Islam Setu, A. Mostafa and M. Mia (2014). "Ethnopharmacological Survey of Medicinal Plants Used by Traditional Health Practitioners in Thanchi, Bandarban Hill Tracts, Bangladesh." *Journal of Ethnopharmacology* **155**(1): 495–508.

Fatima, N., U. Pingali and R. Pilli (2014). "Evaluation of *Phyllanthus emblica* Extract on Cold Pressor Induced Cardiovascular Changes in Healthy Human Subjects." *Pharmacognosy Research* **6**(1): 29-35.

Fernandes, G. and J. Banu (2012). "Medicinal Properties of Plants from the Genus Cissus: A Review." *Journal of Medicinal Plants Research* **6**(16): 3080-3086.

Fongod, A., L. Ngoh and M. Veranso (2014). "Ethnobotany, Indigenous Knowledge and Unconscious Preservation of the Environment: An Evaluation of Indigenous Knowledge in South and Southwest Regions of Cameroon." *International Journal* **6**(1): 85-99.

Fu, X. and J. Jian-qin (2013). "Chemical Constituents in the Calyx of *Physalis alkekengi* Var. Franchetii." *Pharmaceutical and Clinical Research* **4**(1): 15.

Gandhiraja, N., S. Sriram, V. Meenaa, J. K. Srilakshmi, C. Sasikumar and R. Rajeswari (2009). "Phytochemical Screening and Antimicrobial Activity of the Plant Extracts of *Mimosa pudica* L. Against Selected Microbes." *Ethnobotanical Leaflets* **13**: 618-624.

Gangwal, A., S. Parmar and N. Sheth (2010). "Triterpenoid, Flavonoids and Sterols from *Lagenaria siceraria* Fruits." *Der Pharmacia Lettre* **2**(1): 307-317.

Gautam, G. K. (2013). "A Review on the Taxonomy, Ethnobotany, Chemistry and Pharmacology of *Solanum Lycopersicum* Linn." *International Journal of Chemistry and Pharmaceutical Sciences* **1**(8): 521-527

Ghafar, M., K. N. Prasad, K. K. Weng and A. Ismail (2010). "Flavonoid, Hesperidine, Total Phenolic Contents and Antioxidant Activities from Citrus Species." *African Journal of Biotechnology* **9**(3): 326-330.

Ghildiyal, J., P. Juyal and G. Sadana (2014). "Indigenous Uses of Plants in Different Women Ailments in Garhwal Region." *Indian Journal of Pharmaceutical and Biological Research* **2**(1): 39-44.

Ghisalberti, E. (2000). "*Lantana camara* L. (*Verbenaceae*)." *Fitoterapia* **71**(5): 467-486.

Ghosh, C. and A. Das (2011). "Some Useful and Poisonous Tea Garden Weeds from the Darjiling District of West Bengal, India." *East Himalayan Society for Spermatophyte Taxonomy* **5**(1): 91-114.

Giday, M., T. Teklehaymanot, A. Animut and Y. Mekonnen (2007). "Medicinal Plants of the Shinasha, Agew-Awi and Amhara Peoples in Northwest Ethiopia." *Journal of Ethnopharmacology* **110**(3): 516-525.

Gilani, A. H., N. Aziz, S. M. Ali and M. Saeed (2000). "Pharmacological Basis for the Use of Peach Leaves in Constipation." *Journal of Ethnopharmacology* **73**(1): 87-93.

Gohil, K. J., J. A. Patel and A. K. Gajjar (2010). "Pharmacological Review on *Centella asiatica*: A Potential Herbal Cure-All." *Indian Journal of Pharmaceutical Sciences* **72**(5): 546-556.

Goji, M., T. Gebre-Mariam, K. Asres, H. Lemma, N. Gemeda and K. Yirsaw (2006). "Screening of the Antimicrobial Activities of Some Plants Used Traditionally in Ethiopia for the Treatment of Skin Disorders." *Ethiopian Pharmaceutical Journal* **24**(2): 130-135.

Goshwami, D., M. M. Rahman, M. A. Muhit, M. S. Islam and M. Anasri (2013). "Antioxidant Property, Cytotoxicity and Antimicrobial Activity of *Lasia spinosa* Leaves." *Nepal Journal of Science and Technology* **13**(2): 215-218.

Grosvenor, P. W., A. Supriono and D. O. Gray (1995). "Medicinal Plants from Riau Province, Sumatra, Indonesia. Part 2: Antibacterial and Antifungal Activity." *Journal of Ethnopharmacology* **45**(2): 97-111.

Gu, J.-Q. (2014). Bioactive Constituents of *Hernandia ovigera* and *Tithonia diversifolia*, Diss. University of Illinois at Chicago, Health Sciences Center.

Gulfraz, M., M. Imran, S. Khadam, D. Ahmed, M. J. Asad, K. S. Abassi, M. Irfan and S. Mehmood (2014). "A Comparative Study of Antimicrobial and Antioxidant Activities of Garlic (*Allium sativum* L.) Extracts in Various Localities of Pakistan." *African Journal of Plant Science* **8**(6): 298-306.

Gunasekaran, M. and P. Balasubramanian (2012). "Ethnomedicinal Uses of Sthalavrikshas (Temple Trees) in Tamil Nadu, Southern India." *Ethnobotany Research & Applications* **10**: 253-268.

Guo, Z., Z. Liu, X. Wang, W. Liu, R. Jiang, R. Cheng and G. She (2012). "*Elsholtzia*: Phytochemistry and Biological Activities." *Chemistry Central Journal* **6**(1): 147.

Gupta, D. D. and S. S. Mandi (2013). "Species Specific Aflp Markers for Authentication of *Zanthoxylum acanthopodium* and *Zanthoxylum oxyphyllum*." *Journal of Medicinal Plants Studies* **1**(6): 1-9.

Gupta, G., A. More, R. Kumari, A. Kumar, S. Nabi and S. Tandan (2011). "Effect of *Entada pursaetha* Dc. Against Experimentally Induced Hepatotoxicity in Wistar Rats." *Haryana Veterinarian* **50**(4): 64-67.

Gupta, P. and N. Vasudeva (2010). "*In-vitro* Antiplasmodial and Antimicrobial Potential of *Tagetes erecta* Roots." *Pharmaceutical Biology* **48**(11): 1218-1223.

Gupta, R., N. Sharma and V. Dixit (1990). "Calotropin – A Novel Compound for Fertility Control." *Ancient Science of Life* **9**(4): 224-230.

Hajhashemi, V. and V. Klooshani (2013). "Antinociceptive and Anti-Inflammatory Effects of *Urtica dioica* Leaf Extract in Animal Models." *Avicenna Journal of Phytomedicine* **3**(2): 193-200.

Hamill, F., S. Apio, N. Mubiru, R. Bukenya-Ziraba, M. Mosango, O. Maganyi and D. Soejarto (2003). "Traditional Herbal Drugs of Southern Uganda, Ii: Literature Analysis and Antimicrobial Assays." *Journal of Ethnopharmacology* **84**(1): 57-78.

Haque, M. R., K. M. Rahman, C. M. Hasan and M. A. Rashid (2007). "Antimicrobial and Cytotoxic Activities of *Stereospermum chelonoides*." *Dhaka University Journal of Pharmaceutical Sciences* **5**(1): 71-72.

Haque, M. R., K. M. Rahman, M. N. Iskander, C. M. Hasan and M. A. Rashid (2006). "Stereochenols a and B, Two Quinones from *Stereospermum chelonoides*." *Phytochemistry* **67**(24): 2663-2665.

Hasan, M., P. Gatto and P. Jha (2013). "Traditional Uses of Wild Medicinal Plants and Their Management Practices in Nepal - A Study in Makawanpur District." *International Journal of Medicinal and Aromatic Plants* **3**(1): 102-112.

Hazarika, T. and B. Nautiyal (2012). "Studies on Wild Edible Fruits of Mizoram, India Used as Ethnomedicine." *Genetic Resources and Crop Evolution* **59**(8): 1767-1776.

Hiremath, I., Y.-J. Ahn and S.-I. Kim (1997). "Insecticidal Activity of Indian Plant Extracts against *Nilaparvata lugens* (Homoptera: Delphacidae)." *Applied Entomology and Zoology* **32**(1): 159-166.

Holdsworth, D. and H. Sakulas (1986). "Medicinal Plants of the Morobe Province Part Ii1. The Aseki Valley." *Pharmaceutical Biology* **24**(1): 31-40.

Hossain, K., F. Islam, R. Kuddus and F. Latif (2013). "Actividad Antimicrobiana Preliminar Y Citotoxicidad De Extractos De Hojas De *Mussaenda roxburghii* Hook. F." *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* **12**(6): 612-617.

Hossan, S., A. Hanif, R. Jahan and M. Rahmatullah (2014). "Ethnomedicinal Plants of the Bawm Tribal Community of Rowangchhari in Bandarban District of Bangladesh." *The Journal of Alternative and Complementary Medicine* **20**(8): 581-589.

Hu, Z., Y. Lai, J. Zhang, Y. Wu, Z. Luo, G. Yao, Y. Xue and Y. Zhang (2014). "Phytochemical and Chemotaxonomic Studies on *Phyllanthus urinaria*." *Biochemical Systematics and Ecology* **56**(0): 60-64.

Hunyadi, A., A. Martins, B. Danko, F. Chang and Y. Wu (2014). "Protoflavones: A Class of Unusual Flavonoids as Promising Novel Anticancer Agents." *Phytochemistry Reviews* **13**(1): 69-77.

Hussain, M., U. Farooq, M. Rashid, H. Bakhsh, A. Aziz, A. Majeed and I. A. Khan (2014). "An Investigation of the Antimicrobial Activity of the Aqueous, Dichloromethane, Ethanol and Methanol Extract of the Seeds and Whole Plant of *Ipomoea nil*." *Journal of Pharmacy and Alternative Medicine* **3**(1): 14-25.

Hussain, M. M., M. M. R. Mughal, M. M. Alam, M. G. Dastagir, A. M. Billah and M. Ismail (2010). "Antimicrobial Activity of *n*-Hexane and Ethyl Acetate Extracts of *Erythrina stricta* Roxb." *Bangladesh Journal of Microbiology* **27**(2): 65-66.

Ingale, A. and A. Hivrale (2010). "Pharmacological Studies of *Passiflora* Sp. And Their Bioactive Compounds." *African Journal of Plant Science* **4**(10): 417-426.

Ingawale, G. S. and A. S. Goswami-Giri (2014). "Isolation and Characterization of Bioactive Molecule from *Lantana camara*." *Asian Journal of Research In Chemistry* **7**(3): 339-344.

Inoue, M. and L. E. Craker (2014). Medicinal and Aromatic Plants—Uses and Functions. Horticulture: Plants for People and Places, Volume 2, Springer**:** 645-669.

Ishwori, L., A. D. Talukdar, P. Singh, M. Dutta Choudhury and D. Nath (2014). "Antibacterial Activity of Some Selected Plants Traditionally Used as Medicine in Manipur." *African Journal of Biotechnology* **13**(13): 1491-1495.

Islam, M. K., S. Saha, I. Mahmud, K. Mohamad, K. Awang, S. Jamal Uddin, M. M. Rahman and J. A. Shilpi (2014). "An Ethnobotanical Study of Medicinal Plants Used by Tribal and Native People of Madhupur Forest Area, Bangladesh." *Journal of Ethnopharmacology* **151**(2): 921-930.

Ismail, L. D., M. M. El-Azizi, T. I. Khalifa and F. R. Stermitz (1996). "Iridoid Glycosides from *Thunbergia grandiflora*." *Phytochemistry* **42**(4): 1223-1225.

Iwu, M. M. (2014). Handbook of African Medicinal Plants, CRC press.

Jadhav, P., H. Lal and N. Kshirsagar (2014). "Assessment of Potency of Pc-Complexed *Ocimum sanctum* Methanol Extract in Embryonated Eggs against Influenza Virus (H1n1)." *Pharmacognosy Magazine* **10**(37): 86-91.

Jagtap, N., S. Khadabadi, D. Ghorpade, N. Banarase and S. Naphade (2009). "Antimicrobial and Antifungal Activity of *Centella asiatica* (L.) Urban, Umbeliferae." *Research Journal of Pharmacy and Technology* **2**(2): 328-330.

Jagtap, U. B. and V. A. Bapat (2010). "*Artocarpus*: A Review of Its Traditional Uses, Phytochemistry and Pharmacology." *Journal of Ethnopharmacology* **129**(2): 142-166.

Jain, P. (2014). "Antibacterial Activity of a New Flavone Glycoside from the Seeds of *Dolichos lablab* Linn." *Chemical Science Review and Letters* **3**(10): 252-257.

Jamir, N. and L. Takatemjen (2010). "Traditional Knowledge of Lotha-Naga Tribes in Wokha District, Nagaland." *Indian Journal of Traditional Knowledge* **9**(1): 45-48.

Jansz, E. and I. Uluwaduge (2012). "Biochemical Aspects of Cassava (*Manihot esculenta* Crantz) with Special Emphasis on Cyanogenic Glucosides - A Review." *Journal of the National Science Foundation of Sri Lanka* **25**(1): 1-24.

Jarić, S., Z. Popović, M. Mačukanović-Jocić, L. Djurdjević, M. Mijatović, B. Karadžić, M. Mitrović and P. Pavlović (2007). "An Ethnobotanical Study on the Usage of Wild Medicinal Herbs from Kopaonik Mountain (Central Serbia)." *Journal of Ethnopharmacology* **111**(1): 160-175.

Jash, S., R. Roy and D. Gorai (2014). "Bioactive Constituents from *Bauhinia variegata* Linn." *International Journal of Pharmaceutical and Biomedical Research* **5**(2): 51-54.

Jeeva, S., M. Johnson, J. Aparna and V. Irudayaraj (2011). "Preliminary Phytochemical and Antibacterial Studies on Flowers of Selected Medicinal Plants." *International Journal of Medicinal and Aromatic Plants* **1**(2): 107-114.

Jian, W., Y. Hong-xing, T. Yong-zhen, Y. Pei, T. Rui and L. Bi-chun (2010). "Review on the Chinese Research Articles of *Urticaceae* Plants Chemical Constituents." *Chinese Journal of Ethnomedicine and Ethnopharmacy* **17**: 020.

Joffry, S. M., N. Yob, M. Rofiee, M. Affandi, Z. Suhaili, F. Othman, A. M. Akim, M. M. Desa and Z. Zakaria (2011). "*Melastoma malabathricum* (L.) Smith Ethnomedicinal Uses, Chemical Constituents, and Pharmacological Properties: A Review." *Evidence-Based Complementary and Alternative Medicine* **2012**: 48.

Joharchi, M. R. (2014). "Ethno-Medicinal Plants Used to Cure Jaundice Bytraditional Healers of Mashhad, Iran." *Iranian Journal of Pharmaceutical Research* **13**(1): 157-162.

Joseph, B., J. George and J. Mohan (2013). "Pharmacology and Traditional Uses of *Mimosa pudica*." *International Journal of Pharmaceutical Sciences and Drug Research* **5**(2): 41-44.

Joseph, B. and M. Priya (2011). "Review on Nutritional, Medicinal and Pharmacological Properties of Guava (*Psidium guajava* Linn.)." *International Journal of Pharma and Bio Sciences* **2**(1): 53-69.

Joshi, A. R. and K. Joshi (2007). "Ethnomedicinal Plants Used against Skin Diseases in Some Villages of Kali Gandaki, Bagmati and Tadi Likhu Watersheds of Nepal." *Ethnobotanical Leaflets* **2007**(1): 27.

Joshi, K., R. Joshi and A. Joshi (2011). "Indigenous Knowledge and Uses of Medicinal Plants in Macchegaun, Nepal." *Indian Journal of Traditional Knowledge* **10**(2): 281-286.

Jouad, H., M. Haloui, H. Rhiouani, J. El Hilaly and M. Eddouks (2001). "Ethnobotanical Survey of Medicinal Plants Used for the Treatment of Diabetes, Cardiac and Renal Diseases in the North Centre Region of Morocco (Fez–Boulemane)." *Journal of Ethnopharmacology* **77**(2–3): 175-182.

Juyal, P. and J. Ghildiyal (2013). "Medicinal Phyto-Diversity of Bhabar Tract of Garhwal Himalaya." *Journal of Medicinal Plants Studies* **1**(6): 43-57.

Kabir, M. H., N. Hasan, M. M. Rahman, M. A. Rahman, J. A. Khan, N. T. Hoque, M. R. Q. Bhuiyan, S. M. Mou, R. Jahan and M. Rahmatullah (2014). "A Survey of Medicinal Plants Used by the Deb Barma Clan of the Tripura Tribe of Moulvibazar District, Bangladesh." *Journal of Ethnobiology and Ethnomedicine* **10**(1): 19.

Kadirvelmurugan, V., K. Raju, T. Arumugam, V. Karthik and S. Ravikumar (2014). "Ethnobotany of Medi-Flora of Kolli Hills, Tamil Nadu." *Archives of Applied Science Research* **6**(1): 159-164.

Kala, C. P. (2005). "Ethnomedicinal Botany of the Apatani in the Eastern Himalayan Region of India." *Journal of Ethnobiology and Ethnomedicine* **1**(11).

Kalidhar, S. (2006). "Chemical Constituents of *Crataeva nurvala* (Buch-Ham) Leaves." *Indian Journal of Pharmaceutical Sciences* **68**(6): 804-806.

Kalita, J., S. S. Singh and M. L. Khan (2012). "*Clerodendrum colebrookianum* Walp.: A Potential Folk Medicinal Plant of North East India." *Asian Journal of Pharmaceutical and Biological Research* **2**(4): 256-261.

Kalyan, D. and D. Pallwabee (2014). "Traditional Knowledge of the Women's of Kaibarta Community of Assam About the Application of Phyto-Remedies in Certain Common Childhood Diseases." *International Research Journal of Biological Sciences* **3**(1): 57-63.

Katewa, S. S., B. D. Guria and A. Jain (2001). "Ethnomedicinal and Obnoxious Grasses of Rajasthan, India." *Journal of Ethnopharmacology* **76**(3): 293-297.

Kaur, J. and S. Arora (2013). "Evaluation of Antioxidative and Antiproliferative Potential of Methanol Extract of Leaves of *Ficus benghalensis*, *Ficus benjamina*, *Ficus elastica*, *Ficus infectoria* and *Ficus religiosa*." *International Journal of Pharmacy and Biotechnology* **3**(1): 5-14.

Ke-qun, H. (2013). "Determination of Total Flavonoids from Hedyotis Scandens Roxb. By Uv Spectrophotometry." *Journal of Anhui Agricultural Sciences* **3**: 036.

Khaleequr, R., S. Arshiya and R. Shafeequr (2012). "*Gossypium herbaceum* Linn: An Ethnopharmaceutical Review." *Journal of Pharmaceutical and Scientific Innovation* **1**(5): 1-5.

Khan, A. V., Q. U. Ahmed, M. W. Khan and A. A. Khan (2014). "Herbal Cure for Poisons and Poisonous Bites from Western Uttar Pradesh, India." *Asian Pacific Journal of Tropical Disease* **4**(Supplement 1): 116-120.

Khan, E. U. (2014). Investigation of Phytochemical and Cytotoxic Activity of Methanol Extract of Spondias Pinnata Bark, Diss. East West University.

Khan, F. A., T. Mahmood, M. Ali, A. Saeed and A. Maalik (2014). "Pharmacological Importance of an Ethnobotanical Plant: *Capsicum annuum* L." *Natural Product Research* **28**(16): 1267–1274.

Khan, R. U., S. Mehmood and S. U. K. F. Jaffar (2013). "Ethnobotanical Study of Food Value Flora of District Bannu Khyber Pakhtunkhwa, Pakistan." *Journal of Medicinal Plants Studies* **1**(4): 93-106.

Khuankaew, S., K. Srithi, P. Tiansawat, A. Jampeetong, A. Inta and P. Wangpakapattanawong (2014). "Ethnobotanical Study of Medicinal Plants Used by Tai Yai in Northern Thailand." *Journal of Ethnopharmacology* **151**(2): 829-838.

Khyade, M. S., D. M. Kasote and N. P. Vaikos (2014). "*Alstonia scholaris* (L.) R. Br. And *Alstonia macrophylla* Wall. Ex G. Don: A Comparative Review on Traditional Uses, Phytochemistry and Pharmacology." *Journal of Ethnopharmacology* **153**(1): 1-18.

Kichu, M. (2010). Documentation and Biological and Phytochemical Analysis of Chungtia Medicinal Plants of Nagaland, India, Diss. *Chemistry and Biomolecular Sciences*, Macquarie University, Sydney. **PhD:** 1-179.

Kim, H.-R., I.-D. Kim, S. K. Dhungana, M.-O. Kim and D.-H. Shin (2014). "Comparative Assessment of Physicochemical Properties of Unripe Peach (*Prunus persica*) and Japanese Apricot (*Prunus mume*)." *Asian Pacific Journal of Tropical Biomedicine* **4**(2): 97-103.

Kiranmai, M., S. Kazim and M. Ibrahim (2011). "Combined Wound Healing Activity of *Gymnema sylvestre* and *Tagetes erecta* Linn." *International Journal of Pharmaceutical Applications* **2**(2): 135-140.

Kokila, K., S. Priyadharshini and V. Sujatha (2013). "Phytopharmacological Properties of *Albizia* Species: A Review." *International Journal of Pharmacy and Pharmaceutical Sciences* **5**(Supplement 3): 70-73.

Kono, R., Y. Okuno, M. Nakamura, K.-i. Inada, A. Tokuda, M. Yamashita, R. Hidaka and H. Utsunomiya (2013). "Peach (*Prunus persica*) Extract Inhibits Angiotensin Ii-Induced Signal Transduction in Vascular Smooth Muscle Cells." *Food Chemistry* **139**(1): 371-376.

Konta, E. M., M. R. Almeida, C. L. d. Amaral, J. D. C. Darin, V. V. Rosso, A. Z. Mercadante, L. M. G. Antunes and M. L. P. Bianchi (2014). "Evaluation of the Antihypertensive Properties of Yellow Passion Fruit Pulp (*Passiflora edulis* Sims *F. Flavicarpa* Deg.) in Spontaneously Hypertensive Rats." *Phytotherapy Research* **28**(1): 28-32.

Kotoky, J., B. Dasgupta and N. Deka (2005). "Pharmacological Studies of *Clerodendron colebrookianum* Walp, a Potent Hypotensive Plant." *Indian Journal of Physiology and Pharmacology* **49**(3): 289-296.

Krohn, K., S. Cludius-Brandt, B. Schulz, M. Sreelekha and P. M. Shafi (2011). "Isolation, Structure Elucidation, and Biological Activity of a New Alkaloid from *Zanthoxylum rhetsa*." *Natural Product Communications* **6**(11): 1595-1596.

Kruger, M. J., N. Davies, K. H. Myburgh and S. Lecour (2014). "Proanthocyanidins, Anthocyanins and Cardiovascular Diseases." *Food Research International* **59**(0): 41-52.

Kubde, M. S., S. Khadabadi, I. Farooqui and S. Deore (2010). "*Lagenaria siceraria*: Phytochemistry, Pharmacognosy and Pharmacological Studies." *Report and Opinion* **2**(12): 24-31.

Kuldeep, S., V. Rao Ch, H. Zeashan and P. Ritu (2014). "Evaluation of *in vivo* Antioxidant and Oral Glucose Tolerance Test of Ethanolic Extract of *Calotropis gigantea* Linn. Against Streptozotocin-Induced Diabetic Rats." *Journal of Pharmacognosy and Phytochemistry* **2**(5): 59-65.

Kulkarni, Y. A., R. Panjabi, V. Patel, A. Tawade and A. Gokhale (2013). "Effect of *Gmelina arborea* Roxb in Experimentally Induced Inflammation and Nociception." *Journal of Ayurveda and Integrative Medicine* **4**(3): 152-157.

Kumar, M., S. K. Prasad and S. Hemalatha (2014). "A Current Update on the Phytopharmacological Aspects of *Houttuynia cordata* Thunb." *Pharmacognosy Reviews* **8**(15): 22-35.

Kumar, R. and K. A. Bharati (2014). "Ethnomedicines of Tharu Tribes of Dudhwa National Park, India." *Ethnobotany Research & Applications* **12**: 001-013.

Kumar, R., R. J. Sharma, K. Bairwa, R. K. Roy and A. Kumar (2010). "Pharmacological Review on Natural Antidiarrhoel Agents." *Der Pharma Chemica* **2**(2): 66-93.

Kumar, S. (2014). "Ethnomedicinal Properties of an Ayurvedic Drug Karmaranga (*Averrhoa carambola* Linn.): A Review." *Global Journal of Traditional Medicinal Systems* **3**(1): 10-16.

Kumar, S., B. Bajwa, K. Singh and A. Kalia (2013). "Anti-Inflammatory Activity of Herbal Plants: A Review." *International Journal of Advances in Pharmacy, Biology and Chemistry* **2**(2): 272.

Kumar, V., F. Anwar, D. Ahmed, A. Verma, A. Ahmed, Z. A. Damanhouri, V. Mishra, P. W. Ramteke, P. C. Bhatt and M. Mujeeb (2014). "*Paederia foetida* Linn. Leaf Extract: An Antihyperlipidemic, Antihyperglycaemic and Antioxidant Activity." *BMC Complementary and Alternative Medicine* **14**(1): 76.

Kumari, A., S. K. Yadav, Y. B. Pakade, V. Kumar, B. Singh, A. Chaudhary and S. C. Yadav (2011). "Nanoencapsulation and Characterization of *Albizia chinensis* Isolated Antioxidant Quercitrin on Pla Nanoparticles." *Colloids and Surfaces B: Biointerfaces* **82**(1): 224-232.

Kumari, P., A. M. Otaghvari, H. Govindapyari, Y. M. Bahuguna and P. Uniyal (2011). "Some Ethno-Medicinally Important Pteridophytes of India." *International Journal of Medicinal and Aromatic Plants* **1**(1): 18-22.

Kuruvilla, G. R., M. Neeraja, A. Srikrishna, G. Subba Rao, A. Sudhakar and P. Venkatasubramanian (2010). "A New Quinone from *Maesa indica* (Roxb.) A. Dc (*Myrsinaceae*)." *Indian Journal of Chemistry. Section B, Organic Including Medicinal* **49**(12): 1637-1641.

Kusari, P., M. Spiteller, O. Kayser and S. Kusari (2014). Recent Advances in Research on *Cannabis sativa* L. Endophytes and Their Prospect for the Pharmaceutical Industry. Microbial Diversity and Biotechnology in Food Security, Springer**:** 3-15.

Lai, C.-H., S.-H. Fang, Y. K. Rao, M. Geethangili, C.-H. Tang, Y.-J. Lin, C.-H. Hung, W.-C. Wang and Y.-M. Tzeng (2008). "Inhibition of *Helicobacter pylori* -Induced Inflammation in Human Gastric Epithelial Ags Cells by *Phyllanthus urinaria* Extracts." *Journal of Ethnopharmacology* **118**(3): 522-526.

Lanzotti, V., F. Scala and G. Bonanomi (2014). "Compounds from *Allium* Species with Cytotoxic and Antimicrobial Activity." *Phytochemistry Reviews*: 1-23.

Latheef, A., S. P. Kumar and A. Remashree (2014). "Ethnomedicine Used for Treating Cuts and Wounds by the Tribes of Attappady, Kerala." *International Journal of Herbal Medicine* **2**(2): 1-8.

Leaman, D. J., J. T. Arnason, R. Yusuf, H. Sangat-Roemantyo, H. Soedjito, C. K. Angerhofer and J. M. Pezzuto (1995). "Malaria Remedies of the Kenyah of the Apo Kayan, East Kalimantan, Indonesian Borneo: A Quantitative Assessment of Local Consensus as an Indicator of Biological Efficacy." *Journal of Ethnopharmacology* **49**(1): 1-16.

Lee, K. R., K. H. Kim, K. W. Woo, E. Moon, S. U. Choi, S. Y. Kim, S. Z. Choi and M. W. Son (2014). "Identification of Antitumor Lignans from the Seeds of Morning Glory (*Pharbitis nil*)." *Journal of Agricultural and Food Chemistry* **62**(31): 7746-7752.

Lepcha, S. and A. Das (2011). "Ethno-Medicobotanical Exploration Along the International Borders to Tibet Autonomous Region of China and the Kingdom of Bhutan with Special Reference to the Pangolakha Wildlife Sanctuary, East Sikkim." *Recent Studies in Biodiversity and Traditional Knowledge in India*: 257-270.

Li, X., G. Yang, C. Zhang, D. Wu, L. Tang and Y. Xin (2014). "Improvement of Intestinal Microflora Balance by Polysaccharide from *Physalis alkekengi* Var. *Francheti*." *Molecular Medicine Reports* **9**(2): 677-682.

Lifongo, L. L., C. V. Simoben, F. Ntie-Kang, S. B. Babiaka and P. N. Judson (2014). "A Bioactivity Versus Ethnobotanical Survey of Medicinal Plants from Nigeria, West Africa." *Natural Products and Bioprospecting* **4**(1): 1-19.

Lim, T. (2012). *Citrus X microcarpa*. Edible Medicinal and Non-Medicinal Plants, Springer**:** 865-870.

Lim, T. (2012). *Passiflora edulis*. Edible Medicinal and Non-Medicinal Plants, Springer**:** 147-165.

Lim, T. (2014). *Bauhinia variegata*. Edible Medicinal and Non-Medicinal Plants, Springer**:** 754-765.

Lim, T. (2014). *Chrysanthemum indicum*. Edible Medicinal and Non-Medicinal Plants, Springer**:** 270-286.

Lim, T. (2014). *Mirabilis jalapa*. Edible Medicinal and Non Medicinal Plants, Springer**:** 497-513.

Lim, T. (2014). *Tagetes erecta*. Edible Medicinal and Non-Medicinal Plants, Springer**:** 432-447.

Lim, T. K. (2012). *Passiflora edulis*. Edible Medicinal And Non-Medicinal Plants: Volume 4, Fruits, Springer Netherlands. **4:** 147-165.

Liu, A., S. M. Lee, Y. Wang and G. Du (2007). "*Elsholtzia*: Review of Traditional Uses, Chemistry and Pharmacology." *Journal of Chinese Pharmaceutical Sciences* **16**(2): 73.

Liu, X., M. Zhao, W. Luo, B. Yang and Y. Jiang (2009). "Identification of Volatile Components in *Phyllanthus emblica* L. And Their Antimicrobial Activity." *Journal of Medicinal Food* **12**(2): 423-428.

Lohiya, N., B. Manivannan, P. Mishra, N. Pathak, S. Sriram, S. Bhande and S. Panneerdoss (2002). "Chloroform Extract of *Carica papaya* Seeds Induces Long-Term Reversible Azoospermia in Langur Monkey." *Asian Journal of Andrology* **4**(1): 17-26.

Lokendrajit, N., S. Indira, N. Swapana and C. Singh (2012). "Antioxidant and Antimicrobial Activity of *Croton Caudatus* Geisel." *Asian Journal of Chemistry* **24**(10).

Lone, S. A., A. Yadav, A. K. Sharma and M. Tafazul (2013). "A Review on *Adhatoda vasica* Nees - an Important and High Demanded Medicinal Plant." *Indo American Journal of Pharmaceutical Research* **3**(2): 2600-2615.

Lu, J., T. Shen, Z. Guo, X. Shen and S. Zheng (2000). "The Chemical Constituents of *Elsholtzia blanda*." *Acta Botanica Sinica* **43**(5): 545-550.

Madruga, M. S., F. S. M. de Albuquerque, I. R. A. Silva, D. S. do Amaral, M. Magnani and V. Queiroga Neto (2014). "Chemical, Morphological and Functional Properties of Brazilian Jackfruit (*Artocarpus heterophyllus* L.) Seeds Starch." *Food Chemistry* **143**(0): 440-445.

Mahato, R. and R. Chaudhary (2005). "Ethnomedicinal Study and Antibacterial Activities of Selected Plants of Palpa District, Nepal." *Scientific World* **3**(3): 26-31.

Majaw, S. and J. Moirangthem (2009). "Qualitative and Quantitative Analysis of *Clerodendron colebrookianum* Walp. Leaves and *Zingiber cassumunar* Roxb. Rhizomes." *Ethnobotanical Leaflets* **2009**(5): 3.

Malek, N., S. Baek and A. Asari (2003). "Chemical Components of *Melastoma malabathricum*." *ACGC Chemical Research Communications* **16**: 28-33.

Mali, R. G., S. G. Mahajan and A. A. Mehta (2007). "Rakta Kanchan (*Bauhinia variegata*): Chemistry, Traditional and Medicinal Uses-a Review." *Pharmacognosy Reviews* **1**(2): 314.

Malla, B., D. Gauchan and R. Chhetri (2014). "Medico - Ethnobotanical Investigations in Parbat District of Western Nepal." *Journal of Medicinal Plants Research* **8**(2): 95-108.

Mallavadhani, U. V., A. K. Panda and Y. R. Rao (1998). " Pharmacology and Chemotaxonomy of *Diospyros*." *Phytochemistry* **49**(4): 901-951.

Manandhar, N. P. (1995). "An Inventory of Some Herbal Drugs of Myagdi District, Nepal." *Economic Botany* **49**(4): 371-379.

Manjamalai, A., G. Jiflin and V. Grace (2012). "Study on the Effect of Essential Oil of *Wedelia chinensis* (Osbeck) against Microbes and Inflammation." *Asian Journal of Pharmaceutical and Clinical Research* **5**(2): 155-163.

Mao, A., T. Hynniewta and M. Sanjappa (2009). "Plant Wealth of Northeast India with Reference to Ethnobotany." *Indian Journal of Traditional Knowledge* **8**(1): 96-103.

Mariani, R., E. Y. Sukandar and A. G. Suganda (2014). "Antibacterial Activity from Indonesian *Urticaceae*." *International Journal of Pharmacy and Pharmaceutical Sciences* **6**(4): 191-193.

Maroyi, A. (2012). "Garden Plants in Zimbabwe: Their Ethnomedicinal Uses and Reported Toxicity." *Ethnobotany Research & Applications* **10**: 045-057.

Materska, M. (2014). "Bioactive Phenolics of Fresh and Freeze-Dried Sweet and Semi-Spicy Pepper Fruits (*Capsicum annuum* L.)." *Journal of Functional Foods* **7**(0): 269-277.

Mawere, M. (2014). Culture, Indigenous Knowledge and Development in Africa: Reviving Interconnections for Sustainable Development, Langaa RPCIG.

Medhi, P. and S. Borthakur (2012). "Phytoresources from North Cachar Hills of Assam-Iii: Edible Plants Sold at Haflong Market." *Indian Journal of Natural Products and Resources* **3**(1): 84-109.

Medic, J., C. Atkinson and C. R. Hurburgh Jr (2014). "Current Knowledge in Soybean Composition." *Journal of the American Oil Chemists' Society* **91**(3): 363-384.

Meena, A., G. Nitika, N. Jaspreet, R. Meena and M. Rao (2011). "Review on Ethanobotany, Phytochemical and Pharmacological Profile of *Alstonia scholaris*." *International Research Journal of Pharmacy* **2**(1): 49-54.

Meena, M. C., V. Kumar, R. K. Meena and S. Khandelwal (2014). "Ethnomedicinal Plants Used by Tribal's in Pratapgarh, Rajasthan." *World Journal of Pharmacy and Pharmaceutical Sciences* **3**(7): 1009-1016.

Melguizo-Melguizo, D., E. Diaz-de-Cerio, R. Quirantes-Piné, J. Švarc-Gajić and A. Segura-Carretero (2014). "The Potential of *Artemisia vulgaris* Leaves as a Source of Antioxidant Phenolic Compounds." *Journal of Functional Foods* **10**: 192-200.

Milad, R., S. El-Ahmady and A. N. Singab (2014). "Genus Kalanchoe (*Crassulaceae*): A Review of Its Ethnomedicinal, Botanical, Chemical and Pharmacological Properties." *European Journal of Medicinal Plants* **4**(1): 86-104.

Mishra, K., A. Sharma, A. Khare, R. Nagar and P. Porwal (2013). "*Mimosa pudica*: Traditional Uses, Phytochemistry and Pharmacological Properties: A Review." *Inventi Rapid: Planta Activa* **2013**.

Mohammadhosseini, M., B. Mahdavi and H. Akhlaghi (2013). "Characterization and Chemical Composition of the Volatile Oils from Aerial Parts of *Eryngium bungei* Bioss.(*Apiaceae*) by Using Traditional Hydrodistillation, Microwave Assisted Hydrodistillation and Head Space Solid Phase Microextraction Methods Prior to Gc and

Gc/Ms Analyses: A Comparative Approach." *Journal of Essential Oil Bearing Plants* **16**(5): 613-623.

Mohan G, K. and S. Sanjey J (2014). "Free Radical Scavenging, Immunomodulatory Activity and Chemical Composition of *Luffa acutangula* Var: *Amara* (*Cucurbitaceae*) Pericarp." *Journal of the Chilean Chemical Society* **59**(1): 2299-2302.

Mollik, M. A. H., M. S. Hossan, A. K. Paul, M. Taufiq-Ur-Rahman, R. Jahan and M. Rahmatullah (2010). "A Comparative Analysis of Medicinal Plants Used by Folk Medicinal Healers in Three Districts of Bangladesh and Inquiry as to Mode of Selection of Medicinal Plants." *Ethnobotany Research & Applications* **8**: 195-218.

Morvin, J., S. Prabhu and S. Vijayakumar (2014). "An Ethnobotanical Study of Medicinal Plants Used by Traditional Healers in Silent Valley of Kerala, India." *Journal of Ethnopharmacology* **154**(0): 774–789.

Muhammad, N., M. Saeed, A. Adhikari, K. M. Khan and H. Khan (2013). "Isolation of a New Bioactive Cinnamic Acid Derivative from the Whole Plant of *Viola betonicifolia*." *Journal of Enzyme Inhibition and Medicinal Chemistry* **28**(5): 997-1001.

Muhammad, N., M. Saeed, M. Ibrar and H. Khan (2012). "Pharmacognostic Studies of *Viola betonicifolia*." *African Journal of Pharmacy and Pharmacology* **6**(1): 43-47.

Muhammad, N., M. Saeed and H. Khan (2012). "Antipyretic, Analgesic and Anti-Inflammatory Activity of *Viola betonicifolia* Whole Plant." *BMC Complementary and Alternative Medicine* **12**(1): 59.

Mukherjee, S., A. Dey and T. Das (2012). "*In vitro* Antibacterial Activity of *n*-Hexane Fraction of Methanolic Extract of *Alstonia scholaris* Lr Br. Stem Bark against Some Multidrug Resistant Human Pathogenic Bacteria." *European Journal of Medicinal Plants* **2**(1): 1-10.

Mundepi, U., V. Kumar, S. Gupta, G. S. Bisht, F. C. Garg and V. K. Gupta (2014). "Evaluation of Anticandidal Potential of Some Folklore Plants." *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* **84**(2): 357-363.

Murray, P. R., K. S. Rosenthal and M. A. Pfaller (2013). Medical Microbiology, Online Access, 7: Medical Microbiology, Elsevier Health Sciences.

Nagaraja, T. (2011). "*In vitro* Evaluation of Anti Fungal Properties of *Zanthoxylum rhetsa* (Roxb) Dc." *Trends in Biosciences* **4**(1): 112-113.

Nagori, K., M. K. Singh, A. Alexander, T. Kumar, D. Dewangan, H. Badwaik and D. Tripathi (2011). "*Piper betle* L.: A Review on Its Ethnobotany, Phytochemistry,

Pharmacological Profile and Profiling by New Hyphenated Technique Dart-Ms (Direct Analysis in Real Time Mass Spectrometry)." *Journal of Pharmacy Research* **4**(9): 2991-2997.

Nair, S. S., N. C. Madembil, P. Nair, S. Raman and S. B. Veerabadrappa (2013). "Comparative Analysis of the Antibacterial Activity of Some Phytolectins." *International Current Pharmaceutical Journal* **2**(2): 18-22.

Namsa, N. D., M. Mandal, S. Tangjang and S. C. Mandal (2011). "Ethnobotany of the Monpa Ethnic Group at Arunachal Pradesh, India." *Journal of Ethnobiology and Ethnomedicine* **7**(1): 31.

Nanda, Y., S. Nengnunnem and N. Rao (2013). "Ethnomedicinal Plants of Thadou Tribe of Manipur (India)-1." *Pleione* **7**(1): 138-145.

Nath, M. and M. D. Choudhury (2010). "Ethno-Medico-Botanical Aspects of *Hmar* Tribe of Cachar District, Assam (Part-I)." *Indian Journal Of Traditional Knowledge* **9**(4): 760-764.

Nath, R., S. Roy, B. De and M. Dutta Choudhury (2013). "Anticancer and Antioxidant Activity of Croton: A Review." *International Journal of Pharmacy & Pharmaceutical Sciences* **5**(2): 63-70.

Nath, S. C. and D. N. Bordoloi (1991). "*Clerodendrum colebrookianum*, a Folk Remedy for the Treatment of Hypertension in Northeastern India." *Pharmaceutical Biology* **29**(2): 127-129.

Naveed, M., T. A. Khan, I. Ali, A. Hassan, H. Ali, Z. Ud, Z. H. Din, S. Tabassum, A. M. Saqib and M. U. Rehman (2014). "*In vitro* Antibacterial Activity of *Cannabis sativa* Leaf Extracts to Some Selective Pathogenic Bacterial Strains." *International Journal of Biosciences* **4**(4): 65-70.

Nayeem, A., A. Khatun, M. S. Rahman and M. Rahman (2011). "Evaluation of Phytochemical and Pharmacological Properties of *Mikania cordata* (*Asteraceae*) Leaves." *Journal of Pharmacognosy and Phytotherapy* **3**(8): 111-123.

Negi, J. S., V. Bisht, A. Bhandari, R. Bisht and S. K. Negi (2012). "Major Constituents, Antioxidant and Antibacterial Activities of *Zanthoxylum armatum* Dc. Essential Oil." *Iranian Journal of Pharmacology & Therapeutics* **11**(2).

Negi, V. S. and R. Maikhuri (2013). "Socio-Ecological and Religious Perspective of Agrobiodiversity Conservation: Issues, Concern and Priority for Sustainable Agriculture, Central Himalaya." *Journal of Agricultural and Environmental Ethics* **26**(2): 491-512.

Neuwinger, H. D. (2004). "Plants Used for Poison Fishing in Tropical Africa." *Toxicon* **44**(4): 417-430.

Ngoci, N. S., M. Ramadhan, M. S. Ngari and O. P. Leonard (2014). "Screening for Antimicrobial Activity of *Cissampelos pareira* L. Methanol Root Extract. 2." *Drug Discovery* **32**: 33.

Nguyen, T., M.-O. Parat, M. P. Hodson, P. N. Shaw and A. K. Hewavitharana (2014). Investigation of Compounds with Cytotoxic Activity in *Carica papaya* Leaves. ICDDT 2014: 6th International Conference on Drug Discovery and Therapy.

Nirmala, C., M. S. Bisht and M. Laishram (2014). "Bioactive Compounds in Bamboo Shoots: Health Benefits and Prospects for Developing Functional Foods." *International Journal of Food Science & Technology* **49**(6): 1425-1431.

Njume, C., N. Goduka and G. George (2014). "Indigenous Leafy Vegetables (Imifino, Morogo, Muhuro) in South Africa: A Rich and Unexplored Source of Nutrients and Antioxidants." *African Journal of Biotechnology* **13**(19): 1933-1942.

Nomani, I., A. Mazumder and G. Chakraborthy (2013). "*Wedelia chinensis* (*Asteraceae*) - an Overview of a Potent Medicinal Herb." *International Journal of PharmTech Research* **5**(3): 957-964.

Nono, N. R., K. L. Nzowa, L. Barboni and A. L. Tapondjou (2014). "*Drymaria cordata* (Linn.) Willd (*Caryophyllaceae*): Ethnobotany, Pharmacology and Phytochemistry." *Advances in Biological Chemistry* **4**(2): 160-167.

Nour, V., I. Trandafir and M. E. Ionica (2014). "Evolution of Antioxidant Activity and Bioactive Compounds in Tomato (*Lycopersicon esculentum* Mill.) Fruits During Growth and Ripening." *Journal of Applied Botany and Food Quality* **87**: 97-103.

Nuwamanya, E., P. R. Rubaihayo, S. Mukasa, S. Kyamanywa, J. F. Hawumba and Y. Baguma (2014). "Biochemical and Secondary Metabolites Changes under Moisture and Temperature Stress in Cassava (*Manihot esculenta* Crantz)." *African Journal of Biotechnology* **13**(31): 3173-3186.

Nwodo, U., A. Ngene, C. Iroegbu, O. Onyedikachi, V. Chigor and A. Okoh (2011). "*In vivo* Evaluation of the Antiviral Activity of *Cajanus cajan* on Measles Virus." *Archives of Virology* **156**(9): 1551-1557.

Ohemu, T., A. Agunu, P. Olotu, U. Ajima, D. Dafam and J. Azila (2014). "Ethnobotanical Survey of Medicinal Plants Used in the Traditional Treatment of Viral Infections in Jos, Plateau State, Nigeria." *International Journal of Medicinal and Aromatic Plants* **4**(2): 74-81.

Olowa, L. F., M. A. J. Torres, E. C. Aranico and C. G. Demayo (2012). "Medicinal Plants Used by the Higaonon Tribe of Rogongon, Iligan City, Mindanao, Philippines." *Advances in Environmental Biology* **6**(4): 1442-1449.

Othman, M., S. Genapathy, P. S. Liew, Q. T. Ch'ng, H. S. Loh, T. J. Khoo, C. Wiart and K. N. Ting (2011). "Search for Antibacterial Agents from Malaysian Rainforest and Tropical Plants." *Natural Product Research* **25**(19): 1857-1864.

Pal, D., P. Mishra, N. Sachan and A. K. Ghosh (2011). "Biological Activities and Medicinal Properties of *Cajanus cajan* (L) Millsp." *Journal of Advanced Pharmaceutical Technology & Research* **2**(4): 207-214.

Palaksha, M., K. Ravishankar and V. G. Sastry (2013). "Phytochemical Screening and Evaluation of *in-vitro* Antibactreial and Anthelmintic Activities of *Saccharum officinarum* Leaf Extract." *World Journal of Pharmacy and Pharmaceutical Sciences* **2**(6): 5761-5768.

Panda, S. K. (2014). "Ethno-Medicinal Uses and Screening of Plants for Antibacterial Activity from Similipal Biosphere Reserve, Odisha, India." *Journal of Ethnopharmacology* **151**(1): 158-175.

Pandya, D., S. Doshi and R. Parmar (2013). "Comparative Pharmacognostic Study and Establishment of Quality Parameters of Leaves of Two Species of Karen: *Thevetia peruviana* and *Nerium indicum*." *International Journal of Biomedical Research* **3**(12): 444-447.

Pant, G., O. Prakash, M. Chandra, S. Sethi, H. Punetha, S. Dixit and A. Pant (2014). "Biochemical Analysis, Pharmacological Activity, Antifungal Activity and Mineral Analysis in Methanolic Extracts of *Myrica esculenta* and *Syzygium cumini*: The Indian Traditional Fruits Growing in Uttarakhand Himalaya." *Indian Journal of Pharmaceutical and Biological Research* **2**(1): 26-34.

Pantaleón-Velasco, M. d. R., I. I. Ruiz-López, A. Pérez-Silva, L. Bravo-Clemente, R. Mateos, H. Ruiz-Espinosa and M. d. l. A. Vivar-Vera (2014). "Antioxidant and Functional Properties of a High Dietary Fibre Powder from Carambola (*Averrhoa carambola* L.) Pomace." *International Journal of Food Science & Technology* **49**(9): 2101-2110.

Parekh, J. and S. Chanda (2007). "*In vitro* Screening of Antibacterial Activity of Aqueous and Alcoholic Extracts of Various Indian Plant Species against Selected Pathogens from *Enterobacteriaceae*." *African Journal of Biomedical Research* **1**(6): 92-99.

Partha, P. (2014). "Ethnobotany of the Laleng (Patra) Community in Bangladesh." *Journal of Pharmacognosy and Phytochemistry* **2**(6): 173-184.

Parvin, S., M. A. Kader, A. U. Chouduri, M. Rafshanjani and M. Haque (2014). "Antibacterial, Antifungal and Insecticidal Activities of the *n*-Hexane and Ethyl-Acetate Fractions of Methanolic Extract of the Leaves of *Calotropis gigantea* Linn." *Journal of Pharmacognosy and Phytochemistry* **2**(5): 47-51.

Patel, K., M. Gadewar, V. Tahilyani and D. K. Patel (2012). "A Review on Pharmacological and Analytical Aspects of Diosgenin: A Concise Report." *Natural Products and Bioprospecting* **2**(2): 46-52.

Patel, V. G., S. H. Shukla, B. V. Jogi and H. A. Mistry (2014). "Pharmacognostical Standardization and Preliminary Phytochemical Screening of *Cissampelos pareira* Linn. Var. Hirsuta Roots." *Journal of Pharmacognosy and Phytochemistry* **2**(5): 27-31.

Pathak, A. and A. Argal (2007). "Analgesic Activity of *Calotropis gigantea* Flower." *Fitoterapia* **78**(1): 40-42.

Paul, J., C. Seaforth and T. Tikasingh (2011). " *Eryngium foetidum* L.: A Review." *Fitoterapia* **82**(3): 302-308.

Pavunraj, M., K. Baskar, S. Janarthanan and M. Arumugam (2014). "Antibacterial and Antifeedant Activities of *Spilanthes acmella* Leaf Extract against Gram-Negative and Gram-Positive Bacteria and Brinjal Fruit Borer, *Leucinodes orbonalis* Larvae." *Journal of Coastal Life Medicine* **2**(6): 490-495.

Pawar, V. and P. Pawar (2014). "*Costus speciosus*: An Important Medicinal Plant." *International Journal of Science and Research* **3**(7): 28-33.

Pazyar, N., R. Yaghoobi, E. Rafiee, A. Mehrabian and A. Feily (2014). "Skin Wound Healing and Phytomedicine: A Review." *Skin Pharmacology and Physiology* **27**(6): 303-310.

Pereira, V., C. Dias, M. Vasconcelos, E. Rosa and M. Saavedra (2014). "Antibacterial Activity and Synergistic Effects between *Eucalyptus globulus* Leaf Residues (Essential Oils and Extracts) and Antibiotics against Several Isolates of Respiratory Tract Infections (*Pseudomonas aeruginosa*)." *Industrial Crops and Products* **52**(0): 1-7.

Perumal, G. N., R. B. Shankar, S. Malini, V. Soumya, S. Janardhanan, V. Palanivel and K. S. Kumar (2010). "Anti-Inflammatory and Ulcer Prtotective Activities of Chloroform Extract of *Albizzia chinensis* Bark and Its Phytochemical Studies." *Der Pharmacia Sinica* **1**(1): 95-103.

Pfoze, N. L., M. Kehie, H. Kayang and A. A. Mao (2014). "Estimation of Ethnobotanical Plants of the Naga of North East India." *Journal of Medicinal Plants* **2**(3): 92-104.

Pfoze, N. L., Y. Kumar and B. Myrboh (2012). "Survey and Assessment of Ethnomedicinal Plants Used in Senapati District of Manipur State, Northeast India." *Phytopharmacology* **2**(2): 285-311.

Piccaglia, R., M. Marotti, M. Pesenti, P. Mattarelli, B. Biavati, C. Franz, A. Mathe and G. Buchbauer (1996). Chemical Composition and Antibacterial Activity of *Tagetes erecta* and *Tagetes patula* Essential Oils. Proceeding of the 27 th International Symposium on Essential Oils, Wien, Austria.

Pittler, M. H. and E. Ernst (2007). "Clinical Effectiveness of Garlic (*Allium sativum*)." *Molecular Nutrition & Food Research* **51**(11): 1382-1385.

Ponnusha, B. S., S. Subramaniyam and P. Pasupathi (2011). "Antioxidant and Antimicrobial Properties of *Glycine max* - a Review." *International Journal of Current Biological and Medical Science* **1**(2): 49-62.

Prachayasittikul, V., S. Prachayasittikul, S. Ruchirawat and V. Prachayasittikul (2013). "High Therapeutic Potential of *Spilanthes acmella*: A Review." *EXCLI Journal* **12**: 291-312.

Pradeep, B. V., M. Tejaswini, P. Nishal, G. Pardhu, S. Shylaja and K. K. Ch (2013). "Phytochemical Screening and Antimicrobial Activities of Plant Extract of *Lantana camara*." *Journal of Environmental Biology* **34**(3): 645-649.

Prakash, C. and I. Prakash (2011). "Bioactive Chemical Constituents from Pomegranate (*Punica granatum*) Juice, Seed and Peel - A Review." *International Journal of Chemical, Environmental and Pharmacological Research* **1**(1): 1-18.

Prasobh, G. (2014). "Pharmacological Importance of *Musa species*–a Review." *International Journal of Pharmaceutical Archive* **3**(6): 453-459.

Preeti, K. and A. Verma (2014). "A Review on Ethnopharmacological Potential of *Ricinus communis* Linn." *PharmaTutor* **2**(3): 76-85.

Quazi Majaz, A., S. Nazim, S. Afsar, S. Siraj and P. M. Siddik (2011). "Evaluation of Antimicrobial Activity of Roots of *Kalanchoe pinnata*." *International Journal of Pharma and Bio Sciences* **5**(1): 93-96.

Ragasa, C. Y., R. Dumag and J. A. Rideout (2001). "Antimicrobial Compounds from *Mentha cordifolia*." *Philippine Journal of Science* **130**(1): 39-43.

Raghu, A., S. George, V. Renju Krishna and K. Sindhu (2013). "Bioactive Properties of Phenolics Present in *Oroxylum indicum* – A Review." *Journal of Pharmacognosy and Phytochemistry* **2**(3): 23-27.

Rahman, A. (2013). "Ethno-Medicinal Investigation on Ethnic Community in the Northern Region of Bangladesh." *American Journal of Life Sciences* **1**(2): 77-81.

Rahman, A. M., S. K. Nitu, Z. Ferdows and A. R. Islam (2013). "Medico-Botany on Herbaceous Plants of Rajshahi, Banglabesh." *American Journal of Life Sciences* **1**(3): 136-144.

Rahman, M. A. (2010). "Indigenous Knowledge of Herbal Medicines in Bangladesh. 3. Treatement of Skin Diseases by Tribal Communities of the Hill Tracts Districts." *Bangladesh Journal of Botany* **39**(2): 169-177.

Rahman, S., S. Rahman, M. S. A. Bhuiyan, A. Rahman, F. Mawla, K. Debnath, S. M. Mou, A. A. Bashar and M. Rahmatullah (2013). "Traditional Phytotherapy of Folk and Tribal Medicinal Practitioners in Bangladesh for Treatment of Elephantiasis." *American-Eurasian Journal of Sustainable Agriculture* **7**(3): 143-148.

Rahmatullah, M., D. Ferdausi, M. A. H. Mollik, M. N. K. Azam, M. Rahman and R. Jahan (2009). "Ethnomedicinal Survey of Bheramara Area in Kushtia District, Bangladesh." *American Eurasian Journal of Sustainable Agriculture* **3**(3): 534-541.

Rahmatullah, M., M. S. Hossan, A. Hanif, P. Roy, R. Jahan, M. Khan, M. H. Chowdhury and T. Rahman (2009). "Ethnomedicinal Applications of Plants by the Traditional Healers of the Marma Tribe of Naikhongchhari, Bandarban District, Bangladesh." *Advances in Natural and Applied Sciences* **3**(3): 392-401.

Rahmatullah, M., Z. Khatun, D. Barua, M.-U. Alam, S. Jahan and R. Jahan (2013). "Medicinal Plants Used by Traditional Practitioners of the Kole and Rai Tribes of Bangladesh." *The Journal of Alternative and Complementary Medicine* **19**(6): 483-491.

Rai, B., G. Kumar and R. Diwan (2014). "Collection and Characterization of Latex from *Euphorbia royleana*." *International Journal of Applied Science and Engineering Research* **1**(3): 16-24.

Rai, P. K. and H. Lalramnghinglova (2010). "Ethnomedicinal Plant Resources of Mizoram, India: Implication of Traditional Knowledge in Health Care System." *Ethnobotanical Leaflets* **2010**(3): 6.

Rai, V., P. K. Tandon and S. Khatoon (2014). "Effect of Chromium on Antioxidant Potential of *Catharanthus roseus* Varieties and Production of Their Anticancer Alkaloids: Vincristine and Vinblastine." *BioMed Research International* **2014**: 10.

Rajkumari, R., P. Singh, A. K. Das and B. Dutta (2013). "Ethnobotanical Investigation of Wild Edible and Medicinal Plants Used by the Chiru Tribe of Manipur, India." *Pleione* **7**(1): 167-174.

Rajput, S. B., M. B. Tonge and S. M. Karuppayil (2014). "An Overview on Traditional Uses and Pharmacological Profile of *Acorus calamus* Linn. (Sweet Flag) and Other *Acorus* Species." *Phytomedicine* **21**(3): 268-276.

Rani, D., P. Khare and P. Dantu (2010). "*In vitro* Antibacterial and Antifungal Properties of Aqueous and Non-Aqueous Frond Extracts of *Psilotum nudum*, *Nephrolepis biserrata* and *Nephrolepis cordifolia*." *Indian Journal of Pharmaceutical Sciences* **72**(6): 818-822.

Rao, R. and N. Jamir (1982). "Ethnobotanical Studies in Nagaland. I. Medicinal Plants." *Economic Botany* **36**(2): 176-181.

Rathi, P., C. S. Rajput and S. Singhal (2014). "Evaluation of Total Phenolic Contents, Antioxidant and Antibacterial Capacity of Aqueous Methanolic Extracts Obtained from *Punica granatum* Peel." *International Journal of Pharmaceutical Sciences Review & Research* **25**(2): 92-94.

Rathinamoorthy, R. and G. Thilagavathi (2014). "*Terminalia chebula* - Review on Pharmacological and Biochemical Studies." *International Journal of PharmTech Research* **6**(1): 97-116.

Raveen, R., K. Kamakshi, M. Deepa, S. Arivoli and S. Tennyson (2014). "Larvicidal Activity of *Nerium oleander* L. (*Apocynaceae*) Flower Extracts against *Culex quinquefasciatus* Say (Diptera: *Culicidae*)." *International Journal of Mosquito Research* **1**(1): 38-42.

Ravi, K. and P. Divyashree (2014). "*Psidium guajava*: A Review on Its Potential as an Adjunct in Treating Periodontal Disease." *Pharmacognosy Reviews* **8**(16): 96-100.

Rayne, S. and G. Mazza (2007). "Biological Activities of Extracts from Sumac (*Rhus* Spp.): A Review." *Plant Foods for Human Nutrition* **62**(4): 165-175.

Renuga, F. B. and S. M. M. Bai (2013). "Natural Products Used by the Kanikkars of Kanyakumari District, Tamil Nadu, India." *Journal of Pharmacognosy and Phytochemistry* **2**(2013): 255-261.

Rhama, S. and S. Madhavan (2011). "Antibacterial Activity of the Flavonoid, Patulitrin Isolated from the Flowers of *Tagetes erecta* L." *International Journal of PharmTech Research* **3**: 1407-1409.

Ritthiwigrom, T., S. Laphookhieo and S. G. Pyne (2013). "Chemical Constituents and Biological Activities of *Garcinia cowa* Roxb." *Maejo International Journal of Science and Technology* **7**(2): 212-231.

Rivadeneyra-Domínguez, E., A. Vázquez-Luna, J. F. Rodríguez-Landa and R. Díaz-Sobac (2013). "Neurotoxic Effect of Linamarin in Rats Associated with Cassava (*Manihot esculenta* Crantz) Consumption." *Food and Chemical Toxicology* **59**(0): 230-235.

Rizwana, J. N., I. Nazlina, A. R. M. Razehar, A. Z. S. Noraziah, C. Y. Ling, S. A. S. Muzaimah, A. H. Farina, W. A. Yaacob, I. B. Ahmad and L. B. Din (2010). "A Survey on Phytochemical and Bioactivity of Plant Extracts from Malaysian Forest Reserves." *Journal of Medicinal Plants Research* **4(3)**: 203-210.

Roblot, F., L. Montaz, M. Delcoustal, E. Gaboriau, J. Chavagnat, G. Morichaud, O. Pourrat, M. Scepi and D. Patte (1994). "*Datura stramonium* Poisoning: The Diagnosis Is Clinical, Treatment Is Symptomatic." *La Revue de Medecine Interne/fondee... par la Societe Nationale Francaise de Medecine Interne* **16**(3): 187-190.

Romano, B., F. Borrelli, E. Pagano, M. G. Cascio, R. G. Pertwee and A. A. Izzo (2014). "Inhibition of Colon Carcinogenesis by a Standardized *Cannabis sativa* Extract with High Content of Cannabidiol." *Phytomedicine* **21**(5): 631-639.

Roy, A. and S. Saraf (2006). "Ethnomedicinal Approach in Biological and Chemical Investigation of Phytochemicals as Antimicrobials." *Pharmaceutical Reviews* **4**(2).

Roy, D., M. Shark and H. Faruquee (2013). "A Brief Review on Phytochemistry and Pharmacological Properties of *Adhatoda vasica*." *Journal of Tropical Medicinal Plants* **14**(1/2): 115-124.

Rudrapal, M., N. Sridhar and M. Raghavendra (2012). "Ethnomedicinal Plants Used by Traditional Healers in East Godavari District of Andhra Pradesh, India." *Indian Journal of Natural Products and Resources* **3**(3): 426-431.

Rukachaisirikul, T., A. Saekee, C. Tharibun, S. Watkuolham and A. Suksamrarn (2007). "Biological Activities of the Chemical Constituents of *Erythrina stricta* and *Erythrina subumbrans*." *Archives of Pharmacal Research* **30**(11): 1398-1403.

Rummun, N., J. Somanah, S. Ramsaha, T. Bahorun and V. S. Neergheen-Bhujun (2013). "Bioactivity of Nonedible Parts of *Punica granatum* L.: A Potential Source of Functional Ingredients." *International Journal of Food Science* **2013**: 12.

Sabzar, A. D., A. G. Farooq, S. Poonam, K. Naresh and S. Rambir (2012). "Bioassay Guided Isolation and Identification of Anti-Inflammatory and Anti-Microbial Compounds

from *Urtica dioica* L.(*Urticaceae*) Leaves." *African Journal of Biotechnology* **11**(65): 12910-12920.

Salgado, H., A. F. F. Roncari and R. R. Moreira (2005). "Antidiarrhoeal Effects of *Mikania glomerata* Spreng.(*Asteraceae*) Leaf Extract in Mice." *Revista Brasileira de Farmacognosia* **15**(3): 205-208.

Samuel, J. K. and B. Andrews (2010). "Traditional Medicinal Plant Wealth of Pachalur and Periyur Hamlets Dindigul District, Tamil Nadu." *Indian Journal of Traditional Knowledge* **9**(2): 264-270.

Sanglakpam, P., R. R. Mathur and A. K. Pandey (2012). "Ethnobotany of Chothe Tribe of Bishnupur District (Manipur)." *Indian Journal of Natural Products and Resources* **3**(3): 414-425.

Sangtam, T. L., N. Jamir, C. Deb and S. Jamir (2012). "A Study on the Medicinal Plants Used by the Sangtam Naga Tribe in Kiphire District, Nagaland, India." *International Journal Of Ayurvedic And Herbal Medicine* **2**(2): 267-275.

Sani, H. A., A. Rahmat, M. Ismail, R. Rosli and S. Endrini (2004). "Potential Anticancer Effect of Red Spinach (*Amaranthus gangeticus*) Extract." *Asia Pacific Journal of Clinical Nutrition* **13**(4): 396-400.

Sapkota, P. P. (2014). "Religious Culture and Medicinal Plants: An Anthropological Study." *Dhaulagiri Journal of Sociology and Anthropology* **7**: 197-224.

Saraf, A. (2010). "Phytochemical and Antimicrobial Studies of Medicinal Plant *Costus speciosus* (Koen.)." *Journal of Chemistry* **7**(1): 405-413.

Saranraj, P. and S. Sivasakthi (2014). "Medicinal Plants and Its Antimicrobial Properties: A Review." *Global Journal of Pharmacology* **8**(3): 316-327.

Sarin, B., N. Verma, J. P. Martín and A. Mohanty (2014). "An Overview of Important Ethnomedicinal Herbs of *Phyllanthus* Species: Present Status and Future Prospects." *The Scientific World Journal* **2014**: 12.

Sarker, B., F. Akther, U. Ayman, R. Sifa, I. Jahan, M. Sarker, S. K. Chakma, P. K. Podder, Z. Khatun and M. Rahmatullah (2012). "Ethnomedicinal Investigations among the Sigibe Clan of the Khumi Tribe of Thanchi Sub-District in Bandarban District of Bangladesh." *American Eurasian Journal of Sustainable Agriculture* **6**: 378-386.

Savithramma, N., C. Sulochana and K. Rao (2007). "Ethnobotanical Survey of Plants Used to Treat Asthma in Andhra Pradesh, India." *Journal of Ethnopharmacology* **113**(1): 54-61.

Saxena, M., N. K. Khare, P. Saxena, K. V. Syamsundar and S. K. Srivastava (2014). "Antimicrobial Activity and Chemical Composition of Leaf Oil in Two Varieties of *Piper betle* from Northern Plains of India." *Journal of Scientific & Industrial Research* **73**(2): 95-99.

Sayyed, A. and M. Shah (2014). "Phytochemistry, Pharmacological and Traditional Uses of *Datura stramonium* L." *Journal of Pharmacognosy and Phytochemistry* **2**(5): 123-125.

Scholz, E. P., E. Zitron, H. A. Katus and C. A. Karle (2010). "Cardiovascular Ion Channels as a Molecular Target of Flavonoids." *Cardiovascular Therapeutics* **28**(4): 46-52.

Semwal, D. K., R. B. Semwal, I. Vermaak and A. Viljoen (2014). "From Arrow Poison to Herbal Medicine-the Ethnobotanical, Phytochemical and Pharmacological Significance of *Cissampelos* (*Menispermaceae*)." *Journal of Ethnopharmacology* **155**(2): 1011-1028.

Seraj, S., S. Rahman, N. A. Afsana, M. J. Mahal, A. Swarna and M. Rahmatullah (2014). "Antihyperglycemic Activity Evaluation of a Formulation Consisting of *Phyllanthus emblica*, *Terminalia bellirica* and *Terminalia chebula* Fruits and *Trigonella foenum graecum* Seeds." *Advances in Natural & Applied Sciences* **8**(1).

Shamsi-Baghbanan, H., A. Sharifian, S. Esmaeili and B. Minaei (2014). "Hepatoprotective Herbs, Avicenna Viewpoint." *Iranian Red Crescent Medical Journal* **16**(1): 1-6.

Shankar, R. and R. B. Devalla (2012). "Conservation of Folk Healing Practices and Commercial Medicinal Plants with Special Reference to Nagaland." *International Journal of Biodiversity and Conservation* **4**(3): 155-163.

Sharma, A. (2011). "Antibacterial Activity of Ethanolic Extracts of Some Arid Zone Plants." *International Journal of PharmTech Research* **3**(1): 283-286.

Sharma, A., G. Joseph and R. Singh (2014). "Antioxidant and Antiplatlet Aggregation Properties of Bark Extracts of *Garcinia pedunculata* and *Garcinia cowa*." *Journal of Food Science and Technology* **51**(8): 1626-1631.

Sharma, B. (2013). "*In vitro* Antibacterial Activity of Certain Folk Medicinal Plants from Darjeeling Himalayas Used to Treat Microbial Infections." *Journal of Pharmacognosy and Phytochemistry* **2**(4): 1-4.

Sharma, G. (2011). "Gastroprotective Potential of Some Plants from *Rutaceae* Family: A Review."

Sharma, H. K., L. Chhangte and A. K. Dolui (2001). "Traditional Medicinal Plants in Mizoram, India." *Fitoterapia* **72**(2): 146-161.

Sharma, J., S. Gairola, R. Gaur, R. Painuli and T. Siddiqi (2013). "Ethnomedicinal Plants Used for Treating Epilepsy by Indigenous Communities of Sub-Himalayan Region of Uttarakhand, India." *Journal of Ethnopharmacology* **150**(1): 353-370.

Sharma, K., A. k. Saikia, H. Sharma, B. Sahariah, S. Deka and B. Das (2013). "Chemical Composition and Antimicrobial Study of Essential Oil from the Leaves of *Eupatorium odoratum* Linn. From Upper Assam Region." *Journal of Essential Oil Bearing Plants* **16**(4): 482-488.

Sharma, T. P. and S. Borthakur (2008). "Ethnobotanical Observations on Bamboos among Adi Tribes in Arunachal Pradesh." *Indian Journal of Traditional Knowledge* **7**(4): 594-597.

Sharma, U. K., S. Pegu, D. Hazarika and A. Das (2012). "Medico-Religious Plants Used by the Hajong Community of Assam, India." *Journal of Ethnopharmacology* **143**(3): 787-800.

Shil, S., M. Dutta Choudhury and S. Das (2014). "Indigenous Knowledge of Medicinal Plants Used by the Reang Tribe of Tripura State of India." *Journal of Ethnopharmacology* **152**(1): 135-141.

Shine, V., P. Latha, S. Suja, G. Anuja, B. Sabulal, V. Vilash and S. Rajasekharan (2014). "Anti-Hepatotoxic Effect of Root Ethanol Extract of *Cyclea peltata* against Acetaminophen Induced Oxidative Stress in Wistar Rats and *in vitro* Primary Hepatocyte Culture." *American Journal of Experimental Biology* **1**(1): 1-15.

Shine, V. J., P. G. Latha, S. N. R. Suja, G. I. Anuja, G. Raj and S. N. Rajasekharan (2014). "Ameliorative Effect of Alkaloid Extract of *Cyclea peltata* (Poir.) Hook. F. & Thoms. Roots (Acp) on Apap/Ccl4 Induced Liver Toxicity in Wistar Rats and *in vitro* Free Radical Scavenging Property." *Asian Pacific Journal of Tropical Biomedicine* **4**(2): 143-151.

Shirazi, M. T., H. Gholami, G. Kavoosi, V. Rowshan and A. Tafsiry (2014). "Chemical Composition, Antioxidant, Antimicrobial and Cytotoxic Activities of *Tagetes minuta* and *Ocimum basilicum* Essential Oils." *Food Science & Nutrition* **2**(2): 146-155.

Sindambiwe, J. B., M. Calomme, S. Geerts, L. Pieters, A. Vlietinck and D. Vanden Berghe (1998). "Evaluation of Biological Activities of Triterpenoid Saponins from *Maesa lanceolata*." *Journal of Natural Products* **61**(5): 585-590.

Singh, A. G. and J. P. Hamal (2013). "Traditional Phytotherapy of Some Medicinal Plants Used by Tharu and Magar Communities of Western Nepal, against Dermatological Disorders." *Scientific World* **11**(11): 81-89.

Singh, B. K., K. K. Pillai, K. Kohli and S. E. Haque (2013). "Effect of *Cissampelos pareira* Root Extract on Isoproterenol-Induced Cardiac Dysfunction." *Journal of Natural Medicines* **67**(1): 51-60.

Singh, H., T. Husain, P. Agnihotri, P. Pande and S. Khatoon (2014). "An Ethnobotanical Study of Medicinal Plants Used in Sacred Groves of Kumaon Himalaya, Uttarakhand, India." *Journal of Ethnopharmacology* **154**(1): 98-108.

Singh, K. K., G. P. Rauniar and H. Sangraula (2012). "Experimental Study of Neuropharmacological Profile of *Euphorbia pulcherrima* in Mice and Rats." *Journal of Neurosciences in Rural Practice* **3**(3): 311.

Singh, K. P., S. P. Devi, K. K. Devi, D. S. Ningombam and P. Athokpam (2010). "*Bambusa tulda* Roxb. In Manipur State, India: Exploring the Local Values and Commercial Implications." *Notulae Scientia Biologicae* **2**(2): 35-40.

Singh, P. and A. Singh (2012). "Evaluation of Latex Extract of *Euphorbia royleana* for Its Piscicidal and Muricidal Activities." *World Journal of Agricultural Sciences* **8**(5): 520-524.

Singh, R. K., O. Rallen and E. Padung (2013). "Elderly Adi Women of Arunachal Pradesh:"Living Encyclopedias" and Cultural Refugia in Biodiversity Conservation of the Eastern Himalaya, India." *Environmental Management* **52**(3): 712-735.

Singh, S., S. K. Singh and A. Yadav (2013). "A Review on Cassia Species: Pharmacological, Traditional and Medicinal Aspects in Various Countries." *American Journal of Phytomedicine and Clinical Therapeutics* **1**(3): 291-312.

Singh, T. T., H. M. Sharma, A. R. Devi and H. R. Sharma (2014). "Plants Used in the Treatment of Piles by the Scheduled Caste Community of Andro Village in Imphal East District, Manipur (India)." *Journal of Plant Sciences* **2**(3): 113-119.

Singhal, P., G. Kaushik and P. Mathur (2014). "Antidiabetic Potential of Commonly Consumed Legumes: A Review." *Critical Reviews in Food Science and Nutrition* **54**(5): 655-672.

Sirisha, N., M. Sreenivasulu, K. Sangeeta and C. M. Chetty (2010). "Antioxidant Properties of *Ficus* Species – A Review." *International Journal of PharmTech Research* **2**(4): 2174-2182.

Sivasankari, B., M. Anandharaj and P. Gunasekaran (2014). "An Ethnobotanical Study of Indigenous Knowledge on Medicinal Plants Used by the Village Peoples of Thoppampatti, Dindigul District, Tamilnadu, India." *Journal of Ethnopharmacology* **153**(2): 408-423.

Song, Y., Y. Yang, Y. Zhang, L. Duan, C. Zhou, Y. Ni, X. Liao, Q. Li and X. Hu (2013). "Effect of Acetylation on Antioxidant and Cytoprotective Activity of Polysaccharides Isolated from Pumpkin (*Cucurbita pepo*, Lady Godiva)." *Carbohydrate Polymers* **98**(1): 686-691.

Soni, R. K., R. Irchhaiya, V. Dixit and S. Alok (2013). "*Paederia foetida* Linn: Phytochemistry, Pharmacological and Traditional Uses." *International Journal of Pharmaceutical Sciences and Research* **4**(12): 4525-4530.

Sowndhararajan, K., J. M. Joseph and S. Manian (2013). "Antioxidant and Free Radical Scavenging Activities of Indian Acacias: *Acacia leucophloea* (Roxb.) Willd., *Acacia ferruginea* Dc., *Acacia dealbata* Link. And *Acacia pennata* (L.) Willd." *International Journal of Food Properties* **16**(8): 1717-1729.

Srivastava, R. (2010). "Traditional Knowledge of Nyishi (Daffla) Tribe of Arunachal Pradesh." *Indian Journal of Traditional Knowledge* **9**(1): 26-37.

Srivastava, S., P. Singh, K. Jha, G. Mishra and R. Khosa (2013). "Antiinflammatory, Analgesic and Antipyretic Activities of Aerial Parts of *Costus speciosus* Koen." *Indian Journal of Pharmaceutical Sciences* **75**(1): 83.

Srivastava, S. R., G. Keshri, B. Bhargavan, C. Singh and M. M. Singh (2007). "Pregnancy Interceptive Activity of the Roots of *Calotropis gigantea* Linn. In Rats." *Contraception* **75**(4): 318-322.

Stoewsand, G. (1995). "Bioactive Organosulfur Phytochemicals in *Brassica oleracea* Vegetables — A Review." *Food and Chemical Toxicology* **33**(6): 537-543.

Su, M.-J. and Y.-H. Kuo (2012). Pharmaceutical Composition and Method for Preventing or Treating Chronic Heart Disease, Google Patents.

Subba, B. and P. Basnet (2014). "Antimicrobial Activity of Some Medicinal Plants from East and Central Part of Nepal." *International Journal of Applied Sciences and Biotechnology* **2**(1): 88-92.

Subramanian, S. S. and A. G. R. Nair (1971). "Flavonoids of *Thunbergia grandiflora* and *Asystasia travancorica*." *Cur Sci* **40**(15): 404.

Sumanth, M. V., M. B. Yellanthoor, K. Ravikumar and P. Ravichandran (2013). "Comparative Physicochemical, Phytochemical and Hptlc Studies on Root Species Used as Patala in Ayurvedic System of Medicine." *Journal of Pharmacy Research* **7**(9): 810-816.

Sundararajan, R. and R. Koduru (2014). "*Asclepias currassavica*: A Review of Ethnomedicinal, Phytochemical and Pharmatological Information." *Indo American Journal of Pharmaceutical Research* **4**(4): 1739-1755.

Surse Sunita, N. and B. K. City (2014). "*Celosia cristata*: Potent Pharmacotherapeutic Herb–a Review." *International Journal of Pharmaceutical and Phytopharmacological Research* **3**(6): 444-446.

Sutha, E., P. Sangeetha and K. Rameshkumar (2014). "Screening of Phytochemical and Antimicrobial Activity of *Melia composite* against *Enterobacter aerogenes* and *Shigilla flexneri*." *Journal of Chemical & Pharmaceutical Research* **6**(5): 831-835.

Sutha, S., V. Mohan, S. Kumaresan, C. Murugan and T. Athiperumalsami (2010). "Ethnomedicinal Plants Used by the Tribals of Kalakad-Mundanthurai Tiger Reserve (Kmtr), Western Ghats, Tamil Nadu for the Treatment of Rheumatism." *Indian Journal of Traditional Knowledge* **9**(3): 502-509.

Talukdar, A. (2014). "Gas Chromatography-Mass Spectrometric Analysis of the Essential Oil of Eaglewood (*Aquilaria agallocha* Roxb)." *International Journal of Pharmacy and Pharmaceutical Sciences* **6**(7): 629-631.

Tandon, V., A. Yadav, B. Roy and B. Das (2011). "Phytochemicals as Cure of Worm Infections in Traditional Medicine Systems." *Emerging Trends in Zoology. Narendra Publishing House, New Delhi*: 351-378.

Tangjang, S., N. D. Namsa, C. Aran and A. Litin (2011). "An Ethnobotanical Survey of Medicinal Plants in the Eastern Himalayan Zone of Arunachal Pradesh, India." *Journal of Ethnopharmacology* **134**(1): 18-25.

Tantapakul, C., W. Phakhodee, T. Ritthiwigrom, K. Yossathera, S. Deachathai and S. Laphookhieo (2012). "Antibacterial Compounds from *Zanthoxylum rhetsa*." *Archives of Pharmacal Research* **35**(7): 1139-1142.

Tanti, B., A. K. Buragohain, L. Gurung and D. Kakati (2011). "Assessment of Antimicrobial and Antioxidant Activities of *Dendrocnide sinuata* (Blume) Chew Leaves–a Medicinal Plant Used by Ethnic Communities of North East India." *Indian Journal of Natural Products and Resources* **1**(1): 17-21.

Tao, Y. (2001). Compositions and Methods for Treating Back and Leg Discomfort, Google Patents.

Tapondjou, A. L., T. Miyamoto, J.-F. Mirjolet, N. Guilbaud and M.-A. Lacaille-Dubois (2005). "Pursaethosides Ae, Triterpene Saponins from *Entada pursaetha*." *Journal of Natural Products* **68**(8): 1185-1190.

Tasanarong, A., S. Kongkham and A. Itharat (2014). "Antioxidant Effect of *Phyllanthus emblica* Extract Prevents Contrast-Induced Acute Kidney Injury." *BMC Complementary and Alternative Medicine* **14**(1): 138.

Thangavelu, T., S. Kaminidevi, A. Lakshmanan, D. Gunashekar, H. T. Chirayil, J. K. Sukumaran and S. Murugan (2014). "Preliminary Phytochemical Screening and Antimicrobial Activity of Fresh Plant Extract of Indian Folk Medicinal Plant, *Gnaphalium polycaulon*." *International Journal of Phytomedicine* **6**(1): 82-86.

Thapa, L. B., T. M. Dhakal, R. Chaudhary and H. Thapa (2014). "Medicinal Plants Used by Raji Ethnic Tribe of Nepal in Treatment of Gastrointestinal Disorders." *Our Nature* **11**(2): 177-186.

Torabzadeh, P. and P. Panahi (2013). "Evaluation of Antifungal Activity of *Physalis alkekengi* L. Extracts on *Microsporum canis*, *Candida albicans*, *Trichophyton mentagrophytes* and *Nocardia asteroids*." *Middle-East Journal of Scientific Research* **13**(7): 926-929.

Tripathi, Y., V. Prabhu, R. Pal and R. Mishra (1996). "Medicinal Plants of Rajasthan in Indian System of Medicine." *Ancient Science of Life* **15**(3): 190.

Tsukiyama, M., T. Sugita, H. Kikuchi, Y. Yasuda, M. Arashima, H. Okumura, S. Lhieochaiphant and Y. Shoyama (2010). "Effect of *Duabanga grandiflora* for Human Skin Cells." *The American Journal of Chinese Medicine* **38**(02): 387-399.

Tuxill, J. D. and G. P. Nabhan (2001). Eds People, Plants, and Protected Areas: A Guide to in Situ Management (Vol 3), Earthscan.

Uddin, M. Z. and M. A. Hassan (2014). "Determination of Informant Consensus Factor of Ethnomedicinal Plants Used in Kalenga Forest, Bangladesh." *Bangladesh Journal of Plant Taxonomy* **21**(1): 83-91.

Uddin, S. B., M. O. Faruque and S. Talukder (2014). "A Survey of Traditional Health Remedies of the Chakma Indigenous Community of Rangamati District, Bangladesh." *Journal of Plant Science & Research* **1**(2): 106.

Umbare, R., G. Mate and S. Dongare (2011). "Anti-Ulcer Activity of Crude Alcoholic Extract of Rhizomes of *Cissus repens* Lan." *Research Journal of Pharmacy and Technology* **4**(1): 60-62.

Unnithan, A. R., S. Patil, G. Unnikrishnan and J. Varghese (2014). "Long Chain Fatty Alcohols from *Eupathorium odoratum* as Anti-*Candida* Agents." *International Journal of Pharmacy & Pharmaceutical Sciences* **6**(3): 263-266.

Uprety, Y., R. C. Poudel, H. Asselin and E. Boon (2011). "Plant Biodiversity and Ethnobotany inside the Projected Impact Area of the Upper Seti Hydropower Project, Western Nepal." *Environment, Development and Sustainability* **13**(3): 463-492.

Vadivel, V., W. Stuetz, V. Scherbaum and H. Biesalski (2011). "Total Free Phenolic Content and Health Relevant Functionality of Indian Wild Legume Grains: Effect of Indigenous Processing Methods." *Journal of Food Composition and Analysis* **24**(7): 935-943.

Van Kiem, P., C. Van Minh, N. X. Nhiem, B. H. Tai, T. H. Quang, H. L. T. Anh, N. X. Cuong, T. N. Hai, S. H. Kim and J. K. Kim (2012). "Chemical Constituents of the *Ficus elastica* Leaves and Their Antioxidant Activities." *Bulletin of the Korean Chemical Society* **33**(10): 3461-3464.

Van Minh, V., N. T. K. Yen and P. T. K. Thoa (2014). "Medicinal Plants Used by the Hre Community in the Ba to District of Central Vietnam." *Journal of Medicinal Plants* **2**(3): 64-71.

Velmurugan, C. and A. Bhargava (2014). "Anti-Diabetic Activity of *Gossypium herbaceum* by Alloxan Induced Model in Rats." *PharmaTutor* **2**(4): 126-132.

Venkateswarlu, K. (2014). "Pharmacological Evaluations (Analgesic Activity) of '*Piper betel*'." *International Journal of Pharmamedix India* **2**(2): 688-693.

Venkateswarlu, K., N. Devanna and N. Prasad (2014). "Microscopical and Preliminary Phytochemical Screening of '*Piper betel*'." *PharmaTutor* **2**(4): 112-118.

Vidyalakshmi, K., H. R. Vasanthi and G. Rajamanickam (2008). "Ethnobotany, Phytochemistry and Pharmacology of *Mussaenda* Species (*Rubiaceae*)." *Ethnobotanical Leaflets* **2008**(1): 57.

Vij, T. and Y. Prashar (2014). "A Review on Medicinal Properties of *Carica papaya* Linn." *Asian Pacific Journal of Tropical Disease* **5**(1): 1-6.

Wang, X. and Z. Jia (2004). "Chemical Constituents of *Equisetum ramosissimum*." *Acta Botanica Boreali-occidentalia Sinica* **25**(12): 2524-2528.

Wang, Z.-d., R.-r. Liang and Z.-f. Ll (2009). "Development in Pharmacological Research of *Chrysanthemum indicum*." *Medical Recapitulate* **6**: 38.

Wei, H., X. Zhang, G. Wu, X. Yang, S. Pan, Y. Wang and J. Ruan (2013). "Chalcone Derivatives from the Fern *Cyclosorus parasiticus* and Their Anti-Proliferative Activity." *Food and Chemical Toxicology* **60**(0): 147-152.

Wei, W. (2001). "Advances in Studies on *Houttuynia cordata*." *Chinese Traditional and Herbal Drugs* **4**: 39.

Wiart, C. (2006). Medicinal Plants of Asia and the Pacific, CRC Press.

Wiart, C., S. Mogana, S. Khalifah, M. Mahan, S. Ismail, M. Buckle, A. Narayana and M. Sulaiman (2004). "Antimicrobial Screening of Plants Used for Traditional Medicine in the State of Perak, Peninsular Malaysia." *Fitoterapia* **75**(1): 68-73.

Wong, J. Y., P. Matanjun, Y. B. H. Ooi and K. F. Chia (2014). "Evaluation of Antioxidant Activities in Relation to Total Phenolics and Flavonoids Content of Selected Malaysian Wild Edible Plants by Multivariate Analysis." *International Journal of Food Properties* **17**(8): 1763-1778.

Xu, X., T. Liang, Q. Wen, X. Lin, J. Tang, Q. Zuo, L. Tao, F. Xuan and R. Huang (2014). "Protective Effects of Total Extracts of *Averrhoa carambola* L.(*Oxalidaceae*) Roots on Streptozotocin-Induced Diabetic Mice." *Cellular Physiology and Biochemistry* **33**(5): 1272-1282.

Yadav, A. K. and V. Tangpu (2012). "Anthelmintic Activity of Ripe Fruit Extract of *Solanum myriacanthum* Dunal (*Solanaceae*) against Experimentally Induced *Hymenolepis diminuta* (Cestoda) Infections in Rats." *Parasitology Research* **110**(2): 1047-1053.

Yadav, S. and M. Bhandoria (2013). "Ethnobotanical Exploration in Mahendergarh District of Haryana (India)." *Journal of Medicinal Plants Research* **7** (18): 1263-1271.

Yakubu, A. and M. Mukhtar (2011). "*In vitro* Antimicrobial Activity of Some Phytochemical Fractions of *Euphorbia Pulcherima* L.(Poinsettia)." *Quarterly Journal of Science and Medicine* **54**(1): 55.

Yang, B. and P. Liu (2014). "Composition and Biological Activities of Hydrolyzable Tannins of Fruits of *Phyllanthus emblica*." *Journal of Agricultural and Food Chemistry* **62**(3): 529-541.

Yang, S. K., H. J. Kim and S. W. Oh (2014). "Antimicrobial Activities of Ethanol Extracts of *Rhus javanica* and *Terminalia chebula* against *Shigella* Species." *Journal of Food Safety* **34**(1): 95-102.

Yeo, S.-G., J. H. Song, E.-H. Hong, B.-R. Lee, Y. S. Kwon, S.-Y. Chang, S. H. Kim, S. won Lee, J.-H. Park and H.-J. Ko (2014). "Antiviral Effects of *Phyllanthus urinaria* Containing Corilagin against Human Enterovirus 71 and Coxsackievirus A16 *in vitro*." *Archives of Pharmacal Research*: 1-10.

Yin, H.-L., J.-H. Li, B. Li, L. Chen, J. Li, Y. Tian, S.-J. Liu, Y.-K. Zhao, Y.-H. Xiao and J.-X. Dong (2014). "Two New Coumarins from the Seeds of *Solanum indicum*." *Journal of Asian Natural Products Research* **16**(2): 153-157.

You, Y.-O., N.-Y. Choi, S.-Y. Kang and K.-J. Kim (2013). "Antibacterial Activity of *Rhus javanica* against Methicillin-Resistant *Staphylococcus aureus*." *Evidence-Based Complementary and Alternative Medicine* **2013**: 8.

Yu, S., J. Zhang, S. Ma, R. Xu and C. Ji (2013). Application of *Albizzia chinensis* Extract in Preparation of Medicine for Treatment of Gastric Ulcer, Institute of Materia Medica, Chinese Academy of Medical Sciences, Peop. Rep. China . 19pp.

Yumnam, J. and O. Tripathi (2013). "Ethnobotany: Plants Use in Fishing and Hunting by Adi Tribe of Arunachal Pradesh." *Indian Journal of Traditional Knowledge* **12**(1): 157-161.

Yun, S., B. Choi, H. Ku, M. Lee, H. Nam, K. Lee, S. Park, H. Jang and S. Son (2008). "Antimicrobial Activities of the Flower Extract of *Celosia cristata* L." *Planta Medica* **74**(09): 31.

Yusuf, M., M. Wahab, M. Yousuf, J. U. Chowdhury and J. Begum (2007). "Some Tribal Medicinal Plants of Chittagong Hill Tracts, Bangladesh." *Bangladesh Journal of Plant Taxonomy* **14**(2): 117-128.

Zainudin, M. A. M., A. A. Hamid, F. Anwar, A. Osman and N. Saari (2014). "Variation of Bioactive Compounds and Antioxidant Activity of Carambola *Averrhoa carambola* L. Fruit at Different Ripening Stages." *Scientia Horticulturae* **172**: 325-331.

Zhang, F.-p., M.-q. Zheng and M.-j. WU (2012). "Extraction and Antioxidant Activity of Flavonoids from *Gynura crepidioides*." *Food Science* **22**: 9.

Zhang, J., K. Yuan, W.-l. Zhou, J. Zhou and P. Yang (2011). "Studies on the Active Components and Antioxidant Activities of the Extracts of *Mimosa pudica* Linn. From Southern China." *Pharmacognosy Magazine* **7**(25): 35.

Zhao, G., X. Li, W. Chen, Z. Xi and L. Sun (2012). "Three New Sesquiterpenes from *Tithonia diversifolia* and Their Anti-Hyperglycemic Activity." *Fitoterapia* **83**(8): 1590-1597.

Zhao, Q., X. Lu and B.-m. FENG (2013). "Advances on the Chemical Components and Pharmacological Activities of *Urtica* Genus." *Journal of Shenyang Pharmaceutical University* **2**: 015.

Zheng, X.-l., J.-h. Wei, W. Sun, R.-t. Li, S.-b. Liu and H.-f. Dai (2013). "Ethnobotanical Study on Medicinal Plants around Limu Mountains of Hainan Island, China." *Journal of Ethnopharmacology* **148**(3): 964-974.

Zheng, X.-l. and F.-w. Xing (2009). "Ethnobotanical Study on Medicinal Plants around Mt. Yinggeling, Hainan Island, China." *Journal of Ethnopharmacology* **124**(2): 197-210.

Zheng, Z.-P., Y. Xu, C. Qin, S. Zhang, X. Gu, Y. Lin, G. Xie, M. Wang and J. Chen (2014). "Characterization of Antiproliferative Activity Constituents from *Artocarpus heterophyllus*." *Journal of Agricultural and Food Chemistry* **62**(24): 5519-5527.

Zhou, Z.-h., L.-q. Jiao, M. YU and C.-a. ZHAO (2012). "Gc-Ms Analysis of Essential Oils Chemical Constituents from Rhizome of *Physalis alkekengi* L. Var. Franchetii." *Special Wild Economic Animal and Plant Research* **1**: 022.

Zhu, G.-Y., X.-J. Yao, L. Liu, L.-P. Bai and Z.-H. Jiang (2014). "Alistonitrine a, a Caged Monoterpene Indole Alkaloid from *Alstonia scholaris*." *Organic Letters* **16**(4): 1080-1083.

Zia-Ul-Haqi, M., S. Ahmad, M. Qayum and S. Ercisli (2013). "Compositional Studies and Antioxidant Potential of *Albizia lebbeck* (L.) Benth. Pods and Seeds." *Turkish Journal of Biology* **37**(1): 25-32.

Zou, G.-A., Z.-H. Su, H.-W. Zhang, Y. Wang, J.-S. Yang and Z.-M. Zou (2010). "Flavonoids from the Stems of *Croton caudatus* Geisel. Var. Tomentosus Hook." *Molecules* **15**(3): 1097-1102.

Chapter 3

Selection of Plants with Antimicrobial Potential

This chapter describes the rationale behind the selection of Chungtia medicinal plants for subsequent antimicrobial screening, along with detailed ethnomedicinal, pharmacological and phytochemical information on these species from the published literature.

3.1 Background

As described in Chapter two, thirty five plants were identified following first-hand interviews with Chungtia villagers as being used for skin related treatments that were likely to have a microbial aetiology. The aim of this chapter was to identify which of these plants were worthy of further investigation for their antimicrobial properties and bioactive constituents.

3.2 The choice of plants with potential antimicrobial properties.

Current estimates indicate that nearly 6 million people suffer from chronic wounds worldwide (Vadivel 2014). The prevalence of chronic wounds in the community was reported as 4.5 per 1000 population whereas that of acute wounds was nearly doubled at 10.5 per 1000 population. Chronic wounds are also a major cause of morbidity in developing countries (Kumar *et al.* 2007). Among rural people skin injuries are common, sustained as a result of working in the fields or burns from cooking (Kumar *et al.* 2007).

The presence of pathogenic bacteria and fungi is often a causative or contributing factor for skin infections and the healing of sores and wounds (Edwards and Harding 2004). Thus, plants used customarily for the topical treatment of skin infections, sores and wounds are likely to possess antimicrobial properties (Bowler 2002). The thirty five plants used by Chungtia villagers for skin infections, sores and wounds were therefore selected for a comprehensive literature search on their antimicrobial activities and any antimicrobial chemical constituents reported. This review provided the basis for logical selection of Chungtia plants for subsequent chemical and biological investigations. The literature search results (updated to September 2014) are presented in Table 3.1.

Table 3.1 Reported antimicrobial activities and antimicrobially active extracts/chemical constituents from plants used by Chungtia villagers for skin related conditions of possible microbial aetiology

Plant scientific name	Skin related uses by Chungtia villagers	Antimicrobial activity (Plant part: microbe against which active)	Antimicrobial extracts/compounds (Plant part: active extract/ compound)
Albizia lucidior	Root infusion is applied topically to abscesses and boils	None found	None found
Artocarpus heterophyllus	Sap is applied topically to treat skin diseases	WR Fruits, wood: *P. aeruginosa, B. subtilis, E. coli, S. aureus, C. albicans, A. niger* (Khan *et al.* 2003, Ragasa *et al.* 2004); seeds: *S. aureus, E. coli, B. subtilis* (Nair *et al.* 2013)	Seeds: lectins (Nair *et al.* 2013); fruits: cycloartenone, cycloartenol, diastereomeric 2,3-butanediol (Ragasa *et al.* 2004)
Asclepias curassavica	Leaf paste is applied topically for cuts and wounds	WR Leaves, seeds: *P. aeruginosa* (Lentz *et al.* 1998); aerial parts, roots: *E. coli, K. pneumoniae, P. vulgaris, P. aeruginosa, S. aureus, B. subtilis* (Neto *et al.* 2002, Hemavani and Thippeswamy 2012, Kurdekar *et al.* 2012, Reddy *et al.* 2012)	Aerial parts: ethanol, chloroform, petroleum ether extracts (Hemavani and Thippeswamy 2012); roots: chloroform, water extracts (Kurdekar *et al.* 2012); leaves, roots: petroleum spirit, ethyl acetate, methanol extracts (Reddy *et al.* 2012)
Begonia picta	Leaves are used to cleanse hands by crushing between palms	Aerial parts: *S. aureus* (Kichu 2010)*	Aerial parts: Ethanolic aqueous extract (Kichu 2010)*
Calotropis gigantea	Leaf poultice is used topically for burns	WR Leaves: *S. aureus, K. pneumoniae, B. cereus, P. aeruginosa, E. coli* (Kumar *et al.* 2010); flowers: *S. aureus, E. coli, B. subtilis, S. lutea, S. sonnei, S. shiga, S. dysenteriae, P. aeruginosa, B. cereus, S. agalactiae, S. flexneri, C. albinans, A. niger* (Habib and Karim 2009, Parvin *et al.* 2014); stem, roots: *S. aureus, E. coli, P. aeruginosa, S. typhi, V. cholerae* (Radhakrishnan *et al.* 2013)	Leaves: water extract (Kumar *et al.* 2010), methanol extract - hexane, ethyl acetate partitions (Parvin *et al.* 2014); flowers: ethyl acetate extract - anhydrosophoradiol-3-acetate, di-(2-ethylhexyl)phthalate (Habib and Karim 2009); stem, roots: methanol, ethanol, acetone, hexane, ethyl acetate extracts (Radhakrishnan *et al.* 2013)

Cassia floribunda	Warmed leaves are made into a paste and applied externally for fungal infections and burns	Leaves: *S. aureus* (Kichu 2010)*; seeds: *E. coli, M. pyogenes, S. typhi, S. paratyphi* (Singh *et al.* 2013)	Leaves, seeds: ethanol extract (Kichu 2010, Singh *et al.* 2013)
Celosia cristata	Leaf paste is applied topically for cuts and wounds	WR Flowers: *S. aureus, B. subtilis, C. albicans* (Yun *et al.* 2008); whole plant: *B. subtilis* (Bazzaz and Haririzadeh 2003, Gnanamani *et al.* 2003)	Flowers: ethanol, methanol extract (Yun *et al.* 2008); whole plant: ethanol extract (Bazzaz and Haririzadeh 2003)
Chrysanthemum indicum	Leaf paste is applied topically to treat lip scab (angular cheilitis)	WR Flowers: *S. aureus, E. coli, B. subtilis, S. typhi, P. vulgaris, P. mirabilis, K. pneunoniae, E. faecalis, Candida* sp (Shunying *et al.* 2005); leaves: *P. aeruginosa, C. albicans* (Pradhan *et al.* 2011)	Flowers: essential oils (Shunying *et al.* 2005); leaves: essential oils (Pradhan *et al.* 2011)
Cyclea peltata	Leaf decoction is applied topically for abscesses and boils	Tuber: *E. coli, P. vulgaris, P. mirabilis, S. pyogenes* (Raja *et al.* 2011); whole plant: *E. coli, P. aeruginosa, K. pneumoniae, P. vulgaris, V. cholerae* (Abraham and Thomas 2012)	Tuber: methanol, hexane extracts (Raja *et al.* 2011); whole plant: ethanol, methanol, chloroform, hexane, ethyl acetate, acetone extracts (Abraham and Thomas 2012)
Dendrocnide sinuata	Outer fresh stem is scraped off and the secreted mucilage is applied on fresh cuts and wounds	Leaves: *S. aureus, E. coli, P. aeruginosa* (Tanti *et al.* 2011)	Leaves: methanol, water extracts (Tanti *et al.* 2011)
Drymaria cordata	Toasted whole plants are crushed into a paste and applied topically to treat fungal infection (ringworm)	Whole plant: *S. aureus, E. coli, P. aeruginosa, B. subtilis* (Mukherjee *et al.* 1997, Pulok *et al.* 1997, Nono *et al.* 2014)	Whole plant: methanol extract (Mukherjee *et al.* 1997, Nono *et al.* 2014)
Duabanga grandiflora	Fresh bark is scraped off and applied topically to skin diseases and cuts and wounds	Whole plant: *S. aureus, E. coli* (Othman *et al.* 2011)	Whole plant: aqueous ethanolic, ethyl acetate extracts (Othman *et al.* 2011)
Elsholtzia blanda	Leaf paste is applied to fresh cuts	WR Aerial parts: *S. aureus, E. coli, B. subtilis, S. flexneri, S. typhimurium* (Fang *et al.* 1990, Guo *et al.* 2012)	Aerial parts: essential oils - 1,8-cineole, *R*-(+)-α-phellandrene, bornyl acetate, camphene, linalool, α-terpinene, α-pinene (Fang *et al.* 1990, Guo *et al.* 2012)

Erythrina stricta	Bark paste is applied topically to treat contact dermatitis, eczema and skin infections	WR Stem bark: *S. aureus*, MRSA, MDRSA, *E. coli, P. aeruginosa, B. subtilis, B. cereus, S. paratyphi, S. typhi, S. boydii, S. dysenteriae, V. mimicus, V. parahemolyticus, C. albicans, A. niger* (Kichu 2010, Hussain *et al.* 2011); whole plant: *E. coli, B. subtilis* (Sharma 2013)	Stem bark: maackiaflavanone B, alpinumisoflavone, lupalbigenin, cristacarpin, erystagallin A, erythrabyssin II (Kichu 2010)*, hexane, ethyl acetate fractions (Hussain *et al.* 2011); whole plant: ethanol extract (Sharma 2013)
Eupatorium odoratum	Leaf paste is applied topically to fresh cuts and wounds	WR Leaves: *S. aureus, E. coli, B. subtilis, K. aerogenes* (Inya-Agha *et al.* 1987); whole plant: *S. epidermidis, P. acnes,* MRSA (Chomnawang *et al.* 2005, Chomnawang *et al.* 2009); flowers: *M. tuberculosis* (Suksamrarn *et al.* 2004)	Leaves: essential oils (Inya-Agha *et al.* 1987); whole plant: chloroform, ethyl acetate, methanol fractions (Chomnawang *et al.* 2005), ethanol extract (Chomnawang *et al.* 2009); flowers: isosacuranetin (Suksamrarn *et al.* 2004)
Euphorbia royleana	Milky sap is applied topically to treat skin diseases	WR Whole plant: *S. aureus, B. subtilis, B. cereus, C. albicans* (Shi *et al.* 2008); rhizomes: *S. aureus, E. coli, B. subtilis* (Pratush *et al.* 2013)	Whole plant: *ent*-11-hydroxyabieta-8(14),13(15)-dien-16,12*R*-olide, helioscopinolide A, helioscopinolide B (Shi *et al.* 2008); rhizomes: ethanol, water extracts (Pratush *et al.* 2013)
Eurya acuminata	Leaf paste is applied topically to cuts and wounds	Leaves, stem: *S. aureus* (Grosvenor *et al.* 1995)	Leaves, stem: aqueous ethanolic extract (Grosvenor *et al.* 1995)
Ficus elastica	Root juice or sap is applied topically to cuts and wounds	WR Leaves: *B. cereus, P. aeruginosa* (Almahy *et al.* 2003), *K. pneumoniae, P. mirabilis* (Parekh and Chanda 2007); bark of aerial roots: *S. aureus, S. faecalis* (Mbosso *et al.* 2012)	Leaves: emodin, sucrose, morin, rutin (Almahyl *et al.* 2003); aqueous ethanolic extract (Parekh and Chanda 2007); bark of aerial roots - ficusamide, elasticoside (Mbosso *et al.* 2012)
Gmelina arborea	Mesocarp of the drupe is applied topically to treat skin diseases	WR Fruits: *S. aureus, P. aeruginosa, B. subtilis* (Nayak *et al.* 2012); root bark: *S. aureus, E. coli, P. aeruginosa, B. subtilis* (Audipudi and Chakicherla 2010); leaves, stem bark: *E. coli,*	Fruits: ethanol extract (Nayak *et al.* 2012); root bark: methanol, chloroform extracts (Audipudi and Chakicherla 2010); leaves, stem bark: water, ethanol,

		K. pneumoniae, P. mirabilis, S. dysenteriae, S. typhi (El-Mahmood *et al.* 2010)	hexane, chloroform extracts (El-Mahmood *et al.* 2010)
Hedyotis scandens	Leaf paste is applied topically to cuts and wounds	Stem: *S. aureus, E. coli, P. vulgaris, K. pneumoniae* (Subba and Basnet 2014)	Stem: ethanol extract (Subba and Basnet 2014)
Holboellia latifolia	Foam from crushed leaves is applied topically to fire burns (demulcent)	None found	None found
Ipomoea nil	Leaf paste is applied topically to fire burns (demulcent)	Whole plant, seeds: *S. aureus, E. coli, S. pneumoniae, K. pneumoniae, C. albicans, A. niger* (Hussain *et al.* 2014)	Whole plant, seeds: ethanol, methanol, water, dichloromethane extracts (Hussain *et al.* 2014)
Kalanchoe pinnata	Warmed leaf paste is applied topically to treat ringworms, skin diseases and burns	WR Leaves: *S. aureus, E. coli, B. cereus, B. subtilis, S. pyogenes, S. faecalis, P. aeruginosa, K. pneumoniae, S. typhi, S. dysenteriae, P. vulgaris, P. aeruginosa,* (Biswas *et al.* 2011, Pattewar 2012)	Leaves: methanol extract (Wiart *et al.* 2004), chloroform extract (Muthuvelan and Raja 2008), water, methanol extract (Akinpelu 2000, Pattewar 2012); aerial parts: ethanol extract (Biswas *et al.* 2011)
Lagenaria siceraria	Juice extract of leaves is applied topically to treat skin diseases	WR Leaves, seeds: *S. aureus, P. aeruginosa, S. pyogenes;* fruits: *P. aeruginosa, S. pyogenes* (Goji *et al.* 2006); leaves: *S. aureus. E. coli, P. aeruginosa, K. aeruginosa, C. albicans, A. niger K. pneumoniae, S. typhi, E. faecalis* (Badmanaban 2010, Nagaraja *et al.* 2011)	Leaves, seeds, fruits: methanol extract (Goji *et al.* 2006); leaves: ethanol, water extracts (Badmanaban 2010), petroleum ether, chloroform, ethanol, water extracts (Nagaraja *et al.* 2011)
Lasia spinosa	Leaf paste is applied topically to treat skin diseases	Aerial parts: *S. aureus* (Kichu 2010)*; leaves: *S. aureus, E. coli, P. aeruginosa, B. cereus. B. subtilis, S. paratyphi, S. typhi, S. boydii, S. dysenteriae, V. mimicus, C. albicans, A. niger* (Goshwami *et al.* 2013)	Aerial parts: aqueous ethanolic extract (Kichu 2010)*; leaves: petroleum ether, dichloromethane, ethyl acetate, water extracts (Goshwami *et al.* 2013)
Maesa indica	Leaf paste is applied topically to cuts and wounds	None found	None found
Mentha cordifolia	Leaf paste is applied topically to fresh cuts and skin diseases	WR Leaves: *B. subtilis, P. aeruginosa, C. albicans, A. niger* (Ragasa *et al.* 2001)	Leaves: 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone, piperitenone epoxide (Ragasa *et al.* 2001)

Mikania cordata	Leaf paste is applied topically to treat skin diseases and cuts	WR Leaves: *S. aureus, S. typhi, S. sonnei, S. pyogenes, S. epidermidis, P. aeruginosa, E. coli, C. albicans* (Hamill *et al.* 2003, Nayeem *et al.* 2011)	Leaves: ethanol extract (Hamill *et al.* 2003, Nayeem *et al.* 2011)
Mussaenda roxburghii	Fresh leaf paste is applied topically to cuts and wounds	Leaves: *B. cereus, B. subtilis, S. aureus, S. lutea, E. coli, P. aeruginosa, S. typhi, S. paratyphi, S. boydii, S. dysenteriae, V. mimicus, V. parahemolyticus* (Hossain *et al.* 2013); aerial parts: *S. aureus, E. coli* (De Utpal *et al.* 2012)	Leaves: methanol extract (Hossain *et al.* 2013); aerial parts: shanzhiol (De Utpal *et al.* 2012)
Myrica esculenta	Leaf paste is applied to cuts and wounds	WR Stem bark: *S. aureus, E. coli, P. aeruginosa, B. subtilis S. epidermidis, C. albicans, A. niger* (Mahato and Chaudhary 2005, Agnihotri *et al.* 2012); leaves: *S. aureus, E. coli, S. epidermidis* (Bamola *et al.* 2008)	Stem bark: methanol extract (Mahato and Chaudhary 2005), essential oils (Agnihotri *et al.* 2012); leaves: ethanol extract (Bamola *et al.* 2008)
Nephrolepis cordifolia	Fresh tubers are crushed and applied topically or a slice of tuber is rubbed into the affected areas for the treatment of skin infections	Leaves, tuber: *S. aureus* (Kichu 2010)*; fronds: *E. coli, P. aeruginosa, B. subtilis, B. cereus, S. typhimurium, S. faecalis, K. pneumoniae, E. aerogenes* (Rani *et al.* 2010)	Leaves, tuber: aqueous ethanolic extract (Kichu 2010)*; fronds: water extract (Rani *et al.* 2010)
Piper betel	Leaf paste is applied topically to cuts and wounds	WR Leaves: *S. aureus, E. coli, P. aeruginosa, B. subtilis, B. cereus, P. mirabilis, P. vulgaris, S. agalactiae, S. faecalis, E. aerogenes, S. typhimurium, K. pneumoniae C. albicans, A. niger* (Nair and Chanda 2008, Akter *et al.* 2014, Saxena *et al.* 2014)	Leaves: methanol and ethanol extracts (Nair and Chanda 2008, Datta *et al.* 2011), (Akter *et al.* 2014), essential oil (Saxena *et al.* 2014)
Polygonum hydropiper	Leaf paste is applied topically to treat fungal infections and scabies	WR Aerial parts: *S. aureus* (Kichu 2010)*; roots: *S. aureus, E. coli, P. aeruginosa, B. subtilis, S. typhi, S. sonnei, E. aerogenes, C. albicans, A. niger* (Hasan *et al.* 2009); leaves: *S.*	Roots: chloroform extract (Hasan *et al.* 2009); leaves - confertifolin (Duraipandiyan *et al.* 2010)

		aureus, E. coli, P. aeruginosa, B. subtilis, S. epidermidis, E. faecalis, K. pneumoniae, Erwinia spp., *P. vulgaris* (Duraipandiyan *et al.* 2010)	
Prunus persica	Fresh roots are soaked in water overnight and used to treat skin infections. Leaf juice extract is applied topically to treat skin diseases.	Roots: *S. aureus* (Kichu 2010)*	Roots: aqueous ethanolic extract (Kichu 2010)*
Stereospermum chelonoides	Bark paste is applied to treat cuts and skin diseases	WR Stem bark: *S. aureus, E. coli, P. aeruginosa, B. cereus, B. subtilis, S. lutea, S. typhi, S. paratyphi, S. boydii, S. dysenteriae, V. mimicus, V. parahemolyticus, C. albicans, A. niger* (Haque *et al.* 2007)	Stem bark: hexane, chloroform extracts (Haque *et al.* 2007), stereochenol A, stereochenol B, caffeic acid (Haque *et al.* 2006, Kaisa 2011)
Tagetes erecta	Whole plant infusion used to treat boils and skin infection	WR Whole plant: MRSA; roots: *S. aureus, E. coli, P. aeruginosa, C. albicans, A. niger* (Chomnawang *et al.* 2009, Chatterjee and Ali 2010)	Whole plant: ethanol extract (Chomnawang *et al.* 2009); roots: petroleum ether, chloroform, ethyl acetate, methanol, water extracts - bithienyl (Gupta and Vasudeva 2010, Dasgupta *et al.* 2012, Lim 2014)

*Studies performed within our research group. WR - widely referenced. Bacteria: *B. cereus - Bacillus cereus, B. subtilis - Bacillus subtilis, C. perfringens - Clostridium perfringens, E. aerogenes - Enterobacter aerogenes, E. coli - Escherichia coli, E. tarda - Edwardsiella tarda, H. pyroli - Helicobacter pylori, K. aerogenes - Klepsiella aerogenes, K. pneumoniae - Klebsiella pneumoniae, M. pyogenes - Micrococcus pyogenes, P. acnes - Propionibacterium acnes, P. aeruginosa - Pseudomonas aeruginosa, P. vulgaris - Proteus vulgaris, S. choleraesuis - Salmonella choleraesuis, S. enteritidis - Salmonella enteritidis, S. typhi - Salmonella typhi, S. paratyphi - Salmonella paratyphi, S. typhimurium - Salmonella typhimurium, S. lutea - Sarcinia lutea, S. boydii - Shigella boydii, S. dysenteriae - Shigella dysenteriae, S. flexneri - Shigella flexneri, S. sonnei - Shigella sonnei, S. aureus - Staphylococcus aureus*, MRSA - methicillin resistant *Staphylococcus aureus*, MDRSA - multi drug resistant *Staphylococcus aureus, S. epidermidis - Staphylococcus epidermidis, S. haemolyticus - Staphylococcus haemolyticus, S. agalactiae - Streptococcus agalactiae, S. pneumoniae - Streptococcus pneumoniae, S. pyogenes - Streptococcus pyogenes, S. viridans - Streptococcus viridans, V. cholera - Vibrio cholera, V. mimicus - Vibrio mimicus, V. parahaemolyticus - Vibrio parahaemolyticus*. Fungi: *A. niger - Aspergillus niger, C. albicans - Candida albicans.*

The literature review revealed that thirty two (89%) of the plants used by the Chungtia villagers topically for ailments of a likely microbial aetiology have already been documented for their antimicrobial activities and thirteen of them have had their antimicrobially active compounds isolated. This validates and strongly supports the reliance of Chungtia villagers on these plants as a treatment for sores, wounds and other skin related ailments. This review identified three plants, namely *Albizia lucidior*, *Holboellia latifolia* and *Maesa indica* that have not been described in the literature as possessing antimicrobial properties. A further ten species, namely *Begonia picta, Cyclea peltata, Dendrocnide sinuata, Duabanga grandiflora, Erythrina stricta, Eurya acuminata, Hedyotis scandens, Ipomoea nil, Mussaenda roxburghii* and *Prunus persica* were found to have limited reports on their antimicrobial activities. Moreover, the compounds responsible for these activities have not been determined. These thirteen plants were therefore regarded as valuable for further antimicrobial investigations. Antibacterial activity of *B. picta* (leaves), *C. floribunda* (Leaves), *E. stricta* (bark), *L. spinosa* (leaves) and *P. persica* (roots) 70% aqueous ethanolic extracts have been previously screened within our research group by Kichu (Kichu 2010). *E. stricta* and *P. persica* were found to have very good antibacterial properties against a susceptible strain of *S. aureus* (MIC = 0.156 mg/mL), while *B. picta, C. floribunda* and *L. spinosa* had moderate antibacterial properties (MIC = 0.612 mg/mL) against the same strain. None of the plants showed activity against *E. coli* and *P. aeruginosa*. Chemical constituents responsible for *E. stricta* activity were partly isolated, but active compounds of *P. persica* were not identified (Kichu 2010). Thus, based on this result, both plants remained on the list for further screening against susceptible as well as antibiotic resistant microbial strains.

3.3 Selection of plants for antimicrobial screening

Following the identification of the above thirteen plants of interest, seven species (*D. sinuata, D. grandiflora, E. stricta, E. acuminata, H. latifolia, M. indica* and *P. persica*) were short listed for collection within Nagaland. Their choice was based on their ease of access to the Chungtia villagers for collection and relative availability in reasonable quantities for initiating laboratory investigations. All the chosen plants are highly regarded for their medicinal properties by the Chungtia community members. The next section provides some salient botanical and ethnomedicinal features of these seven plants.

3.4 Detailed literature review of the seven selected plants

3.4.1 *Dendrocnide sinuata*

Figure 3.1 ***Dendrocnide sinuata*** photo Meyanungsang Kichu (Bl.) (Urticaceae) *Zaklojawa* (Kichu 2010).

Dendrocnide is a genus of thirty seven species in the nettle family Urticaceae. It comprises from evergreen shrubs to large trees. The genus is widely distributed across South-East Asia, Australia and the Pacific Islands (Chew 1969, Mabberley 2008). Several of the plants are known to sting violently, producing urticaria and shock (Everist 1972). Young stems and branches of this genus are densely covered with stinging hairs. Leaves are elliptical in shape with dentate margins and sparsely covered with stinging hairs on veins.

Dendrocnide sinuata is a dull green, perennial herb. Its minute rigid hairs or prickles excrete a venomous fluid when pressed. The stem is obtusely four-angled, arising from a branching root with flashy shoots and many fibres. The leaves are opposite and armed with stings. The flowers are white and bell shaped (Tanti *et al.* 2011).

In Chungtia village the local name of the plant is *Zaklojawa.* It is utilised as a treatment for fresh cuts and wounds, with the fresh outer stem applied on affected areas. Caution is recommended when used as the plant produces an extremely painful sting (Kichu 2010). The antibacterial activity of the leaves of this plant was reported fairly recently. The methanol and water extracts were found to be active against *S. aureus, E. coli* and *P. aeruginosa*, but the bioactive compounds responsible for the antibacterial activity are still to be isolated (Tanti *et al.* 2011). Moreover, the stem of the plant, which is used by the Chungtia tribe for their medicinal purposes has not been antimicrobially screened.

D. sinuata has been widely applied for a variety of diseases, including skin related disorders, by many tribes of India. Tribal communities of Western Mizoram use root decoctions boiled with crabs to treat jaundice (Lalfakzuala *et al.* 2007). The plant is well known and utilised by the communities of Arunachal Pradesh. The Nyishi community of this region use the powdered plant roots in the form of a paste applied against swollen muscles. It is also used to treat injuries and itching. Leaves of *D. sinuata* can be mixed with leaves of *Stephania glabra* (2:1) and boiled with water. 1-2 teaspoons of the decoction is administered as a remedy for fever and malaria. The Apani community of Arunachal Pradesh use young boiled leaves. The decoction is administered in case of urinary disorder or reddish urine; it is also used as a remedy for dysentery (Srivastava *et al.* 2010, Tanti *et al.* 2011). The Adibasi community of Assam use leaves and roots of *D. sinuata* ground together to a paste to treat painful boils (Tanti *et al.* 2011). The Karbis community of Assam use juice of the leaves to treat chronic fever. The Khashi community of Meghalaya use a paste of the roots and leaves to cure swelling and blind abscesses (Tanti *et al.* 2011) and the Bodo community of Assam use the roots to expel intestinal worms (Rajeswar Pegu 2013). The community of Sanitpur (Assam) use roots ground to a paste and mixed with curcuma powder to treat septic ulcers (Chutia 2012). The methanol extract of the leaves have been reported to possess antibacterial activity as well as very good antioxidant activity, but chemical constituents responsible for these properties have not been reported (Tanti *et al.* 2011).

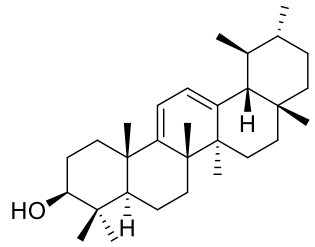
3.4.2 *Duabanga grandiflora*

Figure 3.2 ***Duabanga grandiflora*** (Roxb. ex DC.) Walp. (Sonneratiaceae) *Kisati* photo Meyanungsang Kichu (Kichu 2010).

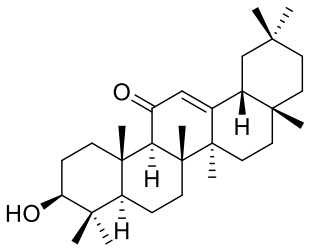
Duabanga is a small genus of lowland evergreen rainforest trees distributed throughout South-East Asia. *Duabanga grandiflora* Roxb. ex DC Walp. (Sonneratiaceae) (Figure 3.2) grows to 10-25 m with an erect undivided trunk. The lower limbs are long, sparingly branched and loosely covered with large spreading leaves. The leaves are often recurved, and are deep green above, and almost white beneath. The large blossoms expand in April, with a rank odour when they first burst, but become inodorous before the petals drop. The fruit is the size of a small apple. The wood is white and soft (Hooker 1855). The local Chungtia name of the plant is *Kisati*.

Chungtia villagers use fresh bark of *D. grandiflora*, which is scraped off and applied topically to cure skin diseases, cuts and wounds (Kichu 2010). Aqueous ethanolic (70%) and ethyl acetate extracts of the whole plant have been shown to possess antibacterial activity against *S. aureus* and *E. coli*, but the compounds responsible have not been isolated (Othman *et al.* 2011). The plant bark has not been screened for its antimicrobial properties.

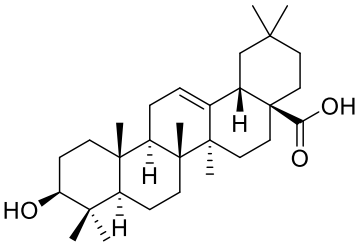
Apart from being utilised as a skin related remedy, *D. grandiflora* has widespread medicinal uses by many Indigenous communities. The stem and root juice is used by the Chittagong community in Bangladesh to treat upset stomach (Biswas *et al.* 2010). It is reported that the hill tribes of Northern Thailand use poultices from its leaves to treat stomach pain (Auamcharoen *et al.* 2009). *D. grandiflora* leaves have also been used as a traditional Thai medicine to treat various ailments of human skin (Tsukiyama *et al.* 2010). It has been reported in pharmacological research that methanol extracts of the leaves cause skin whitening and possess anti-aging and anti-inflammation properties. The compound eugeniin, which has strong activity for type III collagen production, has been isolated from the extract (Tsukiyama *et al.* 2010). The stem bark has been shown to contain 3β-hydroxyursane-9(11),12-diene, 3β-hydroxy-12-oleanen-11-one, oleanolic acid, 3-*O*-(*E*)-coumaroyloleanolic acid, 3β-hydroxyolean-9(11),12-diene, hyptatic acid A, betulinic acid, divergioic acid, (*E*)-5-(2-formylvinyl)-7-methoxy-2 (3,4 methylenedioxyphenyl) benzofuran, vanillin, β-sitosterol, β-sitosterol glucoside and 3-hydroxy-4-methoxycinnamaldehyde (Figure 3.3) (Kaweetripob *et al.* 2012).



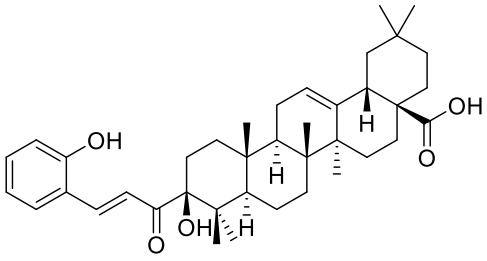
3-beta-Hydroxyursane-9(11),12-diene



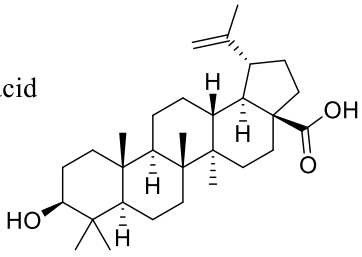
3-beta-Hydroxy-12-oleanen-11-one



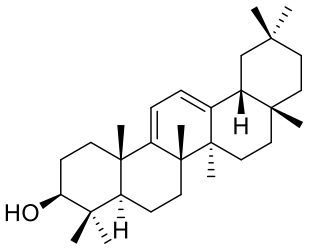
Oleanolic acid



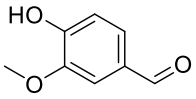
3-O-(*E*)-Coumaroyloleanolic acid



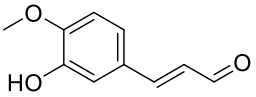
Betulinic acid



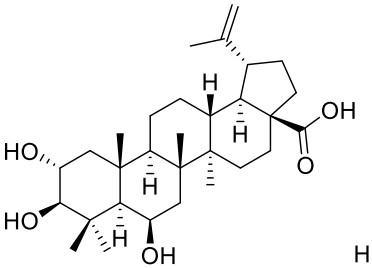
3beta-Hydroxyolean-9(11),12-diene



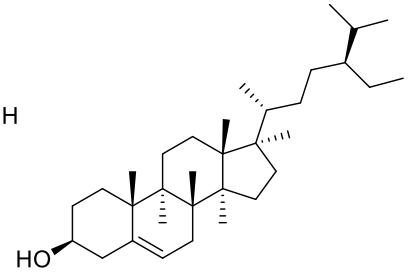
Vanillin



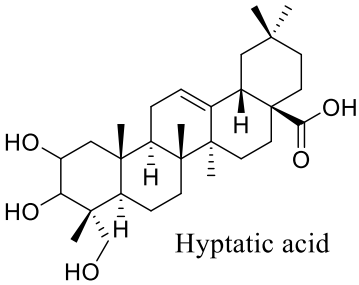
3-Hydroxy-4-methoxycinnamaldehyde



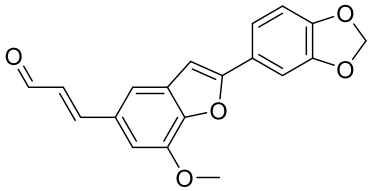
Divergioic acid



beta-Sitosterol



Hyptatic acid



(E)-5-(2-Formylvinyl)-7-methoxy-2 (3,4 methylenedioxyphenyl) benzofuran

Figure 3.3 Chemical constituents of *D. grandiflora*.

3.4.3 *Erythrina stricta*

Figure 3.4 ***Erythrina stricta*** Roxb. (Fabaceae) *Lochet* photo Meyanungsang Kichu (Kichu 2010).

The genus *Erythrina* comprises of more than 100 species distributed in tropical and subtropical regions of the world. *Erythrina stricta* Roxb. (Fabaceae) (Figure 3.4) is a large flowering tree belonging to the family Leguminosae. The bark of *E. stricta* is grey and armed with whitish prickles. Leaves of the plant are stalked and trifoliate and shiny on the upper side. The flowers are arranged into dense clusters with spindle shaped pods. The seeds are red and kidney shaped. *E. stricta* is distributed throughout Nepal, India, Tibet and China (Manandhar 2002).

The plant in Nagaland is locally called *Lochet*. The Chungtia villagers use the stem bark topically as a paste or as an infusion to treat various skin related conditions such as contact dermatitis, eczema and skin infections (Kichu 2010). Antimicrobial activity reports in the published literature are relatively recent and very scarce. *n*-Hexane and ethyl acetate fractions of the stem bark have been reported to be active against *S. aureus, E. coli, P. aeruginosa, B. subtilis, B. cereus, S. paratyphi, S. typhi, S. boydii, S. dysenteriae, V. mimicus, V. parahemolyticus, C. albicans* and *A. niger* (Hussain *et al.* 2011) and the whole plant ethanol extract has been reported to be active against *E. coli* and *B. subtilis* (Sharma 2013).

Our research group has reported antibacterial activity of the plant (stem bark, 70% aqueous ethanolic extract) against antibiotic susceptible, methicillin resistant (MRSA) and multi drug resistant (MDRSA) strains of *S. aureus* and has identified some of the compounds responsible for its antibacterial properties such as erystagallin A and erythrabyssin II. These

compounds have shown very good activity against all tested *S. aureus* strains (Kichu 2010). The plant merits further study, due in part to the wide and diverse range of medicinal uses cited throughout India and south Asia.

The Lotha-Naga tribes of the Wokha district of Nagaland use stem bark paste of *E. stricta* to treat rheumatism, stomach ache, asthma, dysentery and epilepsy (Jamir and Takatemjen 2010). The Mikirs Indigenous people inhabiting the Karbi-Anglong district of India, use the flowers, which are pounded and taken as a tonic. A lotion made by burning the wood is applied on facial inflammation (Jain and Borthakur 1980). The Garasia tribe of the Sirohi district, Rajahstan use extracts of fresh or dried flowers orally to cause abortion (Meena and Yadav 2011). The people of Assam apply the root paste on gout. The root juice mixed with milk is given orally to treat gout (Nath *et al.* 2011). Gorkha district people of Nepal use the squeezed juice of the bark of *Garuga pinnata* mixed with equal parts of the bark of *E. stricta* and *Ficus semicordata* to treat stomach disorders (Manandhar 1990). The leaves of *E. stricta* are frequently used in Indian traditional medicinal systems for the treatment of joint pain and related inflammatory disorders (Umamaheswari *et al.* 2009). These medicinal uses have been validated by pharmacological investigations that have identified some of the active constituents and which have quantified their medicinal effects. For example, *E. stricta* leaves were proven to exhibit cardioprotective effects on isoproterenol induced myocardial infarction in rats (Kuppusamy *et al.* 2010). *n*-Hexane and chloroform extracts of *E. stricta* roots were reported to possess antiplasmodial, antimycobacterial and cytotoxic activity and the compounds responsible for these activities, namely erythrabissin-I, erythrabyssin II, erystagallin A and 5-hydroxysophoranone were isolated (Figure 3.5) (Rukachaisirikul *et al.* 2007).

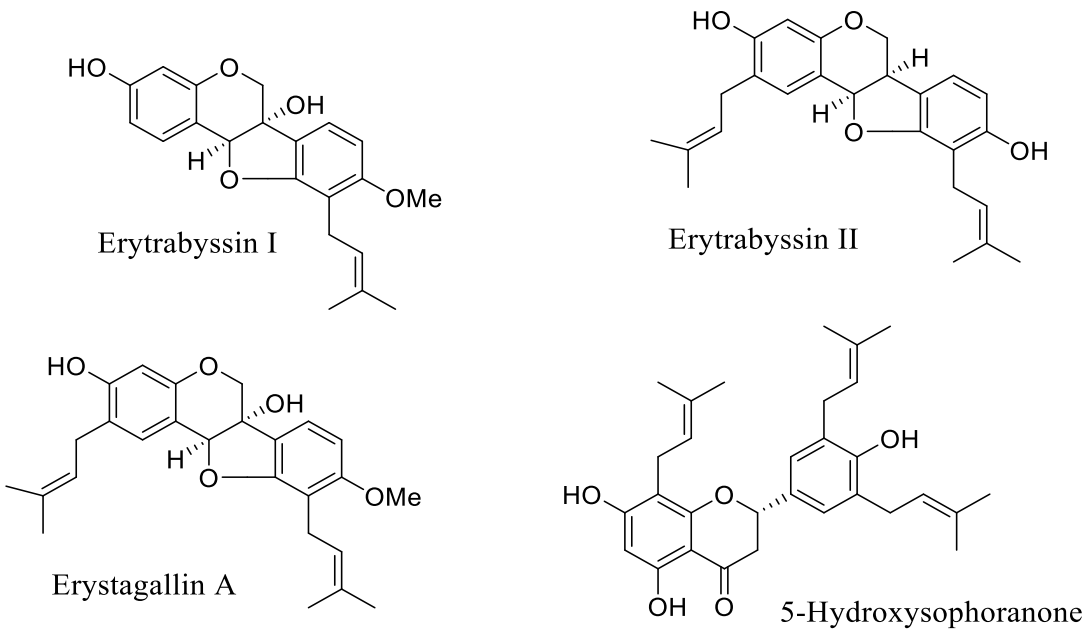


Figure 3.5 Chemical constituents of *E. stricta.*

3.4.4 *Eurya acuminata*

Figure 3.6 ***Eurya acuminata*** DC. (Theaceae) *Mesetwa* photo Meyanungsang Kichu (Kichu 2010).

The genus *Eurya* includes about 50 species distributed throughout South-East Asia and the islands of the Pacific. Members of the genus are commonly dioecious shrubs and small trees. The unisexual flowers are usually in dense clusters in the axils of leaves (Herat and Theobald 1977). *Eurya acuminata* DC. (Theaceae) (Figure 3.6) is a tree growing to a height of 3 m with a straight trunk and reddish brown, smooth to slightly fissured bark. Flowers are dioecious, either male or female borne, on similarly unisexual plants. The fruit is berry-like, fleshy and purple-black. Leaves are alternate and lanceolate (Herat and Theobald 1977).

In Nagaland the plant is known as Masetwa. Chungtia villagers use the plant leaves as an infusion orally to treat dysentery and diarrhoea. In addition, the leaf paste is applied topically to cuts and wounds. Leaves and stem aqueous ethanolic extracts have been shown to be active against *S. aureus* (Grosvenor *et al.* 1995). Even though the plant is well documented for its healing skin properties and has a wide variety of other ethnomedicinal uses, the cited Grosvenor *et al.* publication related to its antibacterial properties has been the only one found. Moreover, the plant chemical composition has not been investigated.

As mentioned above, *E. acuminata* is utilised as a treatment of many types of diseases, including skin disorders by various tribal communities worldwide. The village communities of Kali Gandaki, Bagmati and Tadi Likhu watersheds of Nepal use the plant for the treatment of skin diseases, for example, the juice of the leaves is used to cure itches and scabies (Joshi and Joshi 2007). Communities of Papua New Guinea chew the leaves to prevent cough and in China leaves and fruit are eaten to aid digestion (Holdsworth and Sakulas 1986). People of Uttaranchal State, India use the leaves to treat stomach disorders, dysentery, diarrhoea and cholera (Adhikari *et al.* 2007). The Nyishi community of Arunachal Pradesh use a decoction of the leaves as a permanent dye (Srivastava 2010). The root extract of *E. acuminata* is used to treat diarrhoea by Chakma Communities of Chittagong Hill Tracts of Bangladesh (Khisha *et al.*). The Raji tribal community of Uttaranchal Champawat and Pithoragarh districts use the bark of the plant, ground into a paste and taken as a remedy for scurvy and various skin diseases (Negi *et al.* 2002).

3.4.5 *Holboellia latifolia*

Figure 3.7 ***Holboellia latifolia*** Wall. (Lardizabalaceae) *Mezetsuk* photo Meyanungsang Kichu (Kichu 2010).

Holboellia is a genus of flowering plants in the Lardizabalaceae family. There are twenty species in the genus, mostly perennial, evergreen vines, although some are deciduous; all are restricted to the Himalayas and China. The flowers are monoecious, *ie* separate male and female flowers are produced on the same plant. *Holboellia latifolia* Well. (Lardizabalaceae) (Figure 3.7), locally known by the Chungtia villagers as *Mezetsuk*, is a vigorous, frost resistant, evergreen climber. The trunk of the plant grows up to 15 cm thick with vertically fissured corky bark when old. Branches and twigs are glabrous, striated, often twining at the tips. Leaves are compound, usually 3-5-foliolate and petiolate (La Dell 2008). Clusters of small, greenish-white (male) and purple (female) flowers are produced on the same plant. Flowering occurs in spring. *H. latifolia* is a common horticultural variety, mostly grown for its dark green foliage and edible fruits (Christenhusz 2012). The fruit is a berry, oblong or ovate-oblong, with usually a rounded base and apex, flesh-coloured or rosy-purple with many-seeds. The seeds are almost orbicular and straight, embedded in pulp (Wang *et al.* 2009).

The Chungtia community apply foam from crushed leaves to burns as a demulcent (Kichu 2010). No biological activities or phytochemical studies for *H. latifolia* have been reported. Further study of this species is justified because, in addition to its cited use by the Chungtia villagers, other villagers of the North-Eastern portion of India, comprising the states of Arunachal Pradesh, Assam, Manipur, Meghalaya and Mizoram, use juice from the ground leaves to treat burns (Begum and Nath 2000).

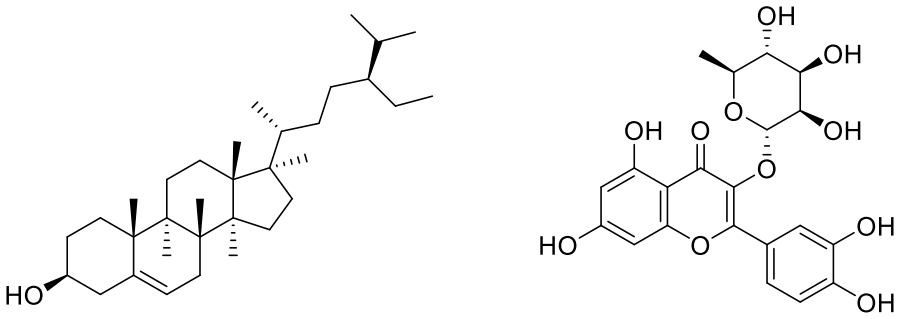
3.4.6 *Maesa indica*

Figure 3.8 ***Maesa indica*** (Roxb.) Wall. (Myrsinaceae) *Kensametong* photo Meyanungsang Kichu (Kichu 2010).

Maesa is a genus in the family Myrsinaceae. Plants of this genus are shrubs or trees. The inflorescence is composed of axillary panicles. The corolla is campanulate (bell-shaped), marked with glandular dots or lines. Fruit can be dry or fleshy, spherical and crowned with persistent sepals and styles. Seeds are numerous (Caris *et al.* 2000, Ståhl and Anderberg 2004). *Maesa indica* Roxb. Wall (Figure 3.8), locally known by the Chungtia villagers as *Kensametong*, is a large, evergreen shrub, with thin bark (Srinivasan 1955). The leaves are ovate to elliptic-lanceolate, serrate, and acute, with the base rounded to narrow. Flowers are bisexual, small, 4-5 mm across, and white (Utteridge and Saunders 2004). The corolla lobes are ovate, obtuse or rotate, minutely dentate at margins. The berries are whitish, surrounded by a persistent calyx. The shrub has soft, brownish wood that is used as a fuel. Flowering occurs from January to April (Caris *et al.* 2000).

The Chungtia villagers apply the leaf paste of *M. indica* topically to treat cuts and wounds (Kichu 2010). No antimicrobial activity of *M. indica* has been reported in the literature to date, but antimicrobial properties of the genus have been reported. Thus, the plant could be worthy of investigation from a chemotaxonomic point of view.

M. indica has widespread medicinal citations. The Yao people of China use a decoction of the leaves to treat numbness of limbs and hepatitis (Long and Li 2004). The Kani tribe from Tamil Nadu apply leaf juice externally to stimulate hair growth. Combined juice of the leaves, roots bark and unripened fruits is applied on the body before bathing to increase body resistance to disease (Ayyanar and Ignacimuthu 2005). The Lotha-Naga tribes of Nagaland eat the fruits to expel intestinal parasites (Jamir and Takatemjen 2010). The Chakma community of Arunachal Pradesh use a decoction of leaves for bathing during fever (Sarmah *et al.* 2008). *M. indica* has been reported to exhibit antiviral activity against Ranikhet disease and vaccinia viruses (Sindambiwe *et al.* 1998). Antibacterial, anti-inflammatory, antiviral, molluscicidal and antioxidant activity have been reported for the *Maesa* genus (Sindambiwe *et al.* 1998). β-Sitosterol and quercetin-3-rhamnoside have been isolated from *M. indica* leaves (Figure 3.9) (Kuruvilla *et al.* 2010).



beta-Sitosterol

Quarcetin-3-rhamnoside

Figure 3.9 Chemical constituents of *M. indica.*

3.4.7 *Prunus persica*

Figure 3.10 ***Prunus persica*** (L.) Stokes (*Rosaceae*) *Mokori* photo Meyanungsang Kichu (Kichu 2010).

Prunus persica L. Stokes (*Rosaceae*) (Figure 3.10), known by Chungtia villagers as *Mokori*, is a deciduous tree of the subfamily Prunoideae of the family Rosaceae. It grows to a height of 5 to 10 m and is commonly cultivated in West Asia, Europe, Himalayas and India up to an altitude of approximately 300 m. The leaves are linear with acute tips and finely serrated margins. The flowers are produced in early spring before the leaves; they are solitary or paired, pink and with five petals. The fruit has yellow or whitish flesh and a delicate aroma. The single, large seed is red-brown, oval shaped and is surrounded by a wood-like husk.

The Chungtia villagers consume the liquid from fresh roots soaked in water to treat typhoid and the seed endosperm to treat dysentery and diarrhoea. The liquid from the roots and aqueous decoctions of the leaves are also used to treat skin related infections (Kichu 2010). All plant parts have been reported for various pharmacological properties, with the

exclusion of the roots. Moreover, antibacterial activity of the plant has not been reported for any part of the species, except by Kichu (Kichu 2010).

Nearly all parts of *P. persica* have been used ethnomedicinally (Lim 2012). In Pakistan, the flowers are considered a purgative, while decoctions of the leaves are used as an anthelmintic, laxative and sedative (Gilani *et al.* 2000). Tribal communities of the North-West Frontier Province of Pakistan apply crushed, fresh leaves directly onto wounds to assist healing and alleviate burning sensations (Abbasi *et al.* 2010). Communities of the Baramulla district of India use a decoction of the leaves to treat abdominal pain (Yousuf *et al.* 2013), while Manipuri folklore healers of India use the leaf decoction to treat hypertension (Lokesh and Amitsankar 2012). The Shinasha, Agew-awi and Amhara peoples of North-West Ethiopia use seeds of the plant to treat malaria (Giday *et al.* 2007). *P. persica* flowers are applied in Asian countries to whiten skin and to treat skin disorders (Kim *et al.* 2002). The seeds are commonly used in Japan and China as an ingredient in Kampo (Chinese medicine) prescriptions to treat a great variety of disorders such as women's diseases including premenstrual syndrome and threatened abortion in early pregnancy (Lee *et al.* 2008), as well as degenerative disorders such as hypermenorrhea, dysmenorrhea, leiomyoma and infertility (Sakamoto *et al.* 1992). The plant seeds are also a part of a Kampo prescription for the treatment of "Oketsu" (stagnation of blood circulation) syndrome (Kosuge *et al.* 1984), rheumatic heart disease, chronic kidney disease, Parkinson's disease (Yao *et al.* 2013) and breast cancer (Liu 2012). The plants' traditional medicinal applications have been validated by the findings of anticancer (Fukuda *et al.* 2003), hepatoprotective, nephroprotective (Lee *et al.* 2008), anti-inflammatory (Rho *et al.* 2007), acetylcholine esterase inhibitory (Suh *et al.* 2006), estrogenic and anti-estrogenic (Kim *et al.* 2008), cardioprotective, anti-hyperglyceamic (Kono *et al.* 2013), spasmogenic, spasmolytic (Gilani *et al*., 2000), UV-protective and antioxidant (Vattem and Shetty 2005, Wu *et al.* 2011) bioactivities. Phytochemical screening has also revealed the presence of many compounds related to the reported activities. These include alkaloids (persicaside), cyanogenic glycosides (amygdalin and prunasin), phenolic acids (caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid), tannins (quinic acid) and flavonol glycosides (isoquercetin, multiflorin B and trifolin) (Figure 3.11).

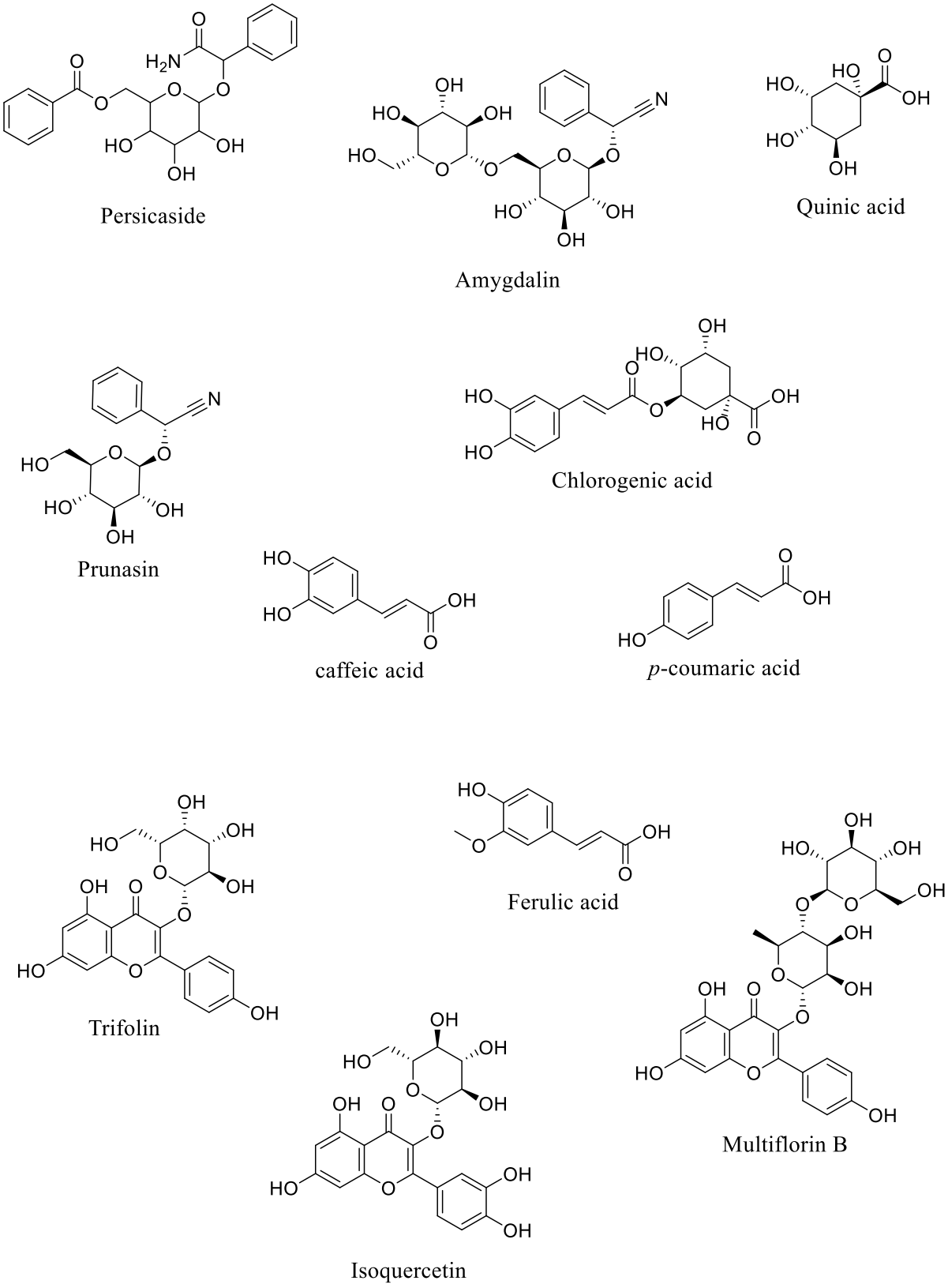


Figure 3.11 Chemical constituents of *P. persica.*

3.5 Conclusions

A literature review of thirty five plants used by Chungtia villagers for skin infections, sores and wounds of a likely microbial origin was conducted on their antimicrobial activities and antimicrobial constituents reported. *Holboellia latifolia* and *Maesa indica* had no reports on antimicrobial activity or their antimicrobial chemical constituents. Although *Dendrocnide sinuata, Duabanga grandiflora* and *Eurya acuminata* are known to possess antibacterial activity, the relevant bioactive constituents have not been isolated. These plants were therefore regarded as worthy of further investigation for their antimicrobial activities and bioactive constituents. For *Prunus persica* and *Erythrina stricta*, antibacterial activity was reported by Kichu (Kichu 2010). Some constituents responsible have been isolated from *E. stricta* but not from *P. persica*.

Abbasi, A. M., M. Khan, M. Ahmad, M. Zafar, S. Jahan and S. Sultana (2010). "Ethnopharmacological Application of Medicinal Plants to Cure Skin Diseases and in Folk Cosmetics among the Tribal Communities of North-West Frontier Province, Pakistan." *Journal of Ethnopharmacology* **128**(2): 322-335.

Abraham, J. and T. D. Thomas (2012). "Antibacterial Activity of Medicinal Plant *Cyclea peltata* (Lam) Hooks & Thoms." *Asian Pacific Journal of Tropical Disease* **2**(2): 280-284.

Adhikari, B. S., M. M. Babu, P. L. Saklani and G. S. Rawat (2007). "Distribution, Use Pattern and Prospects for Conservation of Medicinal Shrubs in Uttaranchal State, India." *Journal of Mountain Science* **4**(2): 155-180.

Agnihotri, S., S. Wakode and M. Ali (2012). "Essential Oil of *Myrica esculenta* Buch. Ham.: Composition, Antimicrobial and Topical Anti-Inflammatory Activities." *Natural Product Research* **26**(23): 2266-2269.

Akinpelu, D. A. (2000). "Antimicrobial Activity of *Bryophyllum pinnatum* Leaves." *Fitoterapia* **71**(2): 193-194.

Akter, K. N., P. Karmakar, A. Das, S. N. Anonna, S. A. Shoma and M. M. Sattar (2014). "Evaluation of Antibacterial and Anthelmintic Activities with Total Phenolic Contents of *Piper betel* Leaves." *Avicenna Journal of Phytomedicine* **4**(5): 320-329.

Almahyl, H. A., M. Rahmani, M. A. Sukari and A. M. Ali (2003). "Investigation on the Chemical Constituents of the Leaves of *Ficus elastica* Roxb. And Their Antimicrobial Activity." *Pertanika Journal of Science & Technology* **11**(1): 57-63.

Auamcharoen, W., A. Chandrapatya, A. Kijjoa, A. Silva and W. Herz (2009). "Chemical Constituents of *Duabanga grandiflora* (*Lythraceae*)." *Biochemical Systematics and Ecology* **37**(4): 535-537.

Audipudi, A. V. and B. V. Chakicherla (2010). "Antioxidative and Antimicrobial Activity of Methanol and Chloroform Extracts of *Gmelina arborea* Roxb." *International Journal of Biotechnology and Biochemistry* **6**(1): 139-144.

Ayyanar, M. and S. Ignacimuthu (2005). "Traditional Knowledge of Kani Tribals in Kouthalai of Tirunelveli Hills, Tamil Nadu, India." *Journal of Ethnopharmacology* **102**(2): 246-255.

Badmanaban, R. (2010). "Studies on Anthelmintic and Antimicrobial Activity of the Leaf Extracts of *Lagenaria siceraria* Mol." *Journal of Global Pharma Technology* **2**(4): 66-70.

Bamola, N., G. Bisht and L. Singh (2008). "*In vitro* Antibacterial Activity of Some Medicinal Plants of Garhwal." *Plant Archives* **8**(1): 133-137.

Bazzaz, B. and G. Haririzadeh (2003). "Screening of Iranian Plants for Antimicrobial Activity." *Pharmaceutical Biology* **41**(8): 573-583.

Begum, D. and S. C. Nath (2000). "Ethnobotanical Review of Medicinal Plants Used for Skin Diseases and Related Problems in Northeastern India." *Journal of Herbs, Spices & Medicinal Plants* **7**(3): 55-93.

Biswas, A., M. Bari, M. Roy and S. Bhadra (2010). "Inherited Folk Pharmaceutical Knowledge of Tribal People in the Chittagong Hill Tracts, Bangladesh." *Indian Journal of Traditional Knowledge* **9**(1): 77-89.

Biswas, S. K., A. Chowdhury, J. Das, U. K. Karmakar and M. C. Shill (2011). "Assessment of Cytotoxicity and Antibacterial Activities of Ethanolic Extracts of *Kalanchoe pinnata* Linn. (*Crassulaceae*) Leaves and Stems." *International Journal of Pharmaceutical Sciences and Research* **2**(10): 2605-2609.

Bowler, P. G. (2002). "Wound Pathophysiology, Infection and Therapeutic Options." *Annals of Medicine* **34**(6): 419-427.

Caris, P., L. P. R. Decraene, E. Smets and D. Clinckemaillie (2000). "Floral Development of Three Maesa Species, with Special Emphasis on the Position of the Genus within Primulales." *Annals of Botany* **86**(1): 87-97.

Chatterjee, S. and A. S. Ali (2010). "Studies on the Antibacterial Activity of *Tagetes erecta* Linn Leaves." *Archives of Pharmacal Research* **2**(2): 360-361.

Chew, W. (1969). "A Monograph of Dendrocnide (*Urticaceae*)." *The Gardens' Bulletin: Singapore* **25**: 111.

Chomnawang, M. T., S. Surassmo, V. S. Nukoolkarn and W. Gritsanapan (2005). "Antimicrobial Effects of Thai Medicinal Plants against Acne-Inducing Bacteria." *Journal of Ethnopharmacology* **101**(1): 330-333.

Chomnawang, M. T., S. Surassmo, K. Wongsariya and N. Bunyapraphatsara (2009). "Antibacterial Activity of Thai Medicinal Plants against Methicillin-Resistant *Staphylococcus aureus*." *Fitoterapia* **80**(2): 102-104.

Christenhusz, M. J. (2012). "An Overview of *Lardizabalaceae*." *Curtis's Botanical Magazine* **29**(3): 235-276.

Chutia, J. (2012). "A Review of Ethno Medicines of Assam: In Special Reference to Sonitpur, Assam." *Journal of Research in Conservation Biology* **1:** 016-028.

Dasgupta, N., S. Ranjan, P. Saha, R. Jain, S. Malhotra and M. Mohamed Saleh (2012). "Antibacterial Activity of Leaf Extract of Mexican Marigold (*Tagetes erecta*) against Different Gram Positive and Gram Negative Bacterial Strains." *Journal of Pharmacy Research* **5**(8): 4201-4203.

Datta, A., S. Ghoshdastidar and M. Singh (2011). "Antimicrobial Property of *Piper betel* Leaf against Clinical Isolates of Bacteria." *International Journal of Research in Pharmaceutical Sciences* **2**(3): 104-109.

De Utpal, C., R. Ghosh, S. Chowdhury and B. Dinda (2012). "New Iridoid from Aerial Parts of *Mussaenda roxburghii*." *Natural Product Communications* **7**(1): 1-2.

Duraipandiyan, V., F. Indwar and S. Ignacimuthu (2010). "Antimicrobial Activity of Confertifolin from *Polygonum hydropiper*." *Pharmaceutical Biology* **48**(2): 187-190.

Edwards, R. and K. G. Harding (2004). "Bacteria and Wound Healing." *Current Opinion in Infectious Diseases* **17**(2): 91-96.

El-Mahmood, A., J. Doughari and H. Kiman (2010). "*In vitro* Antimicrobial Activity of Crude Leaf and Stem Bark Extracts of *Gmelina arborea* (Roxb) against Some Pathogenic Species of *Enterobacteriaceae*." *African Journal of Pharmacy and Pharmacology* **4**(6): 355-361.

Everist, S. (1972). "Personal Communication to Mitchell Jc." *Mitchell J, Rook A (1979) Botanical Dermatology. Plants and plant products injurious to the skin*: 709-710.

Fang, H., Y. Xu, J. Lin, S. Bhattacharyya, N. Sen and K. Sethi (1990). Studies on the Chemical Components of Essential Oil of *Elsholtzia blanda* Benth. Proceedings of the 11th International Congress of essential oils, fragrances and flavours. New Delhi, India, 12-16 November, 1989 Vol. 4 Chemistry-analysis and structure., Aspect Publishing.

Fukuda, T., H. Ito, T. Mukainaka, H. Tokuda, H. Nishino and T. Yoshida (2003). "Anti-Tumor Promoting Effect of Glycosides from *Prunus persica* Seeds." *Biological and Pharmaceutical Bulletin* **26**(2): 271-273.

Giday, M., T. Teklehaymanot, A. Animut and Y. Mekonnen (2007). "Medicinal Plants of the Shinasha, Agew-Awi and Amhara Peoples in Northwest Ethiopia." *Journal of Ethnopharmacology* **110**(3): 516-525.

Gilani, A. H., N. Aziz, S. M. Ali and M. Saeed (2000). "Pharmacological Basis for the Use of Peach Leaves in Constipation." *Journal of Ethnopharmacology* **73**(1): 87-93.

Gnanamani, A., K. Shanmuga Priya, N. Radhakrishnan and M. Babu (2003). "Antibacterial Activity of Two Plant Extracts on Eight Burn Pathogens." *Journal of Ethnopharmacology* **86**(1): 59-61.

Goji, M., T. Gebre-Mariam, K. Asres, H. Lemma, N. Gemeda and K. Yirsaw (2006). "Screening of the Antimicrobial Activities of Some Plants Used Traditionally in Ethiopia for the Treatment of Skin Disorders." *Ethiopian Pharmaceutical Journal* **24**(2): 130-135.

Goshwami, D., M. M. Rahman, M. A. Muhit, M. S. Islam and M. Anasri (2013). "Antioxidant Property, Cytotoxicity and Antimicrobial Activity of *Lasia spinosa* Leaves." *Nepal Journal of Science and Technology* **13**(2): 215-218.

Grosvenor, P. W., A. Supriono and D. O. Gray (1995). "Medicinal Plants from Riau Province, Sumatra, Indonesia. Part 2: Antibacterial and Antifungal Activity." *Journal of Ethnopharmacology* **45**(2): 97-111.

Guo, Z., Z. Liu, X. Wang, W. Liu, R. Jiang, R. Cheng and G. She (2012). "*Elsholtzia*: Phytochemistry and Biological Activities." *Chemistry Central Journal* **6**(1): 147.

Gupta, P. and N. Vasudeva (2010). "*In vitro* Antiplasmodial and Antimicrobial Potential of *Tagetes erecta* Roots." *Pharmaceutical Biology* **48**(11): 1218-1223.

Habib, M. R. and M. R. Karim (2009). "Antimicrobial and Cytotoxic Activity of Di-(2-Ethylhexyl) Phthalate and Anhydrosophoradiol-3-Acetate Isolated from *Calotropis gigantea* (Linn.) Flower." *Mycobiology* **37**(1): 31-36.

Hamill, F., S. Apio, N. Mubiru, R. Bukenya-Ziraba, M. Mosango, O. Maganyi and D. Soejarto (2003). "Traditional Herbal Drugs of Southern Uganda, Ii: Literature Analysis and Antimicrobial Assays." *Journal of Ethnopharmacology* **84**(1): 57-78.

Haque, M. R., K. M. Rahman, C. M. Hasan and M. A. Rashid (2007). "Antimicrobial and Cytotoxic Activities of *Stereospermum chelonoides*." *Dhaka University Journal of Pharmaceutical Sciences* **5**(1): 71-72.

Haque, M. R., K. M. Rahman, M. N. Iskander, C. M. Hasan and M. A. Rashid (2006). "Stereochenols a and B, Two Quinones from *Stereospermum chelonoides*." *Phytochemistry* **67**(24): 2663-2665.

Hasan, M., R. Das, A. Khan, M. Hasan and M. Rahman (2009). "The Determination of Antibacterial and Antifungal Activities of *Polygonum hydropiper* (L.) Root Extract." *Advances in Biological Research* **3**(1): 53-56.

Hemavani, C. and B. Thippeswamy (2012). "Evaluation of Antimicrobial Activity of Root Extract of *Asclepias curassavica*." *Recent Research in Science and Technology* **4**(1).

Herat, T. R. and W. Theobald (1977). "Comparative Studies of Vegetative Anatomy in the Theaceae of Sri Lanka." *Botanical journal of the Linnean Society* **75**(4): 375-386.

Holdsworth, D. and H. Sakulas (1986). "Medicinal Plants of the Morobe Province Part Ii1. The Aseki Valley." *Pharmaceutical Biology* **24**(1): 31-40.

Hooker, J. D. J. F. C. a. W. H. F., Ed. (1855). Illustrations of Himalayan Plants. . London, L. Reeve.

Hossain, K., F. Islam, R. Kuddus and F. Latif (2013). "Actividad Antimicrobiana Preliminar Y Citotoxicidad De Extractos De Hojas De *Mussaenda roxburghii* Hook. F." *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* **12**(6): 612-617.

Hussain, M., U. Farooq, M. Rashid, H. Bakhsh, A. Aziz, A. Majeed and I. A. Khan (2014). "An Investigation of the Antimicrobial Activity of the Aqueous, Dichloromethane, Ethanol and Methanol Extract of the Seeds and Whole Plant of *Ipomoea nil*." *Journal of Pharmacy and Alternative Medicine* **3**(1): 14-25.

Hussain, M. M., M. M. R. Mughal, M. M. Alam, M. G. Dastagir, A. M. Billah and M. Ismail (2011). "Antimicrobial Activity of *n*-Hexane and Ethyl Acetate Extracts of *Erythrina stricta* Roxb." *Bangladesh Journal of Microbiology* **27**(2): 65-66.

Inya-Agha, S., B. Oguntimein, A. Sofowora and T. Benjamin (1987). "Phytochemical and Antibacterial Studies on the Essential Oil of *Eupatorium odoratum*." *Pharmaceutical Biology* **25**(1): 49-52.

Jain, S. and S. Borthakur (1980). "Ethnobotany of the Mikirs of India." *Economic Botany* **34**(3): 264-272.

Jamir, N. and L. Takatemjen (2010). "Traditional Knowledge of Lotha-Naga Tribes in Wokha District, Nagaland." *Indian Journal of Traditional Knowledge* **9**(1): 45-48.

Joshi, A. R. and K. Joshi (2007). "Ethnomedicinal Plants Used against Skin Diseases in Some Villages of Kali Gandaki, Bagmati and Tadi Likhu Watersheds of Nepal." *Ethnobotanical Leaflets* **2007**(1(27)): 235-246.

Kaisa, M. A. (2011). "A Review on Phytochemicals from Some Medicinal Plants of Bangladesh." *Journal of Pharmacy and Nutrition Sciences* **1**(1).

Kaweetripob, W., C. Mahidol, V. Prachyawarakorn, H. Prawat and S. Ruchirawat (2012). "5-Formylfurfuryl Esters from *Duabanga grandiflora.*" *Phytochemistry* **76**: 78-82.

Khan, M., A. Omoloso and M. Kihara (2003). "Antibacterial Activity of *Artocarpus heterophyllus.*" *Fitoterapia* **74**(5): 501-505.

Khisha, T., R. Karim, S. R. Chowdhury and R. Banoo (2012). "Ethnomedical Studies of Chakma Communities of Chittagong Hill Tracts, Bangladesh." *Bangladesh Pharmaceutical Journal* **15**: 59-67.

Kichu, M. (2010). Documentation and Biological and Phytochemical Analysis of Chungtia Medicinal Plants of Nagaland, India, Diss. *Chemistry and Biomolecular Sciences*, Macquarie University, Sydney. **PhD:** 1-179.

Kim, I. G., S. C. Kang, K. C. Kim, E. S. Choung and O. P. Zee (2008). "Screening of Estrogenic and Antiestrogenic Activities from Medicinal Plants." *Environmental Toxicology and Pharmacology* **25**(1): 75-82.

Kim, Y. H., H. E. Yang, B. K. Park, M. Y. Heo, B. K. Jo and H. P. Kim (2002). "The Extract of the Flowers of *Prunus persica*, a New Cosmetic Ingredient, Protects against Solar Ultraviolet-Induced Skin Damage *in vivo*." *Journal of Cosmetic Science* **53**(1): 27-34.

Kono, R., Y. Okuno, M. Nakamura, K.-i. Inada, A. Tokuda, M. Yamashita, R. Hidaka and H. Utsunomiya (2013). "Peach (*Prunus persica*) Extract Inhibits Angiotensin II-Induced Signal Transduction in Vascular Smooth Muscle Cells." *Food Chemistry* **139**(1): 371-376.

Kosuge, T., H. Ishida, H. Yamazaki and M. Ishii (1984). "Studies on Active Substances of the Herbs Used for Oketsu, Blood Coagulation, in Chinese Medicine. I. On Anticoagulant Activities of the Herbs Used for Oketsu." *Yakugaku Zasshi: Journal of the Pharmaceutical Society of Japan* **104**(10): 1050.

Kumar, B., M. Vijayakumar, R. Govindarajan and P. Pushpangadan (2007). "Ethnopharmacological Approaches to Wound Healing—Exploring Medicinal Plants of India." *Journal of Ethnopharmacology* **114**(2): 103-113.

Kumar, G., L. Karthik and K. V. B. Rao (2010). "Antibacterial Activity of Aqueous Extract of *Calotropis gigantea* Leaves–an *in vitro* Study." *International Journal of Pharmaceutical Sciences Review and Research* **4**(2): 141-144.

Kuppusamy, A., S. Varadharajan, C. Josey, H. John, D. Sherin, D. Chirakkan and G. Athiyappan (2010). "Cardioprotective Effect of *Erythrina stricta* Leaves on Isoproterenol-Induced Myocardial Infarction in Rat." *Bangladesh Journal of Pharmacology* **5**(1): 1-4.

Kurdekar, R. R., G. R. Hegde and S. S. Hebbar (2012). "Antimicrobial Efficacy of *Bridelia retusa* (Linn.) Spreng. And *Asclepias curassavica* Linn." *Indian Journal of Natural Products and Resources* **3**(4): 589-593.

Kuruvilla, G. R., M. Neeraja, A. Srikrishna, G. Subba Rao, A. Sudhakar and P. Venkatasubramanian (2010). "A New Quinone from *Maesa indica* (Roxb.) A. Dc (*Myrsinaceae*)." *Indian Journal of Chemistry. Section B, Organic Including Medicinal* **49**(12): 1637-1641.

La Dell, T. (2008). "17 Climbing Plants." *Plant User Handbook: A Guide to Effetive Specifying*: 211.

Lalfakzuala, R., H. Lalramnghinglova and H. Kayang (2007). "Ethnobotanical Usage of Plants in Western Mizoram." *Indian Journal of Traditional Knowledge* **6**(1): 486-493.

Lee, C. K., K.-K. Park, J.-K. Hwang, S. K. Lee and W.-Y. Chung (2008). "The Pericarp Extract of *Prunus persica* Attenuates Chemotherapy-Induced Acute Nephrotoxicity and Hepatotoxicity in Mice." *Journal of Medicinal Food* **11**(2): 302-306.

Lee, C. K., K. K. Park, J. K. Hwang, S. K. Lee and W. Y. Chung (2008). "The Extract of *Prunus persica* Flesh (Ppfe) Attenuates Chemotherapy-Induced Hepatotoxicity in Mice." *Phytotherapy Research* **22**(2): 223-227.

Lentz, D. L., A. M. Clark, C. D. Hufford, B. Meurer-Grimes, C. M. Passreiter, J. Cordero, O. Ibrahimi and A. L. Okunade (1998). "Antimicrobial Properties of Honduran Medicinal Plants." *Journal of Ethnopharmacology* **63**(3): 253-263.

Lim, T. (2014). *Tagetes erecta*. Edible Medicinal and Non-Medicinal Plants, Springer**:** 432-447.

Lim, T. K. (2012). Edible Medicinal and Non-Medicinal Plants: Volume 4, Fruits, Springer.

Liu, Y. (2012). Chinese Medicine Roll for Treating Breast Cancer and Preparation Method Thereof, Southwest Jiaotong University, Peop. Rep. China . 6pp.

Lokesh, D. and D. Amitsankar (2012). "Pharmacognostical Evaluation and Establishment of Quality Parameters of Medicinal Plants of North-East India Used by Folklore Healers for Treatment of Hypertension." *Pharmacognosy Journal* **4**(27): 30-37.

Long, C.-l. and R. Li (2004). "Ethnobotanical Studies on Medicinal Plants Used by the Red-Headed Yao People in Jinping, Yunnan Province, China." *Journal of Ethnopharmacology* **90**(2): 389-395.

Mabberley, D. J. (2008). Mabberley's Plant-Book: A Portable Dictionary of Plants, Their Classifications, and Uses, Cambridge University Press.

Mahato, R. and R. Chaudhary (2005). "Ethnomedicinal Study and Antibacterial Activities of Selected Plants of Palpa District, Nepal." *Scientific World* **3**(3): 26-31.

Manandhar, N. (1990). "Medico Botany of Gorkha District, Nepal - An Elucidation of Medicinal Plants." *Pharmaceutical Biology* **28**(1): 17-25.

Manandhar, N. P. (2002). Plants and People of Nepal, Timber Press.

Mbosso, E. J. T., J. C. A. Nguedia, F. Meyer, B. N. Lenta, S. Ngouela, B. Lallemand, V. Mathieu, P. V. Antwerpen, A. L. Njunda and D. Adiogo (2012). "Ceramide, Cerebroside and Triterpenoid Saponin from the Bark of Aerial Roots of *Ficus elastica* (*Moraceae*)." *Phytochemistry* **83**: 95-103.

Meena, K. and B. Yadav (2011). "Some Ethnomedicinal Plants Used by the Garasia Tribe of District Sirohi, Rajasthan." *Indian Journal of Traditional Knowledge* **10**(2): 354-357.

Mukherjee, P. K., S. Bhattacharya, K. Saha, S. Giri, M. Pal and B. Saha (1997). "Antibacterial Evaluation of *Drymaria cordata* Willd (*Caryophyllaceae*) Extract." *Phytotherapy Research* **11**(3): 249-250.

Muthuvelan, B. and R. B. Raja (2008). "Studies on the Efficiency of Different Extraction Procedures on the Anti Microbial Activity of Selected Medicinal Plants." *World Journal of Microbiology and Biotechnology* **24**(12): 2837-2842.

Nagaraja, Y., K. Geetha and M. Vinay (2011). "Antimicrobial Effect of *Lagenaria siceraria* (Mol.) Standley, against Certain Bacteria and Fungal Strains." *Journal of Applied and Natural Science* **3**(1): 124-127.

Nair, R. and S. Chanda (2008). "Antimicrobial Activity of *Terminalia catappa*, *Manilkara zapota* and *Piper betel* Leaf Extract." *Indian Journal of Pharmaceutical Sciences* **70**(3): 390.

Nair, S. S., N. C. Madembil, P. Nair, S. Raman and S. B. Veerabadrappa (2013). "Comparative Analysis of the Antibacterial Activity of Some Phytolectins." *International Current Pharmaceutical Journal* **2**(2): 18-22.

Nath, K., P. Deka and S. Borthakur (2011). "Traditional Remedies of Joint Diseases in Assam." *Indian Journal of Traditional Knowledge* **10**(3): 568-571.

Nayak, B. S., P. Ellaiah and S. C. Dinda (2012). "Antibacterial, Antioxidant and Antidiabetic Activities of *Gmelina arborea* Roxb Fruit Extracts." *International Journal of Green Pharmacy* **6**(3): 224.

Nayeem, A., A. Khatun, M. S. Rahman and M. Rahman (2011). "Evaluation of Phytochemical and Pharmacological Properties of *Mikania cordata* (*Asteraceae*) Leaves." *Journal of Pharmacognosy and Phytotherapy* **3**(8): 111-123.

Negi, C., S. Nautiyal, L. Dasila, K. Rao and R. Maikhuri (2002). "Ethnomedicinal Plant Uses in a Small Tribal Community in a Part of Central Himalaya, India." *Journal of Human Ecology* **14**(1): 23-31.

Neto, C. C., C. W. Owens, R. D. Langfield, A. B. Comeau, J. S. Onge, A. J. Vaisberg and G. B. Hammond (2002). "Antibacterial Activity of Some Peruvian Medicinal Plants from the Callejon De Huaylas." *Journal of Ethnopharmacology* **79**(1): 133-138.

Nono, N. R., K. L. Nzowa, L. Barboni and A. L. Tapondjou (2014). "*Drymaria cordata* (Linn.) Willd (*Caryophyllaceae*): Ethnobotany, Pharmacology and Phytochemistry." *Advances in Biological Chemistry* **4**(2): 160-167.

Othman, M., S. Genapathy, P. S. Liew, Q. T. Ch'ng, H. S. Loh, T. J. Khoo, C. Wiart and K. N. Ting (2011). "Search for Antibacterial Agents from Malaysian Rainforest and Tropical Plants." *Natural Product Research* **25**(19): 1857-1864.

Othman, M., H. S. Loh, C. Wiart, T. J. Khoo, K. H. Lim and K. N. Ting (2011). "Optimal Methods for Evaluating Antimicrobial Activities from Plant Extracts." *Journal of Microbiological Methods* **84**(2): 161-166.

Parekh, J. and S. Chanda (2007). "*In vitro* Screening of Antibacterial Activity of Aqueous and Alcoholic Extracts of Various Indian Plant Species against Selected Pathogens from *Enterobacteriaceae*." *African Journal of Biomedical Research* **1**(6): 92-99.

Parvin, S., M. A. Kader, A. U. Chouduri, M. Rafshanjani and M. Haque (2014). "Antibacterial, Antifungal and Insecticidal Activities of the *n*-Hexane and Ethyl-Acetate

Fractions of Methanolic Extract of the Leaves of *Calotropis gigantea* Linn." *Journal of Pharmacognosy and Phytochemistry* **2**(5): 47-51.

Pattewar, S. V. (2012). "*Kalanchoe pinnata*: Phytochemical and Pharmacological Profile." *International Journal of Phytopharmacy* **2**(1): pp. 1-8.

Pradhan, C., A. Katara, P. Singh, G. Mishra and R. Khosa (2011). "Chemical Characterization by Glc & Gc-Ms and Antimicrobial Activity of Essential Oil from Leaves of *Chrysanthemum indicum* Linn." *International Journal of Pharmaceutical and Technical Research* **3**(1): 175-179.

Pratush, A., S. Dogra and A. Gupta (2013). "Antimicrobial and Phytochemical Screening of Rhizome Extracts of Some Native Medicinal Plant of Himachal Pradesh (India)." *Applied Biological Research* **15**(2): 149-153.

Pulok, K. M., S. Bhattacharya, S. Kakali, S. N. Giri, M. Pal and B. P. Saha (1997). "Antibacterial Evaluation of *Drymaria Cordata* Willd (*Caryophyllaceae*) Extract." *Phytotherapy Research* **11**(3): 249-250.

Radhakrishnan, K., P. Thangamani and V. Balakrishnan (2013). "Antibacterial and Phytochemical Analysis of Stem and Root Extracts of *Calotropis gigantea* against Selected Pathogens." *Phytotherapy Research* **13**(3): 20-25.

Ragasa, C. Y., R. Dumag and J. A. Rideout (2001). "Antimicrobial Compounds from *Mentha cordifolia*." *Philippine Journal of Science* **130**(1): 39-43.

Ragasa, C. Y., K. Jorvina and J. A. Rideout (2004). "Antimicrobial Compounds from *Artocarpus heterophyllus*." *Philippine Journal of Science* **133**(2): 97.

Raja, R. D. A., S. Jeeva, J. W. Prakash, J. M. Antonisamy and V. Irudayaraj (2011). "Antibacterial Activity of Selected Ethnomedicinal Plants from South India." *Asian Pacific journal of tropical medicine* **4**(5): 375-378.

Rajeswar Pegu, J. G., Ajit K. Tamuli and Robindra Teron (2013). "Ethnobotanical Study of Wild Edible Plants in Poba Reserved Forest, Assam, India: Multiple Functions and Implications for Conservation." *Research Journal of Agriculture and Forestry Sciences* **1**(3): 1-10

Rani, D., P. Khare and P. Dantu (2010). "*In vitro* Antibacterial and Antifungal Properties of Aqueous and Non-Aqueous Frond Extracts of *Psilotum nudum*, *Nephrolepis biserrata* and *Nephrolepis cordifolia*." *Indian Journal of Pharmaceutical Sciences* **72**(6): 818-822.

Reddy, S. H., M. Chakravarthi, K. Chandrashekara and C. Naidu (2012). "Phytochemical Screening and Antibacterial Studies on Leaf and Root Extracts of *Asclepias curassavica* (L)." *IOSR Journal of Pharmacy and Biological Sciences* **2**: 39-44.

Rho, J., C. Jun, Y. Ha, M. Yoo, M. Cui, S. Baek, J. Lim, Y. Lee and K. Chai (2007). "Isolation and Characterization of a New Alkaloid from the Seed of *Prunus persica* L. and Its Anti-Inflammatory Activity." *Bulletin-Korean Chemical Society* **28**(8): 1289.

Rukachaisirikul, T., A. Saekee, C. Tharibun, S. Watkuolham and A. Suksamrarn (2007). "Biological Activities of the Chemical Constituents of *Erythrina stricta* and *Erythrina subumbrans*." *Archives of Pharmacal Research* **30**(11): 1398-1403.

Sakamoto, S., H. Yoshino, Y. Shirahata, K. Shimodairo and R. Okamoto (1992). "Pharmacotherapeutic Effects of Kuei-Chih-Fu-Ling-Wan (Keishi-Bukuryo-Gan) on Human Uterine Myomas." *American Journal of Chinese Medicine* **20**(3-4): 313-317.

Sarmah, R., D. Adhikari, M. Mazumder and A. Arunachalam (2008). "Traditional Medicobotany of Chakma Community Residing in the Northwestern Periphery of Namdapha National Park in Arunachal Pradesh." *Indian Journal of Traditional Knowledge* **7**: 587-593.

Saxena, M., N. K. Khare, P. Saxena, K. V. Syamsundar and S. K. Srivastava (2014). "Antimicrobial Activity and Chemical Composition of Leaf Oil in Two Varieties of *Piper betle* from Northern Plains of India." *Journal of Scientific & Industrial Research* **73**(2): 95-99.

Sharma, B. (2013). "*In vitro* Antibacterial Activity of Certain Folk Medicinal Plants from Darjeeling Himalayas Used to Treat Microbial Infections." *Journal of Pharmacognosy and Phytochemistry* **2**(4): 1-4.

Shi, Q.-W., X.-H. Su and H. Kiyota (2008). "Chemical and Pharmacological Research of the Plants in Genus Euphorbia." *Chemical Reviews* **108**(10): 4295-4327.

Shunying, Z., Y. Yang, Y. Huaidong, Y. Yue and Z. Guolin (2005). "Chemical Composition and Antimicrobial Activity of the Essential Oils of *Chrysanthemum indicum*." *Journal of Ethnopharmacology* **96**(1–2): 151-158.

Sindambiwe, J. B., M. Calomme, S. Geerts, L. Pieters, A. Vlietinck and D. Vanden Berghe (1998). "Evaluation of Biological Activities of Triterpenoid Saponins from *Maesa lanceolata*." *Journal of Natural Products* **61**(5): 585-590.

Singh, S., S. K. Singh and A. Yadav (2013). "A Review on Cassia Species: Pharmacological, Traditional and Medicinal Aspects in Various Countries." *American Journal of Phytomedicine and Clinical Therapeutics* **1**(3): 291-312.

Srinivasan, K. (1955). "Records of the Botanical Survey of India Vol-17."

Srivastava, R. (2010). "Traditional Knowledge of Nyishi (Daffla) Tribe of Arunachal Pradesh." *Indian Journal of Traditional Knowledge* **9**(1): 26-37.

Srivastava, R., R. K. Singh and T. Mukherjee (2010). "Indigenous Biodiversity of Apatani Plateau: Learning on Biocultural Knowledge of Apatani Tribe of Arunachal Pradesh for Sustainable Livelihoods." *Indian Journal of Traditional Knowledge* **9**: 432-442.

Ståhl, B. and A. Anderberg (2004). Myrsinaceae. Flowering Plants· Dicotyledons, Springer**:** 266-281.

Subba, B. and P. Basnet (2014). "Antimicrobial Activity of Some Medicinal Plants from East and Central Part of Nepal." *International Journal of Applied Sciences and Biotechnology* **2**(1): 88-92.

Suh, S.-J., B.-S. Koo, U.-H. Jin, M.-J. Hwang, I.-S. Lee and C.-H. Kim (2006). "Pharmacological Characterization of Orally Active Cholinesterase Inhibitory Activity of *Prunus persica* L. Batsch in Rats." *Journal of Molecular Neuroscience* **29**(2): 101-107.

Suksamrarn, A., A. Chotipong, T. Suavansri, S. Boongird, P. Timsuksai, S. Vimuttipong and A. Chuaynugul (2004). "Antimycobacterial Activity and Cytotoxicity of Flavonoids from the Flowers Ofchromolaena Odorata." *Archives of Pharmacal Research* **27**(5): 507-511.

Tanti, B., A. K. Buragohain, L. Gurung and D. Kakati (2011). "Assessment of Antimicrobial and Antioxidant Activities of *Dendrocnide sinuata* (Blume) Chew Leaves–a Medicinal Plant Used by Ethnic Communities of North East India." *Indian Journal of Natural Products and Resources* **1**(1): 17-21.

Tsukiyama, M., T. Sugita, H. Kikuchi, Y. Yasuda, M. Arashima, H. Okumura, S. Lhieochaiphant and Y. Shoyama (2010). "Effect of *Duabanga grandiflora* for Human Skin Cells." *The American Journal of Chinese Medicine* **38**(02): 387-399.

Umamaheswari, M., K. Asokkumar, A. Sivashanmugam, A. Remyaraju, V. Subhadradevi and T. Ravi (2009). "*In vitro* Xanthine Oxidase Inhibitory Activity of the Fractions of Erythrina Stricta Roxb." *Journal of Ethnopharmacology* **124**(3): 646-648.

Utteridge, T. and R. M. Saunders (2004). "The Genus Maesa (*Maesaceae*) in the Philippines." *Botanical journal of the Linnean Society* **145**(1): 17-43.

Vadivel, E. (2014). Studies on Some Wound Healing Medicinal Plants, Manonmaniam Sundaranar University. **PhD:** 248.

Vattem, D. and K. Shetty (2005). "Biological Functionality of Ellagic Acid: A Review." *Journal of Food Biochemistry* **29**(3): 234-266.

Wang, H.-F., C. R. Friedman, Z.-X. Zhu and H.-N. Qin (2009). "Early Reproductive Developmental Anatomy in Decaisnea (Lardizabalaceae) and Its Systematic Implications." *Annals of Botany* **104**(6): 1243-1253.

Wiart, C., S. Mogana, S. Khalifah, M. Mahan, S. Ismail, M. Buckle, A. Narayana and M. Sulaiman (2004). "Antimicrobial Screening of Plants Used for Traditional Medicine in the State of Perak, Peninsular Malaysia." *Fitoterapia* **75**(1): 68-73.

Wu, H., J. Shi, S. Xue, Y. Kakuda, D. Wang, Y. Jiang, X. Ye, Y. Li and J. Subramanian (2011). "Essential Oil Extracted from Peach (*Prunus persica*) Kernel and Its Physicochemical and Antioxidant Properties." *LWT-Food Science and Technology* **44**(10): 2032-2039.

Yao, X., X. Tang, M. Fan and C. Du (2013). Application of a Traditional Chinese Medicine in Preparation of Drug for Treating Parkinson's Disease Shanghai Traditional Chinese Medicine Technology Co., Ltd., Peop. Rep. China .

Yousuf, J., R. K. Verma and H. Dar (2013). "Traditional Plant Based Therapy among Rural Communities of Some Villages of Baramulla District (Jammu and Kashmir)." *Journal of Phytology* **4**(5).

Yun, S., B. Choi, H. Ku, M. Lee, H. Nam, K. Lee, S. Park, H. Jang and S. Son (2008). "Antimicrobial Activities of the Flower Extract of *Celosia cristata* L." *Planta Medica* **74**(09): 31.

Chapter 4

Nagaland Medicinal Plants and Their Antimicrobial Activity

This chapter describes the results of antimicrobial screening of the chosen Chungtia medicinal plants.

4.1 Introduction

First-hand ethnobotanical documentation of medicinal plants used by Chungtia villagers in Nagaland identified 135 plants out of which 35 were used as treatments for skin related ailments such as sores, wounds and skin infections and as cleansing agents (Kichu 2010). These applications suggested that the plants might possess antimicrobial properties. As detailed in Chapter 3, a thorough literature search for antimicrobial and phytochemical studies on these 35 plants highlighted seven that had not been reported for any antimicrobial properties or the reports have been very scarce for compounds relevant to their medicinal uses. The aim of the research presented in this chapter was to investigate these seven plants for their antimicrobial activity and to use this data to select a plant for further bioassay guided phytochemical studies.

4.2 Selection of microorganisms for antimicrobial activity testing

Antibiotic susceptible and resistant strains of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) as well as *Streptococcus pyogenes* (*S. pyogenes*), *Salmonella typhimurium* (*S. typhimurium*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and the fungus *Candida albicans* (*C. albicans*) were selected for preliminary antimicrobial screening of the plant extracts as they are pathogens commonly isolated from a variety of clinical conditions.

Staphylococcus aureus is a Gram-positive facultatively anaerobic coccus and is the most common cause of wound infections (Zinner 1999, Purohit and Solanki 2013). The characteristic golden yellow colour, which is seen in colonies of this organism, is caused by the carotenoid pigment staphyloxanthin which is responsible for *S. aureus'* virulence (Liu *et al.* 2008). The bacterium can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, toxic shock syndrome (TSS), bacteraemia and sepsis. *S. aureus* is one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections (Thompson *et al.* 1982,

Purohit and Solanki 2013). The spectacular adaptive capacity of this pathogen has resulted in the emergence and worldwide spread of lineages with resistance to the majority of available antimicrobial agents. The choice of therapy against such multidrug resistant *S. aureus* (MDRSA) as well as methicillin resistant *S. aureus* (MRSA) strains has been narrowed to a few antibacterial agents (Mwangi *et al.* 2007). These bacteria are most often acquired in hospitals and aged care facilities, but are becoming increasingly prevalent in community acquired infections (Wertheim *et al.* 2005).

Escherichia coli is a Gram-negative rod-shaped bacterium that is commonly found in the large intestine of warm-blooded organisms (Guarner and Malagelada 2003). Although the pathogenic strains of *E. coli* are regarded primarily as a cause of urinary tract infections and foodborne disease, they can be also responsible for post-surgical wound infections (Shields *et al.* 2013). *E. coli* is the fourth most common organism implicated in surgical site infections (Shields *et al.* 2013). Extended-spectrum beta-lactamase producing *E. coli* (*E. coli* β+) are resistant to many antibiotics including penicillins, narrow- and extended-spectrum cephalosporins and aztreonam. In the past, most infections caused by *E. coli* β+ have been nosocomially acquired or care facilities related. However, infections due to *E. coli* β+ organisms are an increasing problem in the wider community (Doi *et al.* 2012).

Group A Streptococci are extracellular Gram-positive pathogens (Cunningham 2000). *Streptococcus pyogenes* (Group A) exclusively colonises humans and can cause a wide range of primary infections of the skin, throat and other mucosal surfaces, including pharyngitis and impetigo (Smith and Babcock 2010). Occasionally, life-threatening invasive diseases, such as streptococcal toxic shock syndrome and necrotising fasciitis, both associated with high mortality rates, can result from *S. pyogenes* infection. A resurgence of invasive *S. pyogenes* diseases and rheumatic fever has appeared in outbreaks over the past 10 years (Stockmann *et al.* 2012).

Salmonella typhimurium is a Gram-negative bacterium and facultative intracellular pathogen (Fields *et al.* 1986). *S. typhimurium* is known to initiate infection of mammalian hosts by penetrating the intestinal epithelium of the small bowel (Jones *et al.* 1994). Its most common route of infection is oral, but topical wound infections have also been reported (Murphy and Evans 2012). This bacterium is a major cause of enteric infections in humans associated with significant morbidity and mortality, especially in developing countries, however, it has also been isolated from infected burns (Murphy and Evans 2012)

and surgical wound infections (Leaper *et al.* 2011). Overall, it is estimated to cause about 1.2 million illnesses every year in the United States, with around 23,000 hospitalisations and 450 deaths (Murphy and Evans 2012).

Pseudomonas aeruginosa is a Gram-negative bacterium that can be pathogenic for animals and humans. It can be found in soil, water and skin flora. It thrives not only in normal atmospheres, but also in hypoxic atmospheres, thus, it has colonised many natural and man-made environments. It uses a wide range of organic material for growth and it is this versatility that enables the organism to infect damaged tissues. *P. aeruginosa* is most commonly isolated from acute and chronic wounds such as pressure ulcers (bed sores) or diabetic ulcers (Turner *et al.* 2014). The symptoms of *P. aeruginosa* infections are inflammation and sepsis. If the colonisation occurs in body organs such as the lungs, the urinary tract and kidneys, the result can be fatal. Because the bacterium thrives on most surfaces, it can also be found on the surfaces of medical equipment, causing cross-infections in hospitals and clinics (Stover *et al.* 2000).

Candida albicans is a commensal fungus that is a member of the skin and mucosal flora (Sudbery 2011). *C. albicans* is an opportunistic human pathogen that causes fungal skin infections in mainly immunocompromised patients, patients suffering from burns and newborn (especially premature) babies (Sudbery 2011). The fungus is also a common cause of mucosal and cutaneous infections. Oral candidiasis is an infection of the oral cavity that affects immuno-compromised as well as immune-competent patients (Weindl *et al.* 2014).

4.3 Selection of antimicrobial test methods

Two complementary assays, *i.e.* the disc diffusion assay and MTT microdilution assay were chosen for the testing.

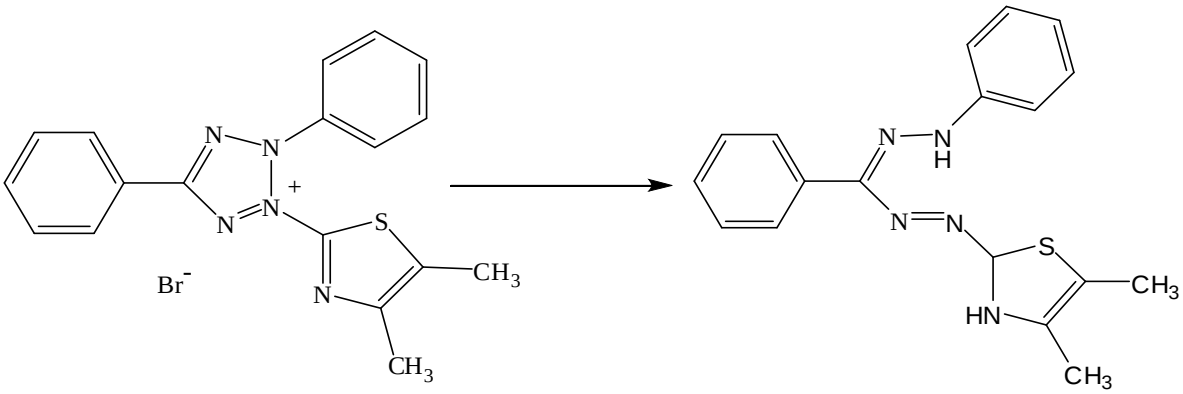
4.3.1 Disc diffusion assay

The disc diffusion assay is one of the most commonly used methods for determination of microorganisms' susceptibility to antibiotics due to its simplicity, rapidity and low cost (Valgas *et al.* 2007). In this method, filter paper discs are impregnated with the test samples, which are then placed on the surface of an agar plate inoculated with the microorganism. The assay is based on the ability of compounds to diffuse into an agar layer and to inhibit microbial growth if the test sample possesses antimicrobial properties. A clear zone of

inhibition appears around the disc if the samples are active. Due to the fact that the method relies on the ability of the test substance to diffuse into the agar layer, this method is not suitable for some types of compounds *i.e.* non polar or large molecules (Valgas *et al.* 2007). The method is also semi-quantitative and is best used as a qualitative method.

4.3.2 MTT microdilution assay

Microdilution assays allow the testing of a range of samples and sample concentrations in a single microtitre plate. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) microdilution assay works on the principle that yellow MTT can be reduced to MTT formazan by living cells (Figure 4.1). This mechanism has been exploited to measure cell proliferation and cytotoxicity (Mosmann 1983) and for antibacterial assays of natural products (Appendino *et al.* 2008, Chérigo *et al.* 2009). Although the mechanism is unknown for bacteria and fungi, the mechanism of cellular MTT reduction to MTT formazan is due to mitochondrial dehydrogenases (Liu *et al.* 1997) and possibly some other non-mitochondrial dehydrogenases or xanthine oxidase (Burdon *et al.* 1993).



MTT MTT Formazan

Figure 4.1 Enzymatic conversion of MTT (yellow) to MTT formazan (blue) by living cells.

In the MTT microdilution assay method for antimicrobial activity, a known volume of liquid medium is dispensed into the wells of a microtitre plate, serial dilutions of the test samples are added and a suspension of known microbial density is dispensed into the wells. After incubation, a solution of MTT is dispensed into each well to detect the microbial growth by a colour change from yellow to dark blue (dark blue wells indicate microbial growth and yellow indicates no microbial growth). The minimum inhibitory concentration

(MIC) is determined as the lowest concentration at which no growth (yellow colour) is observed (Abate *et al.* 1998).

The MTT microdilution assay can be applied to obtain quantitative data and it is generally regarded as a low cost and reliable colorimetric assay (Abate *et al.* 1998). Accuracy of the assay can be compromised by samples that are coloured (such as plant extracts), redox active and/or samples that are not soluble in the medium, which is predominantly aqueous.

4.4 Results and Discussion

As described in Chapter 3, *Dendrocnide sinuata* (stem), *Eurya acuminata* (leaves), *Duabanga grandiflora* (stem bark), *Prunus persica* (roots), *Erythrina stricta* (stem), *Maesa indica* (leaves) and *Holboellia latifolia* (leaves) have been used topically by Chungtia villagers for skin related conditions. These plant parts were collected, air dried and ground by Chungtia villagers, then transported to the research laboratory (Macquarie University, Australia). They were then extracted with 70% aqueous ethanol (Houghton and Raman 1998). The crude extracts were screened against susceptible *S. aureus* and the resistant strains MRSA and MDRSA, *E. coli β+* and *E. coli β-*, *P. aeruginosa*, *S. typhimurium*, *S. pyogenes* and *C. albicans.*

4.4.1 Disc diffusion assay

The ideal concentration of crude plant material for the disc diffusion assay should be 1-5 mg per disc and a microbial density of 1.5×10^6 cfu/mL (Jeevan *et al.* 2004, Kuete *et al.* 2008). In this study, the quantity of 2 mg of extract per disc was used. For interpretation of the results, an arbitrary scoring system was applied. Zones of inhibition larger than 15 mm in diameter (including the 6 mm disc) were considered as high activity, between 10 and 15 mm as moderate activity and less than 10 mm as weak activity. None of the plant extracts inhibited growth of *P. aeruginosa*, *S. typhimurium*, *S. pyogenes* or *C. albicans* in the disc diffusion assay. Only *P. persica* and *E. stricta* showed moderate activity against all three strains of *S. aureus. D. sinuata* extracts showed moderate inhibitory activity against all *S. aureus* strains as well as against *E. coli* β– and *D. grandiflora* showed moderate inhibition against the susceptible strain of *S. aureus* (Table 4.1).

Table 4.1 Positive disc diffusion assay results with the selected plants

Plant sample	Diameter of inhibition zone including disc (in mm)			
	S. aureus	MRSA	MDRSA	*E. coli β-*
[a]*P. persica* roots	10	10	10	na
E. stricta stem	12	10	10	na
D. sinuata stem	12	10	10	10
D. grandiflora stem bark	10	na	na	na
Gentamycin	nt	nt	nt	16
Vancomycin	15	12	12	nt

Sample concentration: 2 mg/disc. Disc size: 6 mm. na = not active, nt = not tested. [a]The results obtained by the author are in agreement with a previously assayed sample (Kichu 2010). Zone of inhibition includes 6 mm disc diameter.

While the disc diffusion assay is a commonly used method for antimicrobial screening of medicinal plants (Das *et al.* 2010, Soković *et al.* 2010, Raja *et al.* 2011), the activity measured as zone of inhibition is influenced by numerous factors including size and polarity of substances (Valgas *et al.* 2007). Moreover, Whatman filter paper discs, which are commonly used and were utilised in this study, can also influence results (Valgas *et al.* 2007). Paper discs are composed of cellulose, which contains many free hydroxyl groups present on glucose and renders the surface of the discs hydrophilic (Burgess et al. 1999). Therefore, polar compounds can adsorb to the surface of the discs and not diffuse into the medium. As a consequence, some polar compounds that possess antimicrobial activity may not show a zone of inhibition in the disc diffusion assay (Valgas *et al.* 2007). Non-polar compounds would not be influenced by the hydroxyls on the surface of the paper, but because of their hydrophobic nature they may not diffuse through the aqueous medium. Large molecules also often diffuse poorly. Thus, some antimicrobial non-polar compounds and large molecules may also have negative results in the disc diffusion assay despite being antimicrobial. A combination of the disc diffusion assay with at least one other assay is therefore often preferred for screening (Valgas *et al.* 2007).

4.4.2 MTT Microdilution assay

The crude extracts of the seven plants were also assayed against all test microbes using the MTT microdilution assay. Unlike for the disc diffusion assay, all of the plants showed activity against susceptible *S. aureus* as well as the resistant MRSA and MDRSA strains, with MIC values ranging from 625 µg/mL for *E. acuminata* and *M. indica* to 156 µg/mL for *P. persica* (all *S. aureus* strains). The *P. persica* extract showed the strongest inhibitory

activity against MRSA and MDRSA (MIC = 156 µg/mL), and the inhibition was more potent than that of the control antibiotic vancomycin (MIC = 312 µg/mL for MRSA and MDRSA). *D. grandiflora* exhibited high antimicrobial potential against all *S. aureus* strains, with MIC values of 156 µg/mL for the susceptible strain and 312 µg/mL for both resistant strains. *D. sinuata* inhibited growth of all *S. aureus* strains, with MIC values 312 µg/mL for the susceptible strain, 1250 µg/mL for MRSA and 2500 µg/mL for MDRSA, respectively, as well as for *E. coli* β- with an MIC of 1250 µg/mL. *M. indica, E. stricta, H. latifolia, D. sinuata* and *E. acuminata* showed activity against *S. typhimurium* with MIC values ranging from 2500 µg/mL for *M. indica* and *E. acuminata* to 625 µg/mL for the remaining plants. None of the plant extracts exhibited activity against *E. coli* β+, *S. pyogenes, P. aeruginosa* and *C. albicans* (Table 4.2, Figure 4.2).

Table 4.2 Positive MTT Microdilution assay results with the selected plants

Plant name	MIC (µg/mL)				
	S. aureus	MRSA	MDRSA	*S. typhimurium*	*E. coli* β-
M. indica leaves	625	625	625	2500	na
E. stricta stem	312	312	312	625	na
H. latifolia leaves	156	1250	1250	625	na
D. grandiflora stem bark	156	312	156	na	na
D. sinuata stem	312	1250	1250	625	1250
E. acuminata leaves	625	625	625	2500	na
P. persica roots	156	156	156	na	na
Kanamycin	156	nt	nt	nt	nt
Gentamycin	nt	nt	nt	312	156
Vancomycin	nt	312	312	nt	nt

na - not active at 2.5 mg/mL, nt - not tested. MIC values were determined as the wells with the lowest sample concentration that showed no change from yellow to dark blue colour after addition of MTT.

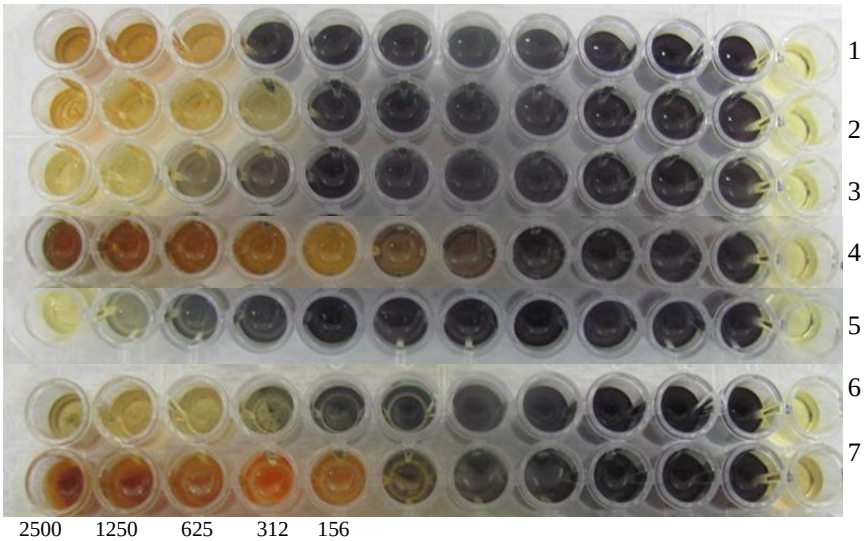


Figure 4.2 The 96 well plate of selected plants tested against MDRSA: 1. *M. indica*, 2. *E. stricta*, 3. *H. latifolia*, 4. *D. grandiflora*, 5. *D. sinuata*, 6. *E. acuminata*, 7. *P. persica*. MIC values were determined as the wells with the lowest sample concentration that showed no change to blue colour after addition of MTT. Concentration range from 156 µg/mL to 2500 µg/mL. The last column to the right is sterile control.

According to several researchers, crude plant extracts with MIC values below 1000 µg/mL are worth further investigation (Rios and Recio 2005, Appendino *et al.* 2008, Chérigo *et al.* 2009), although some researchers suggest that extracts showing activities at concentrations lower than 2000 µg/mL in the preliminary screening should be considered of interest (Palombo and Semple 2001). In this study, MIC values less than 312 µg/mL were considered as good activity, between 612 µg/mL and 1250 µg/mL as moderate activity and greater than 1250 µg/mL as poor activity. The *P. persica* assay results for the MTT microdilution and disc diffusion tests were consistent with those previously obtained in the research group (Kichu 2010) against antibiotic susceptible strains of *S. aureus*, *E. coli* and *P. aeruginosa*.

Based on these assay results, *P. persica* was regarded as the most promising plant for further antimicrobial and phytochemical studies. All the tested medicinal plants showed very good to good activity against susceptible *S. aureus* and good to moderate activity against MRSA and MDRSA, which is consistent with the strong reliance of the Chungtia villagers on the use of these plants for skin related conditions.

4.5 Conclusions

The antibacterial activity of seven plant samples used by Chungtia villagers to treat skin related conditions of a likely microbial aetiology were analysed using the disc diffusion and the MTT microdilution assays. In the disc diffusion assay, *P. persica* and *E. stricta* showed moderate antimicrobial activity against all strains of *S. aureus* and *D. sinuata* displayed weak activity against all strains of *S. aureus* as well as against an antibiotic sensitive strain of *E. coli*. No other plant extracts exhibited activity in the disc diffusion assay. In the MTT microdilution assay all of the plant extracts displayed antibacterial activity against susceptible as well as resistant strains of *S. aureus*. The most potent inhibitory activity was observed with *P. persica* roots, with an MIC of 156 µg/mL for all the strains of *S. aureus*, followed by the stem bark of *D. grandiflora*, with MIC values of 156 µg/mL for the susceptible *S. aureus* and MDRSA and 312 µg/mL for MRSA. The results support the use of the two different assay methods for antimicrobial evaluation. Based on these results, *P. persica* was selected for further investigation of the antibacterial constituents.

Abate, G., R. Mshana and H. Miörner (1998). "Evaluation of a Colorimetric Assay Based on 3-(4, 5-Dimethylthiazol-2-Yl)-2, 5-Diphenyl Tetrazolium Bromide (MTT) for Rapid Detection of Rifampicin Resistance in *Mycobacterium tuberculosis*." *The International Journal of Tuberculosis and Lung Disease* **2**(12): 1011-1016.

Appendino, G., S. Gibbons, A. Giana, A. Pagani, G. Grassi, M. Stavri, E. Smith and M. M. Rahman (2008). "Antibacterial Cannabinoids from *Cannabis sativa*: A Structure − Activity Study." *Journal of Natural Products* **71**(8): 1427-1430.

Burdon, R. H., V. Gill and C. Rice-Evans (1993). "Reduction of a Tetrazolium Salt and Superoxide Generation in Human Tumor Cells (Hela)." *Free Radical Research Communications* **18**(6): 369-380.

Chérigo, L., R. Pereda-Miranda and S. Gibbons (2009). "Bacterial Resistance Modifying Tetrasaccharide Agents from *Ipomoea murucoides*." *Phytochemistry* **70**(2): 222-227.

Cunningham, M. W. (2000). "Pathogenesis of Group a Streptococcal Infections." *Clinical Microbiology Reviews* **13**(3): 470-511.

Das, K., R. Tiwari and D. Shrivastava (2010). "Techniques for Evaluation of Medicinal Plant Products as Antimicrobial Agent: Current Methods and Future Trends." *Journal of Medicinal Plants Research* **4**(2): 104-111.

Doi, Y., Y. S. Park, J. I. Rivera, J. M. Adams-Haduch, A. Hingwe, E. M. Sordillo, J. S. Lewis, W. J. Howard, L. E. Johnson and B. Polsky (2012). "Community-Associated Extended-Spectrum-*β*-Lactamase (Esbl)-Producing *Escherichia coli* Infection in the United States." *Clinical Infectious Diseases* **55**(6): 942-949.

Fields, P. I., R. V. Swanson, C. G. Haidaris and F. Heffron (1986). "Mutants of *Salmonella typhimurium* That Cannot Survive within the Macrophage Are Avirulent." *Proceedings of the National Academy of Sciences* **83**(14): 5189-5193.

Guarner, F. and J.-R. Malagelada (2003). "Gut Flora in Health and Disease." *The Lancet* **361**(9356): 512-519.

Houghton, P. and A. Raman (1998). Laboratory Handbook for the Fractionation of Natural Extracts,(1998), Kluwer Academic Publishers, Boston.

Jeevan, A., L. M. Bhakshu and R. Venkata Raju (2004). "*In vitro* Antimicrobial Activity of Certain Medicinal Plants from Eastern Ghats, India, Used for Skin Diseases." *Journal of Ethnopharmacology* **90**(2): 353-357.

Jones, B. D., N. Ghori and S. Falkow (1994). "*Salmonella typhimurium* Initiates Murine Infection by Penetrating and Destroying the Specialized Epithelial M Cells of the Peyer's Patches." *The Journal of Experimental Medicine* **180**(1): 15-23.

Kichu, M. (2010). Documentation and Biological and Phytochemical Analysis of Chungtia Medicinal Plants of Nagaland, India, Diss. *Chemistry and Biomolecular Sciences*, Macquarie University, Sydney. **PhD:** 1-179.

Kuete, V., B. Ngameni, C. Simo, R. K. Tankeu, B. T. Ngadjui, J. Meyer, N. Lall and J. Kuiate (2008). "Antimicrobial Activity of the Crude Extracts and Compounds from *Ficus chlamydocarpa* and *Ficus cordata* (*Moraceae*)." *Journal of Ethnopharmacology* **120**(1): 17-24.

Leaper, D., O. Assadian, N. O. Hubner, A. McBain, T. Barbolt, S. Rothenburger and P. Wilson (2011). "Antimicrobial Sutures and Prevention of Surgical Site Infection: Assessment of the Safety of the Antiseptic Triclosan." *International Wound Journal* **8**(6): 556-566.

Liu, C.-I., G. Y. Liu, Y. Song, F. Yin, M. E. Hensler, W.-Y. Jeng, V. Nizet, A. H.-J. Wang and E. Oldfield (2008). "A Cholesterol Biosynthesis Inhibitor Blocks *Staphylococcus aureus* Virulence." *Science* **319**(5868): 1391-1394.

Liu, Y., D. A. Peterson, H. Kimura and D. Schubert (1997). "Mechanism of Cellular 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) Reduction." *Journal of Neurochemistry* **69**(2): 581-593.

Mosmann, T. (1983). "Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays." *Journal of Immunological Methods* **65**(1-2): 55-63.

Murphy, P. S. and G. R. Evans (2012). "Advances in Wound Healing: A Review of Current Wound Healing Products." *Plastic Surgery International* **2012**: 4-21

Mwangi, M. M., S. W. Wu, Y. Zhou, K. Sieradzki, H. de Lencastre, P. Richardson, D. Bruce, E. Rubin, E. Myers and E. D. Siggia (2007). "Tracking the *in vivo* Evolution of Multidrug Resistance in *Staphylococcus aureus* by Whole-Genome Sequencing." *Proceedings of the National Academy of Sciences* **104**(22): 9451-9456.

Palombo, E. A. and S. J. Semple (2001). "Antibacterial Activity of Traditional Australian Medicinal Plants." *Journal of Ethnopharmacology* **77**(2): 151-157.

Purohit, S. and R. Solanki (2013). "Microorganisms Responsible for Wound Infection on Human Skin." *Asian Journal of Research in Pharmaceutical Science* **3**(2): 68-71.

Raja, R. D. A., S. Jeeva, J. W. Prakash, J. M. Antonisamy and V. Irudayaraj (2011). "Antibacterial Activity of Selected Ethnomedicinal Plants from South India." *Asian Pacific Journal of Tropical Medicine* **4**(5): 375-378.

Rios, J. and M. Recio (2005). "Medicinal Plants and Antimicrobial Activity." *Journal of Ethnopharmacology* **100**(1): 80-84.

Shields, R., C. Clancy, L. Minces, N. Shigemura, E. Kwak, F. Silveira, R. Abdel-Massih, J. Bhama, C. Bermudez and J. Pilewski (2013). "Epidemiology and Outcomes of Deep Surgical Site Infections Following Lung Transplantation." *American Journal of Transplantation* **13**(8): 2137-2145.

Smith, T. C. and H. Babcock (2010). *Streptococcus* (Group A), Infobase Publishing.

Soković, M., J. Glamočlija, P. D. Marin, D. Brkić and L. J. van Griensven (2010). "Antibacterial Effects of the Essential Oils of Commonly Consumed Medicinal Herbs Using an *in vitro* Model." *Molecules* **15**(11): 7532-7546.

Stockmann, C., K. Ampofo, A. L. Hersh, A. J. Blaschke, B. A. Kendall, K. Korgenski, J. Daly, H. R. Hill, C. L. Byington and A. T. Pavia (2012). "Evolving Epidemiologic Characteristics of Invasive Group a Streptococcal Disease in Utah, 2002–2010." *Clinical Infectious Diseases* **55**(4): 479-487.

Stover, C., X. Pham, A. Erwin, S. Mizoguchi, P. Warrener, M. Hickey, F. Brinkman, W. Hufnagle, D. Kowalik and M. Lagrou (2000). "Complete Genome Sequence of *Pseudomonas aeruginosa* Pao1, an Opportunistic Pathogen." *Nature* **406**(6799): 959-964.

Sudbery, P. E. (2011). "Growth of *Candida albicans* Hyphae." *Nature Reviews Microbiology* **9**(10): 737-748.

Thompson, R. L., I. Cabezudo and R. P. Wenzel (1982). "Epidemiology of Nosocomial Infections Caused by Methicillin-Resistant *Staphylococcus aureus.*" *Annals of Internal Medicine* **97**(3): 309-317.

Turner, K. H., J. Everett, U. Trivedi, K. P. Rumbaugh and M. Whiteley (2014). "Requirements for *Pseudomonas aeruginosa* Acute Burn and Chronic Surgical Wound Infection." *PLoS Genetics* **10**(7): 102-132.

Valgas, C., S. M. d. Souza, E. F. Smânia and A. Smânia Jr (2007). "Screening Methods to Determine Antibacterial Activity of Natural Products." *Brazilian Journal of Microbiology* **38**(2): 369-380.

Weindl, G., J. R. Naglik, D. L. Moyes and M. Schaller (2014). Mucosal Immunology in *Candida albicans* Infection. Human Fungal Pathogens, Springer**:** 161-176.

Wertheim, H. F. L., D. C. Melles, M. C. Vos, W. van Leeuwen, A. van Belkum, H. A. Verbrugh and J. L. Nouwen (2005). "The Role of Nasal Carriage in *Staphylococcus aureus* Infections." *The Lancet Infectious Diseases* **5**(12): 751-762.

Zinner, S. H. (1999). "Changing Epidemiology of Infections in Patients with Neutropenia and Cancer: Emphasis on Gram-Positive and Resistant Bacteria." *Clinical Infectious Diseases* **29**(3): 490-494.

Chapter 5

Chemical and Biological Studies on *Prunus persica* Root Extracts

This chapter describes the studies carried out with the extracts of P. persica *roots to determine their antimicrobial potential and to isolate and identify bioactive fractions and molecules.*

5.1 Introduction

As described in Chapter 4, the crude 70% aqueous ethanolic extract of *Prunus persica* L. Stokes (Peach) roots, collected from Chungtia village, Nagaland, exhibited very good antibacterial activity against antibiotic sensitive as well as antibiotic resistant strains of *S. aureus*. Although *P. persica* is well known for its healing properties, there are no reports concerning the plant's antimicrobial activity. Moreover, of all the examined plant parts, roots seem to be completely left out.

The aim of the work presented in this chapter was to further examine the antibacterial properties of the partitioned extracts of *P. persica* roots with a range of Gram-negative and Gram-positive bacteria, to conduct phytochemical screening, optimise a bioautography method for bioassay-directed fractionation, and to begin to identify the active constituents.

5.2 Results and discussion

5.2.1 Partitioning of *P. persica* root extract and phytochemical screening

Dried powdered roots of *P. persica* (1 kg) were extracted with 70% aqueous ethanol and the extract was partitioned between water and *n*-hexane, DCM and EtOAc to help fractionate the components based on their polarity. The partitions were subjected to qualitative phytochemical screening with vanillin–sulfuric acid, *p*-anisaldehyde and Dragendorff's reagents, iodine, ferric chloride and lead(II) acetate as the staining agents. Vanillin-sulfuric acid reagent is particularly used for the detection of steroids (red or blue spots) (Gibbons 2005, Ebada *et al.* 2008), however some authors suggest that terpenes (red, blue or violet spots) and phenols (pink, red or orange spots) can also be detected (Wagner 1996, Gibbons 2005, Spangenberg 2008, Waksmundzka-Hajnos *et al.* 2008). *p*-Anisaldehyde reagent can be applied for detection of terpenes and steroids (blue or green colouration) (Wagner 1996), while iodine has a high affinity for unsaturated and aromatic compounds (yellow-brown spots) (Waksmundzka-Hajnos *et al.* 2008). Dragendorff's

reagent is a specific reagent for the detection of heterocyclic nitrogen compounds such as alkaloids, which appear as dark orange or red spots (Waksmundzka-Hajnos *et al.* 2008). Ferric chloride and lead acetate are used for detection of flavonoids, phenols and tannins (Waksmundzka-Hajnos *et al.* 2008, Gowri and Vasantha 2010). Formation of blue or green spots with ferric chloride indicates the presence of phenols. Formation of yellow or red spots with lead acetate indicates the presence of tannins (Gowri and Vasantha 2010).

A TLC chromatogram of the *n*-hexane partition stained light blue with vanillin-sulfuric acid reagent and dark blue with *p*-anisaldehyde reagent, suggesting that terpenes and/or steroids might be present in this partition. With the vanillin-sulfuric acid reagent, TLC chromatograms of the DCM, EtOAc and water partitions were predominantly red and pink, suggesting the presence of terpenes (red, violet or blue), steroids (red or blue) and/or phenols (red, pink or orange). Figure 5.1 depicts TLC chromatograms of the *n*-hexane, DCM, EtOAc and water partitions sprayed with vanillin–sulfuric acid reagents and *p*-anisaldehyde. Additional tests were performed to confirm whether steroids, terpenes or phenols were present in the *n*-hexane, DCM, EtOAc and water fractions.

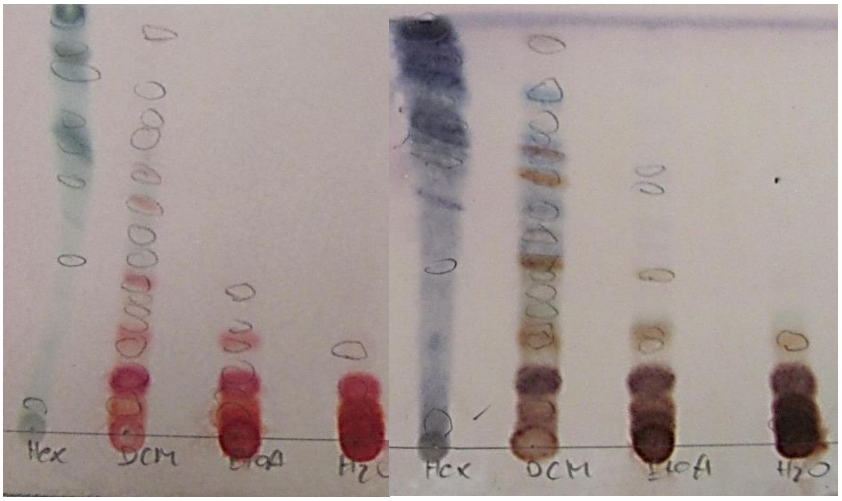


Figure 5.1 *P. persica* partitions run on normal phase TLC plates developed in petroleum ether:diethyl ether 3:7 and stained with vanillin-sulfuric acid (left picture) and *p*-anisaldehyde (right picture) reagents.

After insertion of TLC chromatograms into an iodine chamber, brown spots appeared for all partitions, suggesting the presence of unsaturation. None of the chromatograms showed any orange or red spots after spraying with Dragendorff's reagent, which indicated the absence of alkaloids.

The *n*-hexane, DCM, EtOAc and water partitions as aqueous methanol solutions were treated with $FeCl_3$. The DCM, EtOAc and water partitions turned blue. This indicated the presence of phenols (Gowri and Vasantha 2010). For detection of tannins, the above mentioned partitions were treated with lead acetate. In each case, with the exception of *n*-hexane, a yellow precipitate was produced, which indicated the presence of tannins (Gowri and Vasantha 2010). Similar tests were also carried out using developed TLC plates. Two chromatograms were run under the same conditions (chloroform:methanol 6:4), one treated with $FeCl_3$, the other with lead acetate. The results are depicted in Figure 5.2.

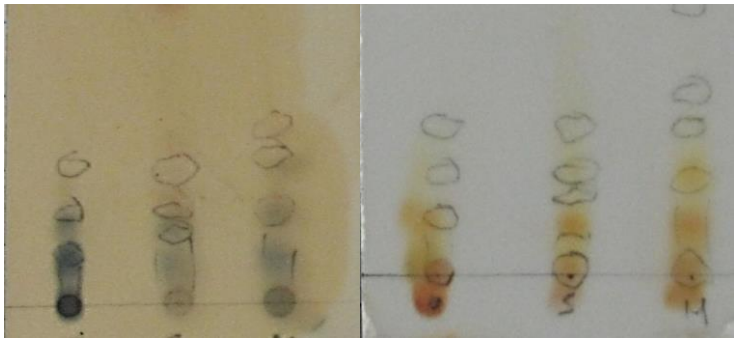


Figure 5.2 TLC chromatograms run on normal phase TLC plates (chloroform:methanol 6:4). Left picture - chromatogram treated with $FeCl_3$. Right picture - chromatogram treated with lead acetate.

Thus, phytochemical testing suggested the presence of terpenes and/or steroids in the *n*-hexane partition of *P. persica*, unsaturated compounds in all partitions, the presence of phenols/tannins in the DCM, EtOAc and water partitions and the total absence of alkaloids in all partitions.

5.2.2 Preliminary antibacterial screening of *P. persica* root extract

The *n*-hexane, DCM, EtOAc and water partitions were tested against *Staphylococcus aureus* (antibiotic sensitive), methicillin resistant *Staphylococcus aureus* (MRSA), multi drug resistant *Staphylococcus aureus* (MDRSA), *Escherichia coli* (β-lactamase negative and positive), *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Streptococcus pyogenes* and *Candida albicans* using the disc diffusion assay and MTT microdilution assay. While the crude 70% aqueous ethanolic root extract had only shown activity against the susceptible and resistant strains of *S. aureus*, the partitions were tested against the suite of microorganisms as partitioning can often lead to concentration of active compounds, revealing further biological activities.

5.2.2.1 Disc diffusion assay

The diameter of zone of inhibition in mm including the 6 mm disc diameter was used as a measure of the antimicrobial activity in the disc diffusion assay. As mentioned in Chapter 4, an arbitrary scoring system was applied for the interpretation of the results where the zones of inhibition larger than 15 mm in diameter (including the disc) are considered as high activity, between 10 and 15 mm as moderate activity and less than 10 mm as weak activity. The results of the disc diffusion assay are shown in Table 5.1.

Table 5.1 Antimicrobial activity of *P. persica* partitioned extracts by disc diffusion assay

Prunus persica partitions	Zone of inhibition (diameter in mm)			
	S. aureus Susceptible	MRSA	MDRSA	*E. coli* (β-, β+), *P. aeruginosa* *S. typhimurium* *S. pyogenes* *C. albicans*
n-Hexane	8	10	10	na
DCM	7	7	7	na
EtOAc	12	12	11	na
Water	12	10	10	na
Vancomycin (2 µg)	15	12	12	na
Gentamycin (2 µg)	nt	nt	nt	16, 16, 12, 18, 10*
Fluconazole	nt	nt	nt	10**

Assays performed in duplicate. Sample amount 2 mg/disc. Zone of inhibition was determined as diameter of complete inhibition, including 6 mm disc. na – not active, nt – not tested. *Values are diameters of zone of inhibition against *E. coli* (β-) and (β+), *P. aeruginosa*, *S. typhimurium* and *S. pyogenes*, respectively. ** Zone of inhibition against *C. albicans*.

All of the tested partitions showed moderate to weak antibacterial activity against antibiotic sensitive as well as antibiotic resistant strains of *S. aureus.* The strongest activity was noted for the EtOAc and *n*-hexane *P. persica* partitions, with interestingly the *n*-hexane partition showing better antibacterial properties against the antibiotic resistant MRSA and MDRSA strains than against the antibiotic sensitive *S. aureus*. None of the extracts possessed activity against antibiotic sensitive and resistant strains of *E. coli* or *P. aeruginosa, S. typhimurium, S. pyogenes* and *C. albicans.* This is consistent with results obtained from the MTT microdilution assay described below.

5.2.2.2 MTT microdilution assay

The *n*-hexane, DCM, EtOAc and water partitions were tested against antibiotic sensitive and resistant strains of *S. aureus* and *E. coli* and antibiotic sensitive strains of *P. aeruginosa*, *S. typhimurium*, *S. pyogenes* and *C. albicans* using the MTT microdilution assay. Partitions with MIC values < 312 µg/mL were considered as good activity, between 612 µg/mL and 1250 µg/mL as moderate activity and > 1250 µg/mL as poor activity (Rios and Recio 2005). The results are shown in Table 5.2.

Table 5.2 Antibacterial activity of *P. persica* partitions by the MTT microdilution assay

Prunus persica partitions	Minimum inhibitory concentration (MIC, µg/mL)			
	S. aureus Susceptible	MRSA	MDRSA	*E. coli (β-, β+), P. aeruginosa S. typhimurium S. pyogenes C. albicans*
n-Hexane	625	312	312	na
DCM	625	625	625	na
EtOAc	312	312	312	na
Water	1250	1250	1250	na
Vancomycin	156	312	312	na
Gentamycin	nt	nt	nt	156*, 312**
Fluconazole	nt	nt	nt	312***

MIC values were determined as the wells with the lowest concentration of the samples that displayed no yellow to blue change of the MTT colour. *MIC values for *E. coli (β-, β+), P. aeruginosa* and *S. typhimurium.* **MIC value for *S. pyogenes.* ***MIC value for *C. albicans.*

All of the tested partitions showed activity against the susceptible and resistant strains of *S. aureus*. None of the partitions were active against *E. coli*, *P. aeruginosa*, *S. typhimurium*, *S. pyogenes* and *C. albicans*. The EtOAc partition showed the strongest activity with MIC values of 312 µg/mL against both antibiotic sensitive and antibiotic resistant strains of *S. aureus.* The DCM and *n*-hexane partitions showed moderate activity against the susceptible strain of *S. aureus* (MIC 625 µg/mL), with the *n*-hexane partition having stronger and considered good antibacterial properties against MRSA and MDRSA (MIC 312 µg/mL). The water partition showed poor antibacterial activity in the microdilution assay and good antibacterial activity in the disc diffusion assay. The discrepancy between those two assays might be due to factors such as size, polarity and solubility of substances assayed, which

affect the two assays in different ways. This is discussed in details in chapter four, section 4.3. None of the partitioned extracts possessed activity against the antibiotic sensitive and resistant strains of *E. coli* or *P. aeruginosa, S. typhimurium, S. pyogenes* and *C. albicans*.

The greater resistance of Gram-negative bacteria to plant extracts is expected. For example, a study on 56 extracts of 39 Australian medicinal plants for their antimicrobial activities found only one extract with partial inhibition of Gram-negative bacteria, while all extracts were active against at least one Gram-positive bacterium (Palombo and Semple 2002). The reason for the different sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences in the bacterial cell wall structures. Gram-negative bacteria are resistant to many antibiotics due to the presence of a lipopolysaccharide-containing additional outer membrane with low permeability that functions as an extra barrier preventing the entrance of antibiotics into the cell (Bernal *et al.* 2013). The Gram-positive bacteria should be more susceptible, having only an outer peptidoglycan layer that is more permeable (Santos *et al.* 2013).

5.2.3 GS-MS analysis of *n*-hexane partition

Following the promising antibacterial activity, the *n*-hexane partition was selected for further analysis to identify the components responsible for its bioactivity. As described earlier, *p*-anisaldehyde treatment of the TLC chromatogram of the *n*-hexane partition indicated the possible presence of terpenes (Wagner 1996). A GC-MS (DB-Wax) study on the volatile oil composition of *P. persica* fruit reported the presence of a number of terpenols such as linalool, terpinen-4-ol, hotrienol, α-terpineol and 3,7-dimethyl-1,5-octadien-3,7-diol (Aubert and Milhet 2007). These compounds have all been reported for possessing antimicrobial properties except for 3,7-dimethyl-1,5-octadien-3,7-diol. Linalool and hotrienol have been shown to be active against *S. aureus, E. coli, P. aeruginosa, S. typhimurium, S. flexnerii* and *V. cholerae* (Pattnaik *et al.* 1997). Terpinen-4-ol was reported to possess activity against *Candida* species and α-terpineol has been reported to be active against *S. aureus* and *P. aeruginosa* (Jirovetz *et al.* 2005).

GC-MS analysis was performed on the *P. persica* roots *n*-hexane partition at the School of Chemistry, University of New South Wales by Dr Joseph Brophy. Eight phytochemicals were identified (Table 5.3 and Figure 5.3) by comparing their GC retention times relative to *n*-alkanes (C5-C26) and also by comparison of their mass spectra (*m/z* values) with either known compounds or published spectra. The major constituents identified were hexadec-

1-ene, octadec-1-ene, palmitic acid, ethyl palmitate, linoleic acid, oleic acid, stearic acid and ethyl stearate. As described in Section 5.2.2.2, the *n*-hexane partition showed moderate antibacterial properties against susceptible *S. aureus* and good antibacterial properties against MRSA and MDRSA in the MTT microdilution assay. According to the literature, palmitic acid (Kurtulmus *et al.* 2009), linoleic acid (Dilika *et al.* 2000) and oleic acid (Dilika *et al.* 2000) possess good activity against *S. aureus*. The antimicrobial activity of the *n*-hexane partition could therefore be at least partly ascribed to the presence of these bioactive compounds.

Table 5.3 GS-MS analysis of *P. persica n*-hexane partition using DB-5 column

Compound	Retention time (min)	*m/z* value	Reference
Hexadec-1-ene	15.433	224	(Oros and Simoneit 2001)
Octadec-1-ene	17.800	252	(Oros and Simoneit 2001)
Palmitic acid	19.617	256	(Hayyan *et al.* 2011)
Ethyl palmitate	19.933	284	(Politi *et al.* 2011)
Linoleic acid	21.333	280	(Hayyan *et al.* 2011)
Oleic acid	21.383	282	(Hayyan *et al.* 2011)
Stearic acid	21.600	284	(Hayyan *et al.* 2011)
Ethyl stearate	21.867	312	(Politi *et al.* 2011)

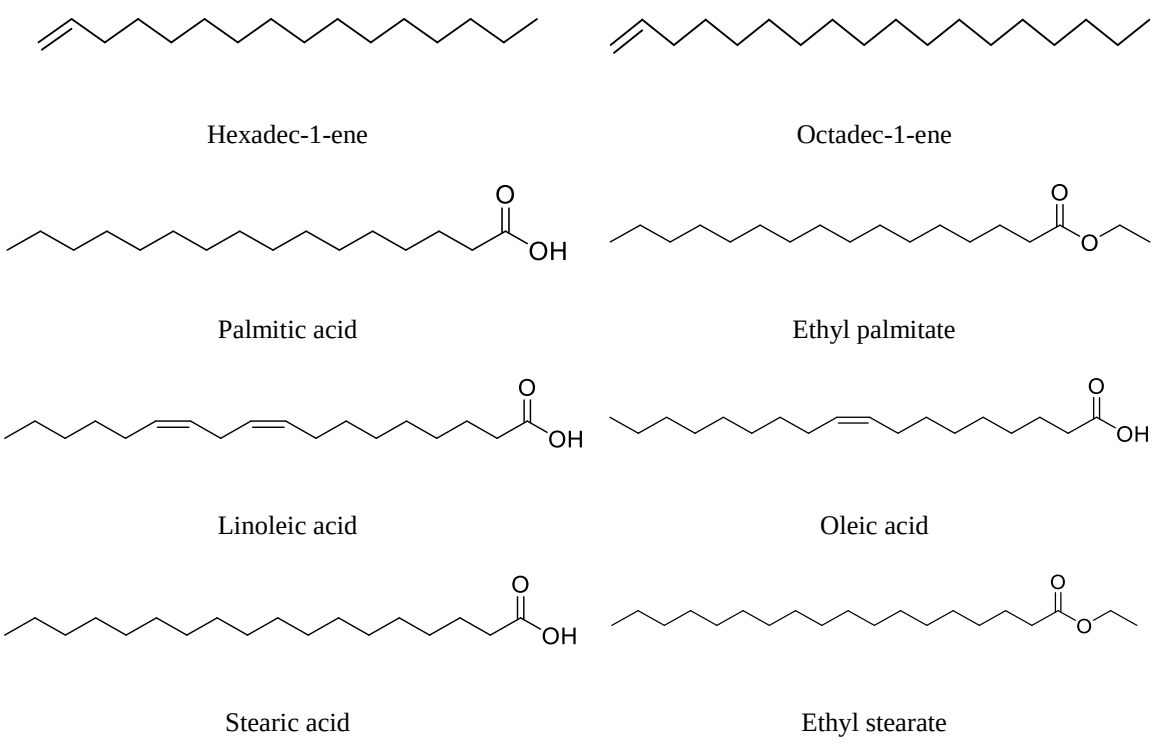


Figure 5.3 Structures of major components of *n*-hexane partition identified by GC-MS analysis.

5.2.4 TLC bioautography guided isolation studies of partitions

Preliminary antibacterial screening showed that the *n*-hexane and EtOAc partitions possessed the most promising antibacterial activity against antibiotic sensitive *S. aureus*, MRSA and MDRSA. Thus, these partitions were chosen for further investigation. The preliminary phytochemical screenings gave insight into the possible chemical compositions of the partitions, suggesting that the *n*-hexane partition contained terpenes (supported by GC-MS studies) and steroids and the EtOAc partition contained phenols and tannins.

TLC bioautography can be considered as one of the most effective methods for targeting active compounds, such as antibacterial and antifungal constituents (Islam *et al.* 2003), present in a mixture. This method combines thin layer chromatographic (TLC) separation and *in situ* activity determination, facilitating the localisation and directed isolation of active constituents (Shahverdi *et al.* 2007). TLC bioautography was therefore chosen as a method for bioassay guided isolation studies of the antibacterial *n*-hexane and EtOAc partitions. In order to determine the optimal TLC bioautography method for studies on the partitions, approaches reported in the literature were trialed and modified. The susceptible strain of *S. aureus* was used for the method development.

5.2.4.1 TLC bioautography method development

A number of variations of the TLC bioautography method have been reported in the literature, however the basic common principles are as follows: i) the fractions or pure compounds are run in duplicate on TLC plates with an appropriate solvent system; ii) one of these plates is retained as a reference chromatogram and visualised for detection of compounds, the other plate (on which the bioautography is performed) is brought in contact with the microorganism; iii) after incubation an appropriate dyeing agent is added to provide a visible zone of inhibition.

According to the method of how the TLC plate is exposed to the microorganisms, TLC bioautography can be divided into direct bioautography (Hamburger and Cordell 1987) where the microorganisms are grown directly on the TLC plate; contact bioautography (Goodall and Levi 1947) where antimicrobial compounds are transferred from the TLC plate to an inoculated agar plate through direct contact; and agar overlay bioautography (Harborne 1984) where an inoculated agar medium is poured onto the TLC plate. For this study MTT was chosen as the staining agent due to its ability to convert yellow MTT into blue MTT formazan in the presence of viable cells (Abate *et al.* 1998).

Direct TLC bioautography

For direct TLC bioautography, the microorganisms suspended in broth are grown directly on TLC plates, and after incubation, visualised with an appropriate dye (Islam *et al.* 2003). Different procedures have been described in the literature referring to the ways of bringing the bacterial suspensions in contact with the TLC plates (Valgas *et al.* 2007). Some authors recommend spraying bacterial suspensions on the TLC plates (Islam *et al.* 2003), and some suggest dipping the TLC plates in bacterial inoculums (Valgas *et al.* 2007). The dipping method was opted for in this study to keep bacteria in a closed system and limit their contact with the environment. For this technique, before spotting and running up the sample, it was necessary to precondition the TLC plates by preheating them at 120^0C for at least 3 hours. This prevented detachment of the constituents from the plates when soaked (Horváth *et al.* 2002). The plates were then incubated for 24 hours and the microorganisms grown directly on them. The literature recommendation for the incubation of inoculated plates is to store them in a water-vapour chamber to prevent drying out (Horváth *et al.* 2002). In this study, empty petri dishes were used to provide a sterile environment for the inoculated TLC plates. The vapour chamber was made of a plastic box lined with wet cotton wool. The petri dishes

with the TLC plates were closed inside and placed in the incubator. Visualisation of inhibition zones was performed directly on the TLC plates, using MTT as a staining agent. The bacterial growth on the plates was very poor, which was most likely due to the drying out of the plates, suggesting that the water-vapour chamber was not effective enough. In the next attempt, petri dishes with sterile agar were used to provide a moist environment for the TLC plates, which were then placed directly in an incubator without the aid of a vapour chamber. The petri dishes provided adequate conditions for bacterial growth. After staining, the spots where bacteria did not grow (the zones of inhibition) were seen as white areas against a purple background. The method was easy to perform and gave accurate localisation of active compounds. However, it was noticed that dipping the TLC plates in broth inoculated with bacteria left uneven patterns on the plates that could obscure the zones of inhibition (Figure 5.4). This effect was decreased by an extra 2 hours incubation of the stained plates, however this led to more intense dyeing of the plates, which also affected visibility of the zones.

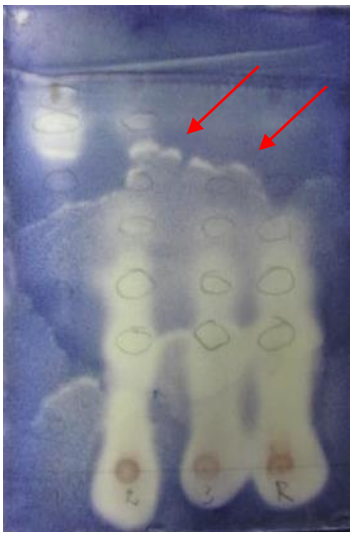


Figure 5.4 Direct bioautography method on TLC chromatogram. Red arrows pointing at the places where the "wavy" pattern of the background could be confused with the inhibition zones.

Contact bioautography

For contact bioautography, developed chromatograms are placed face down onto an inoculated agar layer to enable diffusion of active compounds from the TLC plates into the agar. After removal of the chromatograms, the agar plates are incubated and visualised with the appropriate dye (Rahalison *et al.* 1991). This method resembles a disc diffusion assay, where the inhibition zones are observed on the agar surface in the places where the active

substances diffused into the agar. In the course of this study, it was found that for this technique, the bacteria should be added to the molten agar and poured onto a petri dish rather than streaked on the agar surface. This ensured even growth and staining with the dye. Moreover, the streaked bacteria tended to be washed off while staining and the results were unreadable. This method was found to be very time consuming. To favor diffusion of the test compound over bacterial growth, it has been suggested that the developed TLC plates need to be left face down on the inoculated agar plates in the fridge for at least 6 hours prior to the incubation (Valgas *et al.* 2007). This indeed enabled better diffusion and improved the results, however this method was found less sensitive than the direct one. The sensitivity issue has been also noted by Rahalison *et al.* and has been stated to be due to the transfer process of the phytochemicals from the chromatogram into the agar layer resulting in larger inhibition zones that decrease the ability to discriminate between active compounds with similar R_f values (Rahalison *et al.* 1991).

Overlay bioautography

For the overlay TLC bioautography method, developed TLC plates are placed face up on sterile petri dishes and molten, warm, inoculated agar is rapidly distributed over each of them. After solidification of the medium, the TLC plates are incubated overnight and stained with the dye (Rahalison *et al.* 1991). This method is a hybrid between contact bioautography where antimicrobials are diffusing from the TLC plates into an agar layer and direct bioautography where a microbial suspension in broth is distributed over the developed TLC plates. In principle, the overlay technique resembles the disc diffusion assay and therefore the results might be influenced by the ability of compounds to diffuse into the agar overlay. The agar medium needs to be cooled down before mixing with bacteria and it was found that such agar is difficult to distribute evenly due to its rapid solidification. A uniform surface is crucial for this method - too thin a layer of agar dries out during the incubation, and too thick a layer interferes with the diffusion process. To prevent quick solidification and ensure an even distribution of the agar layer, the agar was poured onto the petri dishes whilst on a hot plate (approximately 40 ºC). An agar-broth mix was also trialed. The mixture was very easy to distribute, solidified more slowly and the layer achieved was thin and even, but did not dry out. After incubation and staining with MTT, very clear zones of inhibition were observed (Figure 5.5).

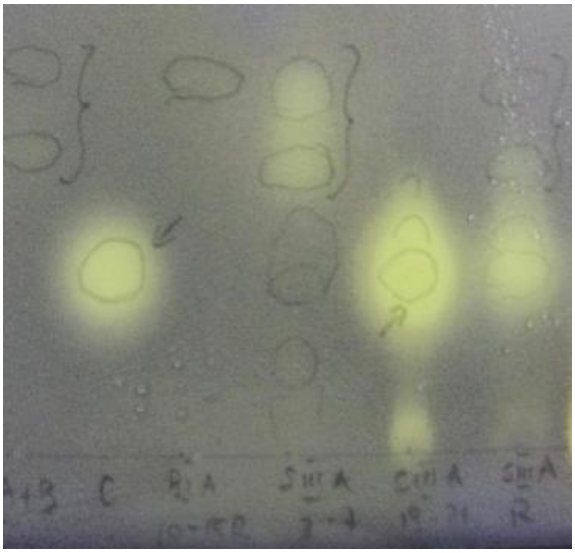


Figure 5.5 The broth-agar mix variant of the overlay TLC bioautography.

In summary, contact TLC bioautography was decided inadequate for the purpose of this study due to its low sensitivity and extended time. The final choice was made between direct TLC bioautography, which was found to be very sensitive, quick and easy to perform but limited by the ability of the microorganism to grow directly on the TLC plates (Rahalison *et al.* 1991) and the overlay method which was more demanding manually but gave clearer inhibition zones. It was decided that the broth-agar mix variation of the overlay technique would be the most suitable for the purpose of this study. This technique is time efficient, sensitive and the results are very clear and readable. Because the chromatograms are overlaid with a thin layer of transparent, inoculated agar, it is possible to discern which R_f regions caused the inhibition of bacterial growth. The broth-agar mix of the overlay method is presented above (Figure 5.5).

5.2.4.2 Bioautography guided isolation studies of the *n*-hexane partition of *P. persica*

As described in Section 5.2.3.2, the *n*-hexane partition of *P. persica* showed good antibacterial activity against the susceptible *S. aureus* strain as well as MRSA and MDRSA. Normal phase silica gel flash column chromatography was performed on the partition (~2 g) and the fractions were pooled together according to their similar normal phase TLC profiles. This yielded 12 major sub-fractions, out of which Hex-2, -7, -8, -9, -10 -11 and -12 showed activity against susceptible *S. aureus* by TLC bioautography, and Hex-7, -10, -11 and -12 showed activity by the MTT microdilution assay against the same bacterial strain. Antibacterial properties of these seven sub-fractions were further investigated by

TLC bioautography as well as the MTT microdilution assays against the MRSA and MDRSA strains. They were not tested against antibiotic sensitive or resistant strains of *E. coli* (β+ and β-), *P. aeruginosa, S. typhimurium, S. pyogenes* or *C. albicans* as the *n*-hexane partition had shown no activity against them. The results of the TLC bioautography and MTT microdilution assays are summarised in Table 5.4.

Table 5.4 MTT microdilution and TLC bioautography assay results (petroleum ether:diethyl ether 3:7) of sub-fractions of the *n*-hexane partition against susceptible *S. aureus*, MRSA and MDRSA

Fraction No.	Yield mg	Antibacterial activity					
		Susceptible *S. aureus*		MRSA		MDRSA	
		MIC values (µg/mL)	R_f values	MIC values (µg/mL)	R_f values	MIC values (µg/mL)	R_f values
Hex-2	15	na	0.8	na	na	na	na
Hex-7	80	625	0.75	1250	0.75	na	na
			0.65		0.65		na
Hex-8	30	na	0.4	na	0.4	na	0.4
			0.35		0.35		0.35
Hex-9	33	na	0.3-0.4*	na	na	na	na
Hex-10	26	1250	bl-0.35*	625	bl-0.45*	625	bl-0.35*
Hex-11	20	1250	bl-0.30*	1250	bl-0.40*	1250	bl-0.30*
Hex-12	24	312	bl-0.35*	312	bl-0.45*	312	bl-0.35*
Vancomycin		156		312		312	

*R_f range where inhibition zones were observed. Samples were run on normal phase TLC plates using (petroleum ether 40-60 ºC:diethyl ether 3:7). na - not active at 2.5 mg/mL, bl - baseline.

An arbitrary system was applied where the MIC values < 312 µg/mL were considered as good activity, between 625 µg/mL and 1250 µg/mL as moderate activity and > 2500 µg/mL as poor activity (Dickson *et al.* 2007, Appendino *et al.* 2008, Cherigo *et al.* 2009). Although fractions Hex-2, -8 and -9 showed activity by TLC bioautography, they were not active with the MTT microdilution assay at 2500 µg/mL, therefore the fractions were not included for further studies. Hex-10 and -11 showed moderate activity against susceptible as well as resistant strains of *S. aureus*. Hex-10 had an MIC value of 1250 µg/mL against susceptible *S. aureus* and of 625 µg/mL against MRSA as well as MDRSA. Hex-11 had MIC values of 1250 µg/mL for all *S. aureus* strains. Hex-12 showed good activity against all three strains of *S. aureus*, each with MIC values of 312 µg/mL. TLC bioautography of the Hex-10, Hex-11 and Hex-12 fractions showed large overlapped inhibition zones, consistent with

a number of active compounds being present (Figure 5.6). The fractions, however, had relatively small amounts of samples (26 mg for Hex-10, 20 mg for Hex-11 and 24 mg for Hex-12), with each sample being a complex mixture showing 6 to 12 distinct spots by TLC. Purification of them was therefore not attempted.

Hex-10 Hex-11 Hex-12

Figure 5.6 TLC bioautography of Hex-10, -11 and -12 against antibiotic sensitive *S. aureus*, developed in petroleum ether:diethyl ether 3:7.

Hex-7 (80 mg) was the only other fraction that showed activity in both the MTT microdilution and TLC bioautography assays. TLC bioautography of Hex-7 showed two active spots against *S. aureus* and MRSA and an MIC value of 625 µg/mL for the susceptible *S. aureus* and 1250 µg/mL for MRSA (Table 5.4). Consequently, the fraction was considered to be moderately active and was of sufficient quantity to warrant further investigation. Hex-7 was found to be stable to normal phase silica by 2D TLC and was thus subjected to normal phase silica column chromatography. The separation yielded three sub-fractions Hex-7a (8 mg), Hex-7b (20 mg) and Hex-7c (10 mg), which were analysed by TLC bioautography against all three strains of *S. aureus*. Hex-7a showed four spots, out of which one (R_f 0.75, petroleum ether:diethyl ether 3:7) was active against both susceptible *S. aureus* and MRSA (Figure 5.7). The MIC test against the sensitive strain of *S. aureus*, however, showed no activity at 2500 µg/mL. Attempted further purification by PTLC yielded a fraction (~0.1 mg) that appeared as one spot by TLC and was active against susceptible *S. aureus* as well as MRSA, but was not of sufficient quantity to conduct structural analysis.

Figure 5.7 Hex-7a sub-fraction, developed in petroleum ether:diethyl ether 3:7. On the left side: TLC bioautography result (MRSA); red arrow points to the possible target compound. On the right side: the actual view of the developed chromatogram; the red arrow points at the same, active compound.

Hex-7b showed two distinct spots (R_f 0.55 and 0.65, petroleum ether:diethyl ether 3:7) and a smear from R_f 0.65-0.75. The spot of R_f 0.65 was active against the susceptible *S. aureus* and MRSA by TLC bioautography and Hex-7b was moderately active in the MTT microdilution assay, with an MIC 1250 µg/mL. Purification by recrystallisation with methanol gave a white precipitate (7b1, 1 mg) and soluble part (7b2, 12 mg when dried). TLC chromatography of the soluble part with different solvent system revealed it contained at least four compounds and further separation was not attempted.

The white precipitate (7b1) was recrystallised twice with diethyl ether to give a white needle-like solid (compound 5.1, 0.5 mg) with R_f 0.55 and staining blue with *p*-anisaldehyde spray reagent. The sample was subjected to EI-MS analysis and the spectrum was compared with National Institute of Standards and Technology (NIST) library data. The molecular ion peak of *m/z* 414 and the fragmentation pattern was found to be consistent with β-sitosterol, $C_{25}H_{50}O$. The structure was confirmed by other spectroscopic analyses as well as by spectroscopic data comparison with an authentic sample of β-sitosterol (Sigma-Aldrich). This is discussed in Section 5.2.3.4.

Hex-7c showed one distinctive spot on the TLC chromatogram of the same R_f as β-sitosterol, along with some lower R_f impurities. The spot stained blue with *p*-anisaldehyde, was not active against any of the *S. aureus* strains in the TLC bioautography assay and was not tested by the MTT microdilution assay. Precipitation with methanol and

recrystallisation with diethyl ether yielded further β-sitosterol (compound 5.1, 1.5 mg). The isolated β-sitosterol did not show any antibacterial activity when tested against susceptible *S. aureus*, MRSA and MDRSA in the TLC bioautography assay (Figure 5.8), despite literature reports on the compound noting antibacterial activity against *S. aureus* (tested by disc diffusion and microdilution assays) (Mokbe and Hashinaga 2005, Cho *et al.* 2012).

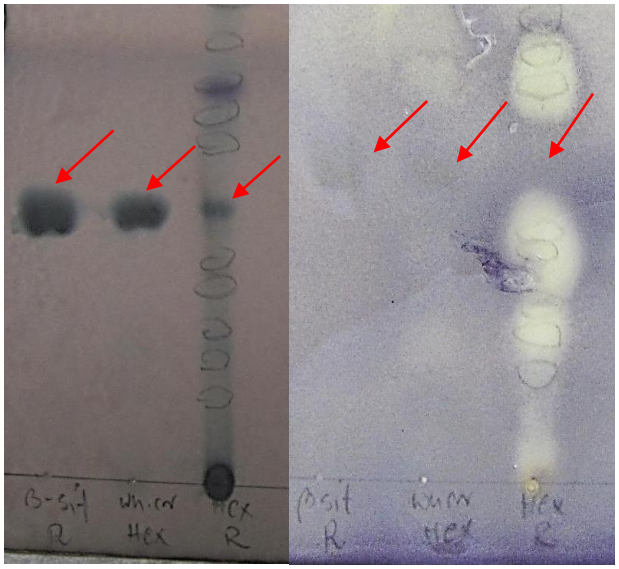


Figure 5.8 Left picture: TLC chromatogram following staining with *p*-anisaldehyde. Red arrows indicate the dark blue colour of Sigma-Aldrich reference sample of β-sitosterol (R) and compound 5.1 (isolated β-sitosterol), with the same R_f values. Right picture: TLC bioautography test result against susceptible *S. aureus*. Red arrows indicate the lack of inhibition zones of R and 5.1. Hex: *n*-hexane partition. Chromatograms run in petroleum ether:diethyl ether 4:7.

5.2.4.3 Antimicrobial studies of β-sitosterol

Literature reports concerning antimicrobial properties of β-sitosterol are inconsistent. It has been found to be active against *S. aureus, E. coli, B. subtilis, B. cereus* and *S. enteritidis* in the disc diffusion assay (Mokbe and Hashinaga 2005, Cho *et al.* 2012) and in the MTT microdilution assay (MIC 0.33 mg/mL for *S. aureus* and MIC 0.30 mg/mL for *E. coli* (Mokbe and Hashinaga 2005). On the other hand, Beltrame *et al.* reported that β-sitosterol exhibited no antibacterial activity against either *S. aureus*, *E. coli* or *P. aeruginosa* in the MTT microdilution assay at 0.1 mg/mL, but inhibited growth of *Bacillus subtilis* at 0.05 mg/mL (Beltrame *et al.* 2002), while Hess *et al.* found it was resistant to *E. coli* and *S. aureus* at doses ≤ 5 mg/mL in a microdilution assay (Hess *et al.* 1995). Hamburger and Cordell *et al*. found β-sitosterol inactive against *B. subtilis* and *E. coli* in a direct TLC bioautography assay (Hamburger and Cordell 1987). Given the conflicting previous reports

of antibacterial activity for β-sitosterol, it was of interest to explore further if the presence of β-sitosterol could be contributing to the antibacterial activities of Hex-7 and Hex-7b sub-fractions, using the MTT assay. Due to the low amount of the isolated compound (2 mg), only the reference sample of β-sitosterol (Sigma-Aldrich) was tested. It was tested against susceptible and resistant strains of *S. aureus*, susceptible and resistant strains of *E. coli*, *P. aeruginosa*, *S. typhimurium*, *S. pyogenes* and *C. albicans*. Positive results are presented in Table 5.5.

Table 5.5 Antimicrobial properties of β-sitosterol (Sigma-Aldrich) tested by MTT microdilution assay

Compound	Antibacterial activity (µg/mL)			
	S. aureus	*S. typhimurium*	*E. coli* β-	*P. aeruginosa*
β-Sitosterol	2500	2500	2500	1250
Kanamycin	156	nt	nt	nt
Gentamycin	nt	312	312	312

nt - not tested

In this study, β-sitosterol was found to possess moderate activity against *P. aeruginosa* with MIC 1250 µg/mL and weak activity with MIC 2500 µg/mL against susceptible strains of *S. aureus* and *E. coli* as well as weak activity against *S. typhimurium* in the MTT microdilution assay. Due to both limited time and plant material, further studies on the *n*-hexane partition were not investigated.

5.2.4.4 Structural elucidation of β-sitosterol (compound 5.1)

Compound 5.1 (Figure 5.9) was isolated as a white crystalline solid. The structure was tentatively identified from the electron-impact mass spectrum (EI-MS) of the compound, which showed a molecular ion at *m/z* 414 and a fragmentation pattern consistent with β-sitosterol ($C_{29}H_{50}O$) based on the NIST library, as well as by comparison with the EI-MS of an authentic sample of β-sitosterol from Sigma-Aldrich (Appendices 3 and 4). HR-MS supported the molecular formula of $C_{29}H_{50}O$ (found: 414.3855, calculated: 414.3862) and the melting point (138 ºC) was consistent with the literature melting point for β-sitosterol (136-139 ºC) (Kircher and Rosenstein 1973).

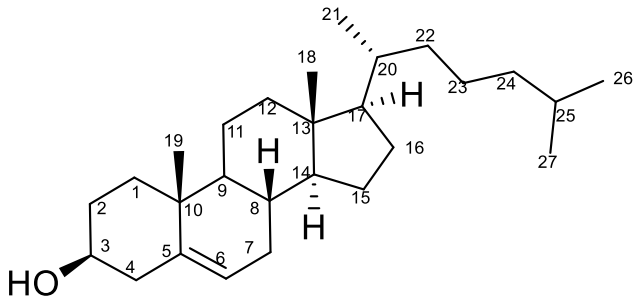


Figure 5.9 The structure of compound 5.1 (β-sitosterol).

The EI-MS showed fragment ions consistent with loss of water (*m/z* 396, M-HOH), plus loss of a methyl group (381, M-CH_3-HOH), the loss of the side chain of β-sitosterol (273, M-side chain), and loss of the side chain and water (255, M-side chain-HOH) (Jamaluddin *et al.* 1994).

The IR spectrum showed a band at 3400 cm^{-1}, indicative of an OH group (Joshi and Poudel 2011, Rani *et al.* 2014). No other characteristic functional group bands were seen (Appendix 5).

The ^{13}C NMR spectrum showed the presence of twenty nine aliphatic signals. The DEPT spectrum showed the presence of six methyl, eleven methylene, nine methine and three quaternary carbon atoms. The ^{1}H-NMR spectrum showed four singlets of three hydrogens each, at δ 0.66, 0.82, 0.84 and 0.99, consistent with shielded methyl groups attached to quarternary carbons, along with doublets of three hydrogens each at δ 0.91 (*d, J* 6.5 Hz); 0.80 (*d, J* 6.9 Hz), consistent with methyl groups next to methines. These were in agreement with ^{1}H NMR signals reported for the methyl groups of β-sitosterol (Joshi and Poudel 2011). A doublet at δ 5.33 (*d, J* 5.3 Hz) was consistent with an olefinic proton. No other olefinic proton signal was present, confirming the double bond was trisubstituted. A multiplet at δ 3.50 equivalent to a single proton was assigned to a proton adjacent to an OH group. Three multiplets, equivalent to two protons each, appeared at δ 1.80, 1.97 and 2.25 and were assigned to three methylene groups. The remaining protons appeared as multiplets at δ 1.05-1.64. These proton and carbon signals were all in agreement with literature reports for β-sitosterol (Joshi and Poudel 2011, Rani *et al.* 2014) and identical with the proton and carbon signals obtained for the authentic sample. The NMR data of compound 5.1 and comparison with the authentic sample of β-sitosterol is presented in Table 5.6 and Appendix 1 and 2.

Table 5.6 NMR data of compound 5.1 and a reference sample of β-sitosterol (Sigma-Aldrich) (600 MHz, $CDCl_3$)

position	δ_C 5.1	δ_C reference	δ_H 5.1 (*J* in Hz)	δ_H reference (*J* in Hz)	multiplicity
1	37.22	37.12	1.13 *m*	1.12 *m*	CH_2
2	31.64	31.63	1.81 *m*	1.81 *m*	CH_2
3-OH	71.80	71.80	3.50 *m*	3.50 *m*	CH
4	42.27	42.27	2.27 *m*	2.27 *m*	CH_2
			2.21 *m*	2.21 *m*	
5	140.73	140.73	-	-	-
6	121.72	121.72	5.33 *d* (5.3)	5.33 *d* (5.3)	CH
7	31.87	31.87	1.49 *m*	1.49 *m*	CH_2
8	31.89	31.89	1.97 *m*	1.97 *m*	CH
9	50.09	50.09	0.91 *m*	0.90 *m*	CH
10	36.48	36.48	-	-	-
11	21.06	21.05	1.47 *m*	1.45 *m*	CH_2
12	39.74	39.74	1.97 *m*	1.96 *m*	CH_2
13	42.30	42.29	-	-	-
14	56.74	56.74	0.94 *m*	0.94 *m*	CH
15	24.28	24.28	1.55 *m*	1.55 *m*	CH_2
16	28.23	28.23	1.81 *m*	1.81 *m*	CH_2
17	56.01	56.01	0.94 *m*	0.94 *m*	CH
18	11.84	0.66 *s*	0.65 *s*	0.65 *s*	CH_3
19	19.81	0.99 *s*	0.81 *d* (8.6)	0.81 *d* (8.6)	CH_3
20	36.13	36.12	1.33 *m*	1.33 *m*	CH
21	18.76	18.75	0.89 *d* (6.5)	0.89 *d* (6.5)	CH_3
22	33.91	33.90	1.29 *m*	1.29 *m*	CH_2
23	26.01	26.00	1.13 *m*	1.12 *m*	CH_2
24	45.79	45.79	0.91 *m*	0.90 *m*	CH
25	29.10	29.10	1.64 *m*	1.64 *m*	CH
26	19.00	19.00	0.79 *d* (8.6)	0.79 *d* (8.6)	CH_3
27	19.38	19.38	0.98 *s*	0.98 *s*	CH_3
28	23.03	23.03	1.24 *m*	1.24 *m*	CH_2
29	11.96	11.96	0.83 *d* (8.6)	0.83 *d* (8.6)	CH_3

5.2.4.5 Bioautography guided isolation studies of the EtOAc partition of *P. persica*

The EtOAc partition of *P. persica* was the most active fraction with MIC value of 312 µg/mL for all tested strains of *S. aureus*. Normal phase silica gel column chromatography of the partition (8 g) resulted in collection of four major sub-fractions based on their TLC profiles (EtOAc-1 to EtOAc-4). Antibacterial activity of the sub-fractions was determined by the MTT microdilution and TLC bioautography assays tested initially against susceptible *S. aureus* (Table 5.7). The EtOAc partition did not show any activity against *E. coli, P. aeruginosa, S. typhimurium, S. pyogenes* and *C. albicans* in preliminary antibacterial screening and the sub-fractions were therefore not tested against these bacteria.

Table 5.7 MTT microdilution and TLC bioautography assay results (chloroform:methanol: water 7:3:0.4) of sub-fractions EtOAc-1-4 tested against susceptible *S. aureus*

Fraction	Yelds (g)	Antibacterial activity	
		MIC values (µg/mL)	R_f values†
EtOAc-1	0.2	na	na
EtOAc-2	1.8	2500	0.63-0.66*
EtOAc-3	3	312	bl-0.45*
EtOAc-4	2	625	bl-0.3*
Vancomycin		156	

na - not active at 2500 µg/mL. bl - baseline. *Merged inhibition zones. †Chloroform:methanol:water 7:3:0.2.

EtOAc-3 showed the most promising activity against susceptible *S. aureus* (MIC 312 µg/mL), followed by EtOAc-4 (MIC 625 µg/mL) and EtOAc-2, which showed weak activity against the tested bacteria (2500 µg/mL). All three (EtOAc-2-4) fractions that showed activity were further investigated.

EtOAc-2 showed two distinct spots of R_f 0.45 and 0.63 and two faint spots of R_f 0.60 and 0.66 (methanol:chloroform:water 7:3:0.2) on the TLC chromatogram, suggesting purification would be relatively straightforward. The distinct spots (R_f 0.45 and 0.63) stained pink with vanillin- sulfuric acid spray reagent and the faint spots of R_f values 0.60 and 0.66 gave no colour with this dye (Figure 5.10). In the TLC bioautography assay against susceptible *S. aureus*, the spots of R_f values 0.45, 0.63 (stained pink) and 0.66 (no colour with dye) gave strong inhibition zones. Despite the weak activity seen in the MTT assay

for EtOAc-2, further separation was of interest due to the observed strong and well defined inhibition zones by TLC bioautography. EtOAc-2 was subjected to normal phase flash silica column chromatography. This afforded sub-fractions EtOAc-2a and EtOAc-2b. These were tested by TLC bioautography as well as the MTT microdilution assay against susceptible *S. aureus*, MRSA and MDRSA.

EtOAc-2a showed two distinct spots of R_f values 0.63 and 0.66 (chloroform:methanol:water 7:3:0.2) that were active against the susceptible strain of *S. aureus* by TLC bioautography. The fraction was also weakly active against this strain by the MTT microdilution assay (MIC 2.5 mg/mL). It was not active against MRSA and MDRSA. The spot of R_f 0.63 stained pink with vanillin-sulfuric acid dye, while the spot of R_f 0.66 was colourless.

EtOAc-2a was subjected to size exclusion column chromatography (SEC) with LH-20 sephadex, eluting with methanol. This yielded three sub-fractions: EtOAc-2a1 (200 mg, one spot of R_f 0.66, chloroform:methanol:water 7:3:0.2, no colour with vanillin dye), EtOAc-2a2 (150 mg, one spot of R_f 0.63 chloroform:methanol:water 7:3:0.2, pink with vanillin dye) and EtOAc-2a3 (220 mg, two spots on TLC plate, 0.66 and 0.63). Even though none of these sub-fractions showed activity against susceptible *S. aureus* either by TLC bioautography or the MTT microdilution test, further purification was attempted due to the relatively clean nature of these fractions and their reasonable quantities.

EtOAc-2a2 was subjected to purification by preparative TLC and yielded compound 5.2 (2 mg, R_f 0.63, chloroform:methanol:water 7:3:0.2, stained pink with vanillin-sulfuric dye). Decomposition of the compound, however, was observed after 10 months in the freezer, as confirmed by NMR. Structural elucidation of compound 5.2 (see Section 5.2.4.6) identified it as afzelechin.

EtOAc-2a1 was subjected to purification by preparative TLC but the initially clean spot of R_f 0.66 subsequently appeared as three smeared spots of R_f values ranging from 0.55 to 0.70. 2D TLC of EtOAc-2a1 had been performed prior to the preparative TLC and had implied the compounds present were stable in the silica gel environment, but the results of the attempted purification suggested decomposition had occurred.

Neither compound 5.2 or EtOAc-2a1 were active by either the TLC bioautography or the MTT microdilution assays against the susceptible *S. aureus*. This was despite spots at their R_f values having shown activity for the EtOAC-2 fraction in the previous TLC bioautography assay. Thus, EtOAc-2a1 and compound 5.2 were co-spotted and tested against susceptible *S. aureus* in the TLC bioautography assay (Figure 5.10). No antibacterial activity was observed, ruling out synergistic antibacterial effects of the compounds in these samples. This implies that either other compounds were responsible for the activities and were lost or the compounds decomposed during the purification steps for EtOAc-2.

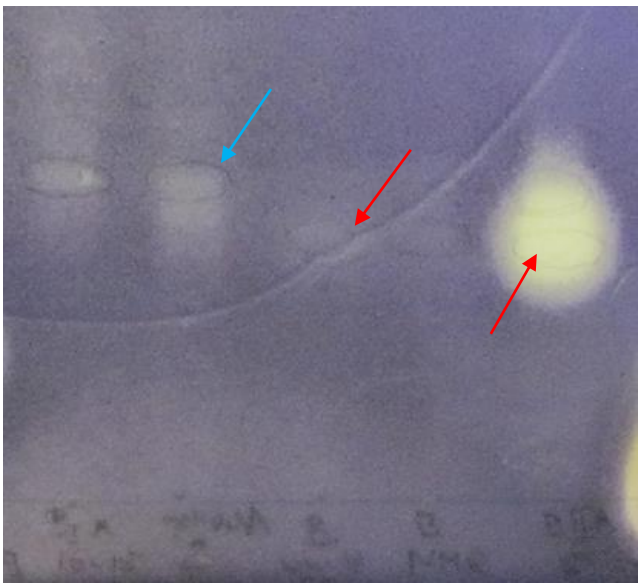


Figure 5.10 TLC chromatogram developed in chloroform:methanol:water 7:4:0.2, tested against susceptible strain of *S. aureus*. Fractions EtOAc-2a1 (2a1), EtOAc-2a (2a) and compound 5.2 spotted, along with a co-spot of compound 5.2 and EtOAc-2a1 (2a1+5.2). Blue arrow points to the co-spotted fraction, red arrows point to the R_f values consistent with compound 5.2.

EtOAc-2b showed two faint spots of R_f 0.63 and 0.66 and one distinct spot of R_f 0.45 (chloroform:methanol:water 7:3:0.2). The spots of R_f 0.63 and 0.66 showed very faint inhibition zones against the susceptible strain of *S. aureus* and no activity for MRSA and MDRSA, while the spot of R_f 0.45 showed strong inhibition zones by TLC bioautography against all three tested strains of *S. aureus*. The fraction was also moderately active against susceptible *S. aureus* by the MTT microdilution assay (MIC 1250 µg/mL). Purification by crystallisation with methanol yielded yellow crystals (one distinct spot of R_f 0.45, stained pink with vanillin-sulfuric acid reagent). The crystals (1 mg) were collected, washed with

chloroform and subjected to LC-MS (ESI-MS) negative ion mode analysis which showed the presence of one compound (5.3) with a molecular ion at *m/z* 543 $[M-H]^-$ consistent with the formula $C_{30}H_{24}O_{10}$ and consistent with the mass of the compound previously isolated from the EtOAc fraction by Kichu (Kichu 2010). The compound isolated by Kichu (*ent*-epiafzelechin-(2α→O→7',4α→8')-(-)*ent*-afzelechin) was not active in the MTT microdilution test at the concentration of 2.5 mg/mL, but was not tested by TLC bioautography or the disc diffusion assay. Compound 5.3 showed strong and well defined inhibition zones against all strains of *S. aureus* by TLC bioautography (Figures 5.11 and 5.12). The MTT microdilution assay was not attempted due to the small amount of sample.

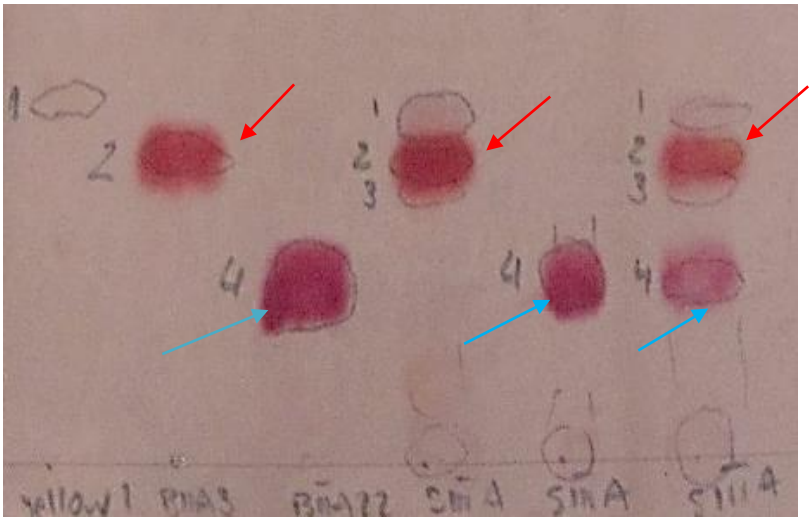


Figure 5.11 TLC chromatogram developed in chloroform:methanol:water 7:3:0.2 and stained with vanillin-sulfuric acid dye. Fraction EtOAc-2a1 (2a1), isolated compound 5.2 (5.2), isolated compound 5.3 (5.3), fraction EtOAc-2a (2a), isolated crystals of fraction 2b (Cr 2b) and fraction EtOAc-2 (2). Red arrows point to the R_f values consistent with compound 5.2 and blue arrows point to the R_f values consistent with compound 5.3.

Compound 5.3

Figure 5.12 TLC bioautography result of compound 5.3 with visible well defined inhibition zone. Chromatogram developed in chloroform:methanol:water 7:4:0.4, tested against susceptible strain of *S. aureus.*

The mother liquor remaining after crystallisation of EtOAc-2b showed no antibacterial activity against the susceptible *S. aureus* as well as MRSA and MDRSA by the MTT microdilution test. This suggested that the moderate activity (MIC 1250 µg/mL) of EtOAc-2b was likely to be due to the presence of the crystallised compound 5.3. Compound 5.3 was also isolated from EtOAc-3a, as described below.

The most active sub-fraction EtOAc-3 (MIC 312 µg/mL against susceptible *S. aureus*), was subjected to normal phase silica column chromatography. This yielded two further sub-fractions EtOAc-3a and EtOAc-3b based on their normal phase TLC profiles and bioautography test results.

Sub-fraction EtOAc-3a appeared on the TLC chromatogram as one major spot with R_f 0.45 (chloroform:methanol:water 7:3:0.4). This was consistent with compound 5.3 isolated from the EtOAc-2b fraction. EtOAc-3 also showed on TLC a faint smear within R_f 0.48-0.55. The major spot stained pink with vanillin-sulfuric acid reagent and was active against all three strains of *S. aureus* in the TLC bioautography assay (Figures 5.10 and 5.11). EtOAc-3a was recrystallised with methanol (2x) and the solid washed with chloroform to give yellow crystals (further compound 5.3, 40 mg). Spectral analysis confirmed the structure as *ent*-epiafzelechin-(2α→O→7',4α→8')-(-)*ent*-afzelechin (see Section 5.2.4.6). Antimicrobial testing by the MTT microdilution assay was also conducted and are described in Section 5.2.4.5.

Sub-fraction EtOAc-3b showed two smeared spots of R_f ranging from the baseline to 0.30 on a normal phase TLC chromatogram run in chloroform:methanol:water 7:3:0.4. Both spots gave positive results when treated with $FeCl_3$ and lead acetate reagents for the presence of phenols and tannins (blue and yellow colour, respectively) and were active against susceptible and resistant strains of *S. aureus* by TLC bioautography and moderately active with the MTT microdilution assay (MIC 625 µg/mL for susceptible *S. aureus* and MRSA and MIC 1250 µg/mL for MDRSA).

Various approaches were trialled for the separation of the major components. The most often cited in the literature method for the isolation of phenols and tannins is size exclusion chromatography (SEC) Sephadex LH-20 (Yanagida *et al.* 1999, Yanagida *et al.* 2003, Herderich and Smith 2005, Sarneckis *et al.* 2006, Kaufman *et al.* 2009). After application on a Sephadex LH-20 SEC column, EtOAc-3b adsorbed to the gel and was unable to be eluted with methanol. Elution with acetone recovered the sample (Murphy and D'Aux 1975), but separation was not achieved. Okuda *et al.* (1989) observed that Sephadex LH-20 tends to absorb large molecular weight condensed tannins (CTs), and possibly some large hydrolysable tannins (HTs) so strongly that they cannot be eluted subsequently (Okuda *et al.* 1989). Normal phase and reversed phase silica gel chromatography failed to separate the mixture. Preparative thin layer chromatography provided some separation, but the isolated compounds had different R_f values to those originally observed in EtOAc-3b when spotted as a reference mixture. 2D TLC confirmed that the compounds were decomposing. CTs and HTs are highly reactive and their chemistry might be influenced by many factors, one of them being extracting solvents. For example methanol cleaves the depside bonds in gallotannins (Harborne 1989) and water easily degrades large and complex tannins into smaller compounds (Okuda *et al.* 1990). The other possibility was that the separated compounds oxidised rapidly. Okuda *et al*. (1989) commented that isolated condensed tannins oxidise fast in air at room temperature (Okuda *et al.* 1989).

Sub-fraction EtOAc-4 was active against the susceptible strain of *S. aureus* in both the MTT assay (MIC 625 µg/mL) and by TLC bioautography (R_f baseline-0.3, merged inhibition zones) and stained pink with vanillin-sulfuric acid reagent. The attempted purification by SEC and normal phase silica gel chromatography met the same challenges as for fraction EtOAc-3b and separation of the compounds was not achieved.

5.2.4.6 Antibacterial activity of isolated compound 5.3

Antimicrobial activity of compound 5.3 was tested against susceptible as well as resistant strains of *S. aureus* and *E. coli* and susceptible strains of *P. aeruginosa*, *S. typhimurium*, *S. pyogenes* and *C. albicans* by the MTT microdilution assay. The positive results are presented in Table 5.8. Compound 5.3 was found to have good activity against the susceptible strain of *S. aureus* (MIC 156 µg/mL) and both resistant strains of *S. aureus* (MIC 312 µg/mL) and weak activity for the susceptible strain of *E. coli*, *S. typhimurium* and *P. aeruginosa*. The compound was inactive against the resistant strain of *E. coli* as well as *S. pyogenes* and *C. albicans*.

Table 5.8 Antimicrobial activity of compound 5.3 by MTT microdilution assay

Compound	Antibacterial activity (µg/mL)					
	S. aureus	MRSA	MDRSA	*E. coli β-*	*S. typhimurium*	*P. aeruginosa*
Compound 5.3	156	312	312	2500	2500	2500
Vancomycin	156	312	312	nt	nt	nt
Gentamycin	nt	nt	nt	312	312	312

nt - not tested

This is the first report of compound 5.3 possessing antibacterial activity.

Due to limited time, further studies on the EtOAc partition were not investigated. A summary of the bioassay-guided fractionation is provided in Figure 5.13.

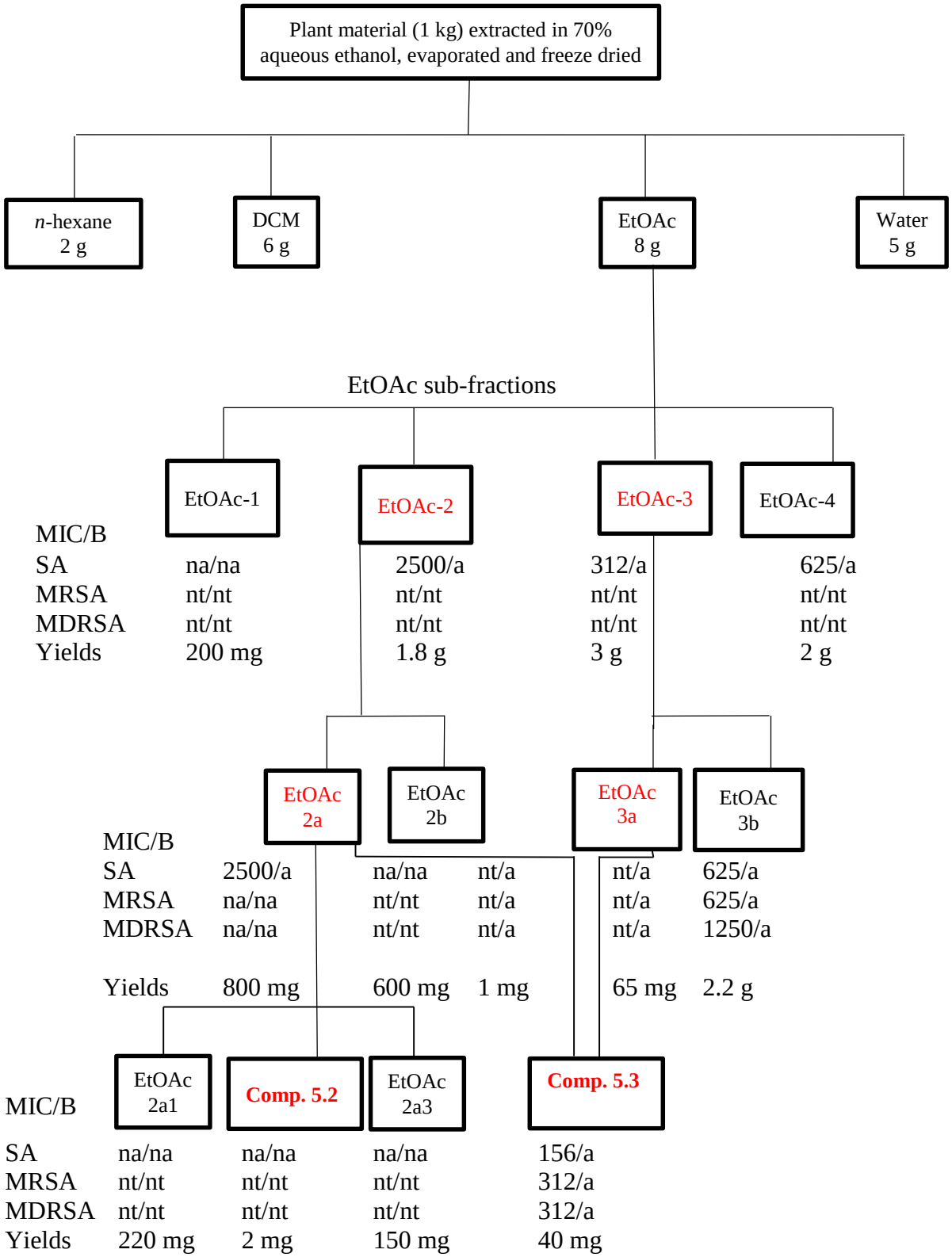


Figure 5.13 Large scale bioautography guided fractionation of EtOAc partition. a: active, na: not active, MIC: minimum inhibitory concentration in MTT microdilution assay (µg/mL), B: overlay bioautography method.

5.2.4.7 Structural elucidation of isolated compounds 5.2 and 5.3

Structural elucidation of compound 5.2

Compound 5.2 (Figure 5.14) was obtained as a creamish amorphous solid. Electrospray ionisation mass spectrometry (ESI-MS) (negative ion mode) indicated a molecular ion at *m/z* 273 $[M+H]^+$, consistent with the molecular formula $C_{15}H_{14}O_5$ (Appendix 9). A UV absorption maxima was observed at 273 nm, consistent with the presence of a flavan structure (Bilia *et al.* 1996).

The ^{13}C NMR (Appendix 6) spectrum showed the presence of three aliphatic (CH_2 x 1, CH x 2, C-O-H x 1) and 12 aromatic (CH x 6, C x 3, C-O x 3) signals. The DEPT spectrum revealed the presence of one methylene, six methine (two of double intensity) and six quaternary carbon atoms. The ^1H NMR spectrum of compound 5.2 exhibited characteristic signals for two highly shielded *meta* coupled aromatic protons [δ_H 5.83 and 5.91 (each *d*, *J* 2.2 Hz)], and a pair of *para*-disubstituted aromatic protons [δ 7.21 (*d*, *J* 8.5 Hz) and 6.77 (*d*, *J* 8.5 Hz)]. The proton signals at δ 4.58 (*d*, *J* 8.0 Hz), 3.97 (*m*), 2.87 (*dd*, *J* 5.5, 16.1 Hz) and 2.49 (*dd*, *J* 8.5, 16.1 Hz) were characteristic of a flavan C ring of a flavan-3-ol skeleton with a phloroglucinol pattern for ring A [δ 5.91 (*d*, *J* 2.1 Hz) and 5.83 (*d*, *J* 2.1 Hz)] (Hussein *et al*., 1999; Zhou *et al*., 2005).

A calculation of index of hydrogen deficiency (IHD) for the molecular formula of $C_{15}H_{14}O_5$ gave a total IHD of nine. Twelve aromatic carbons accounted for two separate aromatic rings, which gives an IHD of eight. The ^1H NMR and ^{13}C NMR spectra showed the molecule does not contain any further double bonds. The presence of three aliphatic carbons, out of which two are CH, one a CH_2, and one C-OH suggested the presence of another heterocyclic ring to account for the remaining IHD. An HSQC experiment established the bond correlation between the H and C nuclei (Table 5.9, Appendix 7).

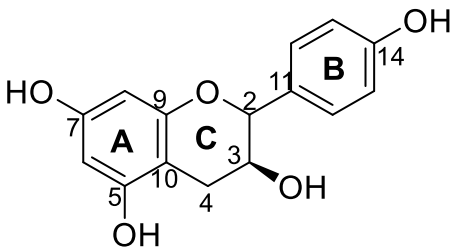


Figure 5.14 The structure of compound 5.2.

The ^{1}H-^{13}C long range coupling information obtained from the heteronuclear multiple bond connectivity (HMBC) experiment allowed the rings to be connected (Figure 5.15, Appendix 8). The proton peak at δ_H 2.87 (H-4) correlated with δ_C 68.87 (C-3), δ_C 157.88 (C-5), δ_C 152.28 (C-9), δ_C 100.8 (C-10) and the proton peak at δ_H 4.58 (H-2) correlated with δ_C 68.87 (C-3), δ_C 157.28 (C-9), δ_C 131.5 (C-11) and δ_C 159.58 (C-12, 16).

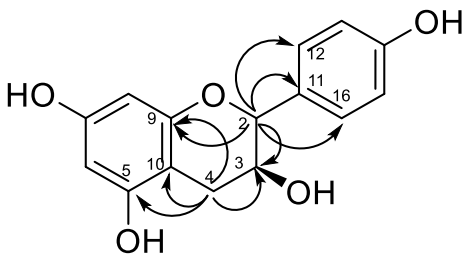


Figure 5.15 Key HMBC correlations of flavonoid rings of compound 5.2.

The coupling constant (*d, J* 8.0 Hz) for δ_H 4.58 indicated a flavan H-2 signal with a 2,3-*trans* configuration (Min-Won *et al*., 1992) and this was confirmed by the chemical shift appearance of a signal at δ 82.7 (C-2) (Vdovin *et al*., 1997). NMR data are provided in Table 5.9.

Based on this spectroscopic evidence and by comparison with the literature, the compound was identified as afzelechin (Wan and Chan 2004, Saijyo *et al.* 2008). This was first isolated from *Corymbia calophylla* (formerly *Eucalyptus calophylla*) from Australia (Hillis and Carle 1963) and since then has been isolated from various other plants such as *Saxifraga ligulata* (Tucci *et al.* 1969, Reddy *et al.* 1999). This compound has also been synthesised (Wan and Chan 2004).

Table 5.9 NMR data of compound 5.2 (400 MHz, CD_3OD)

Position	δ_C	δ_H (*J* in Hz)	HMBC	Multiplicity
2	82.77	4.58 *d* (8.0)	C-3, C-9, C-11, C-12, C-16	CH
3	68.87	3.97 *m*		CH
4	29.15	2.87 *dd* (5.5, 16.1) 2.49 *dd* (8.5, 16.1)	C-3, C-5, C-9, C-10	CH_2
5	157.88			C
6	95.48	5.83 *d* (2.1)	C-5, C-8	CH
7	157.8			C
8	96.41	5.91 *d* (2.1)	C-6, C-9, C-10	CH
9	157.28			C
10	100.8			C
11	131.5			C
12	129.58	7.21 *d* (8.5)	C-2, C-16, C-14	CH
13	116.21	6.77 *d* (8.5)	C-11, C-14, C-15	CH
14	156.86			C
15	116.21	6.77 *d* (8.5)	C-11, C-14, C-13	CH
16	129.58	7.21 *d* (8.5)	C-2, C-12, C-14	CH

Structural elucidation of compound 5.3

Compound 5.3 (Figure 5.16) was obtained as white crystals. The compound changed colour when tested for melting point (decomposition at 238 ºC). ESI-MS (negative ion mode) indicated a molecular ion at *m/z* 543 $[M-H]^-$ (Appendix 15). HRMS confirmed the molecular formula of $C_{30}H_{24}O_{10}$ (found: 567.1266 [M+Na], calculated: 567.1267).

The IR spectrum exhibited strong absorption bands for OH groups (3319 cm^{-1}) as well as a band at 1067 cm^{-1} indicative of the presence of a C-O stretch in the molecule (Appendix 16). It also showed characteristic bands for aromatic groups. An UV absorption maxima observed at 273 nm suggested the presence of a flavan structure (Bilia *et al.* 1996).

The molecular formula of $C_{30}H_{24}O_{10}$ gave a total IHD of nineteen. Twenty four aromatic carbons accounted for four separate aromatic rings, which gives an IHD of sixteen. The remaining number of IHDs in the molecule was three. The ^1H NMR and ^{13}C NMR spectra showed the molecule does not contain any further double bonds. The presence of six aliphatic carbons, out of which one is a CH, one a CH_2, three CHOs and one a O-C-O suggested the presence of another three heterocyclic rings to account for the remaining three IHDs.

The ^{1}H NMR and ^{13}C NMR spectra were done in deuterated acetone (CD_3COCD_3) as well as in deuterated methanol (CD_3OD). Seven protons at δ_H 8.64, δ_H 8.55 (2 H), δ_H 8.22, δ_H 6.92, δ_H 4.33 and δ_H 4.22 were observed in CD_3COCD_3 that were not present in the ^{1}H NMR spectrum in deuterated methanol (CD_3OD). Based on this data, the peaks were assumed to account for exchangeable protons assigned to seven OH groups. This was consistent with the IR spectrum indicating the presence of OH functionality in the molecule.

The ^{1}H NMR spectrum (CD_3COCD_3) (Table 5.10) of compound 5.3 exhibited characteristic peaks for two *meta*-coupled doublets [δ_H 6.06 and 5.92 (each *d, J* 2.2 Hz)] and an aromatic singlet (δ_H 6.13). Two pairs of A_2B_2 aromatic system protons [δ_H 7.35 and 6.85 (each *d, J* 8.6 Hz) and δ_H 7.39 and 6.89 (each *d, J* 8.6 Hz)] revealed the presence of *para*-disubstituted aromatic rings that were identified as *p*-hydroxyphenyl systems from the ^{13}C-NMR chemical shifts of carbon signals at δ 129.3 and δ 115.2 (Bilia *et al.* 1996, Prasad 2000). Additionally, an isolated AB system [δ_H 4.22 and 4.24 (each *d, J* 3.5 Hz)] in the heterocyclic proton region was observed, characteristic of the C-ring protons of A-type proanthocyanidins, consisting of two flavanyl units. Such a structural type was supported by the typical acetal carbon resonance at δ_C 100.55 in the ^{13}C NMR spectrum (Table 5.11). Moreover, the ^{1}H NMR spectrum showed striking similarity to compound 5.2 (afzelechin).

The ^{13}C NMR spectrum showed the presence of six aliphatic (CH_2 x 1, CH x 1, CHO x 3, O-C-O x 1) and twenty four aromatic signals (CH x 11, C x 5, C-O x 8). The DEPT spectrum revealed the presence of one methylene, fourteen methine (two of double intensity) and thirteen quaternary carbon atoms. An HSQC experiment established the bond correlation between H and C nuclei (Table 5.10).

The ^{1}H-^{13}C long range coupling information obtained from the HMBC experiment allowed the rings to be connected. δ_H 7.39 (H-12) correlated with δ_C 100.1 (C-2), δ_C 158.5 (C-14) and δ_C 129.3 (C-16); δ_H 4.24 (H-4) correlated with δ_C 67.1 (C-3), δ_C 100.1 (C-2), δ_C 103.7 (C-10), δ_C 106.3 (C-8'), δ_C 150.6 (C-9'), δ_C 151.8 (C-7'), δ_C 153.8 (C-9) and δ_C 156.5 (C-5); δ_H 7.53 (H-16) correlated with δ_C 100.1 (C-2), δ_C 129.3 (C-12) and δ_C 158.5 (C-14); δ_H 7.39 (H-12') correlated with δ_C 83.6 (C-2'), δ_C 129.87 (C-11') and δ_C 158.5 (C-14'); δ_H 4.81 (H-2') correlated with δ_C 29.2 (C-4'), δ_C 67.7 (C-3'), δ_C 129.87 (C-11'), δ_C 129.80 (C-12') and δ_C 150.6 (C-9'); and δ_H 3.04 (H-4') correlated with δ_C 67.7 (C-3'), δ_C 83.6 (C-2'),

δ_C 102.7 (C-10'), δ_C 150.6 (C-9') and δ_C 155.5 (C-5'). This data supported the structure shown in Figure 5.16.

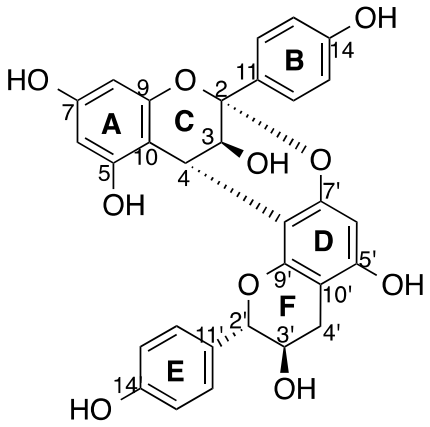


Figure 5.16 Tentative structure of compound 5.3.

The existence of a (2→O→7′) rather than a (2→O→5′) ether linkage was deduced from the HMBC correlations between the phenolic proton at δ_H 8.64 attached to δ_C 155.5 (C-5′) and both δ_C 96.4 (C-6′) and δ_C 102.7 (C-10′). The (4→8′, 2→O→7′) interflavonoid linkage of compound 5.3 was supported by observation of the HMBC cross-peaks of δ_H 4.22 (H-3) with δ_C 106.3 (C-8′) and of δ_H 4.24 (H-4) with δ_C 151.8 (C-7′), δ_C 106.3 (C-8′), and δ_C 150.6 (C-9′) (Figure 5.18). HMBC experiments assigned the OH groups to C-5', C-14, C-14', C-7, C-5 and C-3 respectively. The HMBC correlations between the rings are depicted in Figure 5.17 and the exchangeable hydrogen HMBC correlations are presented in Table 5.10.

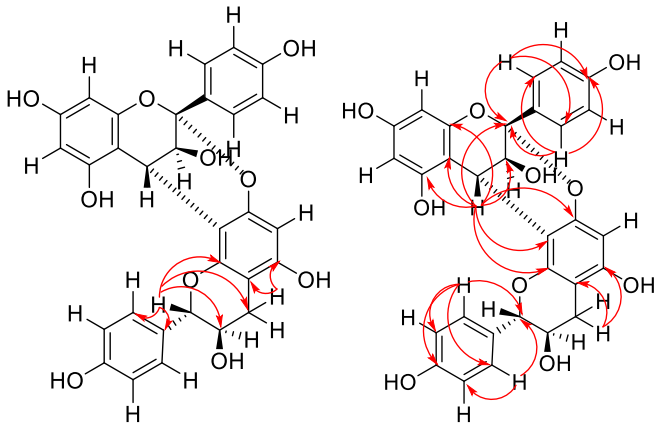


Figure 5.17 Key long–range coupling correlation between the flavanoid rings of proposed compound 5.3 identified by HMBC experiment.

Circular dichroism (CD) measurements allowed the establishment of absolute configurations at C-2 and C-4. A strong negative Cotton effect in the diagnostic wavelength region (232 nm) led to assignment of a 4*S*-configuration of the C-ring, which indicated a (2α, 4α)-configuration of the C-ring since the linkage of the two units of A-type proanthocyanidins must be *cis* (Hikino *et al.*, 1982). Moreover, the absence of NOESY cross-peaks between the δ_H 4.24 (H-4) proton and δ_H 7.39 (H-12′ and H-16′) was in agreement with the α-orientation of the two flavane moieties (Prasad 2000). Figure 5.18.

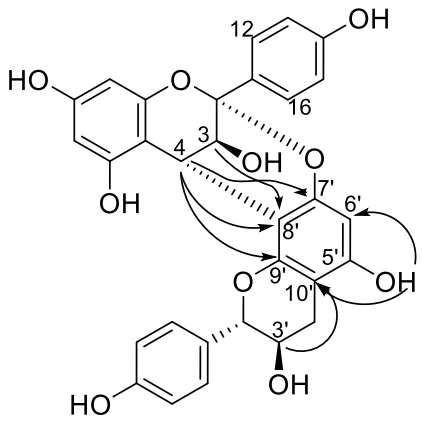


Figure 5.18 Key NOESY correlations of flavonoid rings of compound 5.3.

The absolute configuration at C-2′ of the F-ring was established from the CD spectrum (Appendix 17). Flavan-3-ols with 2*R* and 2*S* absolute configuration give rise to negative and positive Cotton effects, respectively, in the 260–280 nm region of their CD spectra (Bilia *et al.* 1996, Rawat *et al.* 1998, Prasad 2000). Thus, the weak positive Cotton effect around 270 nm ($[\Theta]_{271}$ +580) observed in compound 5.3 indicated that the absolute configuration at C-2 of the F-ring differed from that of the C-ring, indicating a 2*S*-configuration of the F-ring (Bilia *et al.* 1996).

The large coupling constant (*J* 8.0 Hz) between H-2′ and H-3′ of the F-ring reflected a relative *trans*-configuration (Bilia *et al.* 1996, Rawat *et al.* 1998, Prasad 2000). Consequently, the absolute configurations at C-2 and C-3 of the F-ring were deduced to be *S* and *R*, respectively, and the lower flavanyl unit of compound 5.3 was thus confirmed as *ent*-afzelechin (Figure 5.19).

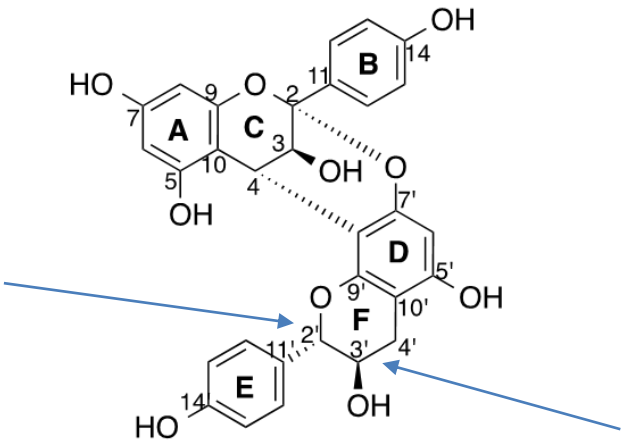


Figure 5.19 *trans*-Configuration of the lower flavanyl moiety.

Table 5.10 NMR data of compound 5.3 (600 MHz, CD_3COCD_3)

C #	δ_C	δ_H (*J* in Hz)	HMBC	Multiplicity	Key NOESY
2	100.1			C	
3	67.1	4.22 *d* (3.5)		CH	C-8’
3-OH		4.33 *d* (4.8)	C-2, C-3, C-4		
4	28.8	4.24 *d* (3.5)	C-3, C-2, C-10, C-8', C-9', C-7', C-9, C-5	CH	C-7’, C-8’, C-9’
5	156.5			C	
5-OH		6.92 *s*	C-4, C-5, C-6		
6	97.8	5.91 *d* (2.2)	C-10, C-5, C-7	CH	
7	157.9			C	
7-OH		8.22 *s*	C-6, C-7, C-8		
8	96.1	6.06 *d* (2.2)	C-6, C-10, C-9	CH	
9	153.8			C	
10	103.7			C	
11	131.4			C	
12	129.3	7.53 *d* (8.6)	C-2, C-14, C-16	CH	
13	115.2	6.85 *d* (8.6)	C-15, C-11, C-14	CH	
14	158.5			C	
14-OH		8.55 *s*	C-13, C-15, C-14		
15	115.2	6.85 *d* (8.6)	C-13, C-11, C-14	CH	
16	129.3	7.53 *d* (8.6)	C-2, C-12, C-14	CH	
1'					
2'	83.6	4.81 *d* (8.5)	C-4', C-3', C-11', C-12', C-9'	CH	
3'	67.7	4.13 *m*		CH	C-10’
3’-OH		4.24 *s*	C-3’, C-4’		
4'	29.2	3.04 *dd* (5.5, 16.5) 2.61 *dd*	C-3', C-2', C-10', C-9', C-5'	CH_2	
5'	155.5			C	
5’-OH		8.64 *s*	C-5’, C-6’, C-10’		C-6’, C-10’
6'	96.4	6.13 *s*	C-10', C-8', C-7', C-5'	CH	
7'	151.8			C	
8'	106.3			C	
9'	150.6			C	
10'	102.7			C	
11'	129.87			CH	
12'	129.80	7.39 *d* (8.6)	C-2', C-11', C-14'	CH	
13'	116.0	6.89 *d* (8.6)	C-11', C-14', C-15'	CH	
14'	158.5			C	
14’-OH		8.55 *s*	C-13’, C-14’, C-15’		
15'	116.0	6.89 *d* (8.6)	C-11', C-13', C-14'	CH	
16'	129.8	7.39 *d* (8.6)	C-2', C-11', C-14'	CH	

Based on the gathered evidence, the compound was characterised as *ent*-epiafzelechin-(2α→O→7’,4α→8’)-(-)*ent*-afzelechin. This is consistent with the compound previously isolated from *P. persica* roots by Kichu (Kichu 2010), although he found it inactive against

susceptible and resistant strains of *S. aureus* (Kichu 2010), while it was found active in these studies. The NMR data of this compound is presented in Appendices 10-14.

5.2.4.7 Comparison of antimicrobial activities between local *Prunus persica* plant material and the Nagaland sample of the plant

Due to the difficulties that were met when processing and transporting samples from Nagaland to Australia, and the desire for further plant material for further testing and active compound isolation, a locally collected sample of *P. persica* roots was examined as a prelude to possible future investigations. The roots (300 g) were collected from Macquarie University grounds (Herring Rd, Sydney, NSW) and freshly extracted with 70% aqueous ethanol. Antibacterial activity was measured using the MTT assay against susceptible as well as resistant strains of *S. aureus*. An MIC of 156 µg/mL was seen for all of the *S. aureus* strains. The results indicated the local plant material possessed strong and comparable antibacterial activity to that observed for the Nagaland plant material, suggesting it could be a good alternative for future studies.

5.3 Conclusions and future directions

An aqueous ethanolic extract of the roots of *P. persica* was partitioned with *n*-hexane, DCM and EtOAc. Phytochemical studies were conducted to give an idea of the type of compounds present in the partitions and these included terpenes, steroids and phenolics. The *n*-hexane and EtOAc partitions showed the most promising antibacterial properties, with activity against susceptible *S. aureus* as well as MRSA and MDRSA strains. GC-MS analysis of the *n*-hexane partition identified three compounds known to be active against *S. aureus*, namely palmitic acid (Kurtulmus *et al.* 2009), linoleic acid (Dilika *et al.* 2000) and oleic acid (Dilika *et al.* 2000).

TLC bioautography methods in the literature were examined to find the most appropriate conditions to provide guidance as to which compounds should be targeted in the isolation process. This identified overlay TLC bioautography as an appropriate method. Chromatographic methods allowed isolation of three compounds, β-sitosterol from the *n*-hexane partition, and afzelechin and *ent*-epiafzelechin-(2α→O→7',4α→8')-(-) *ent*-afzelechin from the EtOAc partition, respectively. All compounds were characterised using spectroscopic methods.

β-sitosterol was found to possess weak activity against susceptible *S. aureus* as well as *S. typhimurium* and *E. coli* β- and moderate activity against *P. aeruginosa*. *ent*-Epiafzelechin-(2α→O→7',4α→8')-(-)*ent*-afzelechin was found to possess strong antibacterial activity against all tested strains of *S. aureus* and weak activity against *E. coli β-, S. typhimurium* and *P. aeruginosa*. Afzelechin was antibacterially tested only by TLC bioautography due to insufficient quantity and was found inactive against susceptible *S. aureus*. It was also observed to decompose upon storage.

There were other active compounds detected by TLC bioautography especially in the *n*-hexane partition, but due to the low amount of Nagaland *P. persica* plant material, and in some cases issues of possible instability, they were not isolated. Preliminary examination of a local (Sydney, Australia) sample of *P. persica* roots, showed comparable activity to the Nagaland sample. This suggests that local *P. persica* would be worthy of exploration for future studies, including the isolation of further bioactive compounds.

Abate, G., R. Mshana and H. Miörner (1998). "Evaluation of a Colorimetric Assay Based on 3-(4, 5-Dimethylthiazol-2-Yl)-2, 5-Diphenyl Tetrazolium Bromide (MTT) for Rapid Detection of Rifampicin Resistance in *Mycobacterium tuberculosis*." *The International Journal of Tuberculosis and Lung Disease* **2**(12): 1011-1016.

Appendino, G., S. Gibbons, A. Giana, A. Pagani, G. Grassi, M. Stavri, E. Smith and M. M. Rahman (2008). "Antibacterial Cannabinoids from *Cannabis sativa*: A Structureal-Activity Study." *Journal of Natural Products* **71**(8): 1427-1430.

Aubert, C. and C. Milhet (2007). "Distribution of the Volatile Compounds in the Different Parts of a White-Fleshed Peach (*Prunus persica* L. Batsch)." *Food Chemistry* **102**(1): 375-384.

Beltrame, F. L., G. L. Pessini, D. L. Doro, B. P. Dias Filho, R. B. Bazotte and D. A. G. Cortez (2002). "Evaluation of the Antidiabetic and Antibacterial Activity of *Cissus sicyoides*." *Brazilian Archives of Biology and Technology* **45**(1): 21-25.

Bernal, P., C. Molina-Santiago, A. Daddaoua and M. A. Llamas (2013). "Antibiotic Adjuvants: Identification and Clinical Use." *Microbial Biotechnology* **6**(5): 445-449.

Bilia, A., I. Morelli, M. Hamburger and K. Hostetmann (1996). "Flavans and A-Type Proanthocyanidins from *Prunus prostrata*." *Phytochemistry* **43**(4): 887-892.

Cherigo, L., R. Pereda-Miranda and S. Gibbons (2009). "Bacterial Resistance Modifying Tetrasaccharide Agents from *Ipomoea murucoides*." *Phytochemistry* **70**(2): 222-227.

Cho, E. J., J. Y. Choi, K. H. Lee and S. Lee (2012). "Isolation of Antibacterial Compounds from *Parasenecio pseudotaimingasa*." *Horticulture, Environment, and Biotechnology* **53**(6): 561-564.

Dickson, R. A., P. J. Houghton and P. J. Hylands (2007). "Antibacterial and Antioxidant Cassane Diterpenoids from *Caesalpinia benthamiana*." *Phytochemistry* **68**(10): 1436-1441.

Dilika, F., P. Bremner and J. Meyer (2000). "Antibacterial Activity of Linoleic and Oleic Acids Isolated from *Helichrysum pedunculatum*: A Plant Used During Circumcision Rites." *Fitoterapia* **71**(4): 450-452.

Ebada, S. S., R. A. Edrada, W. Lin and P. Proksch (2008). "Methods for Isolation, Purification and Structural Elucidation of Bioactive Secondary Metabolites from Marine Invertebrates." *Nature Protocols* **3**(12): 1820-1831.

Gibbons, S. (2005). An Introduction to Planar Chromatography. Natural Products Isolation, Springer**:** 77-116.

Goodall, R. and A. Levi (1947). "A Micro-Chromatographic Method for the Detection and Approximate Determination of the Different Penicillins in a Mixture." *Analyst* **72**(856): 277-290.

Gowri, S. S. and K. Vasantha (2010). "Phytochemical Screening and Antibacterial Activity of *Syzygium cumini* (L.) (*Myrtaceae*) Leaves Extracts." *International Jurnal of Pharmaceutical and Technical Research* **2**(2): 1569-1573.

Hamburger, M. O. and G. A. Cordell (1987). "A Direct Bioautographic Tlc Assay for Compounds Possessing Antibacterial Activity." *Journal of Natural Products* **50**(1): 19-22.

Harborne, J. B. (1984). Phytochemical Methods, Springer.

Harborne, J. B. (1989). Methods in Plant Biochemistry. Volume 1. Plant Phenolics, Academic Press Ltd.

Hayyan, A., M. Z. Alam, M. E. Mirghani, N. A. Kabbashi, N. I. N. M. Hakimi, Y. M. Siran and S. Tahiruddin (2011). "Reduction of High Content of Free Fatty Acid in Sludge Palm Oil Via Acid Catalyst for Biodiesel Production." *Fuel Processing Technology* **92**(5): 920-924.

Herderich, M. and P. Smith (2005). "Analysis of Grape and Wine Tannins: Methods, Applications and Challenges." *Australian Journal of Grape and Wine Research* **11**(2): 205-214.

Hess, S. C., R. L. Brum, N. K. Honda, A. B. Cruz, E. Moretto, R. B. Cruz, I. Messana, F. Ferrari and R. A. Yunes (1995). "Antibacterial Activity and Phytochemical Analysis of *Vochysia divergens* (*Vochysiaceae*)." *Journal of Ethnopharmacology* **47**(2): 97-100.

Hillis, W. and A. Carle (1963). "The Chemistry of Eucalypt Kinos. Iv. *Eucalyptus Hemiphloia* Kino." *Australian Journal of Chemistry* **16**(1): 147-159.

Horváth, G., B. Kocsis, L. Botz, J. Németh and L. Szabo (2002). Antibacterial Activity of Thymus Phenols by Direct Bioautography. Proceedings of the 7th Hungarian Congress on Plant Physiology. Acta Biologica.

Islam, N., S. Parveen, N. Nakazawa, A. Marston and K. Hostettmann (2003). "Bioautography with the Fungus *Valsa ceratosperma* in the Search for Antimycotic Agents." *Pharmaceutical Biology* **41**(8): 637-640.

Jamaluddin, F., S. Mohamed and M. N. Lajis (1994). "Hypoglycaemic Effect of *Parkia speciosa* Seeds Due to the Synergistic Action of β-Sitosterol and Stigmasterol." *Food Chemistry* **49**(4): 339-345.

Jirovetz, L., G. Buchbauer, Z. Denkova, A. Stoyanova, I. Murgov, E. Schmidt and M. Geissler (2005). "Antimicrobial Testings and Gas Chromatographic Analysis of Pure Oxygenated Monoterpenes 1, 8-Cineole, A-Terpineol, Terpinen-4-Ol and Camphor as Well as Target Compounds in Essential Oils of Pine (*Pinus pinaster*), Rosemary (*Rosmarinus officinalis*), Tea Tree (*Melaleuca alternifolia*)." *Scientia Pharmaceutica* **73**(1): 27-38.

Joshi, S. and T. N. Poudel (2011). "Isolation and Characterization of the Chemical Constituents of *Sonchus wightianus* of Nepalese Origin." *Journal of Nepal Chemical Society* **28**(7): 115-120.

Kaufman, R., M. Tilley, S. Bean and M. Tuinstra (2009). "Improved Characterization of Sorghum Tannins Using Size-Exclusion Chromatography." *Cereal Chemistry* **86**(4): 369-371.

Kichu, M. (2010). Documentation and Biological and Phytochemical Analysis of Chungtia Medicinal Plants of Nagaland, India, Diss. *Chemistry and Biomolecular Sciences*, Macquarie University, Sydney. **PhD:** 1-179.

Kircher, H. W. and F. U. Rosenstein (1973). "Purification of Sitosterol." *Lipids* **8**(3): 97-100.

Kurtulmus, A., T. Fafal, T. Mert, H. Saglam, B. Kivcak, T. Ozturk, B. Demirci and K. H. Baser (2009). "Chemical Composition and Antimicrobial Activity of the Essential Oils of Three Anthemis Species from Turkey." *Chemistry of Natural Compounds* **45**(6): 900-904.

Mokbe, M. S. and F. Hashinaga (2005). "Antibacterial and Antioxidant Activities of *Banana musa*, Cv. Cavendish Fruits Peel." *American Journal of Biochemistry and Biotechnology* **1**(3): 125.

Murphy, B. and R. D'Aux (1975). "The Use of Sephadex Lh-20 Column Chromatography to Separate Unconjugated Steroids." *Journal of Steroid Biochemistry* **6**(3): 233-237.

Okuda, T., T. Yoshida and T. Hatano (1989). "New Methods of Analyzing Tannins." *Journal of Natural Products* **52**(1): 1-31.

Okuda, T., T. Yoshida and T. Hatano (1990). "Oligomeric Hydrolyzable Tannins, a New Class of Plant Polyphenols." *Heterocycles* **30**(2): 1195-1218.

Oros, D. R. and B. R. Simoneit (2001). "Identification and Emission Factors of Molecular Tracers in Organic Aerosols from Biomass Burning Part 1. Temperate Climate Conifers." *Applied Geochemistry* **16**(13): 1513-1544.

Palombo, E. A. and S. J. Semple (2002). "Antibacterial Activity of Australian Plant Extracts against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-Resistant *Enterococci* (Vre)." *Journal of Basic Microbiology* **42**(6): 444-448.

Pattnaik, S., V. Subramanyam, M. Bapaji and C. Kole (1997). "Antibacterial and Antifungal Activity of Aromatic Constituents of Essential Oils." *Microbios* **89**(358): 39-46.

Politi, L., F. Mari, S. Furlanetto, E. Del Bravo and E. Bertol (2011). "Determination of Fatty Acid Ethyl Esters in Hair by Gc–Ms and Application in a Population of Cocaine Users." *Journal of Pharmaceutical and Biomedical Analysis* **54**(5): 1192-1195.

Prasad, D. (2000). "Two A-Type Proanthocyanidins from *Prunus armeniaca* Roots." *Fitoterapia* **71**(3): 245-253.

Rahalison, L., M. Hamburger, K. Hostettmann, M. Monod and E. Frenk (1991). "A Bioautographic Agar Overlay Method for the Detection of Antifungal Compounds from Higher Plants." *Phytochemical Analysis* **2**(5): 199-203.

Rani, M. P., K. G. Raghu, M. S. Nair and K. P. Padmakumari (2014). "Isolation and Identification of A-Glucosidase and Protein Glycation Inhibitors from *Stereospermum colais*." *Applied Biochemistry and Biotechnology*: Ahead of Print.

Rawat, M. S. M., G. Pant, D. Prasad, R. K. Joshi and C. B. Pande (1998). "Plant Growth Inhibitors (Proanthocyanidins) from *Prunus armeniaca*." *Biochemical Systematics and Ecology* **26**(1): 13-23.

Reddy, U. D. C., A. S. Chawla, M. Deepak, D. Singh and S. S. Handa (1999). "High Pressure Liquid Chromatographic Determination of Bergenin and (+)-Afzelechin from Different Parts of Paashaanbhed (*Saxifraga ligulata* Yeo)." *Phytochemical Analysis* **10**(1): 44-47.

Rios, J. and M. Recio (2005). "Medicinal Plants and Antimicrobial Activity." *Journal of Ethnopharmacology* **100**(1): 80-84.

Saijyo, J., Y. Suzuki, Y. Okuno, H. Yamaki, T. Suzuki and M. Miyazawa (2008). " Alpha-Glucosidase Inhibitor from *Bergenia ligulata*." *Journal of Oleo Science* **57**(8): 431-435.

Santos, T. G., J. Dognini, I. M. Begnini, R. A. Rebelo, M. Verdi, A. L. d. Gasper and E. M. Dalmarco (2013). "Chemical Characterization of Essential Oils from *Drimys angustifolia* Miers (*Winteraceae*) and Antibacterial Activity of Their Major Compounds." *Journal of the Brazilian Chemical Society* **24**(1): 164-170.

Sarneckis, C., R. G. Dambergs, P. Jones, M. Mercurio, M. J. Herderich and P. A. Smith (2006). "Quantification of Condensed Tannins by Precipitation with Methyl Cellulose:

Development and Validation of an Optimised Tool for Grape and Wine Analysis." *Australian Journal of Grape and Wine Research* **12**(1): 39-49.

Shahverdi, A. R., F. Abdolpour, H. R. Monsef-Esfahani and H. Farsam (2007). "A Tlc Bioautographic Assay for the Detection of Nitrofurantoin Resistance Reversal Compound." *Journal of Chromatography* **850**(1): 528-530.

Spangenberg, B. (2008). "Derivatization, Detection (Quantification), and Identification of Compounds Online." *Thin layer Chromatography in Phytochemistry*: 175.

Tucci, A., F. Delle Monache and G. Marini-Bettòlo (1969). "The Occurrence of (+) Afzelechin in *Saxifraga ligulata* Wall." *Annali Dell'Istituto Superiore di Sanità* **5**(5): 555.

Valgas, C., S. M. d. Souza, E. F. Smânia and A. Smânia Jr (2007). "Screening Methods to Determine Antibacterial Activity of Natural Products." *Brazilian Journal of Microbiology* **38**(2): 369-380.

Wagner, H. (1996). Plant Drug Analysis: A Thin Layer Chromatography Atlas, Springer.

Waksmundzka-Hajnos, M., J. Sherma and T. Kowalska (2008). Thin Layer Chromatography in Phytochemistry, CRC Press.

Wan, S. B. and T. H. Chan (2004). "Enantioselective Synthesis of Afzelechin and Epi-Afzelechin." *Tetrahedron* **60**(37): 8207-8211.

Yanagida, A., T. Kanda, T. Shoji, M. Ohnishi-Kameyama and T. Nagata (1999). "Fractionation of Apple Procyanidins by Size-Exclusion Chromatography." *Journal of Chromatography* **855**(1): 181-190.

Yanagida, A., T. Shoji and Y. Shibusawa (2003). "Separation of Proanthocyanidins by Degree of Polymerization by Means of Size-Exclusion Chromatography and Related Techniques." *Journal of Biochemical and Biophysical Methods* **56**(1): 311-322.

Chapter 6

Experimental

6.1 Reagents and equipment

All the solvents used for extractions and chromatographic separations were of analytical HPLC grade. Analytical normal phase thin layer chromatography (TLC) was performed on fluorescent Merck silica gel F_{254} plates (Germany). Preparative TLC (PTLC) was carried out using Uniplate preparative TLC plates (Sigma-Aldrich). The TLC plates were visualised using UV light (254 nm and 365 nm) and different spray reagents. Normal phase column chromatography was performed using silica gel 60 (0.040-0.063 mm). Size exclusion chromatography (SEC) was carried out using Sephadex LH-20 (Sigma-Aldrich). Mueller Hinton II agar, horse blood agar, sabouraud dextrose (SAB) broth, potato dextrose agar, Todd Hewitt (TH) broth and Mueller Hinton (MH) II broth were purchased from Bacto laboratories Pty Ltd (Australia). Filter paper discs (6 mm) used for disc diffusion assays were from Whatman (UK). Kanamycin, gentamycin, vancomycin and fluconazole were obtained from Amresco (USA). Flat bottom 96-well microtitre plates were from Greiner. MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) was purchased from Sigma-Aldrich. β-Sitosterol (95%) was purchased from Sigma-Aldrich.

The freeze dryer system was from Labconco (USA). The ^{1}H, ^{13}C, HSQC, COSY, HMBC and NOESY NMR spectra were recorded on Bruker Avance AMX 400 and Bruker DRX600K 600 MHz NMR Spectrometers (Germany) using standard pulse sequences. Chemical shifts were calculated relative to the chloroform (^{1}H δ 7.24 and ^{13}C δ 77.2), acetone (^{1}H δ 2.04 and ^{13}C δ 205.8 and 30.6) and methanol (^{1}H δ 3.31 and ^{13}C δ 49.0) solvent peaks. A Shimadzu 2010 LC-MS system was used for electrospray ionisation mass spectrometry (ESI-MS) analysis. A Shimadzu GC-17 system was used for electron impact mass spectrometry (EI-MS) analysis. Analytical gas chromatography (GC) was carried out on a Shimadzu GC17A gas chromatograph. High resolution mass spectrometry (HR-MS) was determined using a Bruker Apex 3 instrument.

6.1.1 Collection, preparation and importation of plant materials to Australia

Seven plants, namely *Dendrocnide sinuata*, *Eurya acuminata*, *Duabanga grandiflora*, *Maesa indica, Holboellia latifolia*, *Prunus persica* and *Erythrina stricta* were selected to be imported and subjected to antimicrobial screening.

All plant materials were collected from Chungtia village, Nagaland, India, by the villagers with the assistance of Mr Anungba Jamir, a Chungtia Senso Mokokchung Town (CSMT) representative. The collections were done between the months of June and August 2007 and 2009.

All plant materials were thoroughly inspected by Mr Anungba Jamir for precise identification of the species and dried in the shade for 10 to 20 days. Voucher specimens for each plant were deposited at Botanical Survey of India (BSI), Shillong Branch, India. For further processing, *D. sinuata, E. acuminata, D. grandiflora, M. indica, H. latifolia* and *E. stricta* were transported to Chennai, India to Dr Velmurugan, while *P. persica* (which was collected and dried at a different time), was transported to Dr Udaya Sankar of Central Food Technological Research Institute (CFTRI), located in Mysore, India. Upon receipt by Dr Velmurugan or Dr Sankar, the plant materials were separated from foreign particles, washed with clean water, dried in the shade for 24 hours then dried in a vacuum drier at 75 to 85 ^{o}C. After 48 hours of drying under vacuum, the plants were kept in the shade for three days and rechecked for foreign particles. After confirming that there were no foreign particles, the plant materials were chopped and passed through a micropulveriser for grinding. The process was repeated until 130-200 mesh size was obtained. The powders were then sieved and dried again under vacuum to ensure that they did not contain any moisture. The plant materials were allowed to cool in the shade and packed into plastic bags, which in turn were packed in plastic containers and sealed. The sealed containers were couriered to Australia, under the import permit IP12012991from Department of Agriculture, Fisheries and Forestry (DAFF).

A local plant specimen of *P. persica* roots and bark was collected from Macquarie University grounds (North Ryde, NSW, Australia) by IBRG ethnobotanist Mr David Harrington in June 2014. A voucher specimen was lodged at the IBRG Herbarium, voucher number 0001.

6.1.2 Preparation of plant materials for antimicrobial screening

Nagaland Plant materials were prepared in Nagaland as described above. Freshly collected roots of the local (Australian) *P. persica* specimen were chopped with a heavy duty Waring blender (John Morris scientific) to produce the ground material. All plant materials were suspended in 70% aqueous ethanol, shaken overnight at room temperature and vacuum filtered to collect the filtrates. The materials were re-extracted as above, two more times, and the combined filtrates were rotary evaporated (Büchi) at 37 ^{o}C. The water residues were freeze dried overnight. The results are tabulated in Table 6.1.

Table 6.1 Weight and appearance of freeze dried extracts (small scale extraction) for antimicrobial screening

Plant sample	Voucher no.	Weight and appearance of extracts
E. stricta stem	69629	0.45 g dark brown solid
P. persica root*	69503	2.30 g dark red solid
P. persica root×	0001	2.32 g dark red solid
M. indica leaf	69514	0.9 g green gum
H. latifolia leaf	69500	0.50 g dark green gum
D. grandiflora stem bark	69686	0.30 g very dark red solid
E. acuminata leaf	69612	0.3 g brown solid
D. sinuata stem	69508	0.2 g brown gum

*Plant material collected in Nagaland. × Plant material collected locally (Australia). Initial weight of samples-10g, extracted with 100 mL, 70% aqueous ethanol x 3.

For the disc diffusion assay, the freeze dried, powdered crude extracts (10 mg) were dissolved in 200 µL DMSO and then diluted to a final volume of 1 mL in milliQ water. Discs (6 mm, Whatman) were loaded with the samples and dried with a hair dryer to afford a concentration of 1 mg per disc. For the MTT microdilution assay, the crude extracts (10 mg) were dissolved in 200 µL of DMSO and diluted with MH II broth to give a starting concentration of 10 mg/mL. 100 µL of each sample was transferred into the first well containing 100 µL of broth and 100 µL of bacteria giving a starting concentration of 2500 µg/mL.

6.1.3 Preparation of *P. persica* extracts

6.1.3.1 Preparation of dried plant material of *P. persica* for large scale extraction and partitioning

Powdered dried root of *P. persica* (Nagaland specimen, 1 kg) was suspended in 70% aqueous ethanol (1 L) and shaken overnight at room temperature. The extract was then filtered and the process repeated three times. After removal of ethanol by rotary evaporation (Büchi) at 37 ^{o}C, the remaining aqueous residue (approximately 900 mL) was successively partitioned with *n*-hexane (1 L), DCM (1 L) and EtOAc (1 L). The process was repeated three times. This afforded four partitions, *ie n*-hexane (2 g, green solid), DCM (6 g, fair red solid) and EtOAc (8 g, dark red solid) partitions and the remaining water residue which was deemed the water partition (5 g, blackish red solid).

6.2 Bioassays: methods and materials

6.2.1 Microbes

Table 6.2 Microbial strains used in antimicrobial assays of crude plants materials, partitioned extracts as well as partly purified and purified compounds

Organism	Strain[a]	Characteristics	cfu/mL (A_{600} = 0.08)[b]
Escherichia coli (β-)	ATCC 25922	*β* lactamase negative – sensitive to common antibiotics	2.54 x 10^7
Escherichia coli (β+)	ATCC 35218	*β* lactamase positive	5.32 x 10^7
Pseudomonas aeruginosa	ATCC 27853	sensitive to common antibiotics	6.97 x 10^7
Salmonella typhimurium		clinical isolate	8.59 x 10^7
Streptococcus pyogenes		clinical isolate	3.70 x 10^7
Staphylococcus aureus (susceptible)	ATCC 29213	sensitive to common antibiotics	9.62 x 10^7
Staphylococcus aureus (MRSA)	ATCC BAA 1026	community acquired methicillin resistant *S. aureus* (MRSA)	6.38 x 10^7
Staphylococcus aureus (MDRSA)		wild multidrug resistant (MDRSA) clinical isolate	3.76 x 10^8
Candida albicans		fungus, clinical isolate	4.56 x 10^5

[a]American Type Culture Collection strain designation where applicable. [b]The inoculum sizes (cfu/mL) at optical density 0.08 (λ 600 nm) were estimated using the spread plate colony count method (Willey *et al.*, 2011).

The use of all microbial strains was approved by the Macquarie University Biosafety Committee (approval references 05/14 LAB, TEM170512BHA). All cultures were provided by Dr John Merlino (Department of Microbiology, Concord Hospital, Sydney).

Stock cultures of the bacterial strains were maintained in MH II broth containing 10% *v/v* glycerol. The stock culture of the fungus was maintained in SAB broth containing 10% *v/v* glycerol. Fresh subcultures were made by inoculating the bacterial cultures in MH II broth with the exception of *S. pyogenes* which was inoculated in Todd Hewitt broth and the fungus in SAB broth followed by an overnight incubation at 37 ºC (bacteria) and 30 ºC (fungus), respectively. For all antimicrobial assays, the bacteria were grown overnight in MH II broth and *C. albicans* in SAB broth. After overnight incubation the optical density at 600 nm (OD_{600}) was measured and the density was adjusted to 0.08 with fresh MH II or Todd Hewitt or SAB broths, as appropriate.

6.2.2 Disc diffusion assay

For the disc diffusion assay the freeze dried samples (10 mg) were dissolved in 200 µL DMSO, and then diluted to a final volume of 1 mL in distilled water. Discs were loaded with the samples and dried with a hair dryer to afford a sample concentration of 2 mg per disc. The MH II agar medium was prepared for all bacterial strains (except *S. pyogenes*) as per the manufacturer's protocol and autoclaved at 121 ºC for 20 minutes. Potato dextrose agar was used for *C. albicans* and horse blood agar for *S. pyogenes*. The molten media were poured into petri plates (10 to 15 mL) and allowed to solidify.

Using a sterile cotton swab, the diluted culture of the microorganism was swabbed evenly on the entire surface of the appropriate agar plate to provide a lawn of microbes. Whatman discs (6 mm) impregnated with the samples were then placed on the inoculated plate and pressed down gently. The plates were incubated at 37 ºC for 18 hours (bacteria) and at 30 ºC for 24 hours (*C. albicans*), and the diameter of the zones of inhibition was measured with a ruler (including the 6 mm disc diameter). Negative control discs were prepared with 20% DMSO/H_2O. Positive control discs were impregnated with appropriate antibiotics 2 µg per disc. Vancomycin and kanamycin were used as positive controls for *S. aureus* strains (susceptible *S. aureus*, MRSA, MDRSA) and gentamycin was used for β-, β+ *E. coli*, *P. aeruginosa* and *S. typhimurium* and *S. pyogenes*. Fluconazole was used as a positive control for *C. albicans*.

6.2.3 MTT Microdilution assay

For the MTT microdilution assay, the samples (10 mg/mL) or the antibiotic (1 mg/mL) were dissolved in 200 µL DMSO and diluted with MH II broth for all bacterial strains with the exception of *S. pyogenes* for which Todd Hewitt (TH) broth was used. SAB broth was used for *C. albicans*. Using a 96 well microtitre plate, 100 µL of suitable broth was dispensed into wells 1-11 (from left to right) for each row, 100 µL of the samples or antibiotic was added to well 1 (in different rows for each sample) and mixed thoroughly, after which 100 µL was taken out and dispensed to the next well (*i.e.* well 2). This process of two-fold serial dilution was carried out until well 10, and skipping well 11, with the final volume dispensed into well 12. 100 µL each of the microbial inoculum was dispensed into wells 1 to 11 leaving well 12. Since well 11 was free of the test sample or the antibiotic, this acted as a positive control for the growth of the inoculum and well 12 being free of inoculum served as the sterile control of the assay. 5% aqueous DMSO was also included as a negative control. The plate was incubated at 37 °C for 18 to 20 hours and finally 20 µL of solution of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliium bromide) in methanol (5 mg/mL) was added to each well and again incubated for 30 minutes. The MIC values were determined as the lowest concentration of the test sample or antibiotic that showed no visible colour change from yellow to blue.

6.2.4 Direct bioautography

Normal phase silica gel TLC plates were preconditioned by heating at 120 ºC in the oven for approximately 4 hours. The plant samples (50 µg) in methanol were spotted on the TLC plates (10×10 cm) in duplicate, gently dried, developed with the appropriate solvent systems and dried with a hair dryer (cold air flow) for complete removal of solvents. One of the TLC chromatograms was retained as the reference plate. The inoculum of test bacteria was prepared by overnight incubation and its OD was adjusted to 0.08 with MH II broth (1:100 dilution). The test TLC chromatogram was then dipped in the bacterial inoculum (susceptible *S. aureus*) under sterile conditions, left submerged for approximately 15 seconds, removed and placed on the agar plate facing upward. The chromatogram was incubated overnight at 37 ºC and gently covered with a thin layer of a solution of MTT in methanol (2.5 mg/mL). The stained chromatogram was then incubated for approximately 1 hour to allow visualisation. White zones of inhibition against a purple background indicated antibacterial activity.

6.2.5 Contact bioautography

The test samples were spotted in duplicate and developed as described above. The inoculum of test bacteria was prepared as above. Agar for this test was prepared by mixing it with the bacterial inoculum to achieve 0.08 OD (dilution 1:100). The agar plate was then prepared by pouring molten, inoculated agar into a petri dish. After solidification of the agar, the developed TLC plate (10×10 cm) was placed on the agar surface facing downward and left for approximately 6 hours in the fridge. The chromatogram was then removed and the agar plate was incubated overnight at 37 ºC. Zones of inhibition were visualised by pouring a solution of MTT in methanol (2.5 mg/mL) onto the agar layer and incubating at 37 ºC for approximately 15 minutes.

6.2.6 Overlay bioautography

For spotting and developing TLC plates, the same method as described above was used. The TLC chromatogram was placed on an agar plate with the silica gel facing upwards. An inoculum of test bacteria was prepared in the broth by overnight incubation. After incubation, OD of the culture was measured and adjusted to 0.16 by diluting with the broth (1:50), then an equal volume of molten agar was added, resulting in a final dilution of 0.08 OD. Approximately 20 mL of the inoculum was rapidly distributed over the TLC plate (10×10 cm). After solidification of the medium, the overlayed TLC plate was incubated overnight at 37 ºC. The bioautogram was then gently covered with a methanolic solution (2.5 mg/mL) of MTT with a sterile micropipette, and then incubated for 5 minutes at 37ºC for the visualisation of results.

6.3 Phytochemical testing: methods and materials

Crude and partitioned extracts were tested for the preliminary detection of different classes of compounds. An iodine chamber, vanillin-sulfuric acid, *p*-anisaldehyde and Dragendorff's spray reagents were used for the detection of unsaturated compounds, terpenes, steroids and alkaloids, respectively. Ferric chloride and lead(II) acetate were used for detection of phenols and tannins. The detection of unsaturated compounds, steroids, terpenes and alkaloids was performed on normal phase silica gel TLC plates developed in different solvent systems. The detection of phenols and tannins was performed in glass test tubes as well as on normal phase chromatograms.

Vanillin-sulfuric acid (Johnsson *et al.* 2007): Vanillin-sulfuric acid reagent was prepared by mixing 6 g vanillin (Sigma-Aldrich) with 2.5 mL concentrated H_2SO_4 in 250 mL ethanol. After spraying with vanillin-sulfuric acid reagent, plates were heated at 100 ºC for 1-2 minutes. Terpenes give blue, red or violet spots, phenols colour pink, red or orange and steroids stain red or blue (Wagner 1996, Gibbons 2005, Spangenberg 2008, Waksmundzka-Hajnos *et al.* 2008). The EtOAc, DCM and water tested samples stained red and pink indicating the presence of steroids, terpenes and/or phenols.

***p*-Anisaldehyde** (Simpson 2011): *p*-Anisaldehyde reagent was composed of 95 mL ethanol, 2.5 mL acetic acid, 2.5 mL concentrated sulfuric acid and 0.5 mL *p*-anisaldehyde. Terpenes and steroids appear as blue or green coloured spots with *p*-anisaldehyde reagent (Wagner 1996). The *n*-hexane samples stained blue with this dye, indicating the presence of steroids and/or terpenes.

Dragendorff's reagent (Appendino *et al.* 2008): Dragendorff's reagent was prepared by adding 10 mL of a 40% aqueous solution of KI to 10 mL of a solution of 0.85 g bismuth(III) subnitrate in acetic acid (10 mL) and milliQ water (50 mL). The resulting solution was diluted with acetic acid and water in the ratio 1:2:10. Alkaloids appear as dark orange or red spots (Waksmundzka-Hajnos *et al.* 2008). No alkaloids were detected in the tested samples.

Iodine chamber: An iodine vapor chamber was made by adding a mixture of iodine crystals powdered with dry silica gel in a sealed glass container. After development and evaporation of solvents the TLC plates were inserted into the chamber. Compounds with unsaturated double or triple bonds appear as brown spots. The presence of unsaturation was observed in most tested samples.

Detection of phenols and tannins: The plant material test sample (about 20 mg) was dissolved in 4 mL of 25% aqueous methanol. 1 mL of solution was transferred in to a test tube and a drop of 1% aqueous $FeCl_3$ solution was added. Blue colour of the solution indicated the presence of phenols (Gowri and Vasantha 2010). For detection of tannins, in a test tube containing about 1 mL of plant material test sample, a solution of lead acetate was added. Formation of a yellow precipitate indicated the presence of tannins (Gowri and Vasantha 2010). Similar tests were carried out using developed TLC plates. The samples of plant material were spotted on the normal phase TLC chromatograms and developed in

the chosen solvent system. Overlaying the TLC plate with a 1% solution of $FeCl_3$ and heating in an oven at 100 ºC for 1-2 minutes gave blue spots against a white background. This indicated the presence of phenols in EtOAc, DCM and water fractions. Overlaying the chromatogram with saturated lead acetate solution and heating in the oven under the same conditions gave yellow spots against a white background, which indicated the presence of tannins in DCM, EtOAc and water samples (Gowri and Vasantha 2010).

6.4 GS-MS analysis

Analytical gas chromatography (GC) was carried out by Dr Joseph Brophy at the University of New South Wales on a Shimadzu GC17A gas chromatograph with a BP-5 column (30 m×0.25 mm×25 μm) that was programmed from 35-250 ºC at 3 ºC/min with helium as the carrier gas. The injector and detector were both programmed at 220 ºC. GC integrations were performed on a SMAD electronic integrator. GC-MS was carried out on a Shimadzu GCMS-QP5000 mass spectrometer operating at 70 eV ionisation energy. Mass spectra were recorded in electron impact (EI) mode at 70 eV, scanning the 41-450 *m/z* range. Compounds were identified by their matching GC retention indices relative to *n*-alkanes and by comparison of their mass spectra with either known compounds or published spectra.

6.5 Chemical study: methods and materials

6.5.1 Bioautography guided isolation of active compounds and fractions from *P. persica n*-hexane extract

The *n*-hexane partition (2 g) of *P. persica* was dissolved in petroleum ether 40-60 ºC (3 mL), mixed with silica gel (1 g) and evaporated to dryness by rotary evaporation at 37 ºC. Petroleum ether of 40-60 ºC boiling point was used throughout the whole *n*-hexane fractionation process. The sample was then applied to the top of a normal phase silica column (240 g silica) prepared with 100% petroleum ether. The column was eluted with a gradient of petroleum ether:diethyl ether (100:0 to 0:100%). 100 of 20 mL fractions were collected and spotted in duplicate on TLC chromatograms. The chromatograms were then developed with petroleum ether and diethyl ether 3:7. The presence of compounds was detected under UV light (254 nm and 365 nm) and by *p*-anisaldehyde spray reagent.

The bioactivity of all eluted fractions was examined by an overlay TLC bioautography assay with the susceptible strain of *S. aureus* (ATCC 29213). The fractions were combined into 12 major sub-fractions on the basis of their TLC profiles and bioactivity: Hex-1

(fractions 1-4, 20 mg, orange oil, R_f = 0.9-1, petroleum ether:diethyl ether 3:7, one spot on TLC plate, no antibacterial activity), Hex-2 (fractions 5-7, 15 mg, orange-yellow oil, R_f = 0.8-1, petroleum ether:diethyl ether 3:7, four spots on TLC plate, one spot antibacterially active), Hex-3 (fractions 8-19, 25 mg, yellow oil with white solid particles, R_f = 0.7-0.8, petroleum ether:diethyl ether 3:7, 3 spots on TLC plate, no antibacterial activity), Hex-4 (fractions 20-27, 30 mg, yellow oil with white solid particles, R_f = 0.65-0.77, petroleum ether:diethyl ether 3:7, two spots on TLC plate, no antibacterial activity), Hex-5 (fractions 28-31, 15 mg, white, solid particles, R_f = 055-0.77, petroleum ether:diethyl ether 3:7, two spots on TLC plate, no antibacterial activity), Hex-6 (fractions 32-37, 40 mg, creamish needles, R_f = 0.5-0.75, petroleum ether:diethyl ether 3:7, four spots on TLC plate, no antibacterial activity), Hex-7 (fractions 38-45, 80 mg, white needles, R_f = 0.55-0.75, petroleum ether:diethyl ether 3:7, four spots on TLC plate, two antibacterially active,), Hex-8 (fractions 46-59, 30 mg, white-green solid, R_f = 0.3-0.5, petroleum ether:diethyl ether 3:7, three spots on TLC plate, two antibacterially active), Hex-9 (fractions 60-66, 33 mg, white-green solid, R_f = 0.3.-0.45, petroleum ether: diethyl ether 3:7, merged spots of antibacterial activity), Hex-10 (fractions 67-73, 26 mg, green solid, R_f = baseline-0.45, petroleum ether:diethyl ether 3:7, 6 spots on TLC plate, merged antibacterial activity), Hex-11 (fractions 74-82, 20 mg, dark green solid, R_f = baseline-0.45, petroleum ether:diethyl ether 3:7, more than 8 spots, merged antibacterial activity), Hex-12 (fractions 83-100, 24 mg, green-white solid, R_f = baseline-0.45, petroleum:diethyl ether 3:7, more than 6 spots on TLC plate, merged antibacterial activity).

Only fractions that showed antibacterial activity against susceptible *S. aureus* (Hex-2, Hex-7, Hex-8, Hex-9, Hex-10, Hex-11 and Hex-12) were further tested by TLC bioautography and MTT microdilution assays against methicillin resistant (MRSA) and multi drug resistant (MRDSA) strains of *S. aureus*.

Overlay TLC bioautography of Hex-7 showed two active spots (MIC = 625 µg/mL for susceptible *S. aureus* and 1250 µg/mL for MRSA). Normal phase silica column chromatography of Hex-7 (80 mg) with 90 g of silica and elution with petroleum ether:diethyl ether (100:0 to 0:100%) gave 25 of 20 mL fractions that were pooled together into three sub-fractions based on their normal phase TLC profiles (petroleum ether:diethyl ether 3:7) and TLC bioautography results. The compounds were visualised under UV light (254 nm and 365 nm) and by staining with *p*-anisaldehyde spray reagent. The solvents of

all three sub-fractions were evaporated by rotary evaporation at 37ºC to afford Hex-7a (fractions 1-6, 8 mg, yellow needle-like crystals, four spots on TLC plate, one spot [R_f= 0.75, petroleum ether:diethyl ether 3:7] antibacterially active against susceptible *S. aureus* and MRSA), Hex-7b (fractions 7-21, 20 mg, white solid, two spots on TLC plate: R_f = 0.55 not antibacterially active and R_f = 0.65 antibacterially active against susceptible *S. aureus* and MRSA) and Hex-7c (fractions 22-25, 10 mg, white crystals, one spot on TLC plate: R_f = 0.55 not active against test bacteria).

Methanol (2 mL) was added to Hex-7b (20 mg) and the mixture was separated into the soluble part and the white precipitate by centrifugation. The supernatant afforded Hex-7b2 (12 mg) as a white solid after rotary evaporation. The precipitate was collected to give Hex-7b1 (1 mg) as a white solid. Hex-7b1 was dissolved in diethyl ether, spotted on a TLC plate and developed with petroleum ether:diethyl ether 3:7, then sprayed with *p*-anisaldehyde reagent. It appeared as a single spot of R_f = 0.55, staining blue. Hex-7b1 was recrystallised with diethyl ether to give 0.5 mg of a white needle- like solid that was subjected to EI-MS analysis which identified the compound as β-sitosterol (5.1) from the NIST library.

Methanol (1 mL) was added to Hex-7c (10 mg) and Centrifuged to give a white solid (3 mg) and the supernatant. After rotary evaporation of the supernatant, a white solid (7 mg) was obtained. The original, obtained by centrifugation solid was recrystallised with diethyl ether to give white needles (1.5 mg, R_f = 0.55, stained blue with *p*-anisaldehyde). EI-MS characterised the compound as further β-sitosterol (5.1). This was combined with β-sitosterol from Hex-7b1 and NMR and IR analyses were performed.

β-Sitosterol **(5.1)**: White needlelike crystals, mp. 138 ºC (lit. 136-139 ºC) (Kircher and Rosenstein 1973). IR (neat) υ_{max} (cm^{-1}): 3400 (br strong), 2955, 2918, 2850, 1735, 1463, 1377, 1057. EI-MS *m/z* 55, 57, 81, 95, 105, 119, 133, 145, 159, 173, 185, 199, 213, 231, 241, 255, 273, 288, 303, 315, 329, 341, 354, 367, 381, 396, 414, fragmentation pattern identical with the authentic sample. HREI-MS *m/z* 414.3855 (calc. 414.3862 for $C_{29}H_{50}O$). ^{1}H-NMR (600 MHz $CDCl_3$) δ 0.65 (3H, *s*, H-18), 0.79 (3H, *d*, *J* 8.6 Hz, H-26), 0.81 (3H, *d*, *J* 8.6 Hz, H-19), 0.83 (3H, *d*, *J* 8.6 Hz, H-29) 0.89 (3H, *d*, *J* 6.5 Hz, H-21), 0.91 (1H, *m*, H-9), 0.91 (1H, *m*, H-24), 0.94 (1H, *m*, H-17), 0.94 (1H, *m*, H-14), 0.98 (3H, *s*, H-27), 1.13 (2H, *m*, H-23), 1.13 (2H, *m*, H-1), 1.24 (2H, *m*, H-28), 1.29 (2H, *m*, H-22), 1.33 (1H, *m*, H-20), 1.47 (2H, *m*, H-11), 1.49 (2H, *m*, H-7), 1.55 (2H, *m*, H-15) 1.64 (1H, *m*, H-25), 1.81 (2H, *m*, H-2), 1.97 (2H, *m*, H-12), 1.97 (H, *m*, H-8), 2.21 (1H, *m*, H-4), 2.27 (1H, *m*, H-4),

3.50 (1H, *m*, H-3), 5.33 (1H, *d*, *J* = 5.3 Hz, H-6). ^{13}C NMR (150 MHz, $CDCl_3$) δ 37.22 (C-1), 31.64 (C-2), 71.80 (C-3), 42.27 (C-4), 140.73 (C-5), 121.72 (C-6), 31.87 (C-7), 31.89 (C-8), 50.09 (C-9), 36.48 (C-10), 21.06 (C-11), 39.74 (C-12), 42.30 (C-13), 56.74 (C-14), 24.28 (C-15), 28.23 (C-16), 56.01 (C-17), 11.84 (C-18), 19.81 (C-19), 36.13 (C-20), 18.76 (C-21), 33.91 (C-22), 26.01 (C-23), 45.79 (C-24), 29.10 (C-25), 19.00 (C-26), 19.38 (C-27), 23.03 (C-28), 11.96 (C-29).

6.5.2 Bioautography guided isolation of active compounds and fractions from *P. persica* EtOAc extract

The ethyl acetate extract (8 g) of *P. persica* was dissolved in methanol (10 mL), mixed with silica gel (5 g) and evaporated to dryness by rotary evaporation at 37 ºC. The sample was then applied to the top of a normal phase silica column (270 g silica) prepared with 100% chloroform. The column was eluted with a gradient of chloroform:methanol (100:0 to 0:100%). 120 of 20 mL fractions were collected and spotted in duplicate on TLC plates. For detection of active compounds, the plates were developed with methanol:chloroform:water 3:7:0.2 and 3:7:0.4. One of the TLC plates was then stained with vanillin-sulfuric acid reagent, the other was used for overlay TLC bioautography testing against susceptible *S. aureus*. The fractions were then combined into four major sub-fractions according to similar TLC R_f values and TLC bioautography results to give EtOAc-1 (fractions 1-20, 200 mg, yellow solid, R_f = 0.75-0.9, chloroform:methanol:water 7:3:0.4, two spots on TLC plate, none active), EtOAc-2, (fractions 21-53, 1.8 g, orange solid, four spots of R_f values 0.45, 0.60, 0.63 and 0.66, chloroform:methanol:water 7:3:0.2, 2 inhibition zones merged R_f 0.63-0.66), EtOAc-3 (fractions 54-90, 3 g, red solid, R_f = baseline-0.45 chloroform:methanol:water 7:3:0.4, four spots on TLC plate, two active), EtOAc-4 (fractions 90-120, 2 g, dark red solid, R_f = baseline-0.3, chloroform:methanol:water 7:3:0.4, two spots on TLC plate, two active).

All sub-fractions except EtOAc-1 showed activity against susceptible *S. aureus.* The sub-fractions EtOAc-2 and EtOAc-3 were subjected to further purification.

EtOAc-2 (1.8 g) was dissolved in methanol, mixed with silica (1 g) and rotary evaporated at 37 ºC. The solid was applied on top of a normal phase silica column (200 g silica) prepared with 100% chloroform. Elution with an increasing polarity gradient of chloroform:methanol (100 to 0 and 0 to 100%) yielded 30 sub-fractions which were spotted in duplicate on TLC chromatograms and developed, one being visualised with vanillin-

sulfuric acid reagent, the other tested by TLC bioautography. The fractions were combined based on their TLC R_f values and bioautography results into two sub-fractions EtOAc-2a (800 mg, red solid, two spots, R_f = 0.63-0.66 of merged antibacterial activity, chloroform:methanol:water 7:3:0.2), EtOAc-2b (600 mg, red solid, three spots, R_f = 0.63, 0.66 and 0.45, spots of R_f = 0.63, 0.66 showed very faint inhibition zones and spot of R_f 0.45 showed strong inhibition, chloroform:methanol:water 7:3:0.2). Crystallisation with methanol gave *ent*-epiafzelechin-(2α→O→7, 4α→8)-(-)-*ent*-afzelechin (5.3) as a yellow solid (1 mg).

Sub-fraction EtOAc-2a (800 mg) was dissolved in minimal methanol (0.25 mL) and subjected to size exclusion column chromatography (Sephadex LH-20), eluting with methanol. This afforded three fractions, EtOAc-2a1 (220 mg, yellow solid, one spot, R_f = 0.66, no activity, chloroform:methanol:water 7:3:0.2), EtOAc-2a2 (200 mg, white flecks, one spot, R_f = 0.63, no activity, chloroform:methanol:water 7:3:0.2), EtOAc-2a3 (150 mg, whitish solid, two spots, none active, R_f = 0.63-0.66, chloroform:methanol:water 7:3:0.2). Sub-fraction EtOAc-2a2 was subjected to PTLC to provide afzelechin (5.2) (2 mg, R_f 0.63, no antibacterial activity).

Afzelechin (**5.2)**: Creamish amorphous solid. UV (MeOH): λ_{max} 273. ESI-MS *m*/*z* 273 $[M+H]^+$, consistent with the molecular formula $C_{15}H_{14}O_5$. ^{1}H-NMR (400 MHz, CD_3OD) δ 2.49 (1H, *dd*, *J* 8.5, 16.1 Hz, H-4), 2.87 (1H, *dd*, *J* 5.5, 16.1 Hz, H-4), 3.97 (1H, *m*, H-3), 4.58 (1H, *d*, *J* 8.0 Hz, H-2), 5.83 (1H, *d*, *J* 2.1 Hz, H-6), 5.91 (1H, *d*, *J* 2.1 Hz, H-8), 6.77 (2H, *d*, *J* 8.5 Hz, H-13, 15), 7.21 (2H, *d*, *J* 8.5 Hz, H-12, 16). ^{13}C NMR (75 MHz, CD_3OD) δ 82.77 (C-2), 68.87 (C-3), 29.15 (C-4), 157.88 (C-5), 95.48 (C-6), 157.8 (C-7), 96.41 (C-8), 157.28 (C-9), 100.8 (C-10), 131.5 (C-11), 129.58 (C-12), 116.21 (C-13), 156.86 (C-14), 116.21 (C-15), 129.58 (C-16)

Sub-fraction EtOAc-3 (3 g) in methanol was mixed with silica (1.5 g) and rotary evaporated at 37 ºC. The solid was subjected to normal phase chromatography (240 g silica), eluting with an increasing ratio of chloroform:methanol (100 to 0 and 0 to 100%). This afforded 40 fractions that were combined based on their TLC profiles into EtOAc-3a (fractions 1-10, 65 mg, yellow crystalline solid, one spot, R_f = 0.45, antibacterial, chloroform:methanol:water 7:3:0.4) and EtOAc-3b (11-40, 2.2g, red solid, two spots, both active, R_f = baseline-0.30, chloroform:methanol:water 7:3:0.4).

EtOAc-3a (65 mg) was recrystallised with methanol to give *ent*-epiafzelechin-(2α→O→7', 4α→8')-(-)-*ent*-afzelechin (5.3) as yellow crystals (40 mg).

ent-Epiafzelechin-(2α→O→7',4α→8')-(-)-ent-afzelechin **(5.3):** yellow crystals. mp. decomposition at 238 ºC. UV (MeOH): λ_{max} 273. IR (neat) υ_{max} (cm^{-1}): 3391 (br, strong), 2918, 2849, 1700, 1364, 835. CD (MeOH) $[\theta]_{232}$ – 44.87 $[\alpha]_D$ – 113.7º (MeOH). ESI-MS *m/z* 543 $[M-H]^-$, HREI MS 567.1266 [M+Na], (calc. 567.1267) consistent with the formula $C_{30}H_{24}O_{10}$.^{1}H-NMR (600 MHz, CD_3COCD_3) δ 4.22 (1H, *d, J* 3.5 Hz, H-3), 4.24 (1H, *d, J* 3.5 Hz, H-4), 5.91 (1H, *d, J* 2.2 Hz, H-6), 6.06 (1H, *d, J* 2.2 Hz, H-8), 7.53 (2H, *d, J* 8.6 Hz, H-12, H-16), 6.85 (2H, *d, J* 8.6 Hz, H-13, H-15), 4.81 (1H, *d, J* 8.5 Hz, H-2'), 4.13 (1H, *m*, H-3'), 3.04 (1H, *dd, J* 5.5, 16.5 Hz, H-4'), 2.61 (1H, *dd, J* 5.5, 16.5 Hz, H-4'), 6.13 (1H, *s*, H-6'), 7.39 (2H, *d, J* 8.6 Hz, H-12', H-16'), 6.89 (2H, *d, J* 8.6 Hz, H-13', H-15'), ^{13}C NMR (150 MHz, CD_3COCD_3) δ 100.1 (C-2), 67.1 (C-3), 28.8 (C-4), 156.5 (C-5), 97.8 (C-6), 157.9 (C-7), 96.1 (C-8), 153.8 (C-9), 103.7 (C-10), 131.4 (C-11), 129.3 (C-12), 115.2 (C-13), 158.5 (C-14), 115.2 (C-15), 129.3 (C-16), 83.6 (C-2'), 67.7 (C-3'), 29.2 (C-4'), 155.5 (C-5'), 96.4 (C-6'), 151.8 (C-7'), 106.3 (C-8'), 150.6 (C-9'), 102.7 (C-10'), 129.8 (C-11'), 129.8 (C-12'), 116.0 (C-13'), 158.5 (C-14'), 116.0 (C-15'), 129.8 (C-16').

Appendino, G., S. Gibbons, A. Giana, A. Pagani, G. Grassi, M. Stavri, E. Smith and M. M. Rahman (2008). "Antibacterial Cannabinoids from *Cannabis sativa*: A Structure − Activity Study." *Journal of Natural Products* **71**(8): 1427-1430.

Gibbons, S. (2005). An Introduction to Planar Chromatography. Natural Products Isolation, Springer**:** 77-116.

Gowri, S. S. and K. Vasantha (2010). "Phytochemical Screening and Antibacterial Activity of *Syzygium cumini* (L.) (Myrtaceae) Leaves Extracts." *International Jurnal of Pharmaceutical and Technical Research* **2**(2): 1569-1573.

Johnsson, R., G. Träff, M. Sundén and U. Ellervik (2007). "Evaluation of Quantitative Thin Layer Chromatography Using Staining Reagents." *Journal of Chromatography A* **1164**(1): 298-305.

Kircher, H. W. and F. U. Rosenstein (1973). "Purification of Sitosterol." *Lipids* **8**(3): 97-100.

Simpson, B. S. (2011). Chemical and Pharmacological Investigation of *Dodonaea polyandra*, University of South Australia. **PhD**.

Spangenberg, B. (2008). "Derivatization, Detection (Quantification), and Identification of Compounds Online." *Thin Layer Chromatography in Phytochemistry*: 175.

Wagner, H. (1996). Plant Drug Analysis: A Thin Layer Chromatography Atlas, Springer.

Waksmundzka-Hajnos, M., J. Sherma and T. Kowalska (2008). Thin Layer Chromatography in Phytochemistry, CRC Press.

Chapter 7

Conclusions and Future Directions

The MPhil study presented in this thesis was an extension of a collaborative research partnership between Macquarie University's Indigenous Bioresources Research Group (IBRG) and Chungtia village's authorising body Chungtia Senso Mokokchung Town (CSMT), Nagaland. The aims of this study were documentation of ethnobotanical knowledge of Chungtia village Elders and healers as well as biological and phytochemical investigation and isolation of bioactive constituents from Nagaland medicinal plants.

The project was initiated by Meyanungsang Kichu, a Nagaland man, who conducted an ethnobotanical study of medicinal plants used by Chungtia villagers and documented 135 plants for their various ethnomedicinal and ethnobotanical applications. This MPhil study completed an up to date literature review of the 135 medicinal plants, then investigated the antimicrobial potential of those plants used by Chungtia villagers for skin conditions of a microbial aetiology, conducted antimicrobial screening of a selection of these, and finally investigated in detail one plant for its antimicrobial activity and bioactive constituents.

A comprehensive literature review encompassing traditional usages of all 135 plants by other Indigenous traditional healers' worldwide and phytochemical and biological properties of these plants was conducted. This revealed that 93 plants have been used by communities other than Chungtia for similar purposes. Thirteen species have not been reported for any ethnobotanical uses except for within Chungtia village. For 80 plant species, the traditional uses by Chungtia villagers aligned with biological activities reported for these plants. Moreover, 55 of these plants have had bioactive compounds isolated that with activities consistent with their ethnomedicinal uses by the Chungtia villagers. These findings of bioactivity and bioactive compounds consistent with Chungtia ethnobotanical/ethnomedicinal usage highlight the significance of the knowledge of Elders of Chungtia village. Of the thirteen species exclusively used by the Chungtia villagers, only three plants (*M. cordifolia*, *O. acetosella* and *C. amara*) have been reported for their biological activities and none have been examined for their bioactive compounds. Given the validation of the knowledge of Elders of Chungtia village, further exploration of these plants aligned to their ethnomedicinal uses would be of value.

Thirty five plants used by the Chungtia villagers for skin related conditions of a microbial aetiology were reviewed for their antimicrobial properties. This highlighted twelve plants with either no antimicrobial activities or antimicrobial compounds reported. Out of these, seven species (*Dendrocnide sinuata, Duabanga grandiflora, Erythrina stricta, Eurya acuminata, Holboellia latifolia, Maesa indica* and *Prunus persica*) that were available for collection were selected for antimicrobial screening.

The antimicrobial screening was performed on the 70% aqueous ethanolic extracts of *D. sinuata* stem, *D. grandiflora* stem bark, *E. stricta* stem, *E. acuminata* leaves, *H. latifolia* leaves, *M. indica* leaves and *P. persica* roots using disc diffusion and MTT microdilution assays. Nine human pathogenic microorganisms were screened against, namely, antibiotic susceptible *Staphylococcus aureus* (susceptible *S. aureus*), methicillin resistant *S. aureus* (MRSA) and multi drug resistant *S. aureus* (MDRSA), susceptible beta-lactamase negative *Escherichia coli* (β- *E. coli*), β- lactamase positive (antibiotic resistant) *E. coli* (β+ *E. coli*), *Pseudomonas aeruginosa, Streptococcus pyogenes, Salmonella typhimurium* and *Candida albicans*. The highest inhibitory activities were exhibited by the *P. persica* root extract, with MIC values of 156 µg/mL for all tested *S. aureus* strains. Based on the antibacterial screening results, *P. persica* was selected for further biological and chemical investigations for its antibacterial constituents. All of the remaining six plants showed antibacterial activity against antibiotic susceptible and resistant strains of *S. aureus* in the MTT microdilution assay at concentrations less than 2.5 mg/mL. Moreover *H. latifolia*, the plant which was identified from the literature search as not having been investigated for its biological or phytochemical properties, was found to possess very good antibacterial activity against susceptible *S. aureus* (MIC 156 µg/mL) and moderate activity against MRSA and MDRSA strains (MIC 1250 µg/mL for both antibiotic resistant strains) as well as moderate activity against *S. typhimurium* (MIC 625 µg/mL). *D. grandiflora*, the other plant with very limited reports concerning its antimicrobial and phytochemical properties, was found to be very active against all three strains of *S. aureus* (MIC 156 µg/mL for susceptible and MRDSA and 312 µg/mL for MRSA strains). The results suggest that these two plants have a potential to be a good source of novel, antibacterially active compounds and are worthy of further examination in future studies.

P. persica is a significant plant for Chungtia villagers; its roots have been used for the treatment of typhoid as well as skin related conditions. Even though the plant is very well documented for various traditional medicinal applications as well as for phytochemical and

biological activities, the roots of the plant seem to not have been investigated. Moreover, surprisingly, there are no reports concerning antimicrobial properties of any part of this medicinally important plant. Preliminary antibacterial testing by Kichu revealed that *P. persica* roots (from Nagaland) possessed strong antibacterial properties against a susceptible strain of *S. aureus*, but the phytochemicals responsible for this activity were not determined.

The 70% aqueous ethanolic *P. persica* root extract was subjected to partitioning with *n*-hexane, DCM, EtOAc and the partitionsts were analysed for the presence of their phytoconstituents using sulfuric-vanillin, *p*-anisaldehyde, Dragendorff's reagents, iodine, ferric chloride and lead(II) acetate. The tests revealed the presence of terpenes and steroids in the *n*-hexane partition, unsaturated compounds in all partitions, and phenols/tannins in the DCM, EtOAc and water partitions. The preliminary antimicrobial testing showed the *n*-hexane and ethyl acetate partitions to possess the strongest antibacterial properties, with these being active against susceptible and resistant strains of *S. aureus*. The GS-MS analysis of the *n*-hexane partition revealed the presence of eight constituents, out of which three were reported in the literature as antibacterial against *S. aureus*.

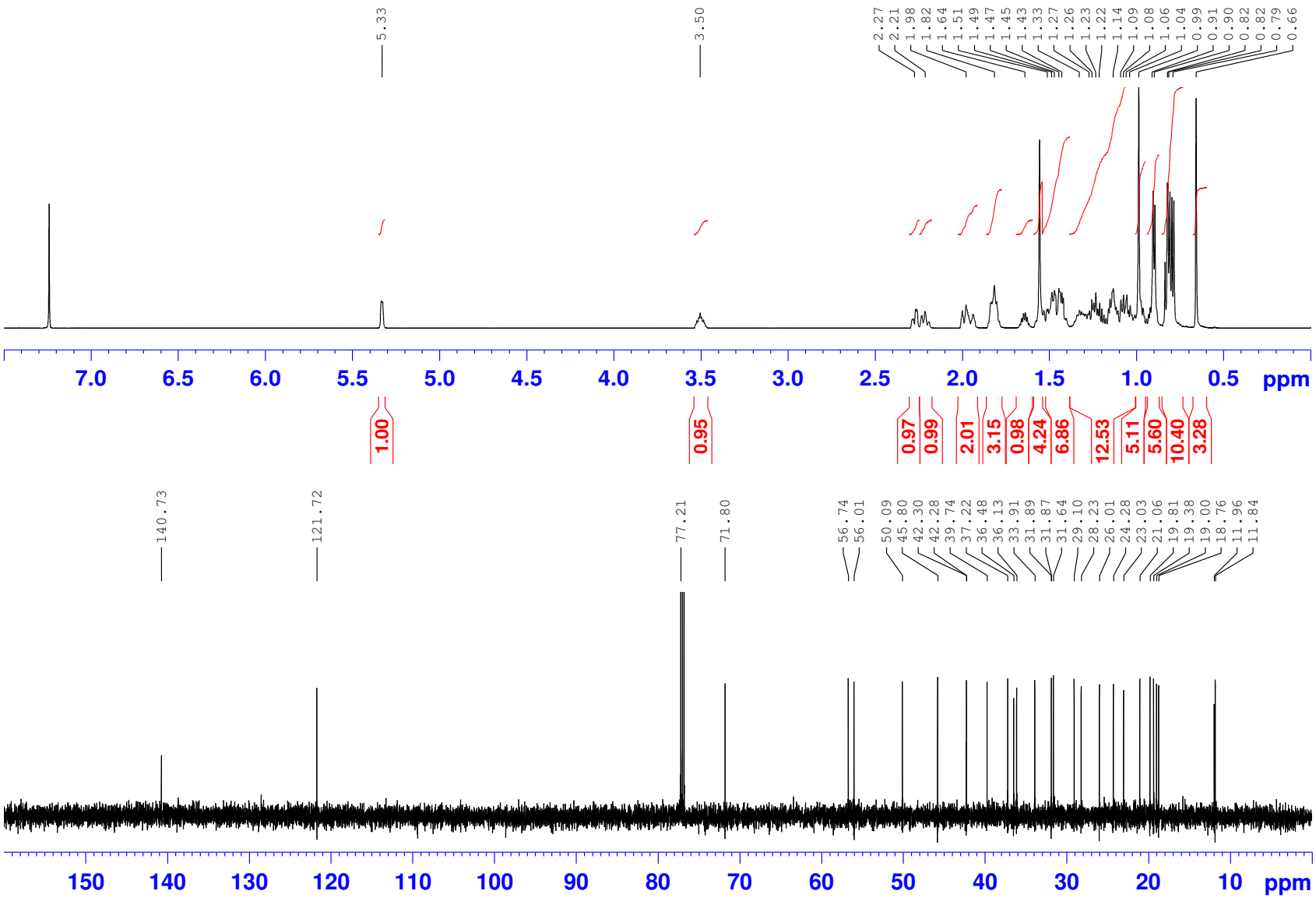
TLC bioautographic methods reported in the literature were trialled with the aim to develop the most appropriate technique for the bioautography guided isolation process. The overlay method was found to be the most effective for the purpose of this study. Normal phase silica gel chromatography and preparative thin layer chromatography (PTLC) of the *n*-hexane partition led to the isolation of β-sitosterol (5.1). TLC bioautography guided isolation by normal phase chromatography, size exclusion chromatography and PTLC led to the isolation of afzelechin (5.2) and *ent*-epiafzelechin-*(2α→O→7',4α→8')*-*(-)ent*-afzelechin (5.3) from the EtOAc partition. The structures of these three compounds were determined based on various spectroscopic methods.

β-Sitosterol was found to be moderately active (MIC 1250 µg/mL) against *P. aeruginosa* as well as weakly active (MIC 2500 µg/mL) against susceptible strains of *S. aureus*, *E. coli* and *S. typhimurium*. *ent*-Epiafzelechin-*(2α→O→7',4α→8')*-*(-)ent*-afzelechin showed good antibacterial activity against all the tested strains of *S. aureus* (MIC 156 µg/mL for susceptible and 312 µg/mL for resistant) as well as weak activity against the susceptible strains of *E. coli*, *P. aeruginosa* and *S. typhimurium* (MIC 2500 µg/mL, for all bacteria).

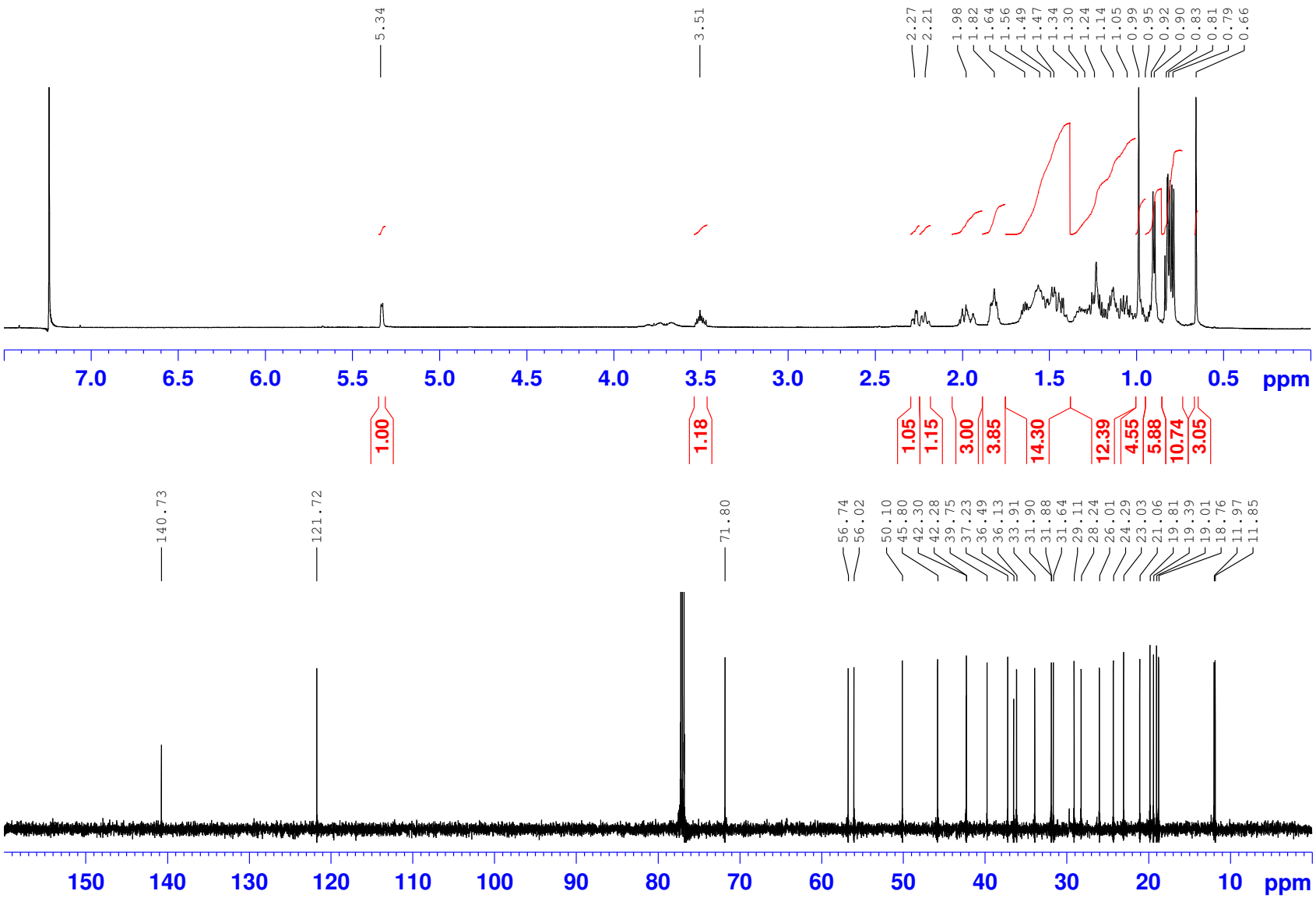
This is the first report of this compound's antibacterial activity. The antimicrobial properties of afzelechin were not tested due to the small quantity of sample.

TLC bioautography detected other antibacterially active compounds, especially in the *n*-hexane partition, but due to the low amount of Nagaland *P. persica* plant material and the project time constraints, they were not isolated. Preliminary examination of a local (Sydney, Australia) sample of *P. persica* roots, gave antimicrobial activity results comparable to the Nagaland sample. This suggests that local *P. persica* would be worthy of exploration for future studies, including the isolation of further bioactive compounds.

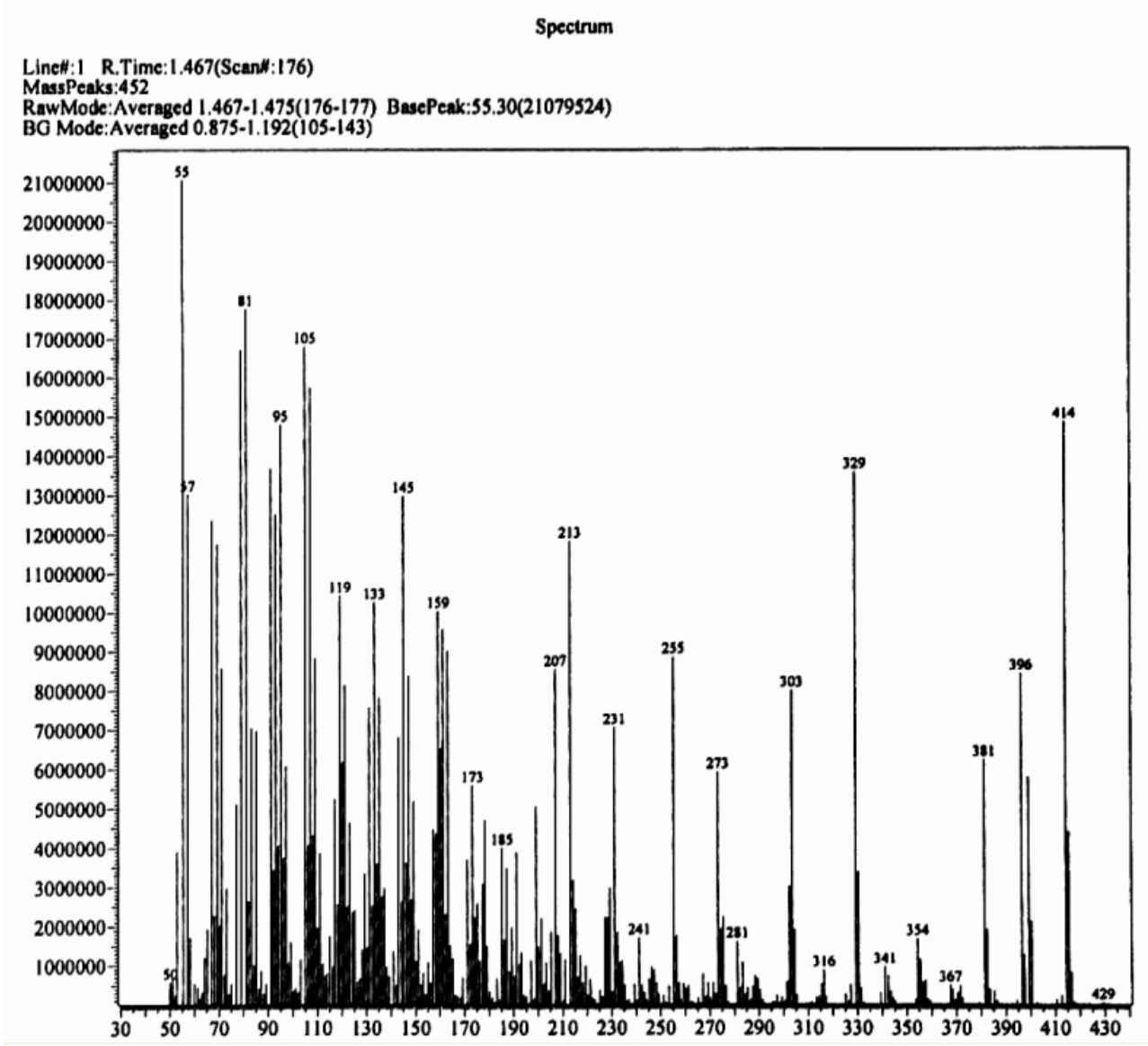
APPENDICES ^{1}H and ^{13}C NMR of reference sample β-Sitosterol (Sigma Aldrich)



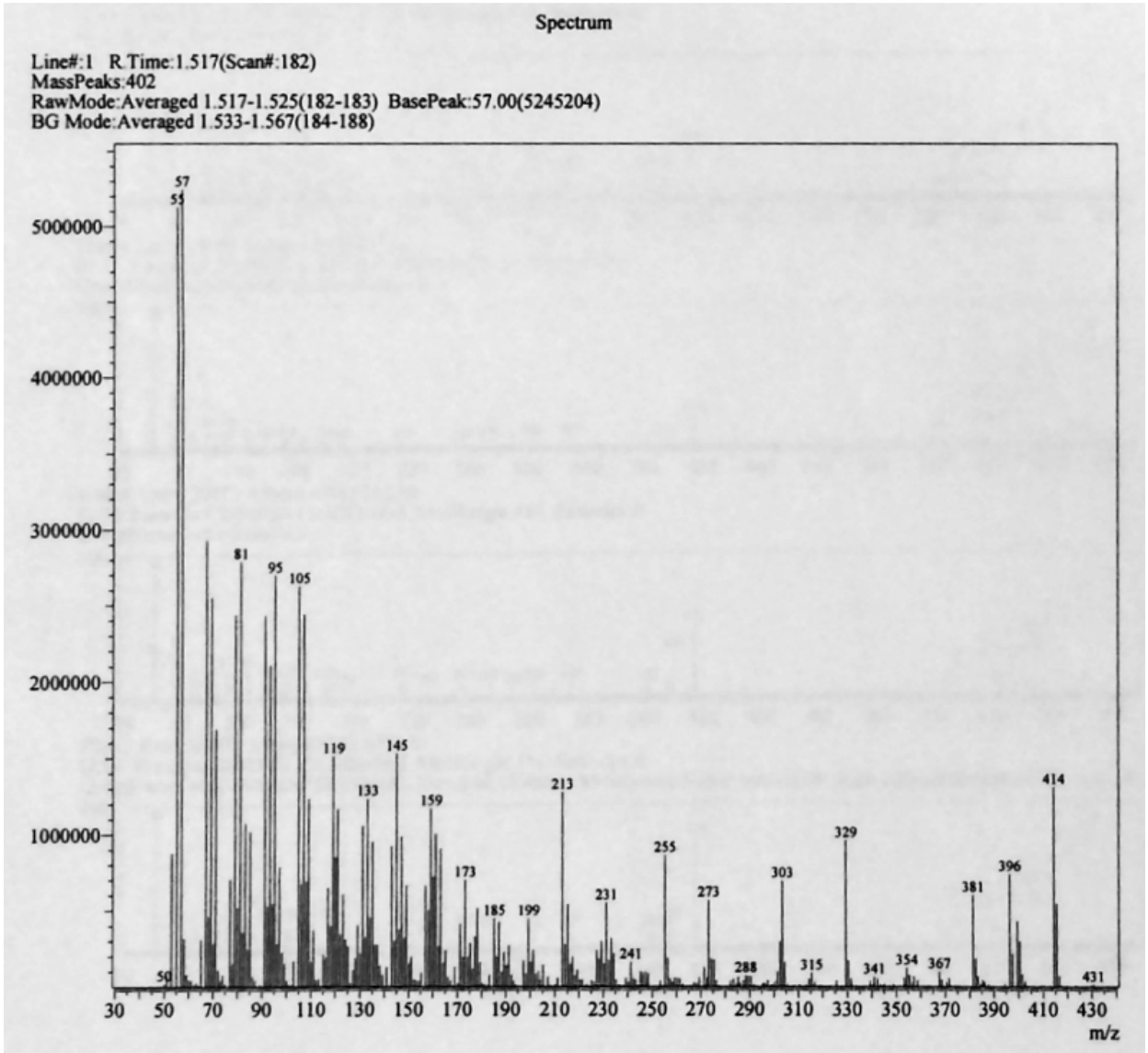
^{1}H and ^{13}C NMR of isolated compound **5.1**



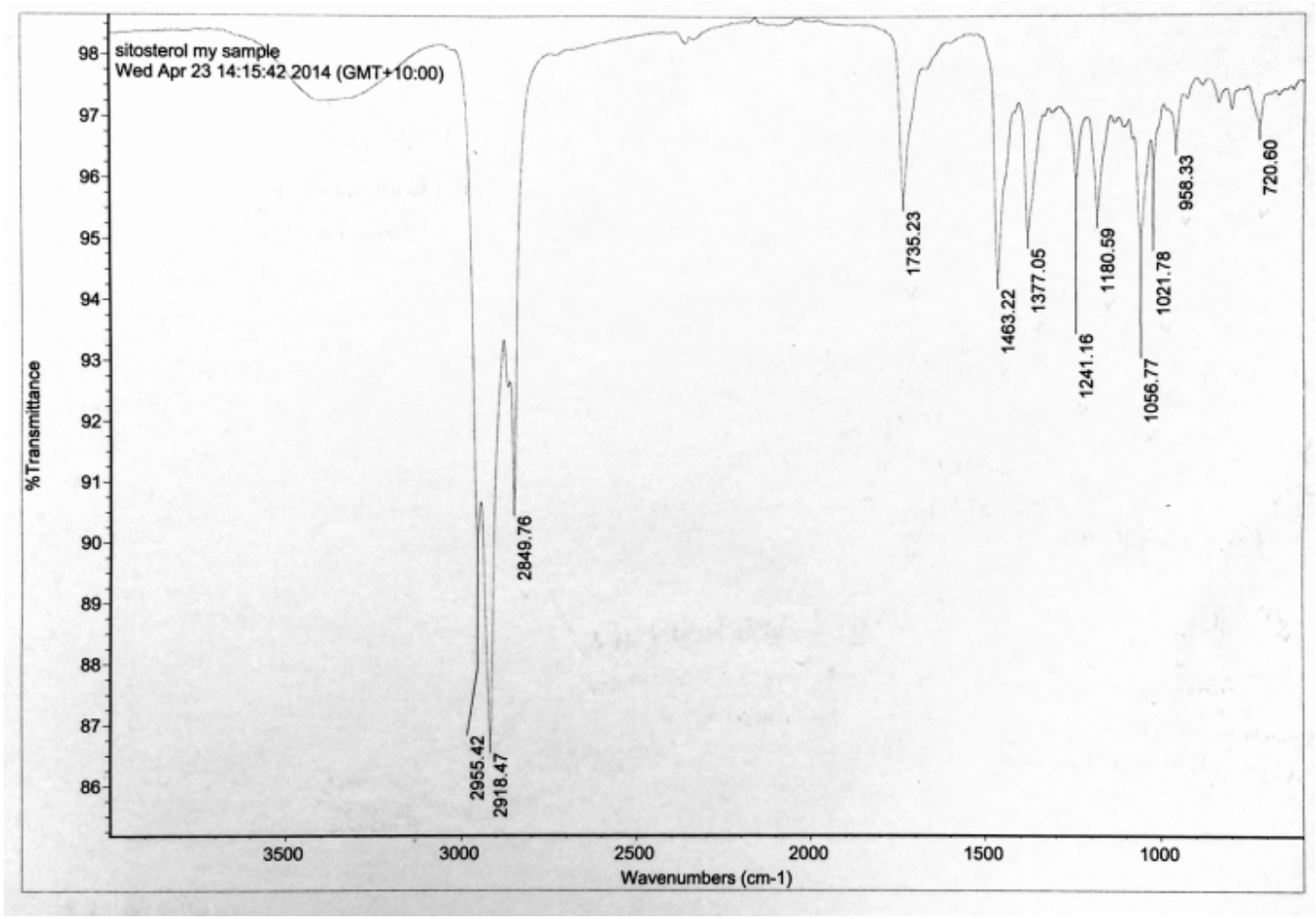
EI mass spectrum of isolated compound **5.1**



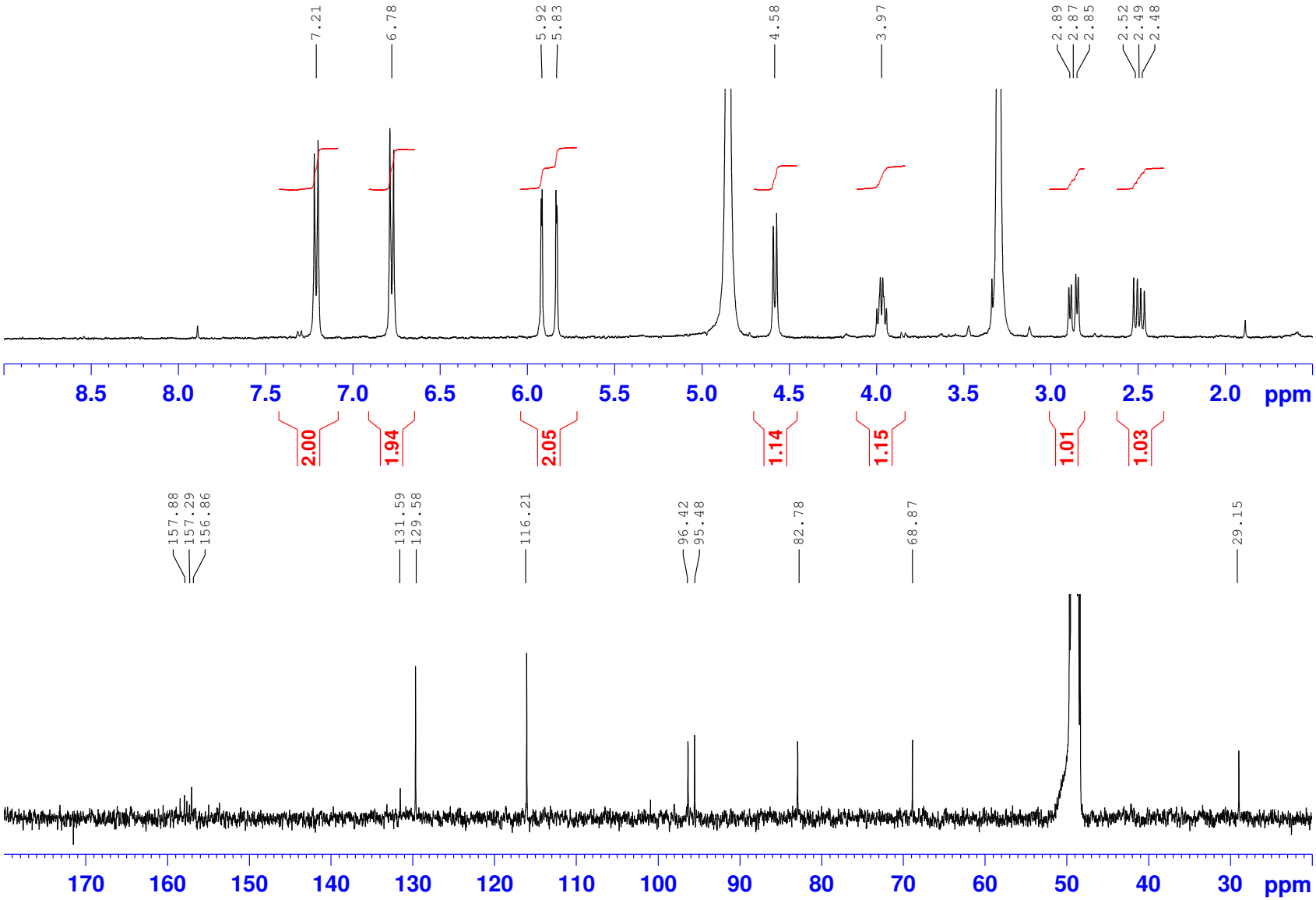
EI mass spectrum of isolated compound **5.1**



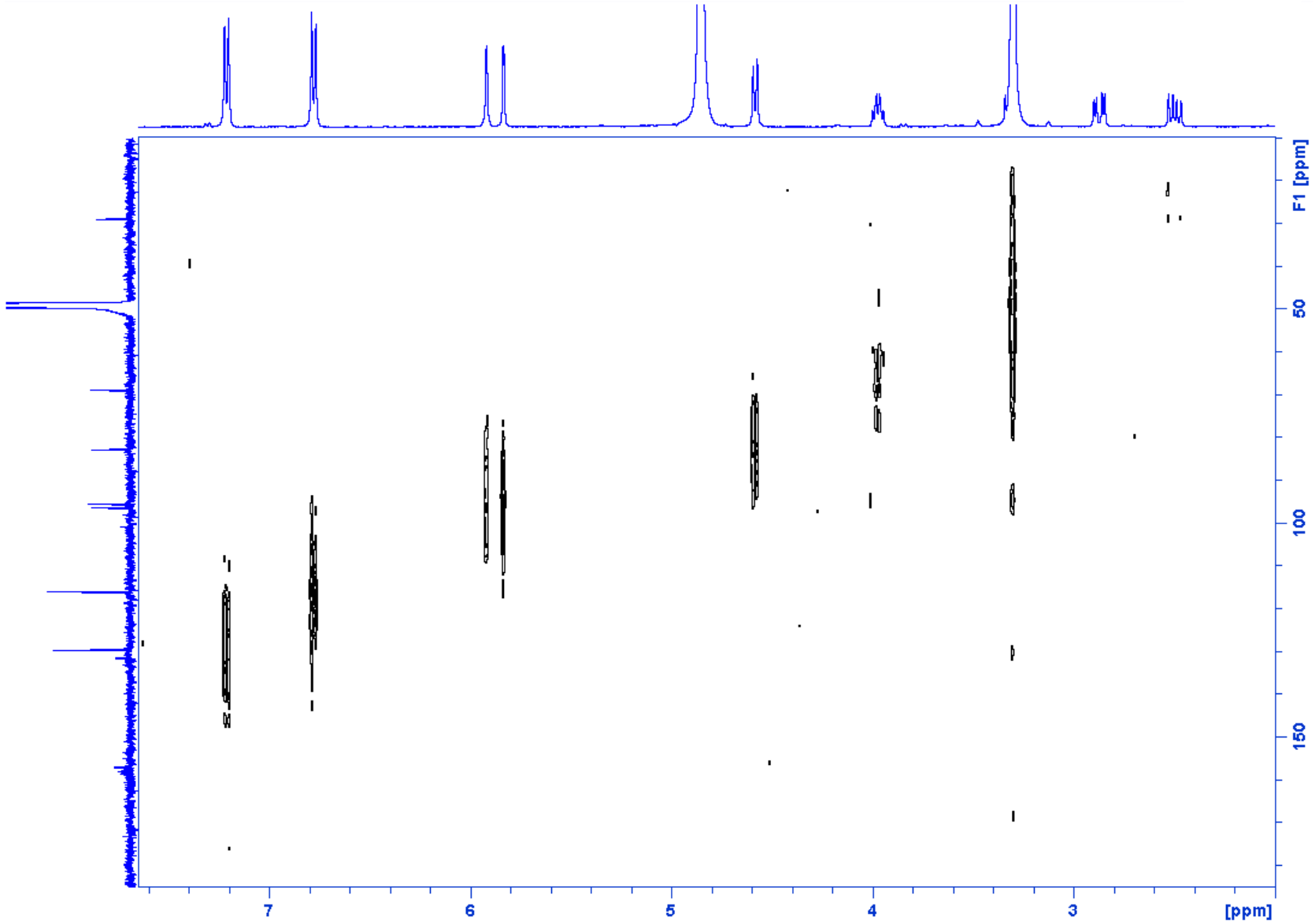
IR of isolated compound **5.1**



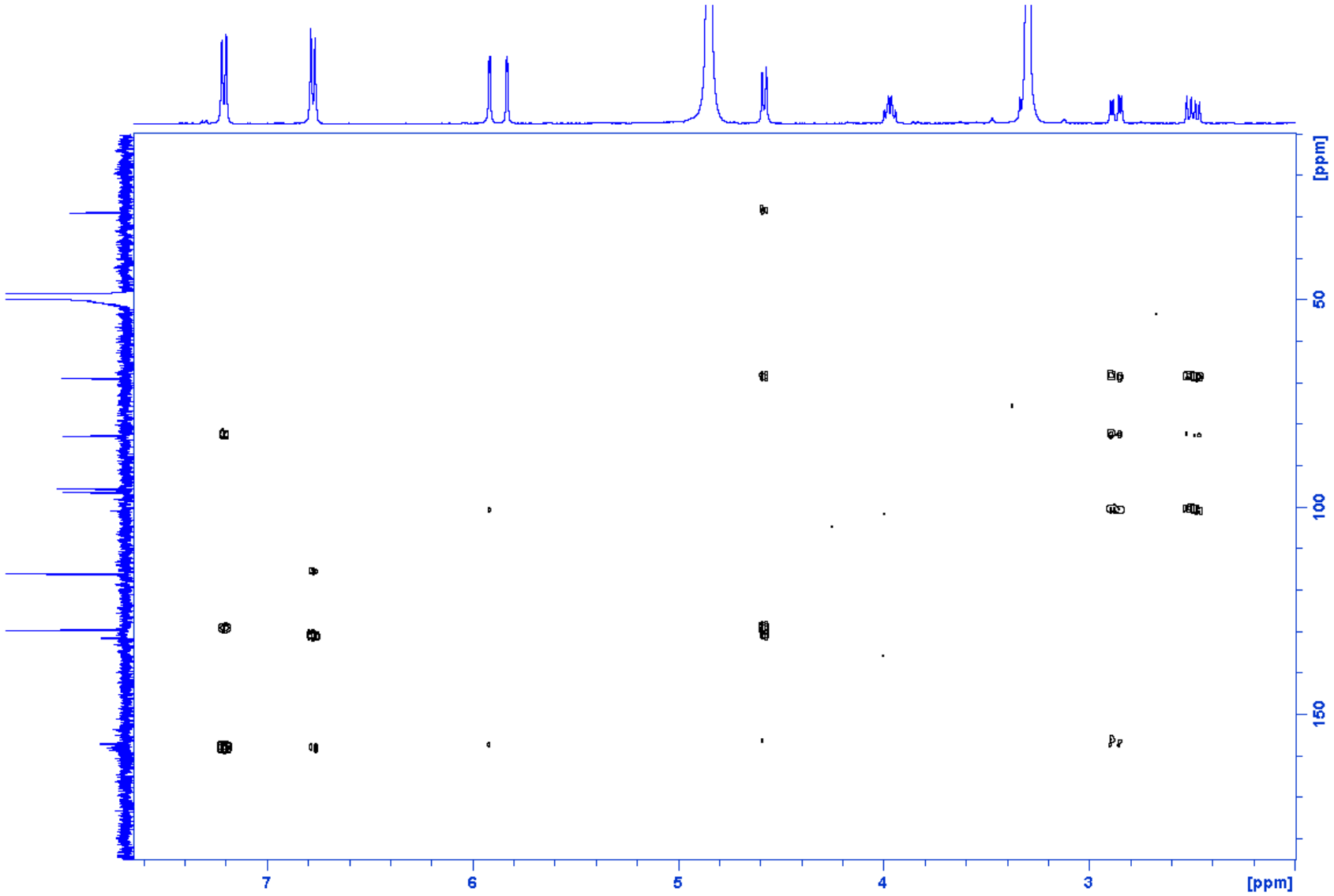
^{1}H and ^{13}C NMR of Isolated Compound **5.2**



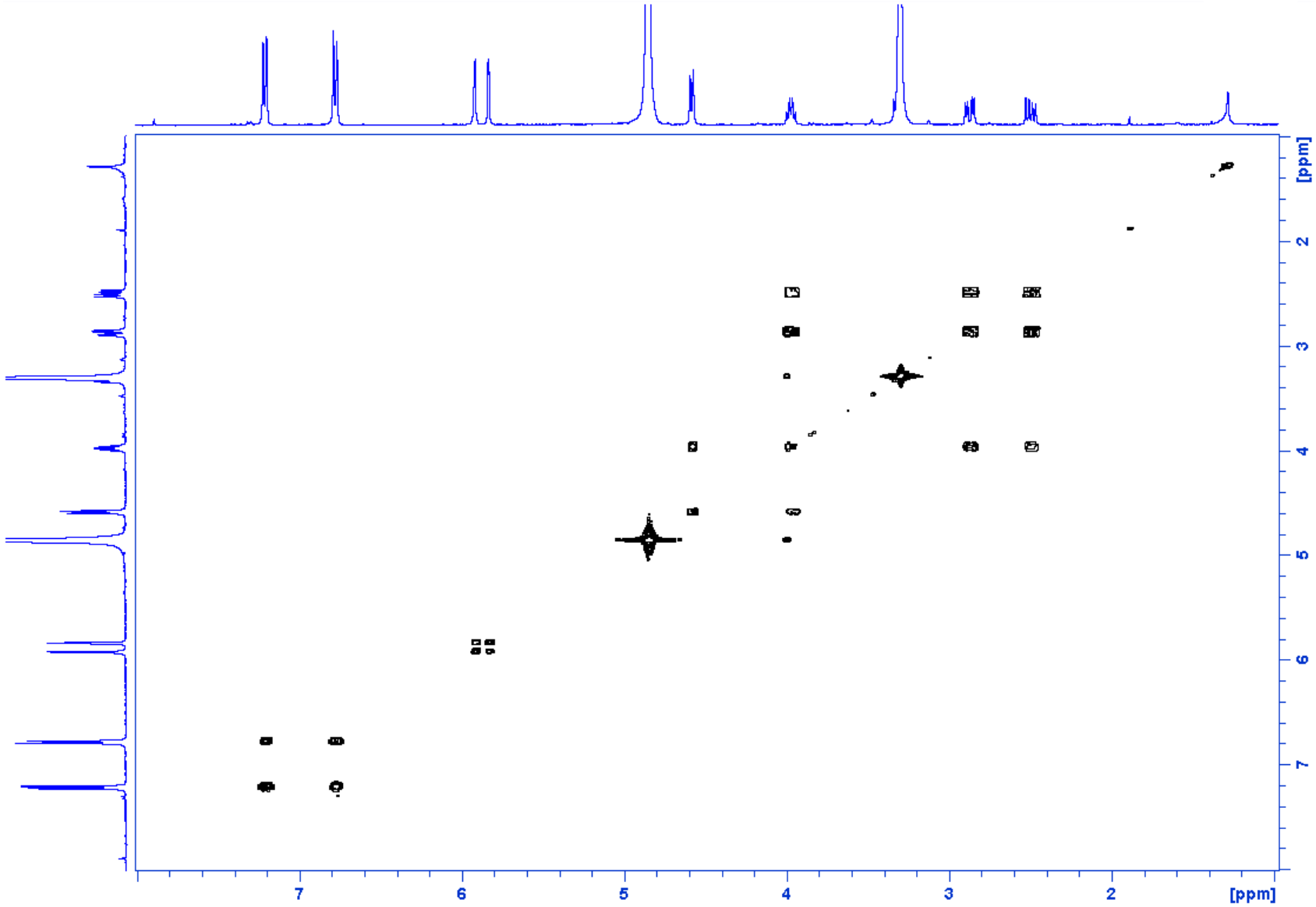
HSQC of isolated Compound **5.2**



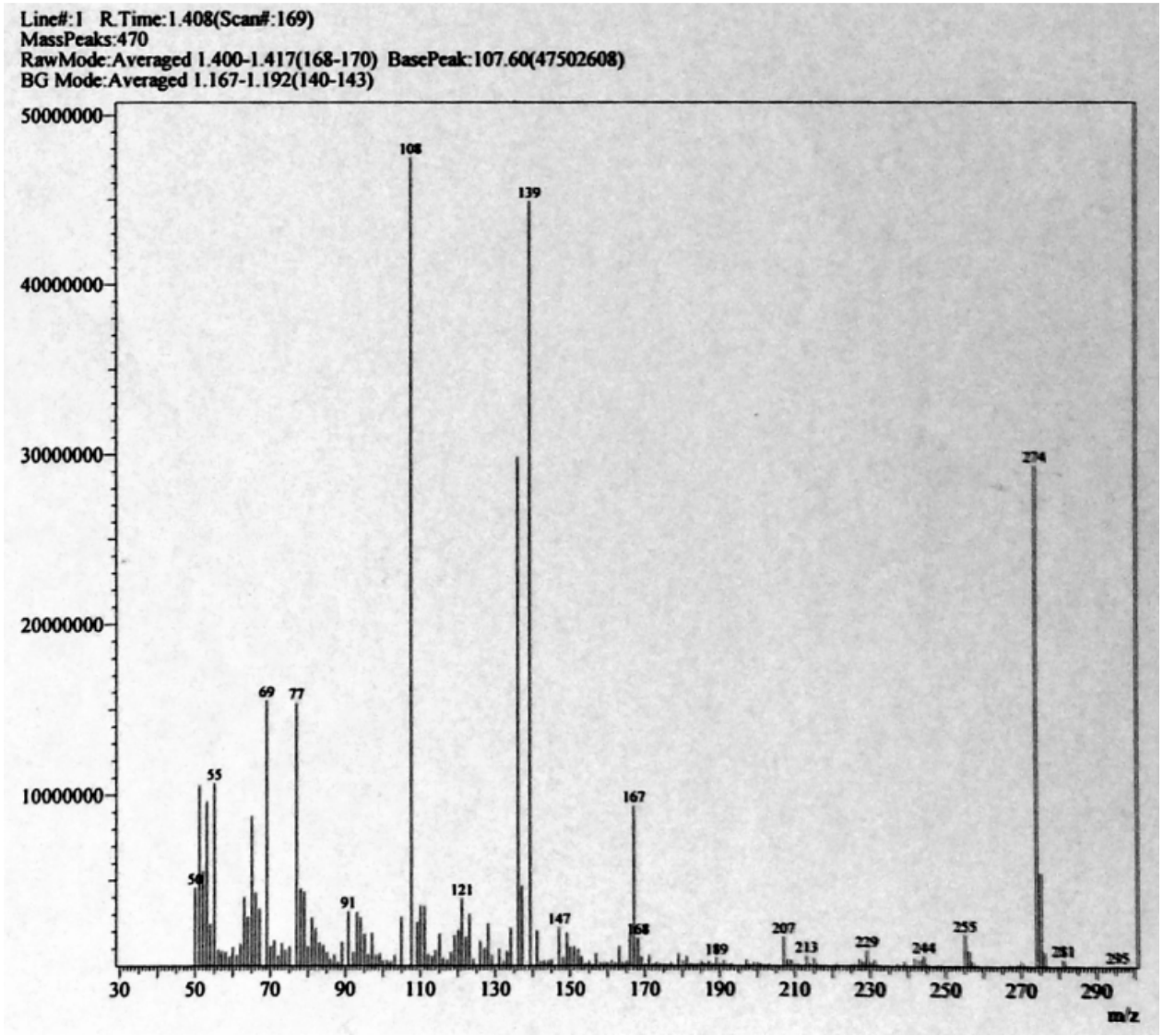
HMBC of isolated Compound **5.2**



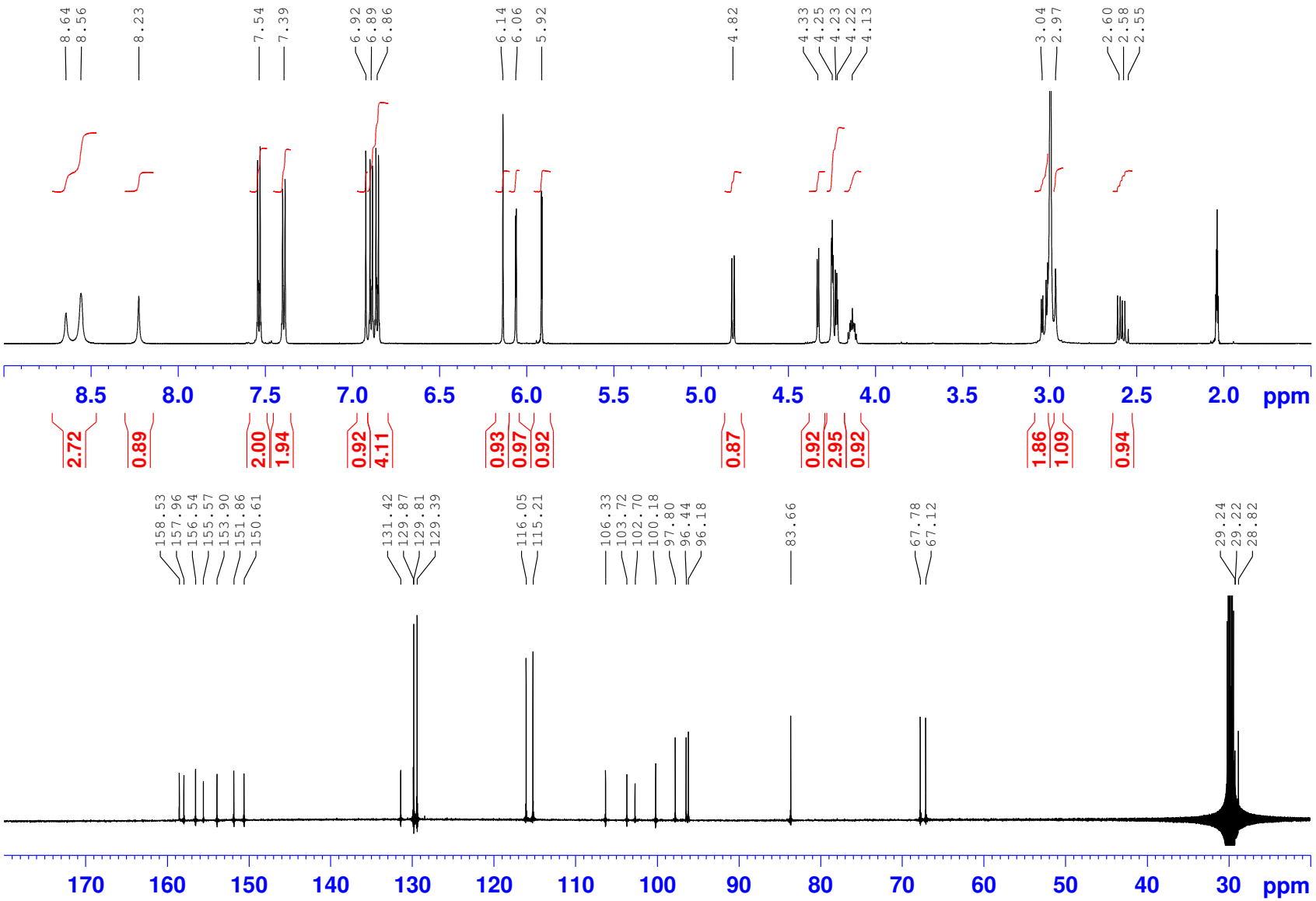
COSY of isolated Compound **5.2**



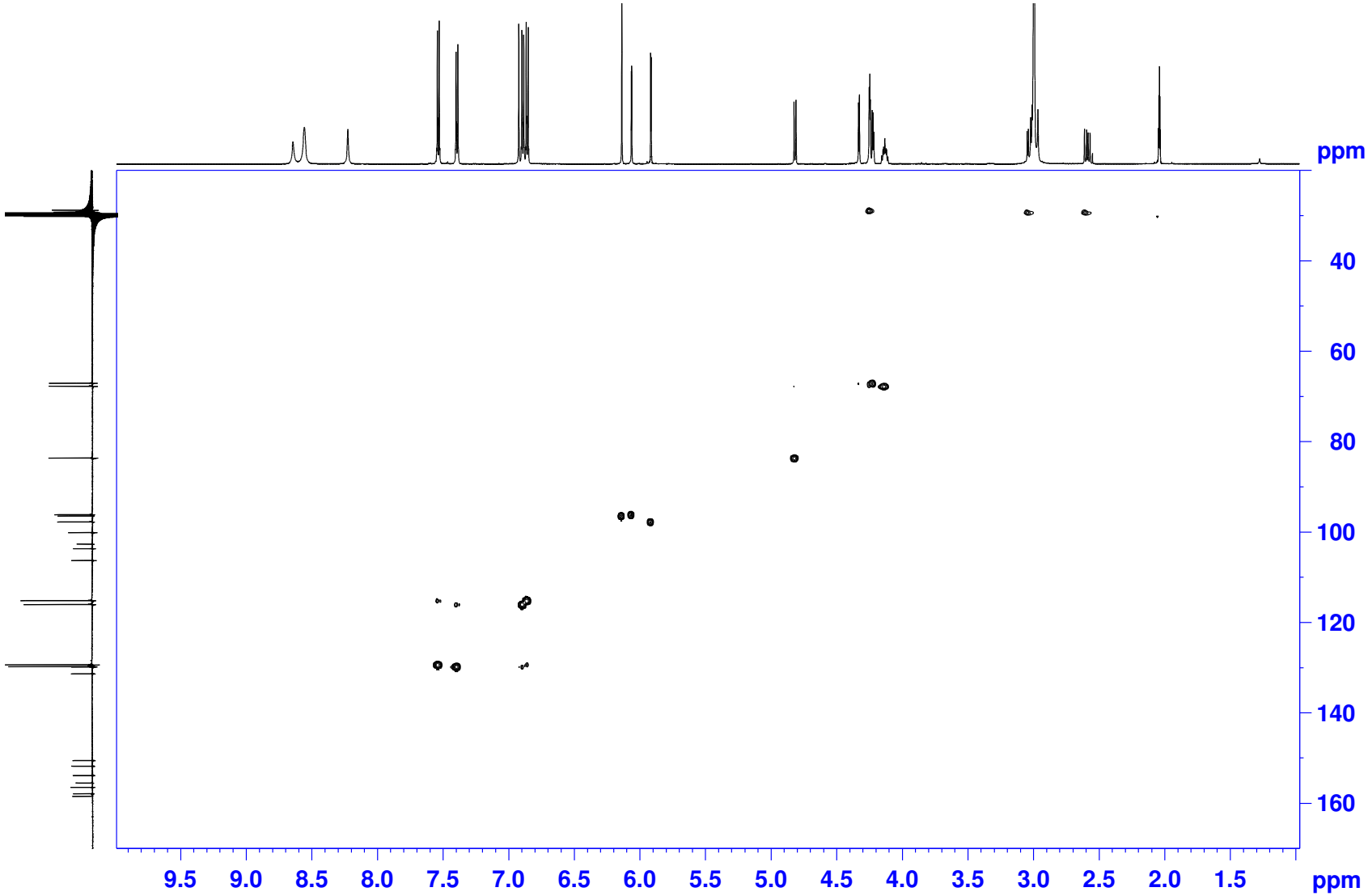
EI mass spectrum of Compound **5.2**



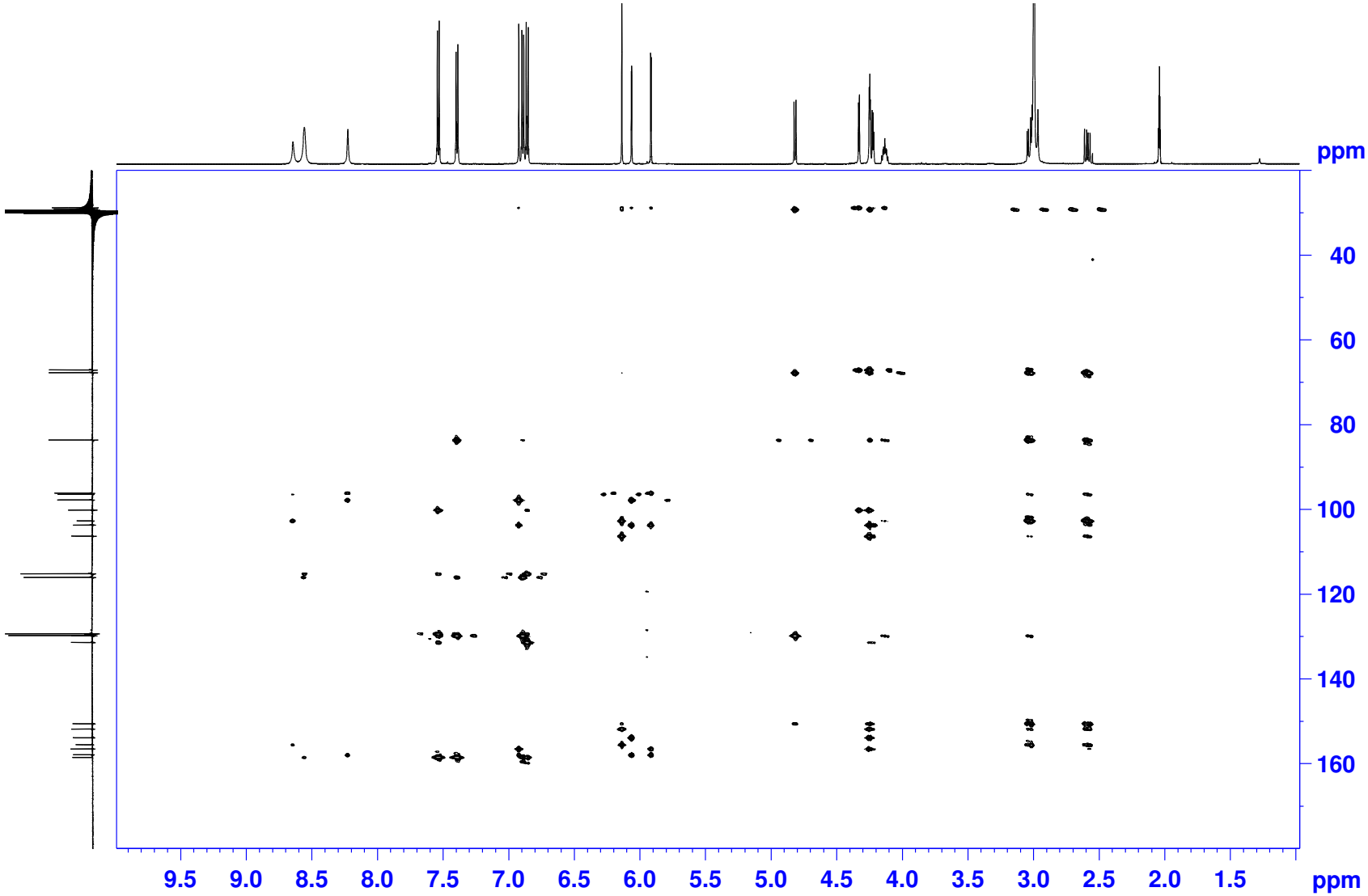
^{1}H and ^{13}C NMR of Isolated Compound **5.3**



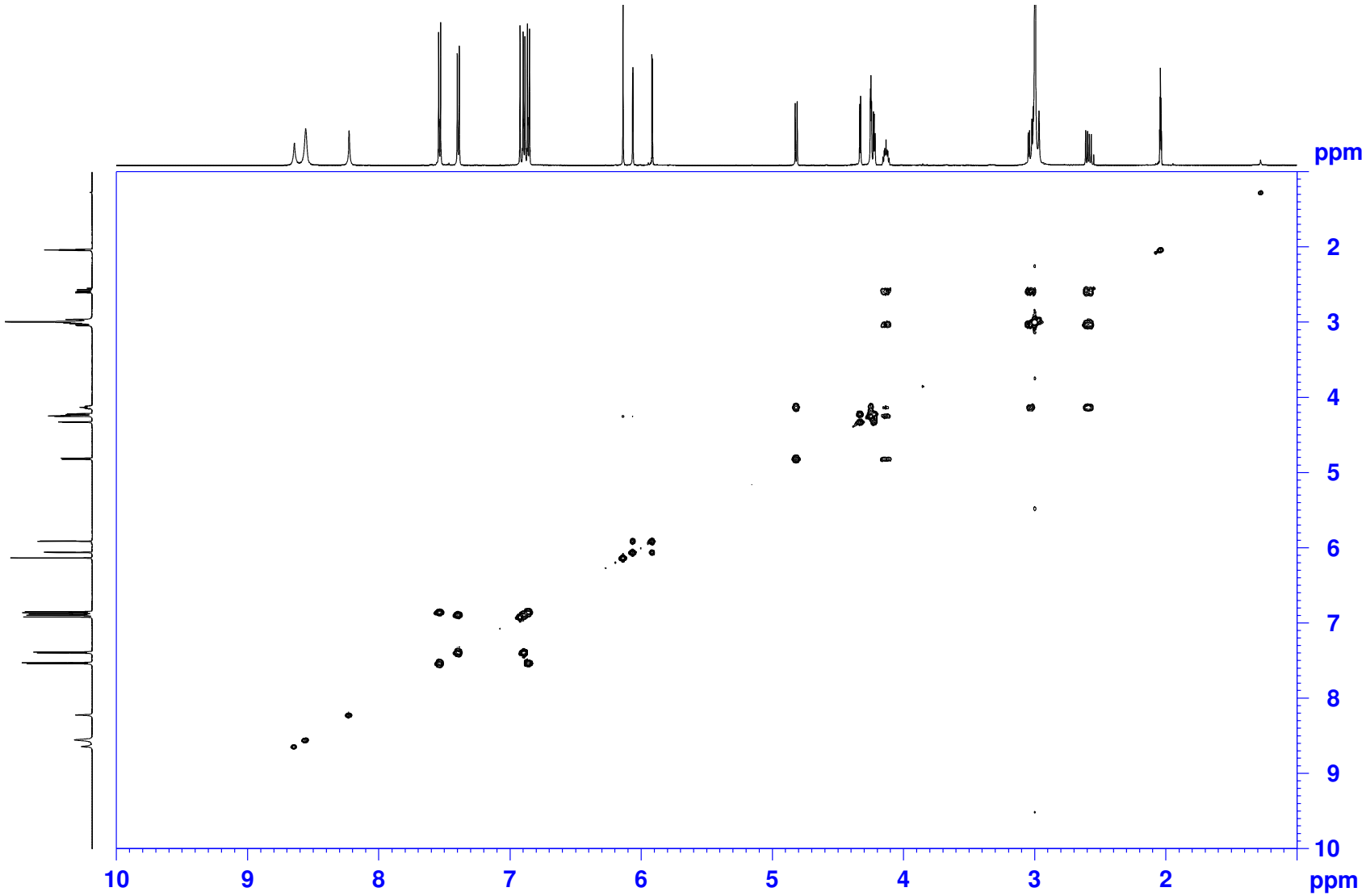
HSQC of isolated Compound **5.3**



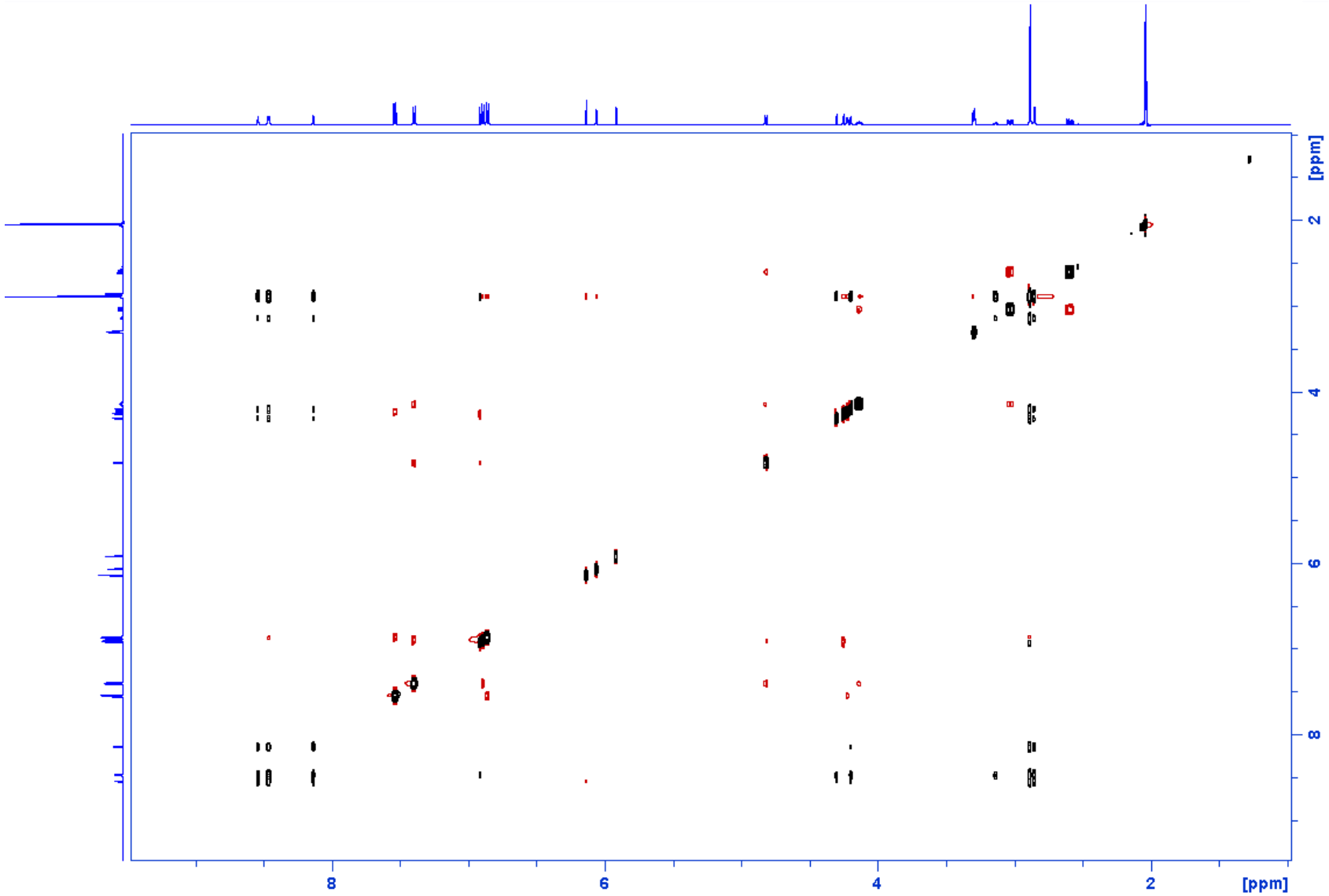
HMBC of isolated Compound **5.3**



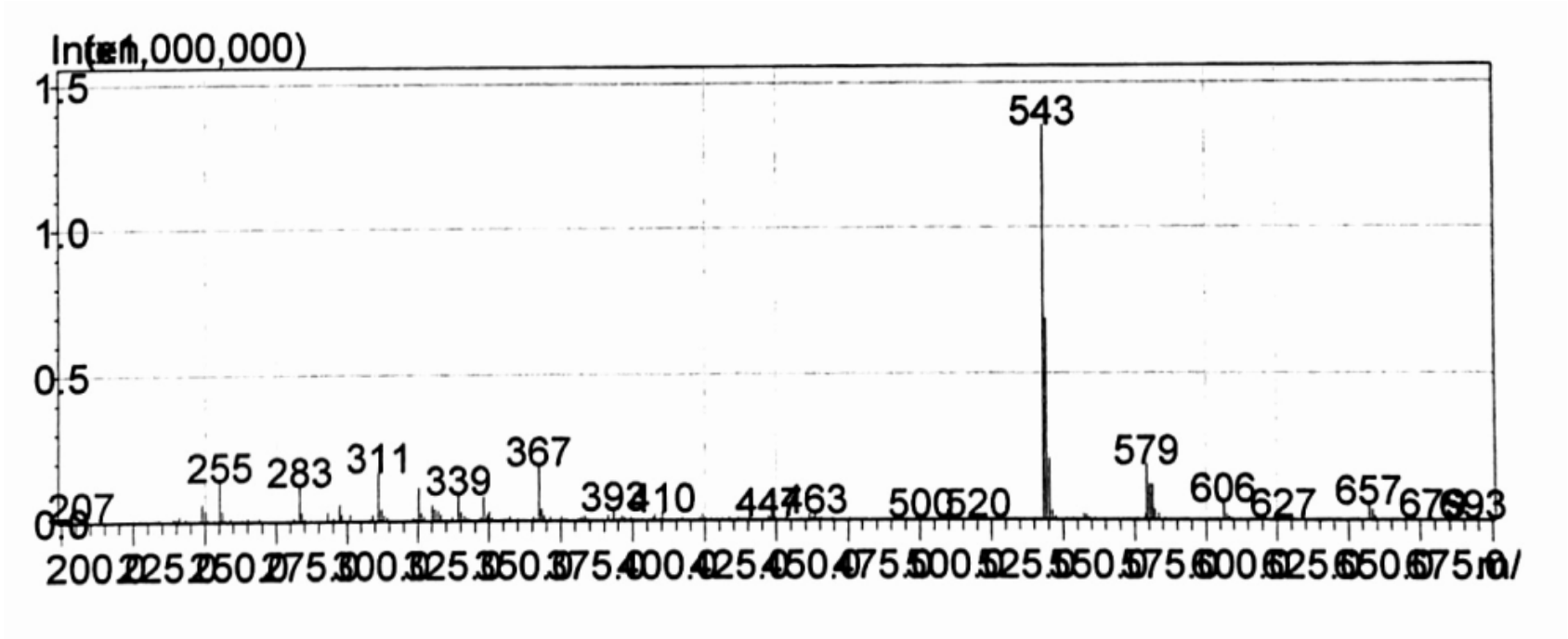
COSY of isolated Compound **5.3**



NOESY of isolated Compound **5.3**

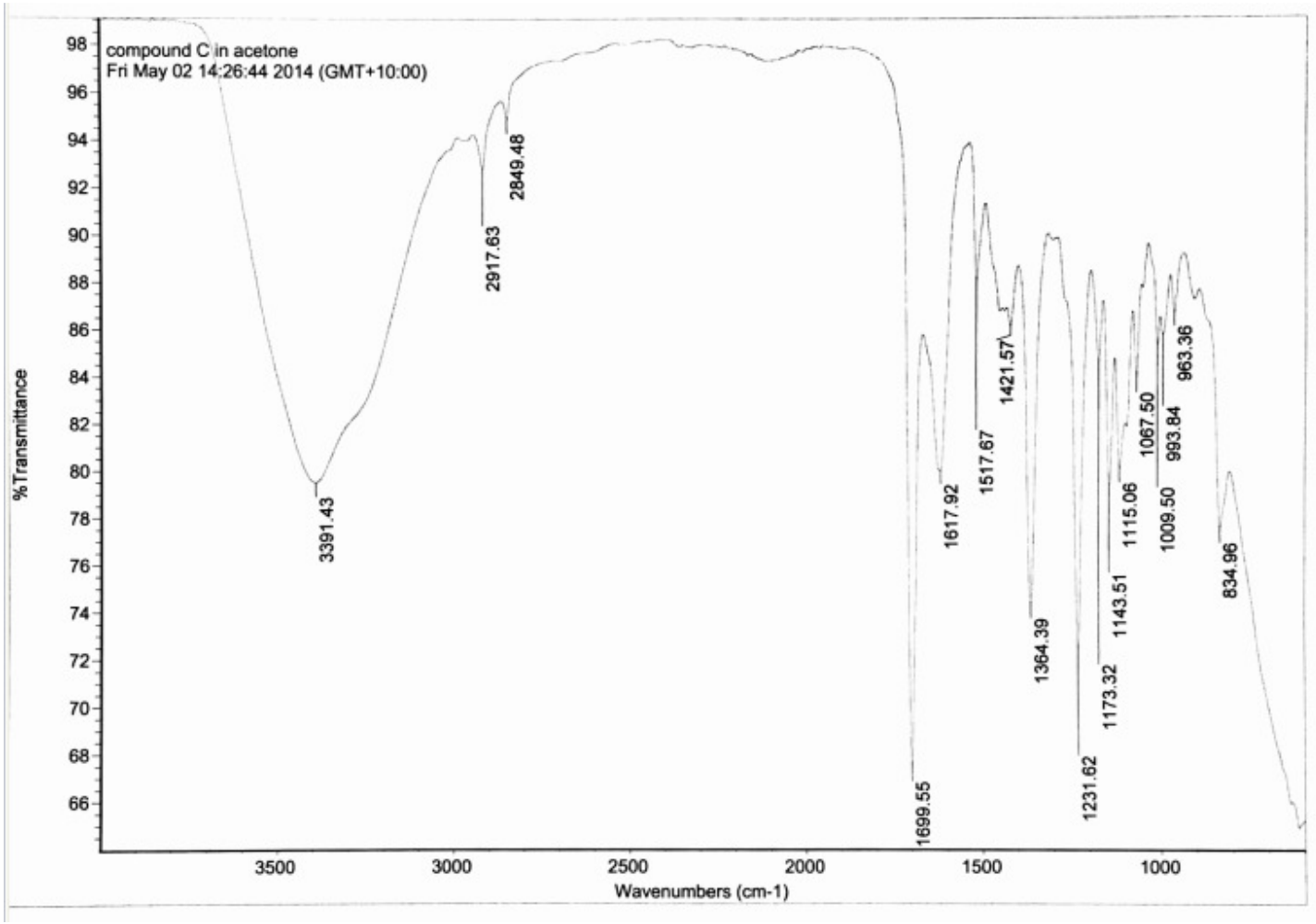


ESI (-ve) mass spectrum of Compound **5.3**



m/z of $[M\text{-}H^{+}]$ is 543

IR of Compound **5.3**



CD of Compound **5.3**