ETHNOBOTANY AND ANTIMICROBIAL STUDY OF SELECTED MEDICINAL PLANTS USED BY MAGAR COMMUNITY IN NAWALPUR, DISTRICT, NEPAL

A THESIS SUBMITTED TO THE

CENTRAL DEPARTMENT OF BOTANY
INSTITUTE OF SCIENCE AND TECHNOLOGY
TRIBHUVAN UNIVERSITY
NEPAL

FOR THE AWARD OF

DOCTOR OF PHILOSOPHY

IN BOTANY

BY

CHANDRA MOHINI NEMKUL

August, 2022

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DECLARATON

Thesis entitled **"Ethnobotany and Antimicrobial Study of Selected Medicinal Plants used by Magar Community in Nawalpur District, Nepal"** which is being submitted to the Central Department of Botany, Institute of Science and Technology (IOST), Tribhuvan University, Nepal for the award of the degree of Doctor of Philosophy (Ph.D.), is a research work carried out by me under the supervision of Prof. Dr. Ila Shrestha, Department of Botany, Patan Multiple Campus, Tribhuvan University and co supervised by Dr. Gan B. Bajracharya, Senior Scientific Officer, Nepal Academy of Science and Technology (NAST).

This research is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

……

Chandra Mohini Nemkul

RECOMMENDATION

This is to recommend that **Chandra Mohini Nemkul** has carried out research entitled **"Ethnobotany and Antimicrobial Study of Selected Medicinal Plants used by Magar Community in Nawalpur District, Nepal"** for the award of Doctor of Philosophy (Ph.D.) in **Botany** under our supervision. To our knowledge, this work has not been submitted for any other degree.

She has fulfilled all the requirements laid down by the Institute of Science and Technology (IOST), Tribhuvan University, Kirtipur for the submission of the thesis for the award of Ph.D. degree.

.....

Dr. Ila Shrestha
Supervisor
Professor
Department of Botany, Patan Multiple Campus,
Tribhuvan University, Nepal.

.....

Dr. Gan B. Bajracharya
Co-Supervisor
Senior Scientific Officer
Nepal Academy of Science and Technology (NAST), Lalitpur, Nepal

July, 2022

TRIBHUVAN UNIVERSITY
INSTITUTE OF SCIENCE AND TECHNOLOGY
CENTRAL DEPARTMENT OF BOTANY

Ref. No.

KIRTIPUR, KATHMANDU
NEPAL

LETTER OF APPROVAL

On the recommendation of **Prof. Dr. Ila Shrestha and Dr. Gan B. Bajracharya**, this Ph. D. thesis submitted by **Chandra Mohini Nemkul,** entitled **"Ethnobotany and Antimicrobial Study of Selected Medicinal Plants used by Magar Community in Nawalpur District, Nepal"** is forwarded by Central Department Research Committee (CDRC) to the Dean, IOST, T.U..

.....

Prof. Dr. Ram Kailash Prasad Yadav
Professor,
Head,
Central Department of Botany,
Tribhuvan University
Kirtipur, Kathmandu
Nepal

January 9, 2022

Phone: 4331322, 4333722 Fax: ++977-1-4332636, Post Box: 26429
E-mail: info@cdbtu.edu.np, Webpage: www.cdbtu.edu.np

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Chandra Mohini Nemkul

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ABSTRACT

Field survey and ethnobotanical data was collected from 2015 to 2017 in Hupsekot , Bulingtar rural municipalities and Kawasoti urban municipality of Gandaki province, Nawalpur district, Nepal focusing on Magar community. To verify traditional use of selected plant species for diarrhea, urinary tract infection, typhoid and pneumonia, laboratory tests of the extracts of the plants were performed. Methods: Data were collected by interviewing local healers and knowledgeable people. Plants were selected for laboratory tests on the basis of factor for informant consensus (FIC) and feudality level (Fl) along with use mention (UM). Plant parts dried at room temperature were used for extraction by using hexane and 70% methanol successively. The extracts in amount 50µl were used for *in vitro* antimicrobial tests by agar well diffusion method. Dimethoxy sulfate (DMSO) was used as negative control; ampicillin and gentamicin were used as positive control. The test bacteria were *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Bacillus subtilis, Shigella dysenteriae* and *Salmonella enterica* subsp. *enterica serovar typhi.* Phytochemical screening was performed by colorimetric method. The effective compounds present were analyzed by GC-MS analysis. Estimation of total phenolic content was done using the Folin-Ciocalteu colorimetric method. Antioxidant assays were done by DPPH free radical scavenging and H_2O_2 scavenging methods. Results: Among 160 plant species recorded 124 species were medicinal use. *Phanera vahlii, Flemingia strobilifera, Lagerstroemia parviflora, Stephania glandulifera, Tectaria coadunata, Woodfordia fruticosa, Rhododendron arboreum and Rhus chinensis* were selected for gastrointestinal category; *Tinospiora sinensis, Cissampelos pareira* and. *Azadirachta indica* for urinary tract infection (UTI) category; *Antidesma acidum* and *Aegle marmelos* for the respiratory category; *Callicarpa macrophylla* for typhoid. Alkaloids, anthocyanosides, flavonoids, saponins, steroids, terpenoids, tannin and polyphenols were detected in the extracts. Gas chromatography and mass spectrometry (GC-MS) analysis showed that antimicrobial compounds such as 2-Methoxy-4-vinylphenol, 5-(Hydroxymethyl)-2-furancarbaldehyde, Phytol, 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, Stigmasterol, 1,2-Benzenedicarboxylic acid, disooctyl estser, vitamin E and squalene were present in the extracts. Methanolic extract of *Rhus chinensis* (RCM) showed largest zone of inhibition (ZOI) (23±0.57mm) against *Escherichi coli* followed by *Woodfordia fruticosa* (18mm) (WFM). The lowest value of minimum inhibition concentration (MIC) against *Escherichia coli* was <1.56mg/mL (WFM). RCM and FSM

showed antimicrobial activities against *Shigella dysenteriae* causal bacteria of dysentery but MIC values of them were bacteriostatic.WFM (18.66±0.66mm) showed the largest ZOI against *Enterococcus faecalis.* The lowest value of MIC was 3.12mg/mL (WFM) against *Enterococcus faecalis* and it was bactericidal. WFM showed the largest ZOI against *Pseudomonas aeruginosa*. The lowest value of MIC against *Pseudomonas aeruginosa* was <1.56 mg/mL (WFM). RCM (14.66±0.33mg/mL) showed larger ZOI against *Klebsiella pneumoniae* and followed by WFM (13.5±0.67mg/mL) and methanolic extract of *Flemingia strobilifera* (FSM) (11.66mg/mL). The lowest MIC value was 6.25mg/mL of FSM against *Klesiella pneumoniae* and it was bactericidal. The result showed that MIC value of methanolic extract of *Antidesma acidum* (AAM) (12.5mg/ml) against *Klebsiella pneumoniae* was bacteridal. MIC of methanolic extract of *Callicarpa macrophylla* leaf (CMML) was 25mg/mL against *Salmonella typhi*. MIC value of CMML against *Salmonella typhi* was bactericidal. RCH (hexane extract of *Rhus chinensis*) (16±0.33mm) showed larger ZOI against *Escherichia coli*. MIC of RCH was 1.56mg/mL against *Escherichia coli* was bacteriostatic and kill the bacteria at 6.25mg/mL. Total phenol content (TPC) in the plant extracts had significant variation ranging from 8.15 to 336.95 mg of GAE/g of dry extract. FSM contained maximum amount of TPC (336.95±14.61 mg of GAE/g of dry extract). 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging range from 4 to 94% at 100 µg/mL concentration of the 70% methanolic extracts and 50% inhibition concentration (IC_{50}) range from 21.59±0.26 to 1434.11±1.17 µg/mL. The best DPPH free radical scavenger was methanolic extract of *Rhododendron arboreum.* The methanolic extract of *Woodfordia fruticosa* was the best H_2O_2 scavenger. Conclusion: The extracts of the plants used for diarrhea by Magars of the study area, have shown antimicrobial activity against *Escherichia coli*, diarrhea causing bacteria. The most effective against *Escherichia coli* was the extract of *Woodfordia fruticosa*. The extract of the plant used for UTI by magars, have shown moderate effect against UTI causing bacteria. The result also verify local us of *Callicarpa macrophylla* against typhoid. These plants also have DPPHradical and H_2O_2 scavenging properties. *Woodfordia fruticosa, Flemingia strobilifera* and *Phanera vahlii* could be used for treatment of UTI along with local use of *Tinospora sinensis, Cissampelos pareira* and. *Azadirachta indica*. In-vitro animal tests are required for these.

LIST OF ACRONYMS AND ABBREVIATIONS

AAH : *Antidesma acidum* hexane exttract

AAM : *Antidesma acidum* 70% methanol exttract

AIH : *Azadirachta indica* hexane exttract

AIM : *Azadirachta indica* 70% methanol exttract

AMH : *Aegle marmelos* hexane exttract

AMM : *Aegle marmelos* 70% methanol exttract

ATCC : American type cell culture

PVH : *Phanera vahlii* hexane exttract

PVM : *Phanera vahlii* 70% methanol exttract

CLSI : Clinical and Laboratory standards Institute

CMHL : *Callicarpa marophylla* hexane exttract

CMHR *: Callicarpa marophylla* hexane exttract

CMML : *Callicarpa marophylla* leaf 70% methanol exttract

CMMR : *Callicarpa marophylla* root 70% methanol exttract

CPH : *Cissampelos pareira* hexane exttract

CPM : *Cissampelos pareira* 70% methanol exttract

DMSO : Dimethylsulphoxide

DPPH : 2,2-Diphenyl-1-picrylhydrazyl

EUCAST : European Committee for Antimicrobial Susceptibility Testing

FSH : *Flemingia strobilifera* hexane exttract

FSM : *Flemingia strobilifera* 70% methanol exttract

GA : Gallic acid

GAE : Gallic acid equivalent

GC-MS : Gas chromatography mass spectrometry

Gram +ve : Gram positive\

Gram –ve : Gram negative

IC_{50} : Concentration for 50% inhibition

LPH : *Lagerstrroemia parviflora* hexane exttract

LPM : *Lagerstrroemia parviflora* 70% methanol exttract

MAPs : Medicinal and aromatic plants

MBC : Minimum bactericidal concentration

MHA	: Mueller Hinton Agar
MHB	: Mueller Hinton Broth
MIC	: Minimum inhibitory concentration
MS	: Mass Spectroscopy
RAH	: *Rhododendron arboretum* hexane exttract
RAM	: *Rhododendron arboretum* 70% methanol extract
RCH	: *Rhus chinensis* hexane exttract
RCM	: *Rhus chinensis* methanol exttract
ROS	: Reactive oxygen species
SEM	: Standard error mean
SGH	: *Stephania glandulifera* hexane exttract
SGM	: *Stephania glandulifera* 70% methanol exttract
Std	: Standard deviation
TCH	: *Tectaria coadunate* hexane exttract
TCME	: *Tectaria coadunata* 70% methanol exttract
TFH	: *Thalictrum foliolosum* hexane exttract
TFME	: *Thalictrum foliolosum* 70% methanol exttract
TM	: Traditional medicine
TPC	: Total phenol content
TSH	: *Tinospora sinensis* hexane exttract
TSM	: *Tinospora sinensis* 70% methanol exttract
UTI	: Urinary tract I nfection
WFH	: *Woodfordia fruiticosa* hexane exttract
WFM	: *Woodfordia fruiticosa* 70% methanol exttract
WHO	: World Health Organization
ZOI	: Zone of inhibition

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CHAPTER 1

1. INTRODUCTION

1.1 Ethnobotany

Nepal is rich in cultural diversity with 126 castes and ethnic groups (CBS, 2011). The ethnic people have indigenous knowledge about the use of local natural resources that had been gained by trial and error methods from remote past. Ethnobotany deals with the use of plants by the ethnic people. The ethnobotanical knowledge of ethnic people is based on the culture and geographical region. Ethnobotanical study is an important tool for recording of plants along with associated traditional botanical knowledge.

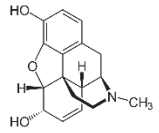
Ethno-botany (ethno = race, botany = plant) means the study of plants used by primitive and aboriginal people, and the term 'ethno-botany' was first used by John W. Harshberger, American, Father of Economic Botany (Gurib-Fakim, 2006). But the definition of ethnobotany has undergone changes and has been significantly amplified in the century since the term was first used in 1895. Ethno-botany is considered to encompass all studies, which concern the mutual relationship between plants and traditional people (Cox & Balick, 1994; Cotton, 1996). Ethno-botany is an interdisciplinary field, combining botany, anthropology, economic botany, phytochemistry, pharmacognosy, medicine and many other areas of study (Hamilton et al., 2003).

1.2 Ethnomedicine

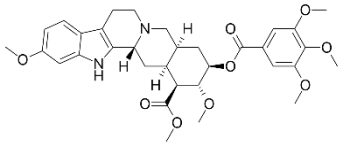
The medicinal plant therapy is based on the knowledge of empirical findings of the traditional uses of the plants and that transmitted orally from one generation to another over hundreds and thousands of years (Gurib-Fakim, 2006). The study of all types traditional medicines, unwritten (folklore medicine) or written (Ayurveda or Traditional Chinese Medicine), is known as ethnomedicine. Even now a day, ethno-medicines are used mostly because ethnomedicinal knowledge is socially and culturally adapted that corresponded to the local views on the diseases and wellbeing (Kunwar & Bussmann, 2008). Ethno-medicine based treatments are gaining a high interest to the public due to several side effects of the synthetic drugs, lacking of curative treatment for several chronic diseases, elevated cost of newly discovered drugs, microbial resistance to the drugs in use and emergence of many new diseases.

About 60% of the world population and 60-90% of people residing in the developing countries rely on traditional medicine (Shrestha & Dhillion, 2003). About 85% of the traditional remedies for primary health care are derived from plants (Farnsworth, 1988). Even today in Nepal, about 85% of the rural hilly population use traditional herbal remedies (Kunwar & Bussmann, 2008) due to the absence of modern medical facilities, poor economic condition along with ease accessibility to the herbs and fate healers (Shrestha et al., 2001-2002; Shrestha & Shrestha 2008). It is estimated that the ratio of traditional healers to the population is 1:100 (Gillam, 1989; Kunwar et al., 2010), whereas the ratio of medical doctors to the population throughout Nepal is 1:1724 and this ratio in rural areas becomes 1:150,000 (Shankar, 2017).

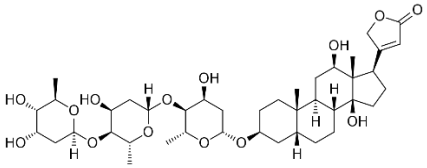
Plant-based traditional knowledge is more efficient means of drug discovery than random plant screening (Heinrich, 2000; Fabricant & Farnsworth, 2001; Sharma & Mujumdar, 2003). Morphine, isolated from opium poppy (*Papaver somniferum*) was the first plant-derived drug (Fabricant & Farnsworth, 2001) (Figure 1). Other examples include reserpine, an antihypertensive drug, from the root of *Rauvolfia serpentina*; digoxin and digitoxin, the cardiac glycosides, from *Digitalis lanata*; and vincristine and vinblastine, the anticancer agents, from *Catharanthus roseus* (Cox & Balick, 1994).



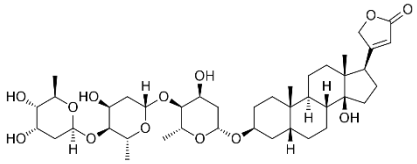
morphine



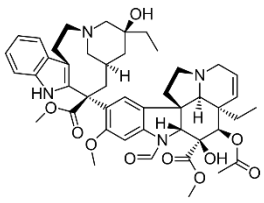
reserpine



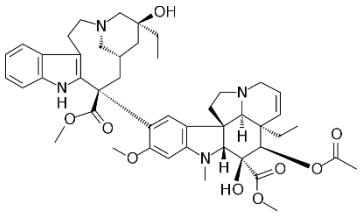
digoxin



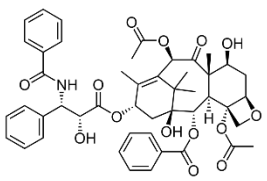
digitoxin



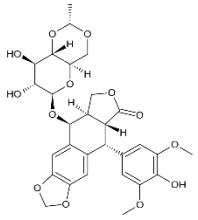
vincristine



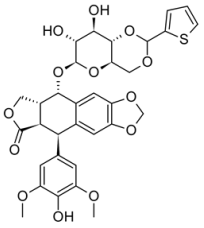
vinblastine



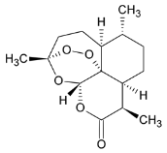
paclitaxel



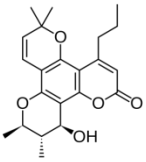
etoposide



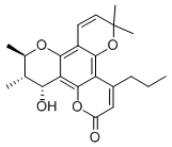
teniposide



artemisinin



calanolide A



calanolide B

Figure 1: Some ethno-medicine derived drugs.

Most of the secondary metabolites of plants employed in modern medicine were first discovered through ethnobotanical investigation. Drugs used for treatment of fatal diseases such as cancer, malaria, AIDS, etc. are discovered from ethno-medicinal plants based on ethnobotanical research. Active complex alkaloid compounds such as vincristine and vinblastine isolated from *Catharanthus roseus* (family Apocynaceae), have been proven to be effective against childhood leukemia, breast cancer and Hodgkin's disease (a cancer of the lymph nodes) (Figure 1) (Gurib-Fakim, 2006). Other anticancer drugs such as paclitaxel is obtained from *Taxus brevifolia* (family Taxaceae), and etoposide and teniposide are obtained from *Podophyllum peltatum* (family Berberidaceae). Antimalarial drug, artemisinin, has been obtained from *Artemisia annua*. The anti-AIDS drugs calanolide A and calanolide B have been isolated from the plant species *Calophyllum lanigerum* (family Clusiaceae) showing anti-HIV activity.

1.3 Pharmacological Evaluation

One of the main motives for increasing use of herbal treatment is an assumption that "natural means safe". Pharmacological evaluation of the plant species have to be performed to ascertain safety and efficacy of the traditional medicinal uses. *In vitro* as well as *in vivo* animal models can be used as the test systems. Pharmacological evaluation provides scientific evidences for the traditional uses of the ethnomedicinal plants.

In vitro antibacterial activity of plant extract is worth harness for evaluation of the traditional uses of medicinal plants curing various infections. In the recent decade, antibiotic-resistant pathogens are an intensifying problem worldwide resulting in an increase of morbidity and mortality, and overall cost of treatments. The main causes of increasing of resistant bacterial strains are indiscriminate and inappropriate uses of antibiotics and disinfectants.

Antimicrobial compounds of plant origin have enormous therapeutic potential without side effects (Cowan, 1999). Therefore, it is essential to search new antimicrobial agents of plant origin. Another driving factor for the renewed interest in plant antimicrobials is the rapid extinction rate of plant species in the past 20 years (Lewis & Elvin-Lewis, 1995). Hence, searching of antibacterial substances derived from natural resources has gained increasing importance alongside the discovery of new synthetic chemical compounds possessing antibiotic and bacteriophage properties (Mandal et al., 2014).

A vast number of medicinal plants have been reported for the presence of antimicrobial phytochemicals (Cowan, 1999; Mahady, 2005). Numerous studies have aimed to describe the chemical composition of plant antimicrobials and the mechanisms involved in microbial growth inhibition (Husain et al., 1992; Sinhababu et al., 1999; Gabbriella et al., 2010; Luitel et al., 2010). Now a days, plant derived compounds have become of great interest for pharmaceutical intermediates and chemical entities for synthetic drugs (Tiwari et al., 2011). About 25% of prescribed drugs contain active principles derived from higher plants (Tiwari & Joshi, 1990).

An antibacterial compound either kills microorganisms or prevents their growth by disturbing cell wall synthesis or inhibits protein biosynthesis (Lambert, 2004). The antibacterial activity is studied by observing the zone of inhibition, the zone of clearing around the extract on the bacterial lawn, using plant extracts, isolates and pure compounds.

Considering the resistance phenomenon, all β-lactam antibacterial (penicillins, cephalosporins, oxacephems, carbacephems) can only be inactivated by bacterial produced enzymes called β-lactamases (e.g. penicillinases, cephalosporinases, cephamycinases, carbapenenases, and so on). Aminoglycosides are characterized by a core structure of amino sugars connected *via* glycosidic linkages to another functional

group. They function as antibacterial by inhibiting protein synthesis in bacterial cell. Some commonly used aminoglycosides are gentamicin, kanamycin A, streptomycin, etc (Figure 2).

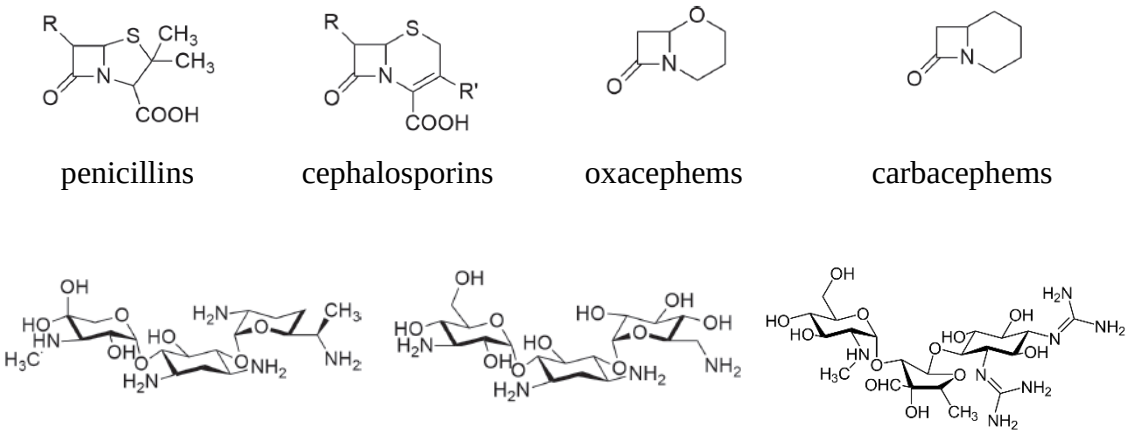


Figure 2: Some antibacterial compounds

Besides these two groups, some compounds belonging to sulphonamides such as sulfadiazine show antibacterial property. Some others are sulfonylureas and thiazide diuretics which proved to be a newer drug group based on the antibacterial sulphonamides. Tetracyclines are four rings hydrocarbon containing compounds.

1.4 Plant Metabolites and Their Importance

Phytochemicals are chemicals produced by plants during primary and secondary metabolisms. Primary metabolism in a plant comprises all metabolic pathways which generate compound (metabolites) such as carbohydrates, lipids, nucleic acids, proteins, etc. These compounds are known as primary metabolites and these are essential for the growth and development of plant. Apart from these compounds, many plants synthesize a number of organic compounds which are not in the mainstream of metabolism and have no direct function of growth and development of plant. These compounds are extremely numerous and chemically diverse in nature and are called as secondary metabolites or secondary plant products or natural products. Alkaloids, terpens, steroids, tannins, flavonoids etc. are examples of secondary metabolites. A common role of secondary metabolites in plants is defense mechanisms. They protect plants against herbivores, pests, and pathogens. These plant metabolites are essential for the survival and proper functioning of plants (Molyneux et al., 2007).

The metabolites in higher plants represent at least 500,000 to 600,000 compounds covering a great number of common plant compounds such as alkaloids, flavonoids, terpenoids, steroids, etc (Berdy, 2005). Biological properties of medicinal plants are mainly due to the presence of these phytochemicals. The medicinal plants possess one or more medicinal properties *viz* antibacterial, antifungal, antiviral, anthelmintic, anticancer, sedative, laxative, diuretic and many more (Taylor et al., 1996; Taylor & Towers, 1998). The phytochemicals within the group of phenolics, terpenoids, essential oils, alkaloids, polyacetylenes, proteins and peptides possess potent antimicrobial activity with varying mode of action (Cowan, 1999). There are many phytochemicals and each works differently. These are some possible actions: antioxidant, antimicrobial effect, hormonal action, stimulation of enzymes, interference with DNA replication and physical action. Ajuru et al. (2017) reported that alkaloids show considerable pharmacological activity including antimicrobial activity. Modern research is not only confined searching of antimicrobial compounds but has also found several enzymatic inhibitors (Mandal et al., 2014).

The first crystalline fungal product mycophenolic acid was discovered in 1896 by Gosio from *Penicillium glaucoma* (Berdy, 2005). It was considered as microbial secondary metabolite. Isolation and identification of biologically active phytochemicals followed by series of tests discovered roles of phytochemicals as medicines. By careful observation of poisoning effect of the plant *Taxus baccata*, alkaloid taxane was isolated (Panter et al., 1993; Molyneux et al., 2007). It was renamed as paclitaxel and later isolated from the barks of Pacific yew (*Taxus brevifolia*). Following many tests, the role of the alkaloid is discovered for the treatment of ovarian, breast, lung and colon cancers. Good muscle-relaxants such as pancuronium, vecuronium, etc. have been developed by the study of alkaloids obtained from *Chondrodendron tomentosum* and *Strychnos sp.* (Bisset, 1991). These alkaloids were used for arrow poisoning by ethnic people. Therefore, researches on phytochemicals led to discover many drugs. Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health conditions.

Along with various toxicities, secondary metabolites exhibit some antimicrobial, antitumor or antiviral activities. More than 13000 antimicrobial, antitumor and antiviral compounds are isolated from algae, lichens and mainly from vascular plants

(Berdy, 2005). Higher plants cover about 12000 antibiotic compounds, which are structurally unique exhibiting antimicrobial as well as antitumor activities. Despite, many phytochemicals are still remaining to be discovered. Employing new more specific methods or re-isolation (sometimes from different species) using more sensitive methods based on absolutely new principles are required to further investigate the plants.

Phytochemicals with antimicrobial action can be divided into several categories such as polyphenols, terpenoids and essential oils, alkaloids, etc. Phenols are aromatic organic compound possessing hydroxyl groups that attached to the benzene structural motif. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane-derived phenolic compounds in the highest oxidation state. Caffeic acid from thyme is reported as effective against viruses as well as bacteria. Catechol and pyrogallol are hydroxylated phenols that reported to be toxic to the microorganisms. Essential oils which constituted of phenolic compounds and terpenoids are often possessing antimicrobial property. Essential oil constituent such as eugenol is a well-characterized representative that found in clove oil. Eugenol is considered bacteriostatic against both fungi and bacteria (Cowan, 1999; Ciocan & Bara, 2007).

Flavones and flavonoids are phenolic compounds having antimicrobial property. Catechins, belonging to flavonoids, inhibited *in vitro Vibrio cholera, Streptococcus mutans, Shigella* and other bacteria (Cowan, 1999; Kumar & Pandey, 2013). Flavonoid compounds also exhibit inhibitory effect against multiple viruses (Cowan, 1999).

Tannin is a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution. Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesive enzymes, cell envelope transport proteins, etc. Tannins in plants inhibit insect growth and disrupt digestive events in ruminal animals (Cowan, 1999; Kumar & Pandey, 2013).

Quinones are other aromatic compounds with two ketonic substituents and with a great antimicrobial effect. For examples, 1,5-dihydroxy-3-methoxy-7-methyl anthraquinone obtained from *Cassia italica* is reported as bacteriostatic for *Bacillus anthracis, Corynebacterium pseudodiphthericum* and *Pseudomonas aeruginosa*, and bactericidal for *Pseudomonas pseudomalliae* (Kazmi et al., 1994). Hypericin, a

naphthodianthrone or an anthraquinone derivative, from *Hypericum perforatum* (St. John's wort), is reported as antidepressant and antimicrobial (Cowan, 1999; Ciocan & Bara, 2007).

Coumarins also possess antimicrobial property. In 1954, R. D. Thornes found that coumarins inhibit *Candida albicans* and he has treated vaginal candidiasis using coumarins in his pregnant patients (Cowan, 1999).

Terpenoids are found active against bacteria, viruses, fungi and protozoa (Kumar & Pandey, 2013). The triterpenoid betulinic acid is just one of several terpenoids which have been shown to inhibit HIV. Another terpenoid, capsaicin, has a wide range of biological activities in human, affecting the nervous, cardiovascular and digestive systems as well as found its use as an analgesic. The evidence for its antimicrobial activity is inconclusive against trypanosomes and plasmodia (Cowan, 1999). Heterocyclic nitrogen compounds are called alkaloids. Diterpenoid alkaloids, commonly isolated from the plants of the Ranunculaceae, are found to possess antimicrobial properties (Cowan, 1999). Berberine is an important representative of the alkaloid group.

A number of phytochemicals present in plants possess antioxidant activity and protect our cells against oxidative damage and reduce the risk of development of certain types of cancer. Some common phytochemicals with antioxidant activity present in foods are allyl sulfides (in onions, leeks, garlic, etc.), carotenoids (in fruits, carrots, etc.), flavonoids (in fruits, vegetables, etc.), polyphenols (in tea, grapes, etc.), etc. Effectiveness of antioxidant is evaluated by either measuring the degree of oxidation inhibition or measuring the free radical scavenging ability (Sanchez-Moreno et al., 1998). There are different *in vitro* methods to evaluate antioxidant activity such as Oxygen radical absorbance capacity (ORAC) method, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity method, method of inhibition of 2,2′-azinobis-(3-ethylbenozothiazoline-6-sulfonate) (ABTS) radical cation, H_2O_2 scavenging method, etc.

The structure of DPPH free radical is shown in Figure 3. It produces a violet solution in ethanol (Huang et al., 2005) also in methanol. It is reduced in the presence of an antioxidant producing a colorless ethanol solution. In the DPPH free radical scavenging capacity method, the reduced form DPPH-H or DPPH-R is produced in

contact with a substance that can donate a hydrogen atom or with another radical ($R^{\bullet}$) (Equations 1 and 2). AH is an antioxidant that acts by donating hydrogen atom producing a stable molecular structure that will stop the chain reaction. The new radical ($A^{\bullet}$) generated can interact with another DPPH free radical to form another stable molecule (DPPH-A). The scavenging of DPPH free radical is then easily evaluated by spectrophotometer.

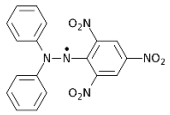


Figure 3: DPPH free radical

$$DPPH^{\bullet} + AH \rightarrow DPPH\text{-}H + A^{\bullet} \quad (1)$$

$$DPPH^{\bullet} + R^{\bullet} \rightarrow DPPH\text{-}R \quad (2)$$

Phytochemicals present in plants can be analyzed qualitatively and quantitatively. Qualitative analysis enables to identify the effective phytochemicals present in plants and quantitative analysis quantifies the phytochemicals present. Gas chromatography-mass spectroscopy (GC-MS) is one of the so called hyphenated analytical techniques. In which Gas chromatography separates the components of mixture in the sample and mass spectrometry characterizes each of the components individually. By combining the two techniques, a sample containing a number of chemicals can be both qualitatively and quantitatively evaluated.

1.5 Rational

The natural resources of Nawalpur district consist of plenty of medicinal plants along with various floras. The first largest indigenous community, Magar is highly populated in this district (CBS, 2011). Magar language speakers are 82.64% in this district (CBS, 2014). The area covers Churia region where loss of biodiversity has occurred due to prone severe landslide. Besides that, deforestation has happened to meet the local demand for pasture, arable land, medicinal plants, wild edible plants and timber. Almost all villages of the study area are found to be relying on traditional healing system with the use of medicinal herbs. Majority of them depend upon the traditional healing system due to their poor economic condition and the geographical situation as well as belief. The knowledge about healing system is transferred orally from generation to generation without any written document. Traditional healing

knowledge is disappearing slowly, generation by generation. The necessity of proper documentation of actual healing system along with techniques, diagnosis and harvesting time of medicinally used parts is equally realized for the enhancement of traditional healing system with the improvement in the use of medicinal herbs available in local areas. Besides this, most of the traditional healing system is based on superstition, which might be unbelievable. The scientific study of traditional healing system is necessary to carry out. Due to all these reasons preparation of inventory of the ethnomedicinal plant is important. Pharmacological evaluation of medicinal plants is important. Antimicrobial and antioxidant assays are worth harness for pharmacological evaluation. This study of ethnobotany with antimicrobial and antioxidant along with the phytoconstituent has been carried out which play an important role of the medicinal plants.

1.6 Hypothesis

The medicinal plants used by Magars in treating of infectious diseases must possess bioactive compounds with antimicrobial property.

1.7 Objectives

1.7.1 General Objectives

The general objectives of this research are: to document ethno-medicinal knowledge and plants of the Magar communities of Nawalpur district and to evaluate antimicrobial activities of the different extracts of plant species (chosen from high prioritized fifteen species of ethno-medicinal plants used by Magar communities in Nawalpur district) with effective phytochemicals present in them to validate the knowledge scientifically.

1.7.2 Specific Objectives.

- To prepare the inventory of the ethnobotanical plants used by Magar community.
- To identify phytochemicals present in the ethnomedicinal plants qualitatively by colour reaction method.
- To analyze phytoconstituents qualitatively & quantitatively by GC-Ms method.
- To assess *in vitro* antimicrobial tests along with MIC and MBC of the high prior 15 ethno-medicinal plant species.

- To estimate total phenol content (TPC) in the ethnomedicinal plants.
- To evaluate antioxidant capacity of the plants by using DPPH free radical scavenging and H_2O_2 scavenging assays.

1.8 Limitation of the Study

Present study covers only two rural municipalities and one urban municipality of Nawalpur district and only 15 species of the ethno-medicinal plants are selected for laboratory investigation because of budgetary constraints.

CHATER 2

2. LITERATURE REVIEW

2.1 Ethnomedicine Focus on Ethnic Group

Plants used in Himalayas as medicine are found in the 6,500 year old texts of the Rigveda followed by Atharveveda (2000-1000 BC) (Kunwar & Bussmann, 2008). Ayurveda is one of the branches of the Veda. It is regarded as upveda of Rigveda or Atharvaveda as it is a stream of the knowledge coming down from generation to generation since parallel to the Vedic period. Ayurveda remains to be the main source of medical knowledge and skill in most part of South Asia including Nepal. Vaidhyas and Kabirajs follow Ayurveda in their pursuit of knowledge and practice in medicine. It has been estimated that Ayurvedic knowledge was accessed by Nepali Vaidhyas as early as about 879 AD when Susruta Samhita Sahotara was hand copied by certain Nepali physician based at Kathmandu (IUCN, 2000). Ayurveda Pharmacy (Baidhya khana) was established in Hanuman Dhoka Palace during the time of King Pratap Malla (Gewali, 2008). Nepal's wealth of Himalayan Herb is reputed in Ayurvedic medicine all over the Indian sub-continent since time immemorial.

'Saushrut Nighantu' was the oldest Nepali medicinal plant book, produced during the rule of the Great King Man Dev in the 5^{th} century (Gewali, 2008). It has three volumes; two are in the National Archives of Nepal and one in Keshar library, describing 278 plants in Sanskrit along with 282 medicines (drabyas) for curing the illness. Nepal Sanskrit University has published 'Saushrut Nighantu' in 2000 (Gewali, 2008).

Ayurvedic treatment was the only way of treatment for health care since time of immemorial. 'Chandra Nighantu' was prepared as medical practitioners of Nepal felt need of a Nepali Pharmacopoeia for a very long time. 'Chandra Nighantu', a hand written herbal encyclopedia with 750 colored plates of plants and its medicinal uses in 8 volumes was compiled at the end of 19th and the beginning of 20^{th} centuries (Malla & Shakya, 1984). It was started as *'Bir Nighantu'* during the prime-minister of Bir Shamsher Jung Bahadur Rana (1885-1901 AD) and the compilation was undertaken by Pandit Ghana Nath Devkota (Devkota, 1968; Gewali, 2008). But on sudden death

of Bir Shamsher the work change into ‘Chandra Nighantu’ depicting the name of reigning prime-minister.

‘Nepali Nighantu’ is compiled by Mr. Koshnath Devkota, son of Pundit Ghana Nath Devkota. It was published by Nepal Academy in 1968. It contains 971 different articles classified under 28 major divisions. This division includes plants, animals, minerals, animal and meat products, cereals and plant products, poisonous and other toxic products, fats and oils, precious metals and minerals. The plants and other materials are named in Sanskrit, Nepali, Newari, Indian languages and Latin. At the end of the book, indices of the plants in different languages in alphabetical order are given (Gewali, 2008).

Besides Ayurvedic, folklore medicine was the way of primary health care in Nepal before introduction of allopathic treatment. It was based on medicinal plants. Therefore, medicinal plants are very much important for Nepal. The Department of Medicinal Plants published Medicinal Plants of Nepal in 1970 enlisting 393 medicinal plants in Nepal. The Department of Plant Resource published revised version of Medicinal plants of Nepal enlisting altogether 701 species in 2007. Later on 2016 altogether 819 medicinal plant species with Nepali name in Devnagari script, scientific name, brief description, uses and distribution are mentioned in Medicinal Plants of Nepal (DPR, 2016). IUCN Nepal (2000) documented 150 medicinal and aromatic plants, which was published in ‘National Register of Medicinal Plants’. This book has been revised and updated in 2004, which describes 187 species of medicinal plants. IUCN (2000) documented 150 plant species with scientific name, family, common name, major documentation, conservation status and traditional uses. Chandra Nighantu and Nepali Nighantu are referred as far as possible for each species of plant in this register.

Magars as the largest ethnic group of Nepal must have their indigenous knowledge on use of medicinal plants. It is essential to document the knowledge of the Magar to explore hidden knowledge of them.

Poudel and Gautam (2008) documented 62 plant species use for 72 medicinal remedies of Magar community of Dhadhing district.

Acharya (2012) documented 161 of plant species belonging to 87 families and 144 genera of Magar community of Badagaun VDC, Gulmi district, Nepal to treat different ailments. Maximum species (57 species) were reported to treat gastro-intestinal problems.

Thapa (2012) documented 75 plant species to treat 39 different ailments including gastrointestinal problem, dermatological infection, and skeleton-muscular problem of Magar in Salija VDC of Parbat district.

Singh et al. (2018) documented 171 new ethno-botanical claims for 70 plant species belonging to 68 genera and 47 angiosperm families and 1 species was fern to treat 104 different ailments of Magar communities in Palpa district, Nepal.

These literatures revealed that Magars of different geographical region of Nepal also have sound knowledge of use of medicinal plants for treatment of different diseases. Bhattarai (1989) reported that same ethnic group at different geographic region may differ in knowledge on use of plants. This research was focus on Magars of Nawalpur district covering mountainous region where government facilitate hospital is not available. Stepp and Moerman (2001) suggested the usefulness of the apparency and resources availability theories in explaining the use and selection of medicinal plants by local people. Easily found plants would offer more possibilities for local populations to experiment with their uses, thus having probability of being introduced into the local culture (Philips & Gentry, 1993) and having its perpetuated by folk knowledge (DeAlbubuque & Farias Paiva de Lucena, 2005). Thus knowledge of use of plants by single ethnic group at different geographic region may differ. So the result of this research would differ from other literatures. It is important to validate the knowledge of use of ethnomedicinal plant scientifically. Though Gewali (2008) had compiled 91 ethnomedicinal plant species with chemical structure and biological properties along with description of basic elements of the traditional medicine, ethnomedicinal plants of Magars have not been tested scientifically yet. Ethnomedicinal plants recorded are planned to be undergone antioxidant, antimicrobial tests and phytochemical screening.

Bhattarai (2009) documented 157 ethnomedicinal plants from Manang and Mustang district to treat 150 ailments by local people. Out of them, 92 extracts of 79 plant species which were used to treat diseases potentially caused by bacteria, were tested

for antimicrobial properties by *in vitro* method. But phytochemical tests were not done.

Rokaya et al. (2014) documented 947 species belonging to 158 families and 586 genera to treat gastrointestinal disorder in Nepal using secondary data such as electronic database, literatures etc. It was found that 348 species were used to treat diarrhea, 340 to treat stomachache, 307 to treat dysentery and pointed out the importance of phytochemical and pharmacological studies on most promising species.

Ethnomedicine is a suitable source of information regarding useful medicinal plants of indigenous people. It gives benefits for humanity in terms of traditional pharmacopoeias. About 60% of the world population and 60-90% of the population of developing countries rely on traditional medicine (Shrestha & Dhillion, 2003) and about 85% of the traditional remedies for primary health care are derived from plants (Farnsworth, 1988). Therefore, indigenous forms of medical treatment are still important.

2.2 Literature Review on the Plant Species used in this Study

2.2.1 *Aegle marmelos* (L.) Correa

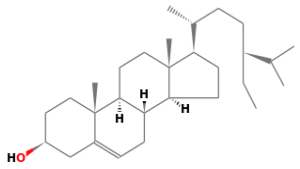
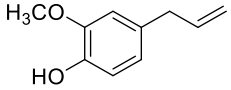
English name: Golden apple, Stone apple, Bengal Quince; Sanskrit name: Bilva, shreephal, Satyaphala, Shailapatra, Shalya, Tripatra; Nepali name: Bel; Magar name: Bel (Annex IE Plate 1)

Ethnomedicinal values: Different parts of Golden apple tree have cultural, religious and medicinal values in Nepal. Ripe and unripe fruits are used to treat stomachache at Dang district (Acharya, 1996); diarrhea and dysentery by the Mooshars of Dhanusha district (Manandhar, 1986a), Limbus of Morang district (Siwakoti & Siwakoti, 1998), Tharus of Chitwan district (Mueller-bocker, 1993); and bowel complaints by Satars of Morang and Jhapa districts (Siwakoti & Siwakoti, 2000). Leaf decoction is used to take bath for sprain by Darai tribe of Chitwan district (Dangol & Gurung, 2000), and taken as an antipyretic drug at Sindhupalchok district (Bhattarai, 1992).

Along with diarrhea and dysentery, the ripe fruits have been used as a tonic for heart and brain (Dutta et al., 2014). Decoction of roots and sometimes the stem barks have been used to get remedies from melancholy, intermittent fevers and palpitation of heart and stomach pain (Patkar et al., 2012; Parmer et al., 2016). The bark juice is

used for diarrhea and stomachache by tribal people in the Seti River of western Nepal (Uprety et al, 2011). Different parts of *Aegle marmelos* have been used to prepare Ayurvedic preparations such as Dashmool, Bilwadi churna, Bilwadi leha, Bilwadi Ghrita, Bilva Kwath, etc. to treat inflammations, diarrhea, dysentery, etc. *Aegle marmelos* has also been used for the treatment of diabetes in Ayurvedic, Unani and Siddha systems (Sharma et al., 2011).

Phytochemicals: Various phytoconstituents such as coumarins, polysaccharides, alkaloids, carotenoids, phenolics, tannins, flavonoids, pectins, fatty acids, steroids etc. have been reported from the various parts of *Aegle marmelos* (Baliga et al., 2011; Rahman & Parvin, 2014; Mathew et al., 2016). Phytochemical such as eugenol, marmesinin, γ-sitosterol and β-sitosterol are reported from the leaf (Duke, 1992; Maity et al., 2009; Vijayalakshmi & Venkatalakshmi, 2017). γ-Sitosterol exhibits strong antifungal, antibacterial and anti-angiogenic activities (Raman et al., 2012). Eugenol exhibits antioxidant, antibacterial, hepatoprotective and antiulcer properties (Duke, 1992; Maity et al., 2009).

	
γ-Sitosterol	Eugenol

Pharmacognosy: The extracts of leaves, roots and fruits have been reported to be active against several bacterial strains (Taylor et al., 1996; Shakya et al., 2008; Saradha & Rao, 2010; Kothari et al., 2011; Mujeeb et al., 2014). The methanolic extract of leaves showed antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae* (Mujeeb et al., 2014), *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi* (Kothari et al., 2011), and *Enteococcus faecalis* (Saradha & Rao, 2010). Antimicrobial activity of the bark of *Aegle marmelos* has been reported (Nandkarni, 1995; Poonkothai & Saravanan, 2008). The leaf and fruit extracts show antioxidant activity (Maity et al., 2009; Baliga et al., 2011). Patra et al. (2015) reported a strong DPPH free radical scavenging activity with 81.1% scavenging at 15 µL/mL concentrations and H_2O_2 scavenging activity with 34.45% at the same

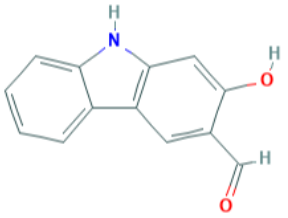
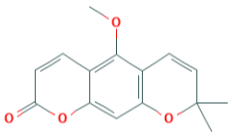
concentration of fermented fruit juice, and reported that fermented juice has increased H_2O_2 scavenging with an increase in its concentration. Wali et al. (2016) reported DPPH free radical scavenging activity of the leaf methanolic extract (IC_{50} = 249.3±9.4 µg/mL) followed by extract of bark (IC_{50} = 268.7±8.5 µg/mL) and then extract of fruit (IC_{50} = 1032.2±7.03 µg/mL). Wali et al. (2016) reported TPCs were 16.5, 14.2 and 10.6 mg GAE/g dry weight of sample in methanolic extract of leaf, bark and fruit, respectively.

2.2.2 *Antidesma acidum* Retz.

English name: Rohitaka; Nepali name: Archal; Magar name: Gwarchal (Annex IE Plate 1)

Ethnomedicinal values: Paste of bark is used to treat mumps by Tamangs of Kabhrepalanchowk district (Manandhar, 1991). Traditionally, leaves of *Antidesma acidum* are used for the treatment of stomachache, dysentery, diabetes, indigestion, Sunstroke (Khan & Yadava, 2010; Horo & Topno, 2015). The decoction of fruit and leaf has long been used for treatment of anemia and stimulation of blood circulation, whereas bark is used as astringent and analeptic, and stem and root are used as diuretics (Sireeratawong et al., 2012). Tender shoot and leaves (dried or fresh) of *Antidesma acidum* are used as vegetables (Patil & Jadhav, 2014; Srivastava & Bhatt, 2018) and fruits are eaten (Horo & Topno, 2015; Kumar et al., 2019a).

Phytochemicals: Phytochemicals such as phenols, anthraquinones, flavones, tannins, coumarins, saponins, alkaloids are reported on phytochemical screening (Patil & Jadhav, 2014). Phan et al. (2016) isolated five carbazole alkaloids such as clauszoline B, clauszoline H, mukonal, 7-methoxymukonal and heptaphyline, and three coumarin derivatives, 5-demethyltoddaculin, xanthoxyletin and alloxanthoxyletin from the leaves; and tested cytotoxic activity of them and concluded that the constituents from *Antidesma acidum* may contain effective compounds which can be used as anticancer agents. Mukonal has been reported to exhibit antimicrobial and antioxidant activity (Tsutsumi et. al., 2016; Liu et al., 2018). Xanthoxyletin showed strong inhibitory effects on expression of the inflammatory-related molecules inducible-nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α) and cyclooxygenase-2 (COX-2) (Nakamura et al., 2009).

	
Mukonal	Xanthoxyletin

Pharmacological activity: Ethanol extract of roots of *Antidesma acidum* did not produce any toxicity in oral acute and sub-acute toxicity studies in male and female rat (Sireeratawong et al., 2012). Aqueous extract of the stem of *Antidesma acidum* showed antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* (Kiran et al., 2014). Anti-HIV and immunomodulating actions of *Antidesma acidum* have been reported (Lousirirojanakul et al., 2003).

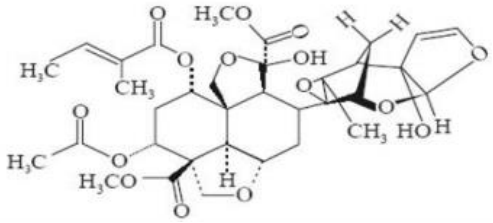
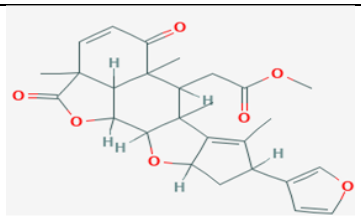
2.2.3 *Azadirachta indica* A. Juss.

English name: Neem, Nimtree, Indian lilac, Margosa tree; Sanskrit: Arishta, Nimba; Nepali name: Neem; Magar name: Neem (Annex IE Plate 1)

Ethnomedicinal values: The leaf decoction is taken in the morning to treat fever by Tharus of Chitwan district (Dangol & Gurung, 1991); leaf juice for night blindness (Rajbhandary & Ranjitkar, 2006). Juice of bark is used for cough, urinary complain and fever, and paste is applied to treat bleeding from gum. Fruit juice is used for skin disease, headache and urinary discharge, and seed oil is used as anthelmintic and antiseptic. A mixture of paste of seed with turmeric and mustard oil applied on leprosy in Kabhrepalanchowk district (Manandhar, 1991). *Azadirachta indica* has been used in Ayurvedic medicine for more than 4,000 years due to presence of its medicinal value. It has been used in Indian traditional medicine for the treatment of diarrhea and cholera (Bhattacharya, 1977; Thakurta et al., 2007). Leaf, bark and seed oil of *Azadirachta indica* have been reported to have antiseptic, wound healing, skin disease curing, antioxidant, antimalarial, antimutagenic, anticarcinogenic and antiulcer activities (Talwar et al., 1997; Chattopadhyay, 1999). Leaves possess anti-inflammatory, antifertility, hypolipidemic, hepatoprotective, antihyperglycemic, antipyretic, antiarthritic, anti-gastritic ulcer, antibacterial and antifungal activities (Sharma et al., 2014). Leaf, bark and seeds are known to contain antibacterial (Banna

et al., 2014; Maleki et al., 2017), antifungal activity against different pathogenic microorganisms and antiviral activity against vaccinia, chikungunya, measles and coxsackie B viruses (Reddy et al., 2013).

Phytochemicals: Phytochemicals such as saponins, glycosides, tannins, flavonoids, alkaloids, terpenes, reducing sugars in the leaves extract (Gupta et al., 2013) are reported. *Azadirchta indica* contain more than 300 chemical constituent such as azadirachtin, nimbolide, gedunin, salanin, nimbin, valassin and many other derivatives. Azadirachtin is the main active component that exhibits as antimalarial. Nimbolide exhibits as antimicrobial (Chauhan et al., 2018).

	
Azadirachtin	Nimbolide

Pharmacognosy: A high level of antibacterial activity of the leaf and bark extracts against *Escherichia coli*, *Staphylococcus spp.* and *Bacillus subtilis* is demonstrated (Ahmad et al., 1995; Sharma & Vaquil, 2018). Fractions of neem oil showed antimicrobial activity against *Escherichia coli* and *Klebsiella pneumoniae* which were not inhibited by the neem oil (Sairam et al., 2000). Ethanolic extract of the leaves showed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* (Gupta et al., 2013). The aqueous extracts of leaves as well as barks showed antibacterial activity against *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and MIC of the aqueous leaf extract was 500µg/mL against each tested bacterial strain (Reddy et. al., 2013).

Autade et al. (2015) studied antimicrobial activity of acetone and chloroform extracts of the leaf, fruit and bark of *Azadirachta zadirachta indica.* The result shows stronger antimicrobial activity by acetone extract of leaf part of the plant than bark and fruit against *Pseudomonas aerugenosa* and *Staphylococcus aureus.*

Thakurta et al. (2007) reported antimicrobial activity of the methanolic extract of leaves against multiple drug resistant *Vibrio cholerae* and MBC was 10µg/mL.

Thakurta et al. (2007) reported that the extract inhibits fluid secretion and hemorrhage in the intestine induced by *Vibrio cholerae* at dose of 450 mg/kg and reported that the extract is not toxic in mice on oral administration at 1800 mg/kg dose.

Maleki et al. (2017) investigated antimicrobial activity of the ethanol, methanol and ethyl acetate extracts of leaves of *Azadirachta indica* against *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa* and *Escherichia coli,* and reported that methanol extract showed strongest inhibition against *Pseudomonas aeruginosa* whereas ethyl acetated and ethanol extracts showed strongest inhibition against *Staphylococcus aureus.* Ethanol and methanol extracts showed strongest inhibitory against *Enterococcus faecalis*. None of the extracts showed antibacterial activity against *Escherichia coli.*

Subapriya et al. (2004) reported antioxidant potential of extracts of *Azadirachta indica* leaves by the reduction of DPPH, ABTS, superoxide, hydroxyl, and nitric oxide radicals and reported that extracts enhanced the antioxidant enzymes like (glutathione-S-transferase), and protected against MNNG-induced (N-methyl-N'-nitro-N-nitrosoguanidine) genotoxicity and oxidative SOD (superoxide dismutase), CAT (catalase), GSH-px (glutathione peroxidase) and GST stress in male rats (Sharma et al., 2017).

Bandyopadhyay et al. (2004) investigated antisecretory and antiulcer effect of the aqueous extract of bark of *Azadirachta indica* in human, and reported that extract completely healed the duodenal ulcers at the dose of 30-60 mg twice daily for 10 weeks, and completely healed esophageal and gastric ulcer at the dose of 30 mg twice daily for 6 weeks.

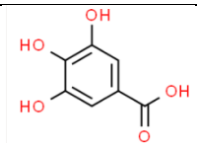
2.2.4 *Phanera vahlii* (Wight & Arn.) Benth

English name: Camel's foot creeper, Cow's paw; Sanskrit name: Asmantaka, Malanjhana; Nepali name: Bhorla, seed is called "tata"; Magar name: Bhorla, fruit is called as "pakshya" in Magar language. (Annex IE Plate 1)

Ethnomedicinal values: Fruits are consumed by Darai tribe of Chitwan district (Dangol & Gurung, 2000) whereas Tharus of Dang district applied them on boils (Manandhar, 1985). Root bark is used in diarrhea and dysentery in Dang district (Manandhar, 1985). Root is given to cure bloody dysentery by Dhanuwar of Sindhuli

distrct (Manandhar, 1990a) whereas it is given to cure gastritis by Limbus of Morang district (Siwakoti & Siwakoti, 1998). Tharus of Chitwan use the leaves to make plates and hats; the barks to make rope (Manandhar, 1994). Seeds are eaten raw or fried and cooked as pulse. *Phanera vahlii* is mostly used by tribal sovereignty for fever, diarrhea, bone fracture, skin irritancy, tonic and as vermifuge medicine (Pattanaik et al., 2007). The roots are used for the pulmonary tuberculosis, dysentery, fever and virus induced disease with specific activity toward herpes simplex. In some places, root is used as tooth brush to cure pyorrhea. Fruits are used in anti-fertility in woman and use as aphrodisiac. Bark is used in skin disease, diarrhea and pods are taken orally as anti-diarrhea, anti-dysentery and seed is use in the treatment of pimple, boils and blister. A paste of the seed is given to children suffering from indigestion (Chauhan & Saklam, 2013).

Phytochemicals: Higher amount of phenolics, tannins and flavonoids was reported in the methanol extract than chloroform, hot water and acetone extracts (Sowndhararajan & Kang, 2013). Compounds taraxerol and β-sitosterol were isolated from the hexane extract of *Phanera vahlii* leaves (Elbanna et al., 2016). Other compounds isolated from the leaves of *Phanera vahlii* are avicularin, quercetin-3-O-β-sophoroside, luteolin, hyperoside, quercetin, quercitrin and gallic acid. The leaf extract of this plant was also reported to contain agathisflavone, quercetin, kaempferol, isoquercitin, campesterol, β-stigmasterol and saturated fatty acids along with vitamin E, α-amyrin and methyl salicylate (Panda et al., 2018).



Gallic acid

Pharmacological activity: The methanolic extract of roots of *Phanera vahlii* show antimicrobial activity against *Escherichia coli, Pseudomonas aerugenosa, Bacillus subtilis, Enterococcus faecalis* and *Staphylococcus aureus* (Dugasani et al., 2010). MIC for all the bacterial strains, except *Staphylococcus aureus*, was 125 µg/mL. The ethanolic extract of root, bark and seed showed antimicrobial activities against *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* and *Staphylococcus aureus*.

The seed extract showed the largest ZOI amongst all (Geetha & Padal, 2014). Singh and Singh (2014) demonstrate antimicrobial activity of leaves using various solvents such as petroleum ether, benzene, chloroform, ethyl acetate, acetone, and ethanol. The result showed that only ethyl acetate and acetone extract gave ZOI against *Klebsiella pneumoniae* and *Staphylococcus aureus*. Panda et al. (2015) demonstrated antimicrobial activity of the leaves extract using methanol, n-hexane, chloroform and ethyl acetate as the solvents against *Enterococcus faecalis*, *Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The result showed antimicrobial activity of all the extracts against the tested bacteria except chloroform and ethyl acetate extracts against *Escherichia coli.* Panda et al. (2018) reported that the compounds presents in extract of *Phanera vahlii* were mainly responsible for antioxidant as well as tyrosinase inhibitor, which is used as skin whitening agents. Panda et al. (2018) compared tyrosinase inhibitory activity of this extract with kojic acid and suggested that the *Phanera vahlii* leaves could be exploited as potential source of natural antioxidant and tyrosinase inhibitory agent, as well.

2.2.5 *Callicarpa macrophylla* Vahl

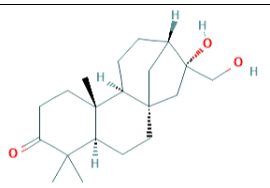
English name: Beautyberry; Sanskrit name: Gandhaphali, Phalini, Asthibandhana, Cochandana, Shyama, Pitatandula, Karamba, Priya, Durjara, Kanta, Priyahva, Vanita, Lata, Kangu, Govandani, Shreyasi; Nepali name*:* Dahigaula, Dahijalo; Magar name*:* Malaburu, Dangali. (Annex IE Plate 1)

Ethnomedicinal values: The juice secreted from the leaf, shaped into cone and into which a burning coal is placed, is used for treating earache by Tharus at Chitwan district; the roots of the plants are boiled in water at night and the decoction is given to the patient to drink early in the morning to lower fever (Dangol & Gurung, 1991). In Chitwan, root juice is given for indigestion (Manandhar, 1990b); roots are chewed to treat rash on the tongue; the paste of young buds is given for stomachache; and paste of root is given to control fever (Manandhar, 2002).

Leaves are used in rheumatism (Anjum et al., 2013), mouth sores and gingivitis (Arya & Agarwal, 2008), stomach disorders, gastritis (Soni et al., 2014), as anti-inflammatory, analgesic and antipyretic (Yadav et al., 2012). Roots of the plant has

been used as anti-pyretic, analgesic, anti-ulcer and also used for stomach disorders, diarrhea, urinary complaints, irregular menstruation, rheumatic pain, heart palpitation and pneumonia (Pandey et al., 2014), the fruit extract of fruit used to treat rheumatic pain and mouth ulcers (Semwal et al., 2010).

Phytochemicals: Phytochemicals such as flavonoids, tannins, steroids, triterpenes, saponins, but not alkaloids, are reported in the leaves and bark (Patel et al., 2016). Phytochemical screening of the leaves revealed the presence of flavonoids, terpenoids and steroids in the petroleum ether extract; flavonoids, terpenoids, steroids and glycosides in the chloroform extract; flavonoids, terpenoids, steroids, carbohydrates, proteins, amino acids, glycosides and saponins in the methanolic extract; and flavonoids, tannins, carbohydrates, proteins, amino acids, glycosides and saponins in the aqueous extract. None of the extracts showed the presence of alkaloids. Over 40-53 compounds were identified in the essential oils of the leaves, seeds and fruits of *Callicarpa macrophhylla* which contributed to 96.55, 94.56, and 95.35% of the total oil, respectively (Chandra et al., 2017). Extract of the stems showed the presence of glycosides, flavonoids, tannins, carbohydrates, steroids saponins, and absence of alkaloids and amino acids (Yadav et al., 2012). Diterpenes such as calliterpenone and calliterpenone-monoacetate along with β-salinene and α-salinene in the essential oil of the aerial parts of *Callicarpa macrophylla* have been reported (Singh et al., 2010). *Callicarpa macrophylla* leaf essential oil showed DPPH free radical scavenging activity, and significant analgesic and antipyretic activities on Swiss albino mice (Chandra et al., 2017). These activities may be due to the synergistic effect of the plant constituents.



Calliterpanone

Pharmacological activities: Yadav et al. (2012a) evaluated antimicrobial activity of the ethanolic and aqueous stem extracts of *Calliarpa macrophylla* against *Staphylococcus aureus*, *Bacillus subtilis*, *C. sporogens*, *S. epidermidis*, *M. lutus*, *B.*

cereus, *Streptococcus pyogens*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Agrobacterium tumifaciens*, and reported moderate growth inhibitory activity against all the bacterial strains, but aqueous was exceptionally inactive against all strains except *Salmonella typhimurium.* Yadav et al. (2012) evaluated analgesic and anti-inflammatory potentiality of the aqueous and ethanolic extracts of roots of *Callicarpa macrophylla* using tail immersion test and carrageenan paw edema method in albino rats, respectively. The aqueous extract of roots is having better analgesic activity than that of its ethanolic extract. Whereas ethanolic root extract has superior anti-inflammatory spectrum than aqueous one. Results are highly promising and ascertain that roots of *Callicarpa macrophylla* have analgesic and anti-inflammatory potential, comparable to that of standards. The aqueous and ethanolic extract of leaves have analgesic as well as anti-pyretic effect (Yadav et al., 2012). Chandra et al. (2017) investigated antioxidant activity of the essential oils of leaves, fruits and seeds of *Callicarpa macrophylla* and reported that the essential oils showed good to moderate DPPH radical scavenging activity. The DPPH free radical scavenging activity was observed between 55.67-62.63%. Jayaraman and Variyar (2015) evaluated antioxidant activity of polysaccharides isolated from leaves of *Callicarpa macrophylla* and showed that the value of DPPH scavenging activity at a concentration of 2 mg/mL concentration was 40.11±0.005%.

2.2.6 *Cissampelos pareira* L.

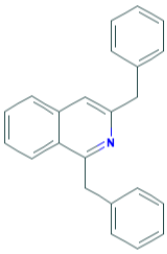
English name: Velvet-leaf, Abuta; Sanskrit name: Laghu patha, Patha, Abuta, Ambashtha, Piluphala, Ekashthila, Dipani, Varatiktata, Tiktapushpa, Atisaranashani, Ambashtaki, Pracchina; Nepali name: Batulepate; Magar name: Batulpate, Thigenphayap. (Annex IE Plate 1)

Ethnomedicinal values: Root juice of *Cissampelos pareira* is given to treat stomachache and diarrhea at Dang district (Acharya, 1996); to treat peptic ulcer at Dhading district (Manandhar, 1992); to treat gastritis at Chitwan district by Darai tribe (Dangol & Gurung, 2000); to treat cough at Gorkha district (Manandhar, 1990c). Juice of the aerial part of plant is given to treat fever and used to induced abortion in human by the Tharus of Chitwan district (Dangol & Gurung, 1991); given as energy supplement after child birth at Rasuwa district (Manandhar, 1980); to stop bleeding

and counteract of the loss of blood after childbirth by the Tamang of Kabhrepalanchok district (Manandhar, 1991). Root paste is given by Chepangs of Makwanpur district to treat stomachache (Manandhar, 1989). Root bulbs are eaten to clear stool at Gorkha district (Manandhar, 1990c). Leaves are taken as tonic (Shrestha & Shrestha, 2000)

Stems, leaves and rhizomes are used to cure diarrhea, dysentery, ulcers, colic, infertility and miscarriage (Samanta & Bhattacharya, 2011).

Phytochemicals: Phytochemicals such as alkaloids, flavonoids, glycosides, coumarins, tannins, terpenoids and steroids are reported on phytochemical screening (Anupa et al., 2014; Ngoci et al., 2014). Alkaloids, especially bisbenzylisoquinoline alkaloid and cissampareine alkaloid, have been isolated from whole plant (Samanta & Bhattacharya, 2011; Arora et al., 2012).



Bisbenzylisoquinoline

Pharmacological activities: Ngoci et al. (2014) investigated the antibacterial activity of the methanolic extract of *Cissampelos pareira* roots against two Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and four Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus vulgaris*). The methanolic root extract demonstrated antibacterial activity against four of the six tested bacteria. Ngoci et al. (2014) reported that the highest inhibition was demonstrated against *Staphylococcus aureus* (20 mm), *Salmonella typhimurium* (17 mm), *Klebsiella pneumoniae* (14 mm) and *Escherichia coli* (9 mm). . *proteus vulgaris* and *Streptococcus pneumoniae* were not sensitive to the extract at all.

Hema et al. (2013) investigated antimicrobial activity of acetone, ethanolic and propanolic extracts of *Cissampelos pareira* leaves and reported that acetone extract

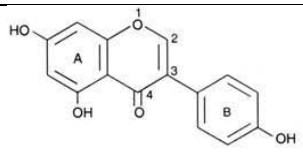
did not show antimicrobial activity against tested bacteria whereas ethanolic extract showed antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Shigella spp.*, *Staphylococcus aureus* and *Streptococcus spp.* but not against *Proteus vulgaris* and *Salmonella typhi*. Hema et al. (2013) also reported that propanolic extract showed better antimicrobial activity among the three extract but it did not showed antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus.*

2.2.7 *Flemingia strobilifera* (L.) W.t. Aiton

English name: Luck plant, Wild hops, Kidney bush; Nepali name: Bhatmase, Bhatwasi, Bharkauli jhar, Bhatte; Magar name: Bhatte. (Annex IE Plate 2)

Ethnomedicinal values: It is used in folklore medicine, such as leaves and flowers for tuberculosis, and roots for ulcers, body swellings, epilepsy, insomnia, fever, diarrhea and dysentery (Manandhar, 2002; Ghalot et al., 2011; Kumar et al., 2011b), and as digestant (Bhattarai, 1991). It is used as fodder by Chepang communities in mid hills of Nepal (Rijal, 2011). Root powder is applied on the body by Darai tribe of Chitwan district for scabies (Dangol & Gurung, 2000).

Phytochemicals: Phytochemical screening revealed presence of terpenoid, saponin, tannin, steroids, glycosides, phenolics, alkaloid and flavonoids (Madan et al., 2010; Tikadar et al., 2017). Madan et al. (2010) showed the presence phenolics and flavonol in the methanolic extracts of roots and leaves of *Flemingia strobilifera.* Compound isoflavonoids was isolated from *Flemingia strobilifera* roots (Madan et al., 2009). Isoflavonoids exhibits antimicrobial activity.



Isoflavonoid

Pharmacological activity: Madan et al. (2009) have reported antimicrobial activity of some isoflavonoids isolated from the roots of *Flemingia strobilifera* against Gram-

positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*).

Madan et al. (2010) reported that the methanolic extracts of roots and leaves of *Flemingia strobilifera* showed a very good DPPH radical scavenging activity with IC_{50} values of 11.4 and 38.0 μg/mL, respectively, whereas the butanolic extract of *Flemingia strobilifera* roots showed good nitric oxide radical inhibition activity with IC_{50} of 150.0 μg/mL in a dose-dependent manner, and the methanolic extract of roots showed hydroxyl radical scavenging by p-NDA method with IC_{50} value of 378.33 μg/mL.

Pizon et al. (2016) evaluated antioxidant capacity of 80% methanolic extract and aquous extract of the leaves of *Flemingia strobilifera* by DPPH free radical scavenging method. The result showed that the scavenging activity was dose dependent where the percentage increased as the concentration of the leaf extract was increased. Both 80% methanol and aqueous leaf extracts exhibited high activity which is more or less comparable to the standard ascorbic acid. The IC_{50} value of aqueous extract (<0.25 mg/mL) is the same with standard ascorbic acid. The IC_{50} of the 80% methanolic extract is slightly higher (0.299 mg/mL) than standard ascorbic acid. The result indicated that the 80% methanolic and aqueous leaf extracts of *Flemingia strobilifera* are potent antioxidants.

Pizon et al. (2016) estimated TPC of aqueous and 80% methanolic extract of leaves by using Folin-Ciocalteu method and reported that TPC of aqueous and 80% methanolic extracts were 102.98±.003 and 12.49±.02 mg GAE/g extract respectively.

Kumar et al. (2011a) reported a significant anthelmintic activity of the alcoholic and chloroform extracts of the leaves of *Flemingia strobilifera.*

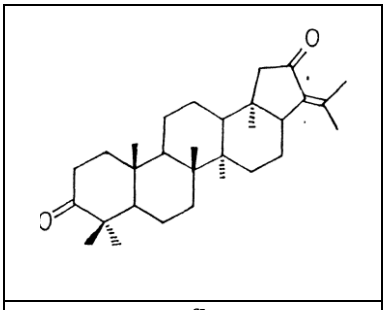
2.2.8 *Lagerstroemia parviflora* Roxb.

English name: Small flower crepe myrtle; Nepali name: Bot daiyero; Magar name: Rangsing, Bot daiyero. (Annex IE Plate 3)

Ethnomedicinal values: Decoction of leaf is used to treat fever at Dhading district (Manandhar, 1992) and Chepang of Makawanpur district (Manandhar, 1989). Wine made of flower is used by the Aathpahariya Rai at Dhankuta district in chronic abdominal pain and dysentery (Dahal & Das, 2000). *Lagerstroemia parviflora* is an

important medicinal plant used for treatment of sores, fever strangulation of intestine, syphilis and carbuncles (Bobade et al., 2013).

Phytochemicals: The whole plant of *Lagerstroemia parviflora* is source of triterpenoid, lageflorin (Sinhababu et al., 1999). Bobade et al. (2013) reported presence of carbohydrates, alkaloids, flavonoids, cardiac glycosides, phytosterols, steroids, tannins, phenolic compound, and coumarines in aqueous and ethanol extract of leaves of *Lagerstroemia parviflora.*



Lageflorin

Pharmacological activity: Mazumder et al. (2002) reported that the benzene extract of *Lagerstroemia parviflora* leaves showed antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Lactobacillus arabinosus*, *Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei*, *Shigella boydii*, *Salmonella typhimurium*, *Proteus mirabilis* and *Vibrio cholerae.* Mazumder et al. (2002) also reported that antimicrobial activity decreased in the following order against the test bacterial strains, *Shiegella dysenteriae*, *Escherichia coli*, *Streptococcus pneumoniae* and *Bacillus cereus*. Similarly, Bobade et al. (2013) reported antimicrobial activity of the ethyl acetate extract of *Lagerstroemia parviflora* leaf against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Propioni bacterium acnes* and *Salmonella typhi.*

Bobade et al. (2013) reported that DPPH free radical scavenging activity of *Lagerstroemia parviflora* methanolic leaf extract is weak in comparison to standard ascorbic acid showing the IC_{50} value for the *Lagerstroemia parviflora* methanolic leaf extract 3482 µg/mL, whereas IC_{50} of the standard ascorbic acid was 2.816 µg/mL.

Mazumder et al. (2005) investigated antipyretic effects of the methanol extract of *Lagerstroemia parviflora* leaves on yeast-induced pyrexia in rats with respect to the control group and reported that the antipyretic activity of the extract was comparable to the standard prototype, paracetamol.

Mazumder et al. (2004) reported significant antitussive activity of the methanol extract of the leaves of *Lagerstroemia parviflora* on a cough model induced by sulphur dioxide gas in mice in a dose-dependent manner. Mazumder et al. (2004) reported that the *Lagerstroemia parviflora* extract (at doses 100, 200, 300 mg/kg) showed maximum inhibition of cough reflex at 90 minutes after drug administration, and the antitussive activity was comparable to that of codeine phosphate, a standard antitussive agent and showed that the result support the claims by the traditional medicine practitioners about the usefulness of the leaves of *Lagerstroemia parviflora* in the treatment of cough.

2.2.9 *Rhododendron arboreum* Sm.

English name: Tree rhododendron; Nepali name: Laligurans; Sanskrit name: Pullasa; Magar name: Pataksar, Rogan (Annex IE Plate 2)

Ethnomedicinal values: Limbus of eastern Nepal use flowers to relieve sore throat (Limbu & Rai, 2013). Flower juice is given to treat bloody dysentery diarrhea and menstrual disorder (Manandhar, 2002). Flowers are chewed in case of fish bone stuck in throat (Manandhar, 2002). Flowers are eaten as appetizer by Sherpas of Helambu district (Bhattarai, 1989). Bark juice is taken in case of diarrhea and dysentery (Manandhar, 2002).

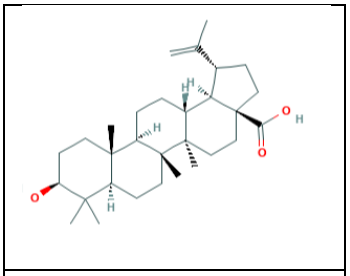
In Homeopathic *Materia Medica*, the tincture of dried leaves has been used in gout and rheumatism (Srivastava, 2012). Young leaves are made into a paste and then applied to the forehead in the treatment of headache and high fever (Bhattacharya, 2011; Manandhar, 2002). The juice of the leaves is spread over cots and beds to get rid of bed lice (Manandhar, 2002). Dried flowers are taken as herbal tea in Rasuwa district (Shrestha, 2014). Leaf paste is applied on forehead for the treatment of headache (Shrestha & Shresth, 2000). Plants are used for various diseases and also detected in the Langtang National Park by using Geographic Information System and

Remote Sensing (Shrestha, 2016). Flowers are eaten as pickled in Rasuwa District (Shrestha & Shrestha, 2004).

The juice of the bark is used in the treatment of cough, diarrhea and dysentery (Manandhar, 2002). In Ayurveda, it is used in jaundice, diabetes, piles, splenomegaly (enlargement of spleen), liver disorder, worms and skin diseases. Ayurvedic preparation, such as Rohitakyadi churna, is prepared from *Rhododendron arboreum*. An extract of the flowers and barks is used as an ingredient in commercial cosmetic preparations as a skin conditioner. Natural edible dye (brown color) is extracted from the flower (Shrestha, 2012)

Phytochemicals: Flavonoids, phenols, saponins, steroids, tannin, alkaloids were reported in the flowers of *Rhododendron arboreum* (Kiruba et al., 2011; Sonar et al., 2012; Kashyap & Ananda, 2016; Saranya & Ravi, 2017). Alkaloids, steroids, terpenoids, tannins, saponins and reducing sugar were reported in bark of *Rhododenddron arboreum* (Nisar et al., 2011; Srivastava, 2012; Kumar et al., 2019b). Kashyap and Ananda (2016) reported high amount of total phenol (65.50±1.12 mg GAE/g) and flavonoid (33.25±0.89 mg rutin equivalent/g), and low amount of alkaloids (0.03±0.002 mg/g) and saponins (0.11±0.009 mg/g) in the ethanolic extract of flower. Kumar et al. (2019b) reported that flavonoids, anthraquinones, phlobatanins and glycosides were absent in bark. Steroids, terpenoids, flavonoids, tannins, phenols, alkaloids, carbohydrate, glycoside, sterols were reported on phytochemical screening of the leaves extract (Nisar et al., 2011; Painuli et al., 2018; Rawat et al., 2018). Alkaloids, steroids, terpenoids, anthraquinones, tannins, glycoside saponins and reducing sugar were reported in stem extract (Nisar et al., 2011; Kumar et al., 2019). Alkaloids, steroids, terpenoids, anthraqunones, tanins and glycosides were reported from the root extract (Nisar et al., 2011).

Biologically active phenolic compounds i.e. quercetin, rutin and coumaric acid have been isolated and reported in flowers of *Rhododendron arboreum* (Sonar et al., 2012; Srivastava, 2012). Kumar et al. (2019b) reported bark is found to be a rich source of taraxerol (triterpenoid), betulinic acid and ursolic acid (Srivastava, 2012). Betulinic acid has anti-retroviral, antimalaria, anti-inflammatory and anticancer properties.



Betulinic acid

Pharmacological activities: Nisar et al. (2013) investigated antibacterial activity of the methanolic extract of flowers, leaves, barks, stems and roots against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella typhi*, and reported that all parts showed significant to low antibacterial activity against the bacteria strains. Flower extract showed significant to low antimicrobial activity in order as *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi* and *Staphylococcus aureus.* Leaf extract showed significant to low antimicrobial activity in order as *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*. Bark extract showed significant to low antimicrobial activity in order as *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. Stem extract showed significant to low antimicrobial activity in order as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhi*. Root extract showed significant to low antimicrobial activity in order as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi.*

Nisar et al. (2013) investigated fungicidal activity of various extracts of *Rhododendron arboreum* bark as well as the isolated compounds. Nisar et al. (2013) reported that the methanolic and the ethyl acetate extracts produce stronger fungicidal effect than the chloroform extract. Hexane extract fail to produce outstanding fungicidal effect. Nisar et al. (2013) concluded that the antifungal activity of the bark extracts may be due to the presence of 3β-acetoxyurs-11,12-epoxy-13β,28-olide, betulin, lupeol and taraxerol compounds.

Nisar et al. (2013) also investigated the cytotoxicity of the crude methanolic extracts of various parts of the plans against *Artemia salina* at 1000, 100 and 10 µg/mL. All parts showed significant activity at concentration of 1000 µg/mL, good activity at 100 µg/mL and no activity at 10 µg/mL, against *Artemia salina.*

Sonar et al. (2012) investigated the anti-microbial activity of ethanolic and aqueous extract of flower against bacteria such as *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Psedomonas aeruginosa* and fungi such as *Agrobacterium tumifaciens*, *Candida albicans* and *Aspergillus niger.* Quercetin was isolated from the ethanolic fraction and was evaluated for antimicrobial activity against the bacteria and the fungi. Sonar et al. (2012) reported that ethanolic extract, aqueous extract and quercetin showed antimicrobial activities against *Staphylococcus aureus* and *Escherichia coli* and no activity against *Bacillus subtilis*, *Pseudomonas aerugenosa*, *Agrobacterium tumifaciens*, *Candida albicans* and *Aspergillus niger.*

Kashyap et al. (2017) analyzed antimicrobial activity of the ethanolic extract of flowers against *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica* and *Shigella flexineri* by agar well diffusion method, and reported the antimicrobial activity against all the tested bacterial strains. Kashyap et al. (2017) also reported that the ethanolic extract of *Rhododendron arboreum* flowers showed maximum ZOI against *Escherichia coli* (17 mm) at extract concentration 50 mg/mL.

Saranya and Ravi (2017) reported that the methanolic extract of flower showed larger ZOI than aqueous extract against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus pyogene*, and showed maximum ZOI against *Staphylococcus aureus* (14 mm), whereas ZOIs against *Escherichia coli* and *Klebsiella pneumoniae* were nearly equal (about 12 mm).

Kashyap et al. (2017) investigated antioxidant activity and reported that the ethanolic extract of the flower extract showed good DPPH free radical scavenging activity (134.1±2.34 mM trolox equivalent/g).

Leaves and bark also showed antioxidant activities (Painuli et al., 2018; Kumar et al., 2019b). Painuli et al. (2018) investigated antioxidant activity of the aqueous and methanolic extracts of leaves and reported that the methanolic extract showed highest radical scavenging activity (91.67%) and also showed high total phenol content (102.7±0.017 mg GAE/g) and total flavonoid content (651.02±1.42 mg rutin equivalent/g).

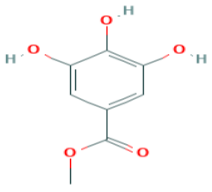
2.2.10 *Rhus chinensis* Mill.

English name: Chinese sumac, Chinese gall, Nutgall tree; Nepali name: Bhaki amilo, Chuk Amilo, Bhangil; Magar name: Muruk, Bhatcaulo. (Annex IE Plate 2)

Ethnomedicinal values: In Chitwan district, fruit powder is given to treat amoebic dysentery (Manandhar, 1990b). Ripe fruit is considered as an appetizer (Manandhar, 2002). Fruit power is used to treat dysentery at Chitwan district (Manandhar, 1990b), Sankhuwasabha district (Parajuli, 2000); and to treat menstruation problem at Lamjung district (Manadhar, 1987a). Decoction of fruit is drunk to cure dysentery and stomach complaint at Manang district (Manadhar, 1987b). Ripe fruits are eaten at Manang (Manadhar, 1987b) as well as other districts of Nepal as appetizer or pickle.

Rhus chinensis has been used by folk medicine practitioners for a long time in Asia (Djakpo & Yao, 2010). Fruits are used in stomachache, profuse bleeding in menstruation, bloody dysentery, diarrhea, gastrointestinal disorder, foot and mouth diseases of animals (Parajuli, 2000; Manandhar, 2002). Roots have been used in folk medicines as antitussive, and for the treatments of anasarca, jaundice and snake bite (Lee et al., 2005). Gallarhois on the leaf of *Rhus chinensis* has been used for treating diarrhea, seminal emission, excessive sweating, bleeding, chronic cough and polyuria; and possesses anti-thrombotic and anti-anaphylactic effects (Djakpo & You, 2010).

Phytochemicals: Phytochemicals such as flavonoids, triterpenoids, phenolics, tannins, aromatic alkanes are reported (Djakpo & Yao, 2010) in different parts of the plant. Isolation of gallic acid, gallicin, betulin, betulonic acid, moronic acid, rhuscholide A, benzofuranones, phenolics, etc. has been reported from different parts (root, stem, gallarhois) of *Rhus chinensis* (Lee et al., 2005; Wang et al., 2008). Gallic acid and methyl gallate observed as major antimicrobial agents and have been isolated from the methanolic extract of *Rhus chinensis* (Djakpo & Yao, 2010).



Methyl gallate

Pharmacological activities: Antibacterial activity of *Rhus chinensis* against methicillin-resistant *Staphylococcus aureus* (You et al., 2013), *Shigella* spp and *Streptococcus iniae* (Choi et al., 2005) was reported. *Rhus chinensis* extract was identified as the most effective anti-inflammatory and anti-photoaging agent (Ha et al., 2016). Zhang et al. (2018) have reported that fruits of *Rhus chinensis* are enriched with phenolics and the extracts have exhibited strong antioxidant and pancreatic lipase inhibitory activities. Antioxidant activity of aqueous, acetone, ethanol and methanol extract of fruit of *Rhus chinensis* was reported (Sharma et al., 2015; Heirangkhongjam & Ngaseppam, 2019).

2.2.11 *Stephania glandulifera* Miers

English name: Elephant dropping plant; Nepali name: Gurjogano; Magar name: Kokardel. (Annex IE Plate 2)

Ethnomedicinal values: In Chitwan district, root juice is eaten to treat peptic ulcer (Manandhar, 1990). Root powder is given in stomach problem at Jumla district (Manandhar, 1986a). A decoction of about 5 g of the root is prescribed twice a day for 2-5 days as a febrifuge. It is also considered effective to cure dysentery and gastritis at Karnali district (Bhattarai, 1992b). Tuberous root are given to cattle as nutritious diet as well as to treat diarrhea (Manandhar, 2002). Root juice is given to treat peptic ulcer (Manandhar, 1990b), gastritis (Dangol & Gurung, 2000).

The plants traditionally have been used for the treatment of asthma, tuberculosis, diarrhea, dysentery, urinary diseases, dyspepsia, hyperglycemia, cancer, fever, intestinal complaints, sleep disturbances and inflammation (Gaur, 1999).

Phytochemicals: The reported data showed that alkaloids are the main and common phytochemicals of the genus *Stephania*. More than 200 alkaloids have been isolated from this genus together with flavonoids, lignans, steroids, terpenoids and coumarins (Semwal et al., 2010).

Alkaloid, glabradine isolated from tuber of *Stephania glabra* (Roxb.) Miers., showed antimicrobial activity against *Staphylococcus aureus*, *Streptococcus mutans*, *Microspporum gypseum*, *Microsporum canis* and *Trichophyton rubrum* showing potent antimicrobial activity superior to novobiocin and erythromycin (Semwal & Rawat, 2009).

Adhikary et al. (2011) reported that the rhizome of *Stephania glandulifera* was used in Dhunkharka village of Kavrepalanchowk district for the post-natal abdominal pain as well as stomach disorders. Adhikary et al. (2011) investigated phytochemicals present and antimicrobial properties of the methanolic extract of rhizomes of *Stephania glandulifera*, and reported presence of alkaloids, tannins, flavonoids, coumarins and glycosides, and showed antibacterial activity against *Escherichia coli* only but not against *Klebsiella sp*. and *Serratia sp.*

Stephania spp. such as, *S. japonica, S. glabra, S. succifera* showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* (Hullatti and Sharada, 2007; Semwal et al., 2010). The ethanolic and aqueous extracts of corms of *Stephania hernandifolia* (Willd.) Walp. strongly scavenged DPPH free radicals with IC_{50} values of 265.33 and 217.90 µg/mL, respectively (Semwal et al., 2010; Sharma et al., 2010).

Along with antimicrobial and antioxidant activities of different species of *Stephania* have been found as antimalarial, anthelmintic, antiviral, anticancer, anti-inflammatory, analgesic and anti-hyperglycemic (Semwal et al., 2010).

2.2.12 *Tectaria coadunata* (J.Sm.) C. Chr.

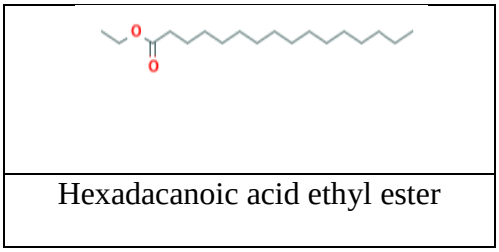
Nepali name: Kali nyuro; Magar name: Kali nyuro. (Annex IE Plate 3)

Ethnomedicinal values: Limbus of eastern use extracts from the rhizome of kaalo unyu (*Tectaria coadunata*) in dental problem (toothache) (Limbu & Rai, 2013). Decoction of rhizome is given in bloody dysentery by the Chepang of Makawanpur district (Manandhar, 1989) and diarrhea in Gorkha district (Manandhar, 1990c). Paste of rhizome is applied on sore throat and headache at Gorkha district (Manandhar, 1990c). Root juice is given in case of stomach pain and giardiasis in Macchegaun, Kathmandu district (Joshi et al., 2011). Tender parts are cooked as green vegetable (Manandhar, 2002).

The fresh rhizome and frond of *Tectaria coadunata* are used on insect bites, while the extraction of dried rhizome, stem and leaves is used for cold, cough, asthma, bronchitis, stomachache and colitis (Joshi et al., 2011; Malviya et al., 2012). In Terai regions of Nepal and Madhya Pradesh in India, some species of *Tectaria* are used as green vegetables (Sukumaran et al., 2012).

Phytochemicals: Pawar et al. (2016) reported presence of tannins, caumarins and anthraquinones in the extract of rhizome of *Tectaria coadunata.* Dubal et al. (2013) reported octadec-9-enoic acid, palmetic acid, stearic acid, hexadecanoic acid ethyl ester in *Tectaria coadunata*. Hexadecanoic acid ethyl ester exhibits antioxidant activity (Alagammal et al., 2011).

Alkaloids are absent in all solvent extraction (Sukumaran et al., 2012; Poudyali, 2013) or present at very less amount. Alkaloids are absent or infrequently found in pteridophytic plants or ferns (Harborne, 2007).



Hexadacanoic acid ethyl ester

Pharmacological activity: The methanolic, ethanolic and aqueous extracts of *Tectaria coadunata* exhibited potential antimicrobial activity against different test microorganisms associated with diarrhea, gastroenteritis (*Escherichia coli*), pneumonia (*Klebsiella pneumoniae),* food borne diseases (*Bacillus cereus*) and general infectious diseases (*Staphylococcus aureus* and *Proteus vulgaris*) (Poudyali & Singh, 2019). The increase in antibacterial efficiency observed with raising the concentration of crude tannins. Several plants that are rich in tannins have exhibited antibacterial activity against several microorganisms (Banso et al., 2007). The methanol and acetone extracts contained tannins and showed the antibacterial effect against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* (Parihar et al., 2010).

Upadhayay (2014) evaluated the antioxidant activity of crude extracted phytochenicals from *Tectaria coadunata* by DPPH free radical scavenging method. Upadhyay (2014) reported 58.23 and 54% DPPH free radical inhibition by crude flavonoid and crude tannin respectively at 100 µg/mL, whereas control (ascorbic acid) inhibited 94% at 100 µg/mL. Upadhyay (2014) also reported that crude saponin and steroid exhibited 50% inhibition but crude alkaloid could not show 50% inhibition event at high concentration. Extract of rhizome of *Tectaria coadunata* showed concentration dependent free radical scavenging activity equivalent to that of the

positive control, ascorbic acid in the respective models (Ghoghari et al., 2006). The total phenolic content in the methanolic extract of rhizome was reported to be 146.4 mg GAE/g dry extract and IC_{50} value was 30 µg/mL with 53.26±0.33% inhibition of DPPH radical (Poudyali & Singh, 2019).

2.2.13 *Thalictrum foliolosum* DC

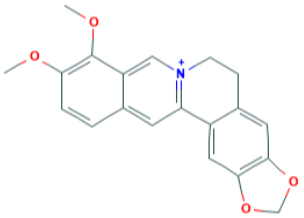
English name: Leafy Meadow-Rue; Nepali name: Bajaraa, Dampatee, Mamiraa; Magar name: Bajuri (Annex IE Plate 3)

Ethnobotanical values: In Chitwan, root juice is given to treat indigestion (Manandhar, 1990b). Root paste is applied to treat itching on skin (Manadhar, 1987a). Root juice of *Thalictrum foliolosum* is given to cure peptic ulcer by the Tamang at Kabhrepalanchok district (Manadhar, 1991). It is given in the case of indigestion at Chitwan district (Manandhar, 1990b). Root and leaf paste is given as anthelmintic by Sherpas of Helambu (Bhattarai, 1989). Root of *Thalictrum foliolosum* is used traditionally as tonic, diuretic, febrifuge, purgative and stomachic (Gangwar et al., 2010; Pandey et al., 2017). Chen et al. (2003) reported that its roots were used to treat virus hepatitis, dysentery, congestion of eyes, heat-type malnutrition of children, chickenpox and inadequate measles eruption in China. The root juice is taken for jaundice by Tamang people in Langtang valley (Shrestha & Shrestha, 2000).

The preliminary antimicrobial activity of leaf, root and rhizome of *Thalictrum foliolosum* was reported (Khera et al., 2012; Joshi & Sati, 2017). Khera et al. (2012) reported antimicrobial activity of the root and stem extracts, using chloroform and methanol as solvent, and also showed that those extracts were better antifungal.

Phytochemicals: *Thalictrum* species have been known to contain a variety of alkaloids of chemical and biological interest (Chattopadhyay et al., 1981; Bhakuni & Singh, 1982). Alkaloids such thalicarpine, thalidasine, thalrugosidine, reticuline, magnoflorine, berberine and palmatine were isolated from root of *Thalictrum foliolosum* (Bhakuni & Singh, 1982). Berberine has therapeutic potential such as antileukaemia, antihepatoma, cardioprotective, anticancer, anti-parasitic, antifungal and antidiarrheal (Pandey et al., 2017). Chen et al. (2003) reported the presence of alkaloids, aporphines, protoberberines, bisbenzylisoquinoline dimers and aporphine-benzylisoquinoline dimers in *Thalictrum foliolosum*.

Pandey et al. (2017) reported that in root of *Thalictrum foliolosum*, berberine content varied inversely with altitude, while phenol and flavonoid contents increased at higher altitudes. They concluded that *Thalictrum foliolosum* from lower elevations can be explored as an alternative source of berberine and the plant has high antioxidant and antimicrobial properties owing to its berberin content. Pandey et al. (2017) also showed direct correlation between the total phenol content and DPPH free radical scavenging activity of the samples and reported that *Thalictrum foliolosum* from higher altitude scavenged free radicals more efficiently than samples from a lower altitude. Pandey et al. (2017) reported moderate to high antimicrobial activities of *Thalictrum foliolosum* against *Candida albicans, Staphylococcus aureus, Escherichia coli* and *Pseudomona aeruginosa*, and showed that extracts with high berberine content were most effective against *Candida albicans* and *Staphylococcus aureus*, and phenol content were effective against *Escherichia coli* and *Pseudomonas aeruginosa.*



Berberine

Pharmacological activity: Joshi and Sati (2017) investigated antibacterial activity of methanol, chloroform, hexane and aqueous extracts of leaves of *Thalictrum foliolosum* collected from Nainital, Kumaun Himalaya. The extracts were tested against five pathogenic bacteria (*Agrobacterium tumefaciens, Bacillus subtilis, Erwinia chrysanthemi*, *Escherichia coli* and *Xanthomonas phaseoli*) using disc-diffusion method with standard antibiotic gentamicin as positive control, and reported that methanol and hexane extracts showed highest activity against all the pathogens tested followed by the chloroform extract, whereas, the aqueous extract had almost no inhibitory effect on the tested organisms (Joshi and Sati, 2017).

Kumar et al. (2016a) isolated two new alkaloids such as thalifendine and berberine from stem of *Thallictrum foliolosum*. These compounds were tested for inhibitory activity against DNA topoisomerase IB of *Leishmania donovani* and found that

compound exhibited almost complete inhibition of the enzyme activity at 50 μM concentrations and it was found to be effective in killing wild type of parasite (Kumar et al., 2016a).

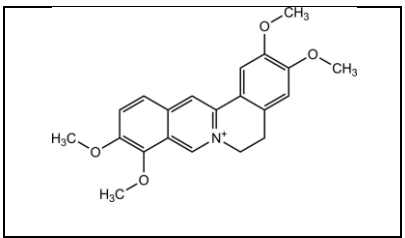
2.2.14 *Tinospora sinensis* (Lour.) Merr.

English name: Chinese tinospora, Malabar gulbel; Sanskrit name: Amrta, Vatsadani; Nepali name: Gurjo-ko-laharaa; Magar name: Chini lahara. (Annex IE Plate 3)

Ethnomedicinal values: Stem is used for stomach problem (Manandhar, 2002; Joshi et al., 2011). Leaves are used by the Darai tribe of Chitwan district for stomach problem (Dangol & Gurung, 2000). Leaves, roots and shoots are used by the Tharus of Nawalparasi district for urinary complaints (Ghimire & Bastakoti, 2009).

Tinospora sinensis has been used in Ayurvedic and Homeopathic medicines, particularly in jaundice, fever, rheumatism, gonorrhea, diabetes and many more as an alternative drug for *Tinospora cordifolia* (Willd.) (Hegde & Jayaraj, 2016). Traditionally, the stem is used to treat debility, dyspepsia, fever, inflammation, ulcer, jaundice, urinary disease, liver disease (Akhar et al., 2000; Devi et al., 2014).

Phytochemicals: Preliminary phytochemical screening of various extracts of leaves and stem of *Tinospora sinensis* revealed the presence of alkaloids, phenolic compounds, tannins, flavonoids, carbohydrates, glycosides, saponins, steroids, mucilage and oxalic acid (Jain et al., 2010; Vijaya & Aruna, 2014). The only alkaloid detected in stem of *Tinospora sinensis* is palmatine (Bisset & Nwaiwu, 1983). Palmatine has broad anti-cancer activity, antioxidant activity and antimicrobial activity, stronger on Gram-positive than Gram-negative bacteria (Long et al., 2019).



Palmatine

Pharmacological activity: Devi et al. (2014) evaluated antimicrobial activity of ethanolic, methanolic, aqueous and chloroform extracts of the leaves, stems and flowers against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*,

Candida albicans, *Aspergillus niger* and *Aspergillus fumigates* by agar well diffusion method. Ethanolic leaf extract had shown antimicrobial activity against *Candida albican, Pseudomonas aeruginosa* (with ZOIs 14 and 13 mm). Ethanolic stem extract had shown antimicrobial activity against *Staphylococcus aureus.* Maximum antifungal activity against *Candida albicans* was exhibited by methanolic flower extract followed by methanolic leaf extract. Aqueous leaves, stems and flowers extracts were not effective against any bacterial and fungal strains. They have concluded that antimicrobial activity depends upon extraction procedure, type of plant parts used, solvents used for extraction and bacterial strain. Aqueous and ethanolic extracts of *Tinospora sinensis* with several concentrations ranging from 50-150 µg/mL were subjected to *in vitro* antioxidant activity screening and reported to be antioxidant (Jain et al., 2010; Hegde & Jayaraj, 2016).

Jain et al. (2010) evaluated *in vitro* antioxidant activity of the aqueous and 95% ethanolic extract of *Tinospora sinensis* stems by DPPH free radical scavenging method using ascorbic acid as standard, and IC_{50} values reported for the aqueous, ethanolic and standard were 100.46±0.36, 94.66±0.049 and 91.53±0.31 µg/mL, respectively.

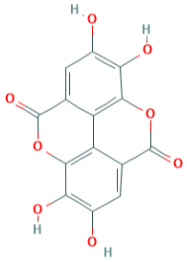
2.2.15 *Woodfordia fruticosa* (L.) Kurz

English name: Fire flame bush; Nepali name: Dhanyaro, Dhataki, Amar phool, Dhaairo; Sanskrit name: Dhaataki, Dhatupushpi; Magar name: Dhainra, Jharek, Chhebok. (Annex IE Plate 3)

Ethnomedicinal values: Flower is used for diarrhea, dysentery and headache (Manandhar, 1990c; Rajbhandary & Ranjitkar, 2006). Decoction of bark is applied on sprain and swelling (Manandhar, 2002). Fruit juice is used to treat urinary trouble at Dhading district (Manandhar, 1992). *Woodfordia fruticosa* has been used traditionally for treatment of diarrhea, dysentery, fever, hemorrhoids, herpes, leprosy, burning sensation, skin diseases, internal hemorrhage, impaired hepatic function and leucorrhea (Das et al., 2007; Kumar et al., 2016). Orange color is extracted from the flowers used for natural dye (Shrestha, 2012).

Phytochemicals: The phytochemicals identified are predominantly phenolics, particularly hydrolysable tannins and flavonoids (Das et al., 2007; Grover and patni

2013). The flavonoids include quercetin glycosides, myricetin glycosides, naringenin 7-glucoside, kaempferol 3-O-glucoside, etc. in flowers and leaves (Kumar et al., 2016). Ellagic acid present in the plant material exhibits antioxidant, anticancer and anti-mutagenesis activities.



Ellagic acid.

Examination of the flowers by Nair et al. (1976) for the orange-red pigment led to the identification of pelargonidin 3,5-diglucoside and anthocyanidin pigment cyanidin 3,5-diglucoside. Various hydrolysable tannins isolated from the flowers by the Yoshida's group include 1,2,3,6-tetra-O-galloyl-β-D-glucose, 1,2,4,6-tetra-O-galloyl-β-D-glucose, 1,2,3,4,6-penta-O-galloyl-β-D-glucose, tellimagrandin, gemin D, heterophylliin A, oenothein B, woodfordins A-D, oenothein A, isoschimawalin A and woodfordins E-I (Kumar et al., 2016b).

Pharmacognosy: Kumar et al. (2013) showed antimicrobial activity of the ethanolic and methanolic extracts of leaves against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Salmonella typhimorium*, *Sigella sonne*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Proteus vulgaris.*

Chougale et al. (2009) investigated antimicrobial activities of petroleum ether, chloroform, diethyl ether and acetone extracts of the leaves and reported that the extracts were found to be effective against *Escherichia coli*, *Bacillus subtilis*, *Staphyloccus aureus* and *Pseudomonas aeruginosa*, and also reported that acetone extract exhibits maximum inhibition compare to other extracts.

Dubey et al. (2014) investigated antimicrobial activity of seven solvent fractions (n-hexane, chloroform, ethyl acetate, dichloromethane, acetone, butanol and methanol) of the leaves against pathogenic bacteria and reported that the butanol leaf fraction showed maximum zone of inhibition against methicillin-resistant *Staphylococcus*

aureus followed by *Citrobacter freundii* and the n-hexane fraction showed very low antibacterial activity compared with the other solvent fractions.

Parekh and Chanda (2007) evaluated the antibacterial activity of the crude methanolic extract of flower by the agar well diffusion method. The methanolic extract of the flower exhibited antibacterial activity at varied levels except against *Bacillus subtilis* and *Micrococcus flavus*. The methanolic extract was active against *Staphylococcus aureus, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa.* The extract was more active against Gram-negative bacteria as compared to Gram-positive.

Grover et al. (2014) investigated antimicrobial activity of the methanol extract of flowers and leaves against *Staphylococcus aureus*, *Micromonospora* sp., *Staphylococcus epidermis, Zymomonas mobilis* and against fungi such as *Alternaria solani* and *Fusarium culmorum,* and reported that flower part showed stronger antimicrobial potentiality as compared to the leaf part. Grover et al. (2014) reported that maximum ZOI was shown by the flower extracts against *S. aureus* (22.4±0.86 mm) amongst the bacteria species, and against *Alternaria solani* (23.5±0.15 mm) amongst the fungal species. Choi et al. (2010) reported *in vitro* anti-enterovirus activity of gallic acid from *Woodfordia fruticosa* flower.

Grover et al. (2014) investigated antioxidant activity of the methanol extracts of the flowers and leaves using DPPH scavenging. Grover et al. (2014) reported that flowers extracts has got profound and significant percent (%) of DPPH scavenging activity (93.48±0.26) at 200 μg/mL concentration followed by leaves (84.17±0.06) at the same concentration.

Khera and Bhatia (2012) investigated the potential role of the methanolic extracts of flower in lowering lipid parameters in mice fed a high cholesterol diet and reported that *Woodfordia fruticosa* serves as a new potential natural product for preventing hyperlipidemia.

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Study Area

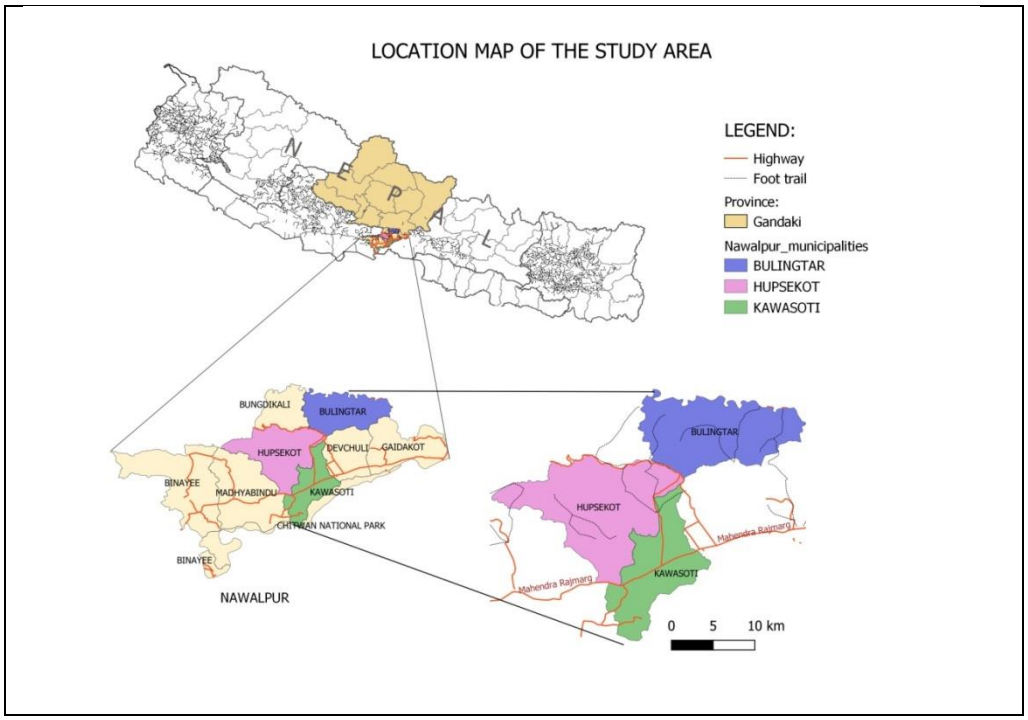
3.1.1 Location

The study area covers Bulingtar, Hupsekot and Kawasoti municipalities at Nawalpur district, Gandaki province (Figure 4). The area is located in the Kerunge and Girubari rivers' watershed. The tentative coordinates of the study area range from 27˚33'17.97" N to 27˚51'7.83" N latitude and 83˚55'43.32"E to 84˚18'14.10"E longitude. The coordinate of Bulingtar range from 27°45'11.09"N to 27°51'7.83"N latitude and 84° 6'19.78"E to 84°18'14.10"E longitude; Hupsekot range from 27°37'31.72"N to 27°46'44.49"N latitude and 83°55'43.32"E to 84° 9'32.02"E longitude; Kawasoti range from 27°37'31.72"N to 27°46'44.49"N latitude and 84°3'49.21"E to 84°11'27.35"E longitude. The altitude ranges from 150 to1900 meters above sea level (masl). Bulingtar rural municipality is bounded by Rhising and Devghat rural municipalities of Gandaki province, Tanahu district at North; Gaidakot municipality at east; Kawasoti municipality and Hupsekot rural municipality at south and Bungdikali rural municipality at west. Hupsekot rural municipality is bounded by Bungdikali and Bulingtar rural municipalities at North; Nisidi rural municipality of Lumbini province, palpa district at west: Devchuli municipality at east; Madhya Bindu and Kawasoti municipality at south; Kawasoti Urban municipality is bounded by Hupsekot rural municipality at North; Devchuli municipality at North-East; Madhya Bindu municipality at west and Bharatpur Metropolitan city, Bagmati province, Chitawan district at south.

Hilly villages of Bulingtar, Hupsekot rural municipalities are homogenously resided by Magars without modern facilitate hospital. They have to walk for six hours or drive for three hours by jeep on hilly gravel road to visit city hospital. They believe on local healers and also there is availability of herbal plants. Focusing on Magar community, hilly villages in these rural areas are selected. And also to know how the indigenous knowledge about use of medicinal plants by Magars is influenced by other community. The Magars of Kawasoti are mixed with other community, so it is

selected. And Kawasoti is located in between these rural municipalities and entry point to reach the hilly villages.

Kawasoti urban municipality is resided by Magars mix with other castes equally. There are facilities of hospitals and private clinics. But most of them believe in traditional herbal treatment. There is altitudinal variation range from 150 masl to 1900 masl between city area and the hilly villages.



Source: Survey department of Nepal

Figure 4: Location Map of the Study area, Nawalpur district, Gandaki province.

3.1.2 Climate

At an average temperature of 28.7 °C during May is the hottest month of the year. January has the lowest average temperature of the year i.e. 15.2 °C. Between the driest and wettest months, the difference in precipitation is 476 mm. During the year, the average temperatures vary by 13.5 °C. The climate/weather graph by month of Nawalpur district is shown in Figure 5. (https://en.climate-data.org/asia/nepal/central-development-region/nawalpur-1025376/#climate-table).

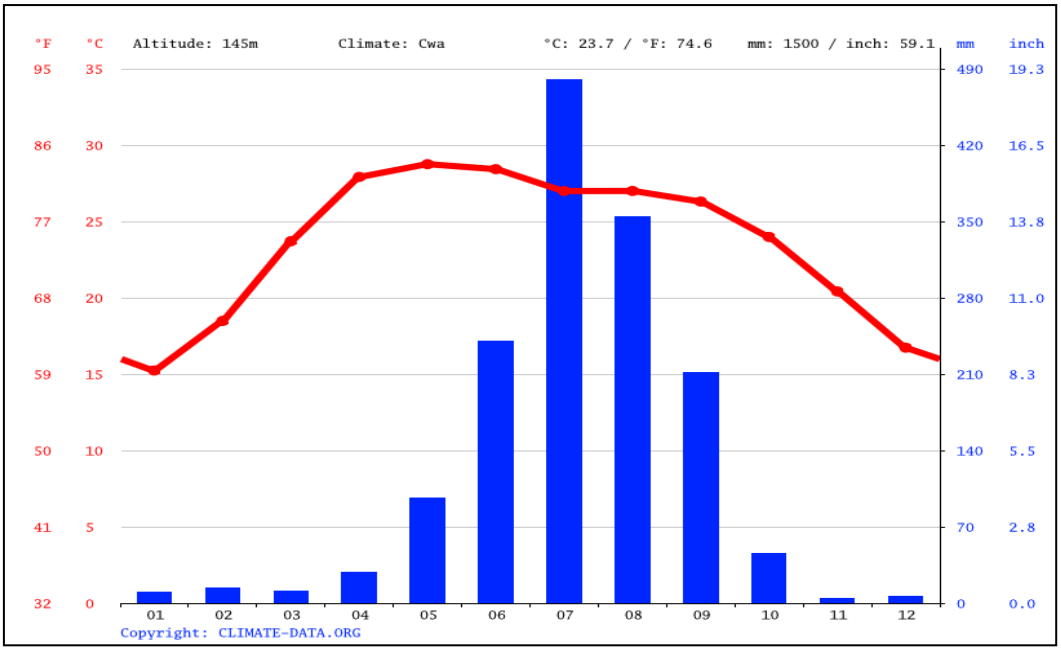


Figure 5: Climate/weather graph by month of Nawalpur.

3.1.3 Vegetation

Sal forest: Luxuriant sal trees in Dun valleys present only as some patches. Sal forest was destroyed by settlement. Patches of Sal forest are remained also at hilly region.

Schima-Castanopsis forest: Stretched from Chure and lower region of Mahabharat mountains. *Schima wallichii* and *Castanopsis indica* are scattered with *Ficus auriculata, Ficus semicordata, Maesa chisia. Anthocephalus chinensis* (kadam) forests are there on upper Chure region. *Rhododendron arboreum* forest present at high hills. *Lagerstroemia parviflora* trees are found mixed with *Madhuca butyracea, Spondias pinnata, Aegle mermelos, Terminalia chebula, Terminalia bellirica* and *Murraya koenigii*. The forests were destroyed extremely for cultivation.

3.1.4 Livelihood

The major occupation in the study sites is agriculture. In hill areas, main agricultural products are *Eleusine indica* (millet), *Zea mays* (maize), *Zingiber officinale* (zinger), *Curcuma longa* (turmeric), *Thysanolaena latifolia* (amriso), etc. In plain areas, the products are *Oryza sativa* (rice), *Triticum vulgare* (wheat), *Zea mays* (maize) , *Eleusine indica* (millet), *Colocasia esculenta* (cocoyam), *Lens culinaris*, *Glycine max* (soya bean), *Vigna mungo* (black gram), *Pisum sativum* (pea), etc., but it is up to subsistence level only. At hillside, production of agriculture does not support even for

six months. Besides agriculture, animal husbandry, small shop operating, labor work at market place or migrate to India as labor are common.

The main sources of energy for cooking are firewood, liquefied petroleum gas (LPG) and bio-gas. There is subsidy provided by the Government of Nepal to install bio-gas plants. Few percentages of households use kerosene and cow dung as cooking fuel. Electricity is used for lightening of houses. Socio-economic factors play an important role in the use of different energy sources.

3.1.5 Caste, Ethnicity and Population

Bulingtar rural municipality: Total population in this rural municipality is 19,122 and 72.31% of them are Magars. The other caste/ethnicity inhabiting in this municipality are Brahman-Hill, Tamang, Newar, Kami, Damai/Dholi, Sanyasi/Dashnami Sarki, Gharti/Bhujel, Kumal, Darai and others.

Hupsekot rural municipallity: Total population is 25,065 in this municipality. Among them 63.82% is Magars. Other castes/ethnicities are Chhetri, Brahman-Hill, Tamang, Newar, Muslim, Kami, Yadav, Gurung, Damai/Dholi, Thakuri, Sarki, Sanyasi/Dashnami, Gharti/Bhujel, Kumal, Darai and Badi.

Kawasoti urban municipality: Total population is 62,421. Among them 12.28% population is Magars. Other castes and ethnicities are Chhetri, Brahman-Hill, Tharu, Tamang, Newar, Kami, Yadav, Rai, Gurung, Damai, Thakuri, Sarki, Teli, Sanyasi/Dashnami, Brahman-Tarai, Kathbaniyan, Gharti/Bhujel, Mallaha, Kalwar, Hajam/Thakur, Kanu, Majhi, Rajput, Braee, Bin and Badi, Musahar, Nuniya, Kumal, Lohar, Bote and Darai.

Population distribution is higher in Kawasoti municipality in the study sites of Nawalpur district. It is followed by Hupsekot municipality and then Bulingtar municipality (Figure 6). Bulingtar municipality and some villages of Hopsekot municipality are located on mountainous region, household distribution as well as population is low compared to the Kawasoti municipality.

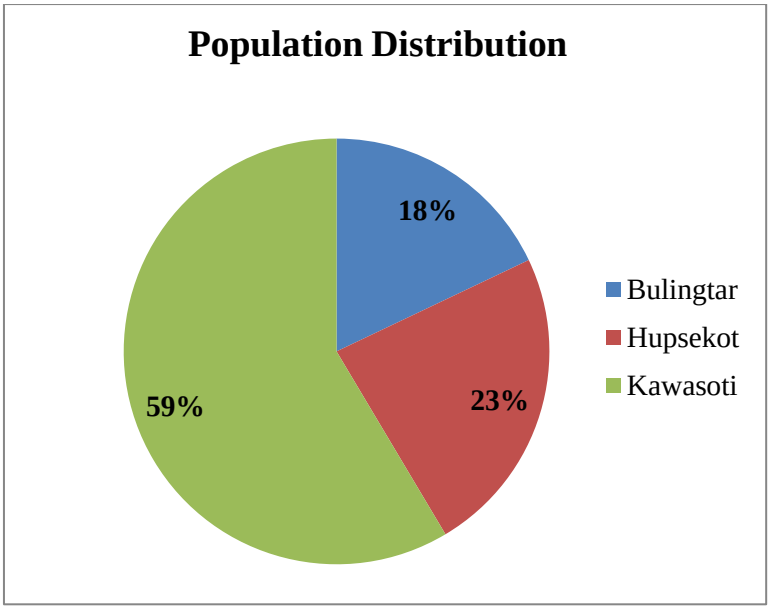


Figure 6: Population distribution in study areas.

3.1.6 Cultural Aspect

The major festivals of the Magar community are Chandi Purnima, Mangsir Purnima, Jestha Purnima, Bhume Puja (worshipping the nature). There are arrangements of informal cultural institution known as Bheja for Chandi worship. In most of the Chandi places built in the forest near Magar village. Pigs and chickens are often sacrificed to Chandi.

Ghatu dance is popular among the Magars. The other Major dances and songs originated from Magar Community are Kauraha, Jhorra, Yanimaya, Sunimaya, Salaijo and many more.

Men wear kachhad or wrap-on-loincloth, a bhangra, a bhoto or a shirt of vest, and the usual Nepali topi (Nepali cap). Women wear the phariya (saree) or lunghi, chaubandhi cholo (Nepali special closed blouse) and the heavy patuka (waistband), and the mujetro (shawl-like garment on the head). The ornaments are the madwari (Special type of ear ring), bulaki (special ring on nose) and the phuli on the left nostril, the haari (silver coin necklace) and the pote (yellow and Green beads) with the tilhari (gold cylinder), jantar and kuntha.

They follow rituals such as rice feeding called Purbhadai and first haircut called Chheawar rituals.

There is Cross -cousin marriage system in Magar community. For a woman, her ideal marriage partner is her father's sister's son (FZD, for a man, his ideal marriage partner is his mother's brother's daughter (MBD). There are three types of marriage such as elopement, capture and arranged.

3.1.7 Healing System

People believe on local healers; so they first visit healer and go to market place to visit doctors only after facing complication on health. Sometimes the situation may be opposite. They visit doctor first for treatment of diseases. However, even after doing lots of laboratory tests, the disease cannot be diagnosed. Then they follow herbal treatment at their village place. Reciting "mantra" is a psychological treatment. This type of therapy is based on physical as well as psychological treatment. The villagers believe on the local familiar healers.

In Magars culture there is strong belief on shamanism. There must be one or more than one shamans in each society. In Magar language they are called 'Dhami' or 'Jhakri'. They become 'Dhami' spontaneously known as 'bhuiphute 'by their language or they become Dhami by learn. The most experienced healers who give drug for treatment of ailments are called as 'Dhami Lama' by the local people in the study area.

3.2 Methods of Data Collection

3.2.1 Field Visit

Study sites were visited on January 2015, March 2015, April 2016, May 2016 and April 2017. Initially, we talked with local people to recognize local traditional healers and people having knowledge on herbal formulations. On this basis, key informants were selected.

Necessary materials such as polythene bags, number tags, old newspapers, plant pressure, hard boards, strings, plant cutter, tool for digging roots, camera, field note book, marker, pencils were taken together during the field visits.

3.2.2 Tools and Technique of Data Collection

We use a multidisciplinary approach which includes preparation of botanical inventories; collection of plant specimens; structured, semi-structured and informal interviews; and participant observation. Informal talks with villagers provide a means for getting to know informants and generating important insights that can be compared with results from our structured techniques.

3.2.2.1 Prior Informed Consent

Prior Informed Consent (PIC) application, written in Nepali language, was submitted to local VDC officer before starting data collection process. At that time VDC offices were not at regular working stage (copy of the application is attached in AnnexIF). The PIC application form included information on details of activity and accessing the plant resources present at the study site seeking the consent from local body. PIC is approved in advance for the use of one's genetic resources and any associated traditional knowledge and information anticipated arising from the research work. Local and indigenous communities have rights over their knowledge and natural resources. Researchers have duty of sharing the benefits of the research, should acknowledge for using local resources and knowledge, and therefore, researches should always respect an established PIC process.

The approved PIC letters given by Deurali VDC (May 10th 2016, Nepali date: 2073-1-27), Kawasoti municipality (May 3rd 2016, Nepali date 2073-1-20) and Dhaubadi VDC (May 9th 2016, Nepali date: 2073-1-26; March 9th 2016, Nepali date: 2072-11-26) are attached in Annex IF.

3.2.2.2 Key Informants Selection and Interview

Informant selection is very important for ethno-botanical research, as people are constantly looked upon for knowledge and information. Only certain people know about herbal medicine in every culture. So, the informant selection must be purposive rather than random in this case. Local herbalists as well as local people having knowledge of herbal treatments (male and female) were selected from the recommendations of knowledgeable people. So, required ethno-medicinal data were collected from purposive sampling. Purposive sampling is an informant selection tool widely used in ethnobotany.

3.2.2.3 Primary Data Collection

In field survey, primary data were collected by (i) observation, (ii) interview, and (iii) questionnaires.

(i) *Observation*: Participant observation is valuable research tool in Ethnobotany. It is independent of respondents' willingness to respond, as happens in the interview or questionnaire methods. Participant observation is highly reliable and valuable technique to documenting plant use. Information were collected during direct participation about gathering of fruit, fuel, forest products; farming, collecting or storing; and using of medicinal plants. Some information was also noted in a notebook without asking the respondents but through direct observation. Some photographs were also taken at the time of direct observation, which would help in documentation of data.

(ii) *Interview*: We use purposive sampling for interview. Ethnomedicinal informations collected through interviewing selected key informents by semistructural method. Ethno-medicinal data were collected by "Specimens display" method. The interviews cover local and Magar names of the ethno-medicinal plants, the used parts of plants, form of drug preparation and uses for the ailments. The unstructural interviews with informants are useful in building initial rapport with informants, approaching sensitive issues, conducting in-depth studies of cultural aspect of plant use and developing formal guidelines for semistructured interviews or questionnaires.

(iii) *Questionnaires*: Social data are collected through structural interview present in questionnaires.

3.2.2.4 Participatory Rural Appraisal

Participatory Rural Appraisal (PRA) is an approach which encourages the community to share their opinions, ideas and experiences pertaining to the local problems, issues and needs. PRA approach is useful for the management and development of drinking water, local roads of villages and so on.

PRA tool is helpful for understanding local villagers ideas about local problems, issues and needs of their every aspect of lives. It helps also for rapport building among villagers. It helps on collection of their knowledge about medicinal knowledge.

3.2.2.5 Secondary Data Collection

Secondary data were collected through Central Bureau of Statistics (CBS, 2011) (for population data of the study area) Village Development Committee Office (village profile of Dhaubadi village). All the data and information have been stored in a computer in the Excel program. The data were ready to tackle the experimental design and statistical analysis. Then the data were transferred to Statistical Package for the Social Sciences (SPSS) program.

3.2.2.6 Field Notes

The interviews, observations and other notable event were noted in a field notebook and also photographs captured.

3.2.3 Plant Collection, Identification and Preservation

3.2.3.1 Plant Collection and Herbaria Preparation

The plant specimens were prepared following Bridson and Forman (Bridson & Forman, 1998). All the plant species were collected in the large polythene bags during transect walks with key informants through vegetation, keeping in mind the conservation of local genetic diversity (Martin, 1995). For herbarium purpose plants were collected in flowering, fruiting condition as far as possible. The collected plant specimens were tagged with collection numbers. The plant specimens are were pressed as soon as possible under wooden plant pressure using cardboard, newspaper, straps to bind the press tightly and dried using flame. The pressed and dried specimens were mounted on standard size (30 × 42 cm) herbarium sheets.

3.2.3.2 Identification and Preservation

The collected specimens were identified by using relevant references (Hara, 1966, 1971; Lawrence, 1967; Stainton, 1972; Malla et al., 1976; GON, 1986, 2001; Polunin & Stainton, 1997; Hooker, 1996; Watson et al., 2013). Prologues and images of type specimens were downloaded from different websites (www.biodiversitylibrary.org., www.theplantlist.org., etc.). The herbaria were confirmed through type specimens and previously authenticated specimens deposited at National Herbarium and Plant Laboratories, Godawari, Lalitpur.

3.2.3.3 Description of Plant Species

The scientific names of the plants were arranged in Table (Annex IA). The list of the scientific names and synonyms was finalized following World Flora Online. Angiosperm Phylogeny Group IV is used for classification. The local (Magar) names are given, which were noted in the field, and some are from the secondary literature (Manandhar, 2002).

3.3 Database Preparation

Database was prepared from all the data obtained during the field visits by interviewing informants, villagers, official persons, etc. These data were layout in the spread sheet, and made for the methods of data handling and analysis. Two-software packages, (1) Microsoft Excel program – a general-purpose spreadsheet program and (2) SPSS Program – a statistical excel, were used for the management of data. Data into excel was ready to tackle the experimental design and statistical analysis. Then, the data were transferred to SPSS program for analysis. The output-analyzed data as medicinal, fodder, marcha making, edible, etc. values are presented in tables.

3.4 Data Processing and Analysis

Data were transferred into SPSS/PC Plus software package for further processing and analysis. As the ethno-medicinal data collected were qualitative, they were changed into string variable for processing and analysis. Then the data are statistically analyzed in pie-chart or bar diagram or tabular form.

3.5 Selection of plant species for laboratory tests

Laboratory testes along with evaluation of biologically active natural products present in ethnomedicinal plants would help in understanding their therapeutic actions of traditionally used. To select the important species used in traditional medicine quantitative ethnobotany *viz*. Factor for informant consensus (FIC) and fidelity level (FL) were used.

The FIC was calculated as the number of use citations in each category (Nur) minus the number of species used (Nt), divided by the number of use citations in each category minus one (Heinrich et al., 1998):

$$\text{FIC} = \frac{\text{Nur} - \text{Nt}}{\text{Nur} - 1}$$

In order to use this tool, the illnesses were classify into broad disease categories (several diseases based on the organ systems in one category) such as: (1) gastrointestinal, (2) respiratory, (3) dermatological, (4) muscular/skeletal, (5) pain (6) reproductive, (7) UTI (8) eye problem (9) kidney stone (10)typhoid (11) jaundice and (12) other.

To assess the importance of individual species in each category fidelity level (FL) (Friedman et al., 1986) was calculated.

$$\text{Fidelity level (FL)} = \frac{\text{Np}}{\text{N}} \times 100$$

Where Np is the number of informants citing the use of the plant for a particular illness and N is the total number of informants citing the species for any illness. Fidelity level (FL) was correlated with use mention (UM) (Andrade-Cetto, 2009) to identify high fidelity. The UM is defined as the number of mentions for one plant given by all of the informants for a specific disease.

3.6 Plant Materials and Extraction

The highly important 15 ethno-medicinal plant species (high FL with UM) used by the Magar community were collected for laboratory experiments. Different parts of the 15 plants species were air dried at room temperature and then ground in an electric grinder to obtain coarse powder.

Soxhlet extraction: It is a continuous extraction method. Grinded plant material was weighed, kept in a thimble and then put inside a Soxhlet apparatus. Heating mantle was used for continuous heating at control temperature.

Solvent: Hexane and 70% methanol were successively used as extracting solvents. Low polar hexane (C_6H_{14}, relative polarity = 0.009) and comparatively high polar methanol (CH_3OH, relative polarity = 0.762) in water were used. Hexane was purchased from Merck Life Science Pvt. Ltd. Methanol was purchased from Fisher Scientific. During extraction, solvents were diffused into the solid plant material and then solubilized compounds. Hexane was used to solubilize non polar compounds,

while 70% methanol was used to solubilize polar compounds that were present in the plant material.

Temperature: Extractions were carried out under reflux temperature of the solvents used.

Period of extraction: About 72 hours or until a clear extract solution was noticed.

Storage of extract: The extracts were concentrated using a rotary evaporator, vacuum dried at 37 °C and then stored in a refrigerator at 4 °C until further use.

3.7 Antibacterial Susceptibility Assay

3.7.1 Materials

Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) were purchased from HiMedia Laboratories Pvt. Ltd.

3.7.2 Bacterial species

The bacteria *Pseudomonas aeruginosa* (ATCC 27263), *Staphylococcus aureus* (ATCC 25923), and *Klebsiella pneumonia* (ATCC 700603) and *Escherichia coli* were collected from Shukraraaj Tropical and Infectious Disease Hospital (STIDH). *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6051), *Shigella dysenteriae* (ATCC 13313) and *Salmonella enteric* subsp. *enteric serovar typhi* were collected from Department of Plant Resources, Thapathali Kathmandu, Nepal. *Salmonella* was clinical isolates. These bacteria were sub-cultured in sterile MHA media.

Leading etiological agents of UTIs include *Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa* (Svanborg & Godaly, 1997; Shankar, et al., 2001).

3.7.3 Inoculum Preparation

The bacteria to be tested were streaked on sterile MHA plates to obtain isolate colonies. The plates were incubated for 18–24 hours at 37 °C. Thereafter, 3-4 isolate colonies were suspended into 5 mL of sterile MHB in a test tube and then vortexed. The turbidity of suspension was adjusted to that of McFarland standard 0.5 by adding sterile MHB when turbidity was high or adding bacterial isolate when the turbidity

was low. Thus prepared inoculum was used within 30 minutes to avoid changes in the cell number.

The $BaSO_4$ turbidity standard equaling the McFarland 0.5 standard can be prepared. So called McFarland 0.5 standard is equal to 1–2 $\times 10^8$ cfu mL^{-1}.

3.7.4 Antibacterial Screening

Dried extracts were dissolved in dimethyl sulfoxide (DMSO) to make 0.1 g/mL concentration for antimicrobial study. The antibacterial screening of these medicinal plant species extracts were evaluated by using the Agar well diffusion technique following Perez et al. (1990). Sterilized MHA media was poured into sterile Petri dishes (20 mL each). After solidification, the prepared inoculums were swabbed on the respective plates. The wells were punched on the agar gel plate using a sterile borer of 6 mm diameter. The wells were filled with 50 µL of the prepared plant extract. Ampicillin and gentamicin (Mast diagnostics) of 10 µg per disc were used as standard reference. DMSO was used as control. The plates were incubated at 37 °C for 18–24 hours following CLSI USA method. Tests were performed in triplicate. Zone of inhibition (ZOI) was measured in mm and indicated as negative when absence.

3.7.5 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Broth dilution technique was used to determine minimum inhibitory concentration (MIC) values of the extracts, which displayed antimicrobial property, following Wiegand et al. (2008). Sterile 96-well micro-titer plates were used for determination MIC value. Each well was labeled for respective concentration of the plant extract. Out of 12 wells in a row, 6 wells were used for the extract of one plant species. The wells labeled for different concentrations and growth control was filled with 50 µL of MHB. The well in the sterility control was filled with 100 µL of broth. 50 µL of the stock solution of the extract (0.1 g/mL concentration) was added in each well, except well for growth control, and series of dilutions of the extracts were adjusted by double dilution method in the respective wells. As only 6 wells were used for each plant extract, the series of concentrations were 50, 25, 12.5, 6.25, 3.12 and 1.56 µg/mL. The bacterial suspension adjusted to 1 $\times$ 10^8 cfu mL^{-1} was diluted to 1:100 and vortexed.

Each well containing the extract dilutions and the growth control were filled with 50 µL of the diluted bacterial suspension. This results in the final desired inoculum of 5×10^5 cfu mL^{-1}.

After incubation for 18–24 hours at 37 °C, the MIC was taken as the lowest concentration of the antimicrobial agent that inhibited visible growth of the tested bacteria as observed no turbidity with the unaided eye.

Minimum bactericidal concentration (MBC) values were then determined as no colony growth (or growth by 10-1%) by direct streaking the content of the wells inhibiting bacterial growth (MIC) and higher concentrations than the MIC value on sterile MHA plates.

3.8 Phytochemical Screening

3.8.1 Materials

Dragendroff's reagent, hydrochloric acid (HCl), sulphuric acid (H_2SO_4), chloroform ($CHCl_3$), amonia (NH_3), amonium hydroxide (NH_4OH), ferric chloride ($FeCl_3$), magnesium granules (Mg), potassium hydroxide (KOH), sodium carbonate (Na_2CO_3) and 3,5-dinitrobenzoic acid were procured from commercial suppliers.

3.8.2 Phytochemical Screening (qualitative)

Dried hexane extract and 70% methanolic extracts were used for the phytochemical screening. Different phytochemical contents present in the hexane and 70% methanolic extracts were identified by the colour reactions with different reagents following the phytochemical screening method developed by Ciulie (1982).

3.8.2.1 Phytochemical Screening of the Hexane Extract

Alkaloids (Dragendroff test): Extract was vigorously shaken with 2% HCl and filtered. The solution was treated with Dragendroff's reagent (3 drops). Formation of orange or red precipitate indicates the presence of alkaloids.

Carotenoids: Extract was vigorously shaken with $CHCl_3$ and filtered. The solution was treated with conc. H_2SO_4. A blue colour at the interface shows the presence of carotenoids.

Steroids (Liebermann-Burchard test): Extract was dissolved in $CHCl_3$ and a few drops of acetic anhydride and conc. H_2SO_4 were added. Formation of a dark pink, red and/or violet to blue or green colour indicates the presence of steroids.

Terpenoids (Salkowski test): Extract was dissolved in $CHCl_3$ and a few drops of H_2SO_4 were added. A reddish brown colouration at interface indicates the presence of terpenoids.

Coumarins (Fluorescence test): Extract was vigorously shaken with hot water and filtered. A blue fluorescence upon treating with 10% NH_4OH under UV light indicates the presence of coumarins.

Anthraquinones (Borntiager test): Extract was boiled with 10% HCl. To this were added $CHCl_3$ and 10% NH_3. Rose-pink colour indicates the presence of anthraquinones.

3.8.2.2 Phytochemical Screening of the Methanolic Extract

Tannins and polyphenols (Braymer test): Extract was heated with water and a few drops of 1% $FeCl_3$ were added. A dark green, deep blue, blue-black or black colouration indicates the presence of phenols. Blue colour for gallic tannins and green black for catecholic tannins.

Alkaloids (Dragendroff test): Extract was vigorously shaken with 2% HCl and filtered. The solution was treated with Dragendroff's reagent (3 drops). Formation of orange or red precipitate indicates the presence of alkaloids.

Anthocyanosides: Extract was heated with 10% HCl and treated with Na_2CO_3. Brownish green or blue colouration indicates the presence of anthocynosides.

Anthracenosides: Extract was mixed with ether (2 mL), conc. H_2SO_4 (1 mL) and 25% ammonia solution (1 mL). Formation of cherished-red solution on the top layer indicates the presence of anthracenosides.

Flavonoids (Shinoda test): Extract was dissolved in methanol and then treated with a piece of magnesium and conc. HCl. Development of a pinkish red colour indicates the presence of flavonoids.

Cardiac glycosides (Kedde's test): Extract was dissolved in methanol and then treated with 1% methnolic KOH and 1% methanolic 3,5-dinitrobenzoic acid. Purple color indicates the presence of cardiac glycosides.

Saponins (Froth test): Extract was vigorously shaken with distilled water and heated to boil. Persistent forth indicates the presence of saponin.

Protein and amino acids (Biuret test): Extract was boiled with water and then treated with 2% copper sulphate solution, ethanol and excess of potassium hydroxide pellets. Development of a pink color indicates the presence of protein.

3.9 Gas Chromatography-Mass Spectrometrya (qualitative and quantitative)

General available method of Gas Chromatography-Mass Spectrometry analyses is applied for all the extracts. The gas chromatography-mass spectrometry (GC-MS) analyses of the hexane and 70% methanolic extracts of the plant materials was analyzed using an Agilent 7890A GC system coupled with an Agilent 5975 C mass selective detector, equipped with a HP-5MS GC column (5% phenyl methyl siloxane, Agilent 19091S-433, 30 m × 250 μm internal diameter, 0.25 μm film thickness). Helium was used as a carrier gas at flow rate of 1.21 mL/min. The instrument was operated in the electron impact (EI) mode at 70 eV and ion source temperature 230 °C in the scan range of 50–500 m/z. The initial column temperature was set at 40 °C held for 2 minutes, ramped at a rate of 4 °C/minute to 270 °C and held for 5.5 minute (total run time 65 minutes). A dilute sample solution of the extracts was prepared either in chloroform or methanol, and a volume of 2 μL was injected in split injection technique. The constituents were identified by comparing the mass spectra available in a MS database (NIST 08).

3.10 Total Phenol Content (Folin-Ciocalteu Colorimetric Method)

3.10.1 Material

Folin-Ciocalteu reagent was purchased from Loba Chemie Pvt. Ltd.

3.10.2 Total Phenol Content (TPC)

Total phenolic content was estimated using the Folin-Ciocalteu colorimetric method (Singleton et al., 1999) with some modifications. A solution of 70% methanolic

extract of concentration 0.4 mg/mL was prepared in distilled water. Thus prepared extract solution (50 µL) was treated with 25 µL of Folin-Ciocalteu reagent and then 100 µL of aq. Na_2CO_3 solution (75 g/L). Distilled water of 175 µL was taken as a blank. The absorbance at 760 nm was measured on spectrophotometer after 1 hour keeping in the dark. To obtain a calibration curve, gallic acid solution of 100 µg/mL in distil water was prepared and 50 µL of this stock solution was filled in the well of 96 well microplate. It was double diluted to make concentrations of 50, 25, 12.5, 6.24, 3.125 and 1.56µg/mL. In each concentration of gallic acid, 25 µL of Folin-Ciocalteu reagent and 100 µL of aqueous Na_2CO_3 solution (75 g/L) were added to make to make total of 175 µL. A linear curve of the standard gallic acid concentrations versus absorbance was constructed. The total phenolic contents were calculated using the formula:

$$C = c\frac{V}{m}$$

Where, C = total phenolic content in mg GAE/g dry extract; c =concentration of gallic acid obtained from calibration curve in mg/mL; c = y/m where y = absorbance of extract solutions, m = gradient of calibration curve. V = volume of extract in mL, m = mass of extract in gram. Total phenolics content of the extract was expressed as mg gallic acid equivalents (GAE) per gram of extract in dry weight (mg/g).

3.11 Antioxidant Assays

3.11.1 DPPH radical scavenging method

3.11.1.1 Materials

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich and Gallic acid from Sisco Research Laboratories Pvt., Ltd. Spectrophotometry was carried out using an Elisa microplate reader (EPOCH2, BioTek Instruments).

3.11.1.2 DPPH Free Radical Scavenging Assay

DPPH scavenging capacity of each extract was determined according to the method of Brand-Williams et al. (1995). DPPH radical solution was prepared by overnight stirring of 3.94 mg DPPH in to 100 ml of distilled methanol (w/v) at 0 $^{\circ}$C and complete dark condition. It gives 0.1 mM concentration.

Microplate assay: The stock solutions of concentration 2000 µg/mL of methanolic extract of the plants were prepared. It was filled in 96 well microplate. The sample solution with concentrations of 1000, 500 and 250µg/mL were filled in its respective well by double dilution method. The concentration 1500, 100 and 10 µg/mL were prepared in separate test tubes and transferred into respective well by micropipette and concentration 750, 50, 25, and 5 were filled in respective well by double dilution of these respective concentrations. The contents in wells were mixed with pipette 4-5 times during double dilution. Every well has 50 µL of sample and 250 µL of the methanol DPPH solution. 300 µL distilled methanol was used as blank. 50 µL distilled methanol was mixed with 250 µL methanol DPPH for control. 50 µL of gallic acid solution of concentrations 20, 10 and 5 µg/mL were used to obtain linear curve of a positive control. The mixture had been shaken well at room temperature for 30 minutes in the dark. The absorbance was determined at 517 nm using an Elisa microplate reader (EPOCH2, BioTek Instruments). The DPPH radical scavenging ability was calculated according to the following equation:

$$\text{DPPH radical scavenging rate (\%)} = 1 - \frac{\text{Absorbance (sample)} - \text{Absorbance (blank)}}{\text{Absorbance (control)} - \text{Absorbance (blank)}} \times 100$$

Each sample was analyzed in triplicates. The concentration of the sample at which 50% inhibition of $DPPH^{\bullet}$ radical was calculated as:

$$IC_{50} = \frac{(50 - c)}{m}$$

Where 'c' is intercept and 'm' is slop of linear curve describing dependence of percentage inhibition with concentration. IC_{50} is expressed in µg/mL.

3.11.2 Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity was measured following the instructions of commercial kit (Radical catch; Hitachi Ltd., Tokyo, Japan) (Maeda et al., 2018). Briefly, 2500 of reagent A (that is 5 mM cobalt chloride solution) was mixed with 2500 of reagent B (luminal solution). Then 10 µL of the experimental sample solution was added (The experimental sample solution was made by dissolving 0.1 mg of aqueous methanol extract in 1 mL of distilled methanol). Subsequently, the mixture

solution was incubated at 37 °C for 5 minutes in an incubator (Varioskan LUX Multimode Microplate Reader, Thermo Fisher Scientific, and Waltham, MA, USA). Then 2500 of reagent C (hydrogen peroxide) was added. After the mixture reacted with hydrogen peroxide solution the luminescence of light for 120 seconds in the incubator was measured. The luminescence was observed to subtract an amount of 120 to 80 seconds. 70% methanol was used as control. Hydrogen scavenging activity was calculated following the equation below.

$$\text{Hydrogen peroxide scavenging activity (\%)} = \frac{\text{Luminescence (control)} - \text{Luminescence (sample)}}{\text{Luminescence (control)}} \times 100$$

3.12 Statistical Analysis

Statistical software SPSS V21 was used for analysis of ethno-botanical data. Statistical analysis of antimicrobial and antioxidant data was done using Microsoft Office Excel 2007. Experiments were performed in triplicates (n = 3) and the results are presented as mean±standard error mean (SEM). Correlation and regression analysis were performed using Microsoft Office Excel 2007 Data analysis tools.

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 Ethnobotany

The present study recorded 160 plant species belonging to 146 genera and 68 families (Annex IA) which are being used in different purposes (medicinal, marcha making, fodder, fruit, vegetables and others) in Nawalpur. The ethnobotanical data have been collected by interviewing 36 key informants (Annex IB) by semi-structured interview (Annex IC). The percentage of the use categories of the documented plants is presented in Figure 7. The chart does not cover multiple purpose of single species where as it is shown on Annex IA.

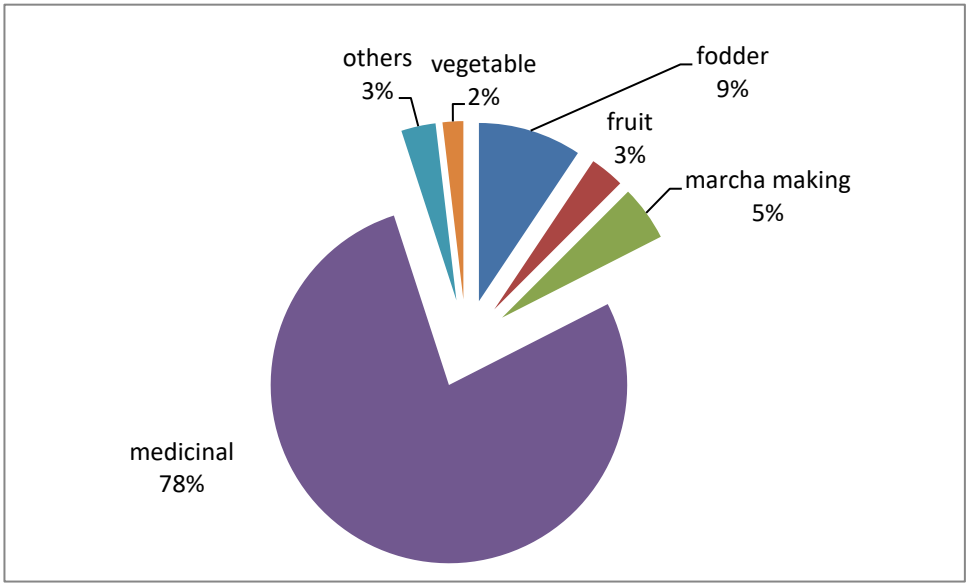


Figure 7: Showing % of use categories of Documented Plants from ethno-botanical study of Nawalpur during 2015-2017.

4.1.1 Marcha making (fermenting cake) plants

In the present study 11 plant species are listed for marcha making (Table 1). Nearly all household prepared liquor using millet *(Eleusine indica*) in the hilly villages for family use or selling. Millet is the main crop in the hill. It has been either used to prepare liquor or exchanged with rice at market place. Manandhar (2002) has also listed marcha making plants used by different ethnic groups in Nepal. But findings of this study added *Ananas comosus, Asparagus racemosus, Cyanthillium cinereum,*

Scoparia dulcis, Spilanthes acmella, Syzygium nervosum and *Tinospora sinensis* as marcha making plants in the list.

Table 1: List of marcha making plants

Scientific name	**Family**	**Magar name**	**Nepali name**	**Part use**
Aerva sanguinolenta (L.) Blume	Amaranthaceae	marchajhar	Mansi ghans	Whole plant
Ananas comosus (L) Merr.	Bromeliaceae	bhuikatahar	Bhuikatahar	leaf
Artocarpus heterophyllus Lam	Moraceae	Katahar	Rookhkatahar	leaf
Asparagus racemosus Willd.	Asparagaceae	Kuril	Kuril, satawari	root
Cyanthillium cinereum (L.) H. Rob.	Asteraceae	Phuli jhar, marchajhar	sahadevi	wholeplant
*Elephantopus scabe*r L.	Asteraceae	saujar	Sahasra buti	whole plant
Scoparia dulcis L.	Plantaginaceae	chinijhar	chinijhar	whole plant
Spilanthes acmella (L.) L	Asteraceae	marati	Bhui timur	leaf
Syzygium nervosum DC.	Myrtaceae	tyamel	kyamuna	leaf
Tinospora sinensis (Lour.) Merr.	Menispermaceae	chinilahara	gurjolahara	stem
Tridax procumbens L (L)	Asteraceae		Kurkure jhar	whole plant

4.1.2 Fodder

The role of tree fodder becomes increasingly important for both livestock productivity and the stability of hill farming systems. The inventory data revealed that a great variety of tree species have been managed on farmland in the study area. Of these, 22 species were recorded as fodder, with their scientific name, family and local name (Table 2). Trees are lopped and carried to stall-fed animals. Leaves and twigs are fed to the animals and the left over branches are used as fuel wood.

The villegers reported increment of ghee portion on milk production on feeding of *Ficus semicordata. Bridelia retusa* was reported to cause labored breathing (dyspnoea) and death of cattle at leaf flushing stage. The mature leaves are recommended as medium to high quality fodder. *Asparagus racemosus* is galactologue for cattle.

Table 2: List of fodder plants

Scientific Name	Family	Local name
Artocarpus lakoocha Wall. Ex Roxb.	Moraceae	barahar,badahar
Asparagus racemosus Willd.	Asparagaceae	ban kuril

Bauhinia purpurea L.	Fabaceae	tanki
Bridelia retusa (L.) A.Juss	Phyllanthaceae	gayo
Cissus repens Lam.	Vitaceae	charchare
Colebrookea oppositifolia Sm.	Lamiaceae	dhusure
Cordia dichotoma G.Forst	Boraginaceae	bohari
Cynodon dactylon (L.) Pers.	Poaceae	duboo
Ficus hispida L. f.	Moraceae	khasreto, thotne
Ficus semicordata Buch.-Ham. ex Sm.	Moraceae	khanayo
Garuga pinnata Roxb.	Burseraceae	dabdabe
Grewia subaequalis Baill	Malvaceae	pharsa
Litsea monopetala (Roxb.) Pers.	Lauraceae	kutmiro
Lygodium japonicum (Thunb.) *Sw.*	Lygodiaceae	thunelo
Maesa chisia Buch.-Ham.ex D. Don	Primulaceae	bilauni
Melia azedarach L.	Meliaceae	bakainno
Morus alba L.	Moraceae	kimbu
Parthenocissus semicordata (Wall.) Planch.	Vitaceaae	charchare
Paspalum scrobiculatum L.	Poaceae	kodo jhar
Quercus semecarpifolia Sm.	Fagaceae	khasru
Saurauia napaulensis DC.	Actinidiaceae	gogun
Uncaria scandens Hutch.	Rubiaceae	rangosing

4.1.3 Ethnomedicinal Plants

The study was mainly focused on the traditional knowledge of Magars on medicinal plants. Altogether 124 ethnomedicinal plant species belonging to 60 families are reported from the study area. The results of the ethnomedicinal study has been presented in (Annex IA) with scientific name (in italics) followed by family names, local names (Nepali/Magar) and detailed uses (mode of preparation, and administration of medicine), voucher number and distribution in the study sites.

These ethnomedicinal plants have been used for treatment of 40 different ailments including gastrointestinal problem, menstrual problem, miscarriage, cough, tonsillitis, sinusitis, hooping cough, pneumonia, common cold, skin burn, cuts, wound, scabies, boils, muscular sprain, bone fracture, rheumatic pain, paralysis, gout, toothache, headache, eye problem, kidney stone, allergy, tuberculosis, fever, food poisoning, measles, controlling blood pressure, cooling, piles etc. Ethnomedicinal knowledge with 87 species of ethnomedicinal plants belonging to 43 families used to cure 36 different ailments, of Magars in Dhaubadi VDC, Nawalpur district has been documented (Nemkul et al., 2018b). Singh et al. (2018), Acharya (2012), Thapa (2012) and Poudel and Gautam (2008) reported herbal remedies prepared by Magars in Palpa, Gulmi, Parbat and Dhading district respectively. But the finding of this study

shows that Magars in the study site has knowledge about herbal treatment for miscarriage also which was not reported by the other studies on Magar community.

The largest number of species have been noted from Fabaceae family (12 species) followed by Asteraceae, Lamiaceae, Zingiberaceae, Apocynaceae etc. (Figure 8). The remaining families cover one or two species each. The maximum number of the species are herb (44 species), followed by tree (43), shrub (28) and climber (9) (Figure 9).

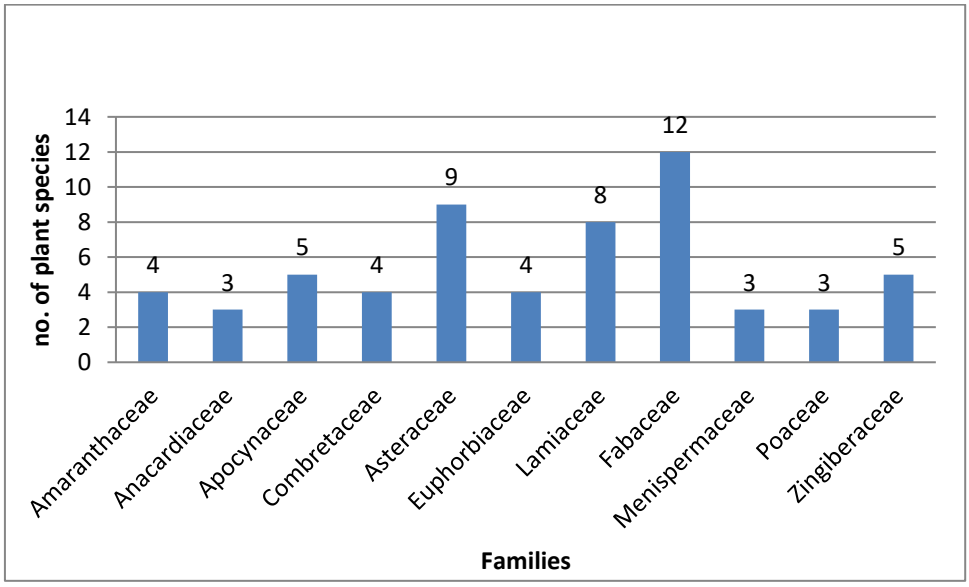
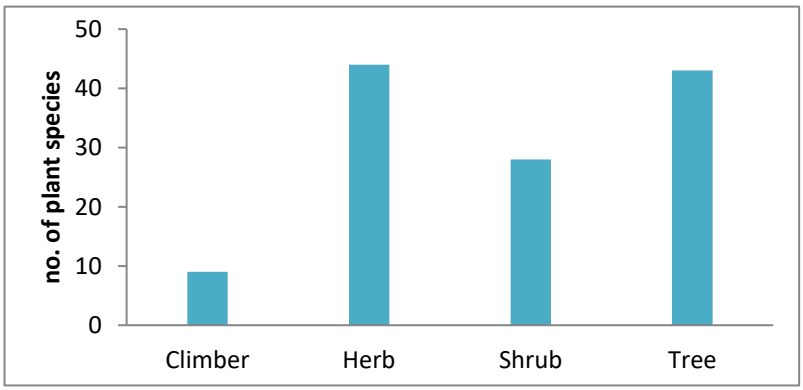


Figure 8: Dominant families of Ethno-medicinally used species by Magars in Nawalpur.



Figue9: Habit of the ethnomedicinal plants

This study showed that many different parts of medicinal plant species are used as medicine. The frequency of use of different plant parts as medicine can be seen in Table 3. Most frequently used plant part was leaf followed by bark and root (Table 3).

The leaves of maximum plant species (37) were used for medicine followed by bark and root. Maximum use of root, rhizome and whole plant destruct the plant population.

Most commonly used plant parts have been selected because they contain more biologically active compound in comparison to the least commonly used parts. Leaves, roots, stems and flowers are physically more vulnerable to attack by herbivores or pathogens than the more hardy bark and therefore it is not surprising that it contains more chemical defence compounds in the form of biologically active secondary metabolites.

Table 3: Plant parts use

Plant parts	Frequency	Percent	Valid Percent	Cumulative Percent
Leaf	37	24.5	24.5	24.5
Bark	24	15.9	15.9	40.5
Root	23	15.2	15.2	55.7
Stem	18	11.9	11.9	67.6
Fruit	15	9.9	9.9	77.5
Whole plant	9	6.0	6.0	83.5
Rhizome	8	5.3	5.3	88.8
Flower	5	3.3	3.3	92.1
Latex	4	2.6	2.6	94.7
seed	4	2.6	2.6	97.3
Tuber	3	2.0	2.0	99.3
Tender shoot	1	0.7	0.7	100.0
Total	151	100.0	100.0	

These plant parts are used in drug in different forms (Table 4). The result showed that maximum used form of the drugs was juice from the medicinal plants followed by decoction.

Table 4: Form of Drugs

Form of drugs	Frequency	Percent	Valid Percent	Cumulative Percent
Juice	43	34.6	34.6	34.6
Decoction	41	33.1	33.1	67.7
Paste	24	19.4	19.4	87.1
Powder	9	7.3	7.3	94.4
Latex	3	2.4	2.4	96.8
Infusion	2	1.6	1.6	98.4
Paste and juice	1	0.8	0.8	99.2
Seeds	1	0.8	0.8	100
Total	124	100.0	100.0	

Out of 124 species, 66 species have been used in different ways than earlier researchers (Malla et al., 1976; Shrestha et al., 2000; Manandhar, 2002; Lama et al., 2001; Shrestha et al., 2001-2002; IUCN, 2000, 2004; Baral & Kurmi, 2006). The uses, mode of preparation of these 66 species have been recorded below. Out of 66, seven species *Acrocarpus fraxinifolius* Wight & Arn., *Ariopsis peltata* Nimmo, *Citrus paradisi* Macfad., *Cryptolepis buchananii* R. Br, ex Roem. & Schult., *Curcuma caesia* Roxb., *Opuntia monacantha* Haw and *Zingiber montanum* (J.König) Link ex A. Dietr were not recorded before as hebal medicine (Manandhar, 2002; Shrestha et al., 2001-2002; IUCN, 2000; Baral & Kurmi, 2006).

1) *Achyranthes aspera* L.: Juice of root is used as appetizer and applied on eye problem. Decoction of root is given to kidney stone. There is challenge of getting free from kidney stone after using six or seven times of the decoction. The decoction is also given for controlling blood pressure. Powder of root mixed with powder of root of *Mimosa pudica*, powder of bark of *Acrocarpus fraxinifolius* and powder of bark of *Oroxylum indicum* is given for gastritis. Similarly, Singh et al. (2018) also reported use of *Achyranthes aspera* for treatment of kidney stone by Magar in Palpa district, but juice of whole plant is used for treatment.

2) *Acrocarpus fraxinifolius* Wight & Arn: Pounded bark with water is applied on the affected part of body by rheumatic pain or paralysis.

3) *Aegle marmelos* (L.) Correa: Fruit juice is given for heat sickness. Concentrated decoction of mixture of bark of *Aegle marmelos*, bark of *Mangifera*, bark of

Shorea robusta, bark of *Acrocarpus fraxinifolius* (Mahadhan lahara) and bark of *Psidium guajava* is given for cough and fever.

Unripe fruit of *Aegle marmelos* are used for preparation of drug use for treatment of gastritis and cough by the Magars in the study area (Nemkul & Shrestha, 2016).

Contrary to this ripe and unripe fruit are used to treat stomachache (Acharya, 1996), diarrhea and dysentery (Manandhar, 1986a) and bowel complain (Siwakoti & Siwakoti, 2000). Decoction of bark has been used to get ride of intermittent fever (Patkar et al., 2012; Parmer et al., 2016).

4) *Angiopteris helferiana* C. Presl: The rhizome is pounded with seeds of *Lindera neesiana* (Wallich ex Nees) Kurz. (Nepali: siltimur), bark of *Prunus cerasoides*, bark of *Cinnamomum tamala* in presence of water. The content is strained and given for cholera.

5) *Antidesma acidum* Retz. Decoction of bark is given for cough and fever. Paste is applied on mumps. In contract to use by Magars in the study site the leaves are reported to use for the treatment of stomachache, dysentery, diabities, indigestion and sunstroke (Khan & Yadava, 2010; Horo & Topno, 2015). Similarly paste of bark is applied on mump by Tamangs of Kabhrepalanchowk district (Manandhar, 1991).

6) *Ariopsis peltata* Nimmo: Pastes of tuber apply on bone fracture and muscular sprain. Medicinal use of this species is not recorded by anyone before.

7) *Artemisia indica* Willd: Seven tender shoots are given for gastritis.

8) *Artocarpus lakoocha* Wall. Ex Roxb.: Latex is applied on boils and wounds. Bark is mixed with bark of *Mangifera indica*, bark of *Acacia catechu*, bark of *Terminalia bellirica* and bark of *Shorea robusta* for preparing concentrated decoction. The decoction is administered orally for treatment of gastritis.

9) *Phanera vahlii* (Wight & Arn.) Benth (Synonym: *Bauhinia vahlii*): Bark decoction is given for menstruation problem. Bark mixed with bark of *Psidium guajava* and *Tectaria coadunata* and pounded. The juice is given on diarrhea.

   Ethnomedicinal uses of *Phanera vahlii* (Wight & Arn.) Benth is usd in different ways by different ethnic groups in different geographic area of Nepal. Fruits are consumed by Darai tribe of Chitwan district (Dangol & Gurung, 2000). Root bark

is used in diarrhea and dysentery in Dang district (Manandhar, 1985). Root is given to cure bloody dysentery by Dhanuwar of Sindhuli distrct (Manandhar, 1990a) whereas it is given to cure gastritis by Limbus of Morang district (Siwakoti & Siwakoti, 1998).

10) *Boerhavia diffusa* L.: Leaf and stem are given as tonic to improve eye power.

11) *Bombax ceiba* L.: Paste of bark is applied topically on cuts and scabies.

12) *Butea butiformis* (Voigt) Grierson: Juice of roots is given for anthelmintic and urine infection.

13) *Caesalpinia decapetala* (Roth) Alston: Root mixed with rhizome of *Kaempferia rotunda* and aerial part of *Urtica dioeca* and pounded to make paste and apply on bone fracture and muscular sprain.

14) *Callicarpa macrophylla* Vahl: Root, leaf and fruit are chewed on tonsillitis, sore throat and rashes on tounge. Decoction is given on typhoid and fever.

There are reports of *Callicarpa macrophylla* used as ethnomedicine by other ethnic people also. The roots of the plants are boiled in water at night and the decoction is given to the patient to drink early in the morning to lower fever (Dangol & Gurung, 1991). In Chitwan, root juice is given for indigestion (Manandhar, 1990b); roots are chewed to treat rash on the tongue; the paste of young buds is given for stomachache; and paste of root is given to control fever (Manandhar, 2002).

15) *Calotropis gigantea* (L.) Dryand: latex on cotton is kept press in between teeth on toothache. Latex is also usd on muscular sprain. Manandher (2002) has described as latex used on muscular sprain.

16) *Cheilanthes dalhousiae* Hook: Decoction of rhizome and leaf is given on gastritis.

17) *Chenopodium album* L.: Young aerial parts are cooked as vegetable and given to newly delivered mother to strengthened uterus.

18) *Cissampelos pareira* L.: Decoction of whole plant is given on typhoid, fever, gastritis and urine infection.

19) *Citrus paradisi* Macfad: Fruits are eaten on menstruation problem.

20) *Cryptolepis buchananii* R. Br, ex Roem. & Schult.: Paste of leaf and stem apply topically on boils.

21) *Curcuma caesia* Roxb.: Juice of rhizome is given on gastritis. This species is native to India and Bangladesh, but the villagers in the study site planted on their farm for medicinal use.

22) *Cyathula tomentosa* (Roth) Moq: Decoction of whole plant is given on diarrhea for human being. The plant is given to cattle to treat diarrhea.

23) *Cyperus rotundus* L.: Root is mixed with leaf of *Reinwardtia indica* and root of *Imperata cylindrica* to make paste. The paste is applied topically on body part having problem with snake bone spine. The paste helps to expel the spine.

24) *Desmodium multiflorum* DC.: Root decoction given on menstruation problem

25) *Dioscorea bulbifera* L.: Tuber is given on urine infection.

26) *Diploknema butyracea* (Roxb.) H.J.Lam: Vegetable butter extracted from seeds is used topically on scabies.

27) *Ficus hispida* L.: Water oozing out after cuttig root is used to treat heating sensation.

28) *Ficus racemosa* L.: One or two drops of latex are given on typhoid. Fruit is given to treat typhoid.

29) *Flemingia strobilifera* (L.) W.T.Aiton: Root is cut into pieces and chewed on gastritis, diarrhea, dysentery and ulcer.

30) *Fraxinus floribunda* Wall: Decoction of bark is given on jaundice.

31) *Holmskioldia sanguinea* Retz: Leaf juice given on dysentery. Manandhar (2002) does not describe as medicine. It is good fodder for goat.

32) *Hypericum cordifolium* Choisy: Flower decoction is used to treat gastritis and indigestion.

33) *Indigofera cassioides* Rottler ex DC.: Decoction of flower is given on hooping cough. Local villagers cook it as vegetable.

34) *Kaempferia rotunda* L.: Paste of rhizome is applied topically on bone fracture and muscular sprain.

35) *Kalanchoe integra* (Medik.) Kuntze: Juice of leaf is applied on skin burn.

36) *Lagerstroemia parviflora* Roxb.: Decoction of bark is given on typhoid, fever, diarrhea and menstruation problem. Yellow leaves are pounded and the extract is applied on forehead of pregnant women.

37) *Lannea coromandelica* (Houtt.) Merr.: Paste of bark is applied topically for healing of scars of cuts. Juice of bark is administered orally for treatment of pneumonia.

38) *Lobelia nicotianifolia* Roth: Chopped root is mixed with yogurt and given on menstruation problem. Root of *Lobelia nicotianifolia* mixed with bark of *Rhododendron arboreum* and fruit of *Citrus paradisi* pounded and given with water on menstruation problem.

39) *Lygodium japonicum* (Thunb.) Sw.: Whole plant is given to cattle for lactation.

40) *Mahonia napaulensis* DC.: Powder of stem is used to apply on teeth to strengthened teeth.

41) *Mangifera indica* L.: Bark of *Mangifera indica* mixed with bark of *Shorea robusta*, bark of *Fraxinus floribunda*, bark of *Acacia catechu* and bark of *Psidium guajava* is pounded and given with water for 2-4 days for gastritis and dysentery. The juice is applied on body for pain due to gout. Inner part of the seed of *Mangifera indica* is given for 2-3 days for stomach problem due to indigestion of oily food.

42) *Myrica esculenta* Buch.-Ham. ex D. Don: Hard part of fruit is pounded and concentrated decoction of it is mixed with wheat flour and given on dysentery. Hard part of fruit of *Myrica esculenta* mixed with bark of *Schima wallichii*, tuber of *Stephania glandulifera*, root of *Leea macrophylla* and stem of *Musa paradisiaca* (ghampe kere) is pounded with water and mixed with lemon juice, *Sesamum orientale* L., *Hordeum vulgare* L. (barley), *Brassica campestris* var. sarson Prain (field mustard), edible *Cinamommum camphora*. Make about 200ml and strain. It is mixed with cow ghee and honey. It is given on fourth day of menstruation to the woman with miscarriage problem.

43) *Nymphaea lotus* L.: Paste of seed is given for jaundice by Magars in study area.Seed is prescribed as stomachic and restorative (IUCN, 2004).

44) *Ocimum basilicum* L.: Seeds are soaked in glass full of water for overnight and given for urine tract infection and also for heat sickness.

45) *Opuntia monacantha* Haw.: Fruit and succulent stem juice is given on fever and heat sickness. This species is native to Brazil, Paraguay and Uruguay, but introduced into Nepal. In the study area this species are found in large number on fallow land.

46) *Phyllanthus parvifolius* Buch.-Ham. Ex D. Don.: Decoction of leaf and stem is given on menstruation problem.

47) *Plumeria rubra* L.: Three drops of latex given on food poisoning for adult and one drop mixed with three drops of milk for child.

48) *Premna barbata* Wall. ex Schauer: The bark, leaf and root are pounded and given on typhoid fever. The paste of leaf applies on forehead on headache.

49) *Prunus cerasoides* Buch.-Ham. ex D. Don : Leaf paste is apply on skin burn.

50) *Psidium guajava* L.: The plant parts of it are used in different way in the study area. It is used for gastritis and dysentery as described in *Mangifera indica*. It is also used for treatment of cough as described in *Aegle marmelos*.

51) *Quercus lanata* Sm.: Root juice is given for food poisoning.

52) *Rhododendron arboreum* Sm.: Similar to described by Manandhar (2002) bark juice is given on diarrhea and dysentery and flower is given on stuck of fish bones in throat by the villagers on the study sites. Along with this flowers are given on urine infection. Barks are used for menstruation problem as described in *Lobelia nicotianifolia*. Bark paste of *Rhododendron arboeum* is applied on bone fracture.

53) *Rumex hastatus* D. Don: Leaf juice is applied inside nostril for sinusitis.

54) *Schima wallichii* Choisy: Root juice is given for food poisoning. Bark is used for miscarriage problem as described on *Myrica esculenta*. Bark decoction is given for seven days to treat menstruation problem. Powder of *Schima wallichii* stem is mixed with diet of pigs for growth of undergrowth pigs.

55) *Shorea robusta* Gaertn. Bark is given for gastritis, dysentery and gout as described in *Mangifera indica*.

56) *Solanum nigrum* L.: Juice of fruit is applied on forehead to treat headache. Paste made of fruit and leaf of *Pogostemon benghalensis* is applied on front part of head of children for fever. Leaf of *Solanum nigrum* mixed with *Centella asiatica, Cuscuta reflexa,* leaf of *Azadirachta indica* and *Spondias pinnata* is pounded and made juice. One glass of juice is given for seven days to treat jaundice.

57) *Solanum torvum* Sw.: Juicce of leaf and stem is given for common cold, headache and fever. Fruits are eaten as pickle.

58) *Spondias pinnata* (L.f) Kurz. Fruits are eaten for sinusitis. Juice of bark and stem are given for uric acid problem and paralysis.

59) *Stephania glandulifera* Miers: Powder made of tuber mixed with fresh yogurt is used for the treatment of diarrhea and gastritis. The mixture of tuber of *Stephania glandulifera,* rhizome of *Acorus calamus,* unripe fruit of *Aegle marmelos,* bark of *Mallotus philippensis,* fruit of *Phyllanthus emblica,* fruit of *Terminalia bellirica,* fruit of *Terminalia chebula,* seed of *Trachyspermum ammi,* flower or bark of *Woodfordia fruticosa* and seed of *Zanthoxylum armatum* are air dried and ground. The powder is mixed with Himalayan black salt (Bide noon) and given as one tea spoonful twice a day for gastritis.

60) *Syzygium cumini* (L.) Skeels: Bark juice is droped in nostril and some drops take as oral medicine for sinusities.

61) *Taraxacum officinale* F.H. Wigg.: Paste of leaf and stem is used topically for fungus diseases on skin.

62) *Terminalia anogeissiana* Gere & Boatwr: Bark is mixed with bark of *Caesalpinia decapetala* and bark of *Rhododendron arboreum* and pounded to make paste and toping on bone fracture, muscular sprain. The strained decoction is given to eat.

63) *Thalictrum foliolosum* DC.: Manandhar (2002) described that the juice of root is given for fever and peptic ulcer and juice leaf is applied to boils and pimples. Dolpo people use root, leaf and flowers for medicine for the treatment of contagiou fever, poisoning, wonds and infection (Lama et al., 2001). The Magars in the study site use the powder of the root with honey for a week to treat rheumatic pain.

64) *Vitex negundo* L.: Leaf juice of *Vitex negundo* mixed with leaf juice of *Justicia adhatoda* and leaf juice of *Nyctanthes arbor-tritis* in an equal amount. Two drops of the mixed juice is given for pneumonia and cough. One drop of water is added to one drop of the mixed juice and put into nostril for sinusitis in adult.

65) *Woodfordia fruticosa* Kurz.: Seven tender shoots are given for gastritis and dysentery. Flower and bark were given for diarrhea, skin burn; flower decoction for gastritis.

66) *Zingiber montanum* (J.König) Link ex A. Dietr.: Juice of rhizome is given on food poisoning. The rhizome is given to treat gastritis and stomach problem. Though this species is native to China, Thailand and Vietnam the villagers in the study area has kept it on kitchen garden and used as medicine.

It shows that the Magar people in the study area know 66 herbals used in different ways from other ethnic group in different geographic area. For treatment of gastritis they have good formulation, mixing *Stephania glandulifera*, *Acorus calamus, Aegle marmelos, Mallotus philippensis, Phyllanthus emblica, Terminalia bellirica, Terminalia chebula*, *Trachyspermum ammi, Woodfordia fruticosa* and *Zanthoxylum armatum* in form of powder (Nemkul & Shrestha, 2016). There are many reports of *Stephania glandulifera* used for treatment of gastritis and peptic ulcer (Manandhar, 1990, 1990b; Bhattarai, 1992b; Dangol & Gurung, 2000).

Powder form of drug retains potency for long time (one year) if preserve in air tight container. Juice form of drug use for instant effect and sometimes as antiseptic (eye drops) and also availability of fresh plants. Cold infusion is generally used for heat sickness disease. For more effective, strong and concentrate drug they mixed more than one ingredients and boil for long time.

Hilly region in Nawalpur there is lacking of modern facilitate hospital. So indigenous people at the region use herbal medicine prepared by healer (Dhami Lama).

4.2 Selection of plant species for laboratory tests

4.2.1 Factors for informant concensus (FIC)

All the illness documented has been classified into 12 disease categories. Diarrhea, dysentery and anthelmintic are included into gastrointestinal category; cough, tonsillitis, sinusitis, hooping cough, pneumonia and commom cold are incuded into respiratory category; burn is classified as dermatological category; muscular sprain and bone fracture are classified as muscular/skeltal category; rheumatic pain, toothache and headache are included in pain category; allergy, fever, removable of germs from poutry, nutritional, expel very fine spines, heart problem, liver problem, control of ectoparasite on cattle, lactation on cattle, strenthenning teeth gum, piles, cooling, food poisoning, blood purifier, blood pressure, tuberculosis and measles are included into other category. Factor for informant consensus (FIC) in each category has been calculated and shown in table 5.

The results of the FIC (Table 5) showed that the muscular/skeletal category has the greatest agreement with a FIC of 0.862, followed by gastrointestinal (0.839), UTI (0.810), jaundice (0.777), respiratory (0.776), dermatological (0.757), reproductive (0.717), others (0.705), typhoid (0.733), eye problem (0.666) and pain (0.666).

Table 5: Factors for informant concensus (FIC)

Illness category	no. of species used (Nt)	use citation (Nur)	FIC(Nur-Nt/Nur-1)
Gastrointestinal	31	188	0.839
Respiratory	18	77	0.776
Dermatological	18	71	0.757
Muscular/skeletal	5	30	0.862
Pain	7	19	0.666
Reproductive	14	47	0.717
UTI	8	38	0.810
Eye problem	4	10	0.666
Kidney stone	1	1	
Typhoid	5	16	0.733
Jaundice	3	10	0.777
others	24	79	0.705

4.2.2 Fidelity level (Fl)

Fidelity level of each species has been calculated to assess important plant species in each category of disease. The Fidelity level (Fl) and use mention of the ethnomedicinal plants are shown in table 6.

Table 6: Fidelity level of ethnomedicinal plants

Scientific name	**disease**	UM	Fl	**Scientific name**	**disease**	UM	Fl
Acacia catechu	cough	1	20	*Alternenthera sessilis*	Menstrual problem	3	100
	diarrhea	2	40				
	rheumatic pain	2	40	*Anaphalis adnata*	Cuts and wounds	2	100
	Eye problem	2	66				
Achyranthes aspera	Kidney stone	1	33	*Angiopterishelferiana*	cholera	1	100
Acorus calamus	cough	3	100	*Antidesma acidum*	cough	4	66
Acrocarpus fracxinifolius	rhumatism paralisis	2	100		fever	1	25
					mumps	1	25
Aegle marmelos	Cough	6	66	*Ariopsis peltata*	fracture, muscular sprain	6	100
	fever	2	22				
	Heat sickness	1	11				
Ageratina adenophora	Cuts and wounds	4	100	*Artemisia indica*	gastrities	3	100
Ageratum conyzoides	Cuts and wounds	5	100	*Artocarpus lakoocha*	gastrities	2	100
Alstonia scholar	Infertility in cattle	2	100	*Azadirachta indica*	boils	6	100

Table 6: Fidelity level of ethnomedicinal plants (contd…)

Scientific name	dissease	UM	FL	Scientific name	disease	UM	Fl
Phanera vahlii	Menstrual problem	6	37	*Cynoglossum lanceolatum*	Eye problem	3	100
	diarrhea	10	63	*Cynoglossum zeylanicum*	Eye problem	3	100
Bergenia ciliata	diarrhea	6	100	*Cyperus rotundus*	Expelsion very fine bone of dead snake	2	100
Boehmeria platyphylla	Cuts & wounds	3	100				
Boenninghausenia albiflora	Remove germs from poultry	2	100	*Desmodium multiflorum*	Menstrual problem	3	100
				Dioscorea bulbifera	UTI	5	100
				Diploknema butyracea	scabies	7	100
Boerhavia diffusa	tonic	4	100	*Drymaria cordata*	sinusities	11	100
Bombax ceiba	cut	2	50	*Eclipta prostrata*	Heart, liver problem	4	100
	scabis	2	50				
Butea butiformis	anthelmintic	3	100	*Elephantopus scaber*	dysentery	2	40
Caesapinia decapetala	fracture, muscular sprain	4	100		diarrhea	3	60
				Euphorbia hirta	Eye problem	2	100
Callicarpa macrophylla	typhoid	4	66	*Ficus auriculata*	Cut, wounds	3	100
				Ficus hispida	cooling	4	100
	Tonsilities, sore throat,rashes on tongue	2	34	*Ficus racemosa*	fever	2	100
				Flemingia strobilifera	desentery	3	16
Calotropis gigantea	toothache				gastritis	2	11
	muscular sprain				ulcer	2	11
Cassia fistula	UTI	3	100		diarrhea	11	61
Catunaregam spinosa	diarrhea	2	66	*Fraxinus floribunda*	jaundice	2	100
	dysentery	1	33				
				Holmskiodia sanguinea	dysentery	2	100
Centella asiatica	typhoid	3	100	*Hypericum cordifolium*	gastritis	1	33
Cheilanthes. dalhousiae	gastrities	4	100				
Chenopodium album	strenthening uterus	3	100		dysentery	1	33
Chromolaena odorata	Cuts & wounds	2	100				
Cinnamomum tamala	dysentery	4	100		indigestion	1	33
Cissampelos pareira	UTI	6	100				
Citrus paradisi	menstruation	4	100	*Imperata cylindrica*	anthelmintic	2	100
Clematis buchananiana	cuts	7	100	*Indigofera cassioides*	hooping cough	3	100
Colebrookea oppositifolia	sinusities	2	100	*Justicia adhatoda*	fever	5	100
Cryptolepis buchananii	boils	3	100	*Kaempferia rotunda*	Fracture, sprain	8	100
Curcuma caesia	gastrities	7	100	*Kalanchoe integra*	burn	3	100
Curcuma longa	allergy	5	100	*Lannea coromandelica*	fill scar of cut	3	100
Cusculta reflexa	jaundice	6	100	*Lagerstroemia parviflora*	Diarrhea	5	71
					Menstruation problem	2	29
Cyathula tomentosa	diarhea	3	100	*Leea macrophylla*	prevent miscarriage	3	100
Cynodon dactylon	nutrition	3	100	*Lens culinaris*	Controlectoparasites on cattle	2	100

Table 6: Fidelity level of ethnomedicinal plants (contd…)

Scientific name	dissease	UM	FL	Scientific name	disease	UM	Fl
Lobelia nicotianifolia	Menstrual problem	2	100	*Rauvolfia serpentina*	Controlling high blood pressure	3	100
Lygodium japonicum	Lactation on cattle	2	100	*Reinwardtia indica*	boils	3	100
Lyonia ovalifolia	scabies	3	100	*Rhododendron arboreum*	UTI	2	33
Mahonia napaulensis	Streenthening teeth gum	3	100		diarrhea	2	33
Mallotus philippensis	gastrities	6	100		desentery	2	33
Mangifera indica	gastrities	2	50	*Rhus chinensis*	dysntery	13	100
	dysentery	1	25	*Rubus niveus*	pneumonia	2	100
	gout	1	25	*Rumex hastatus*	sinusities	4	100
Melia azedarach	anthelmintic	2	100	*Saraca asoca*	Menstrual problem	5	100
Mimosa pudica	piles	2	100	*Schima wallichii*	Tuberculosis	2	50
Mirbilis jalapa	Menstrual problem	4	100		menstrual problem	2	50
Moringa oleifera	anthelmintic	2	100	*Shorea robusta*	diarrhea	3	100
Musa paradisica	bloody dysentery	3	100	*Solanum nigrum*	headache	4	100
				Solanum torvum	common cold	6	100
Myrica esculenta	miscarriage	3	100	*Stephania glandulifera*	gastritis	10	100
Nephrolepis cordifolia	cooling	2	100	*Syzygium nervosum*	cold	1	100
Nymphaea lotus	jaundice	2	100	*Taraxacum officinale*	scabies	2	100
Ocimum basilicum	UTI	2	100	*Tectaria coadunata*	diarrhea	12	100
	cough	2	50	*Terminalia alata*	typhoid	2	100
Ocimum tenuiflorum	cold	2	50	*Terminalia anogeissiana*	Fracture and muscular sprain	2	100
Opuntia monacantha	fever	2	66	*Terminalia bellirica*	cough	6	100
	heat scikness	1	33	*Terminalia chebula*	cough	8	100
Oroxylum indicum	food poisoning	4	100	*Thalictrum foliolosum*	rheumatic pain	2	100
Phyllanthus emblica	blood purifier	7	100	*Thespesia lampas*	sinusities	2	100
Phyllanthus parvifolius	Menstrual problem	3	100	*Thysanolaena latifolia*	boils	6	100
Plumeria rubra	food poisoning	3	100	*Tinospora sinensis*	UTI	9	100
				Urtica dioica	gastritis	3	43
Pogostemon benghalensis	pneumonia	4	57		UTI	4	57
	cold	3	43	*Vitex negundo*	pneumonia	1	33
	headache	3	50		cough	2	55
Premna barbata	typhoid	3	50	*Woodfordia fruticosa*	diarrhea	15	65
Prunus cerasoides	burn	7	100	*Z.ingiber montanum*	gastrities	4	100
	Diarrhea	6	60	*Zingiber officinale*	common cold	12	100
Psidium guajava	desentery	4	40	*Z.iziphus jujuba*	measles	2	100
Quercus lanata	Food poisoning	3	100				

UM= use mention; Fl= fidelity level

The categories gastrointestinal, respiratory, UTI and typhoid have been analyzed to highlight the most important plants in each category. For this analysis, the plants with fedality level more than 60 combine with use mentioned more than 3, are considered. For the gastrointestinal category, the most important species, according to fidelity and

use mention are *Phanera vahlii* (Fl=63, 10), *Bergenia ciliate* (Fl=100, 6), *Cheilanthes dalhousiae* (Fl=100, 4), *Curcuma caesia* (Fl=100, 4), *Flemingia strobilifera* (Fl=100, 18), *Lagerstroemia parviflora* (Fl=71, 5), *Mallotus philippensis* (Fl=100, 6), *Musa paradisica* (Fl=100, 13), *Stephania glandulifera* (Fl=100, 10), *Tectaria coadunata* (Fl=100, 12), *Woodfordia fruticosa* (Fl=100, 28), *Zingiber montanum* (Fl=100, 4), *Rhododendron arboreum* (Fl=100, 8) and *Rhus chinensis* (Fl=100, 21). But only *Phanera vahlii, Flemingia strobilifera, Lagerstroemia parviflora*, *Stephania glandulifera, Tectaria coadunata, Woodfordia fruticosa, Rhododendron arboreum and Rhus chinensis* have been selected for laboratory tests.

For UTI category, the most important species according to fidelity (more than 60) and use mention (more than 3) are *Tinospora sinensis* (FL=100, 9), *Cissampelos pareira* (Fl=100, 6) and. *Azadirachta indica* (Fl=67, 6). For the respiratory category, the most important species, according to fidelity and use mention are *Antidesma acidum* (Fl=66, 4) and *Aegle marmelos* (Fl=66, 6). For typhoid the most important species was *Callicarpa macrophylla* (Fl=66, 4). *Thalictrum foliolosum* (Fl=100, 2) is selected due to request of the local people to do laboratory tests. Use mention of *Thalictrum foliolosum* was two for rheumatic pain and reported to complete relief from pain. Though the medicinal use reporte is not under the infectious disease it was selected for antimicrobial test on their request.

List of selected plant species: *Aegle marmelos* (L.) Correa, *Antidesma acidum* Retzius, *Azadirachta indica* A.Juss., *Phanera vahlii* Wight &Arn,, *Callicarpa macrophylla* Vahl., *Cissampelos pareira L., Flemingia strobilifera* (L.) R.Br., *Lagerstroemia parviflora* Roxb., *Rhododendron arboreum* Sm., *Rhus chinensis* Mill., *Stephania glandulifera* Miers., *Tectaria coadunata* (J.Sm.) C. Chr. , *Thalictrum foliolosum* DC, *Tinospora sinensis* (Lour.) Merr and *Woodfordia fruticosa* Kurz. Short description and synonyms of these plants are given in Annex ID.

4.3 The Plant Extract

The coarsely ground plant materials (100 g each) were successively extracted with hexane and 70% methanol using a series of Soxhlet extractions. Thus obtained hexane and 70% methanolic extracts were concentrated and vacuum dried to yield corresponding extracts (Table 7). Maximum extract yield was obtained from the 70%

methanolic extract of *Phanera vahlii* roots (38%) and minimum yield was noted in the hexane extract from the rhizome of *Tectaria coadunata* (0.2%).

Table 7: Yield of plant extracts

S. N.	Plant species	Plant parts used	Hexane extract		70% Methanolic extract	
			Yield (%)	Colour	Yield (%)	Colour
1	*Aegle marmelos* (L.) Correa	Bark	2.7	Yellowish brown	8.2	Reddish brown
2	*Antidesma acidum* Retzius	Bark	0.5	Greenish	7.5	Brownish
3	*Azadirachta indica* A. Juss	Leaf	2.1	Dark	17.6	Black
4	*Phanera vahlii* Wight & Arn	Root	0.4	Colourless	38.0	Dark red
5	*Callicarpa macrophylla* Vahl	Root	0.6	Pale yellow	15.3	Reddish brown
6	*Callicarpa macrophylla* Vahl	Leaf	3.6	Yellowish	26.0	Dark brown
7	*Cissampelos pareira* L	Whole plant	0.4	Dark green	17.4	Reddish black
8	*Flemingia strobilifera* (L.) R.Br.	Root	0.4	Light pale	10.5	Rock salt
9	*Lagerstroemia parviflora* Roxb.	Bark	0.6	Light pale	17.7	Dark red
10	*Rhododendron arboreum* Sm.	Bark	0.6	Pale white	15.6	Rock salt
11	*Rhus chinensis* Mill. (syn. *Rhus chinensis* var. *chinensis* (Mill.) T.Yamaz.)	Fruit	3.7	Light yellowish	11.5	Reddish brown
12	*Stephania glandulifera* Miers	Tuber	2.3	Yellowish	8.7	Yellowish
13	*Tectaria coadunata* (J.Sm.) C. Chr.	rhizome	0.2	Yellowish	26.3	Reddish black
14	*Thalictrum foliolosum* DC	Root	1.4	Yellowish	11.7	Yellowish brown
15	*Tinospora sinensis* (Lour.) Merr.	Stem	0.5	Greenish	9.7	Dark brown
16	*Woodfordia fruticosa* Kurz	Flower	0.7	Light brown	21.3	Coffee

4.4 Phytochemical Screening

Phytochemical screening of the 70% methanolic extracts of 15 different plant species is presented in Table 8. The names of the tests followed for the detection of phytochemicals are also given. Out of 124 medicinal plant species 16 methanolic extracts of 15 species were taken which have been abbreviated as AMM (*Aegle marmelos*), AAM (*Antidesma acidum*), AIM (*Azadirachta indica*), PVM (*phanera vahlii*), CMMR (*Callicarpa macrophylla*, root), CMML (*Callicarpa macrophylla*, leaf), CPM (*Cissampelos pareira*), FSM (*Flemingia strobilifera*), LPM (*Lagerstroemia parviflora*), RAM (*Rhododendron arboreum*), RCM (*Rhus chinensis*), SGM (*Stephania glandulifera*), TCM (*Tectaria coadunata*), TFM (*Thalictrum foliolosum*), TSM (*Tinospora sinensis*) and WFM (*Woodfordia fruticosa*).

Phytochemical screening of the hexane extracts of different plant species is given in Table 9. The names of the tests followed for the detection of phytochemicals are also given. The hexane extracts of the plant materials are abbreviated as AMH (*Aegle marmelos*), AAH (*Antidesma acidum*), AIH (*Azadirachta indica*), PVH (*Phanera vahlii*), CMHR (*Callicarpa macrophylla*, root), CMHL (*Callicarpa macrophylla*, leaf), CPH (*Cissampelos pareira*), FSH (*Flemingia strobilifera*), LPH (*Lagerstroemia parviflora*), RAH (*Rhododendron arboreum*), RCH (*Rhus chinensis*), SGH (*Stephania glandulifera*), TCH (*Tectaria coadunata*), TFH (*Thalictrum foliolosum*), TSH (*Tinospora sinensis*) and WFH (*Woodfordia fruticosa*).

The result of phytochemical screening showed that AMM contained Tannin, polyphenols and alkaloids and AMH contained alkaloids, steroids and terpenoids. Presence of alkaloids, phenolics, tannins was reported in various part of *Aegle marmelos* previously (Baliga et al., 2011; Rahman & Parvin, 2014; Mathew et al., 2016).

The result of phytochemical screening showed thart AAM contained tannin, polyphenols and alkaloids and AAH contained steroids. Patil and Jadhav (2014) reported presence of phenols, tannins, alkaloids in leaf extract of *Antidesma acidum*.

AIM contained flavonoid and AIH contained steroid, terpenoid and coumarin. Phytochemicals such as tannins, flavonoids, terpenes in the leaves extract are reported previously (Gupta et al., 2013).

PVM contained tannins, polyphenol and alkaloids and PVH contained steroid and terpenoids. Sowndhararajan and Kang (2013) showed higher amount of phenolics, tannins and flavonoids in leaf extract of *Phanera vahlii*.

CMMR andCMML contained tannin, poliphenol, flavonooids and no alkaloid, CMHR and CMML contained steroid and terpenoid. Similarly Patel et al. (2016) reported presence of flavonoids, tannins, steroids, triterpenes, but not alkaloids, in the leaves and bark of *Callicarpa macrophylla*.

CPM and CPH contained alkaloid and steroid. Ngoci et al. (2014) also reported presence of alkaloid in *Cissampelos pareirea*.

FSM and FSH extract showed presence of terpenoid, saponin, tannin, steroids, glycosides, phenolics, alkaloid and flavonoids. Tikadar et al. (2017) showed presence of thses phytochemicals in *Flemingia strbilifera*. The result of phytochemical

screening of *Flemingia strbilifera* (Nemkul et al., 2019) is comparable to the previous finding (Tikader et al., 2017).

Anthocyanoides was detected only in LPM and TCM. Flavonoids were detected in AIM, CMML, RCM and WFM. Saponins were detected in five extracts. Anthracenosides, cardiac glycosides, proteins and amino acids were not detected in the 70% methanolic extract of any of the species. Alkaloids were not detected in CMMR, CMML, LPM, TCM and WFM. Patel et al. (2016) reported absence of alkaloid in methanolic, petroleum ether and aqueous extract of the leaf and bark of *Callicarpa macrophylla.* Bobade et al. (2013) reported presence of alkaloids in the aqueous and ethanolic extracts of the leaves of *Largestroemia parviflora.* But, phytochemicals of methanolic extract of *Largerstroemia parviflora* bark is not reported yet. Other researchers have also reported absence and/or presence of very minute amount of alkaloids in the extracts of *Tectaria coadunata* (Harborne, 2007; Sukumaran et al., 2012; Poudyali, 2013). Harborne (2007) reported that alkaloids was absent and/or infrequently found in pteridophytic plants or ferns. Phytochemicals such as flavonoids, triterpenoids, phenolics, tannins, aromatic alkanes are reported (Djakpo & Yao, 2010) in different parts of the *Rhus chinensis*.

The extract of bark of *Rhododendron arboreum* showed presence of Tanins, Polyphenol, alkaloids, saponins, steroids, terpenoids and absence of flavonoids, anthraquinones and glycosides. Kumar et al. (2019b) also reported presence of alkaloids, steroids, terpenoids, tannins and saponins and absence of flavonoids, anthraquinones and glycosides in bark of *Rhododendron arboreum.*

The extract of fruit of *Rhus chinensis* revealed presence of terpenoids, polyphenols and flavonoids.

SGM showed presence of alkaloid and SGH showed presence of steroids, terpenoids together with alkaloids. Similarly, Semwal et al. (2010) reported that alkaloids are the main and common phytochemicals of the genus *Stephania* and 200 alkaloids have been isolated from this genus.

TFM was found to be contained alkaloids, tannins, polyphenols and saponins. *Thalictrum* species have been known to contain a variety of alkaloids of chemical and biological interest (Chattopadhyay et al**.,** 1981; Bhakuni & Singh, 1982). Pandey et al. (2017) reported that in the roots of *Thalictrum foliolosum*, alkaloid known as

berberine content varied inversely with altitude. Lower the elevation higher was the presence of the alkaloid, berberine.

Tannins, polyphenols, alkaloids and sapponins were detected in TSM. Similarly alkaloids, phenolics, tannins and saponins were reported in various extracts of leaves and stem of *Tinospora sinensis* (Jain et al., 2010; Vijaya & Aruna, 2014; Hegde et al., 2015) previously.

The result showed that WFM contains tannins, polyphenols and flavonoids; but, alkaloid was not detected. Similarly, Grover and Patni (2013) reported that alkaloids were not found in the methanol, ethyl acetate, benzene, ethanol and chloroform extracts of the leaves and flower of *Woodfordia fruticosa*. Grover and Patni (2013) reported presence of tannins and terpenoid predominantly followed by flavonoids in the extracts of *Woodfordia fruticosa*. The flowers of *Woodfordia fruticosa* possess high content of tannins (Kumar et al., 2013).

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Table 8: Phytochemical screening of the 70% methanolic extracts

70% methanolic extract of the Plant species	Tanins & Polyphenol (Braymer Test)	Alkaloids (Dragendroff test)	Anthocyanosides (Sodium chloride test)	Anthracenosides (Ammonia test)	Flavonoids (Shinoda test)	Cardiac glycosides (Kedde's test)	Saponin (Froth test)	Protein & amino acid (Biuret test)
Aegle marmelos	+	+	–	–	–	–	–	–
Antidesma acidum	+	+	ND	ND	ND	ND	ND	ND
Azadirachta indica	ND	–	ND	ND	+	ND	ND	ND
Phanera vahlii	+	+	ND	ND	ND	ND	ND	ND
Callicarpa macrophylla root	+	ND	ND	ND	ND	ND	ND	ND
Callicarpa macrophyla leaf	+	ND	ND	ND	+	ND	ND	ND
Cissampelos pareira	ND	+	ND	ND	ND	ND	ND	ND
Flemingia strobilifera	+	-	-	-	-	-	-	-
Lagerstroemia parviflora	+	ND	+	ND	ND	ND	+	ND
Rhododendron arboreum	+	+	ND	ND	ND	ND	+	ND
Rhus chinensis	+	-	-	-	+	-	-	-
Stephania glandulifera	ND	+	ND	ND	ND	ND	ND	ND
Tectaria coadunata	+	ND	+	ND	ND	ND	+	ND
Thalictrum foliolosum	+	+	ND	ND	ND	ND	+	ND
Tinosppora sinensis	+	+	ND	ND	ND	ND	+	ND
Woodfordia fruticosa	+	ND	ND	ND	+	ND	ND	ND

'+'=present; '-'=absent; ND =not detected

Table 9: Phytochemical screening of the hexane extracts

Hexane extract of the plant species Plant extracts	Alkaloids (Dragendroff test)	Caretenoids (Sulphuric acid test)	Steroids (Liebermann-Burchard test)	Terpenoids (Salkowski test)	Coumarins (Flurescence test)	Anthraquinone (Borntiager test)
Aegle marmelos	+	ND	+	+	ND	ND
Antidesma acidum	ND	ND	+	ND	ND	ND
Azadirachta indica	−	ND	+	+	+	ND
Phanera vahlii	ND	ND	+	+	ND	ND
Callicarpa macrophylla root	ND	ND	+	+	ND	ND
Callicarpa macrophyla leaf	ND	ND	+	+	ND	ND
Cissampelos pareira	+	ND	+	ND	ND	ND
Flemingia strobilifera	ND	ND	+	+	ND	ND
Lagerstroemia parviflora	ND	−	+	+	ND	ND
Rhododendron arboreum	ND	ND	+	+	ND	ND
Rhus chinensis	ND	ND	ND	+	ND	ND
Stephania glandulifera	+	ND	+	+	ND	ND
Tectaria coadunata	ND	ND	+	+	ND	ND
Thalictrum foliolosum	+	ND	ND	ND	ND	ND
Tinosppora sinensis	ND	ND	+	+	ND	ND
Woodfordia fruticosa	ND	ND	+	+	ND	ND

‘+’=present; ‘-’=absent; ND=not detected

4. 5 GC-MS Analysis

The result of GC-MS analysis of 16 methanolic (70%) extracts and 16 hexane extracts of different plant materials are presented below.

As can be seen in Table 10, 70% methanolic extract of *Aegle marmelos* barks mainly constituted steroids *viz*. 3-deoxyestradiol (1.05%), ethyl isoallocholate (0.44%), γ-sitosterol (71.19%), sitostenone (10.14%) and cholest-1-eno[2,1-α]naphthalene, 3',4'-dihydro (0.90%) accounting 83.72% of the total extract. The mass spectrum obtained for γ-sitosterol in GC-MS analysis was carefully compared with the available library spectra of β-sitosterol and γ-sitosterol. A peak appeared at m/z 303 in the spectrum of γ-sitosterol was comparably intense leading us to identify the constituent. Presence of higher percentage of γ-Sitosterol in 70% methanolic extract of bark of *Aegle marmelos* was reported (Maity et al., 2009; Vijayalakshmi & Venkatalakshmi, 2017; Nemkul et al., 2018a). γ-Sitosterol exhibits strong antifungal, antibacterial, antidiarrheal, antiviral and anti-angiogenic activities (Raman et al., 2012). It is being used in traditional medicine to treat ulcers, bronchitis, diabetes and heart diseases. 2-Methoxy-4-vinylphenol exhibits antioxidant, antimicrobial and anti-inflammatory activities (Al-Marzoqi et al., 2016).

Table 10: Phytoconstituents identified from the 70% methanolic extract of *Aegle marmelos* barks (AMM)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	21.116	2-Methoxy-4-vinylphenol	1.26	Phenol
2	28.925	1-(3,5-dimethoxyphenyl)ethan-1-one	2.41	Ketone
3	33.766	(E)-4-(3-hydroxyprop-1-en-1-yl)-2-methoxyphenol	0.38	Phenol
4	39.349	3-Deoxyestradiol	1.05	Steroid
5	42.498	Methyl 7,10,13-hexadecatrienoate	0.34	Ester
6	43.369	Oleic acid	0.76	Fatty acid
7	46.769	Ethyl isoallocholate	0.44	Steroid
8	49.349	γ-Sitosterol	71.19	Steroid
9	58.226	Sitostenone	10.14	Steroid
10	60.892	Cholest-1-eno[2,1α]naphthalene, 3′,4′-dihydro-	0.90	Steroid
		Total	**88.87**	

As indicated in Table 11, the hexane extract of *Aegle marmelos* constituted a number of fatty acids. Out of 15 compounds identified, seven are free fatty acids, three of them unsaturated, and three are fatty acids methyl esters accounting 29.28% of the

total extract. The presence of palmitic acid (10.48%), stearic acid (6.34%) as well as 3-pentadecylphenol (4.59%) may be responsible for the antibacterial activity of the extract (Devakumar et al., 2017; Agoramoorthy et al., 2007). Alkanes such as tetracosane (6.61%) and heptacosane (9.78%) were also identified as major ingredients. Tetracosane was reported as antimicrobial (Devakumar et al., 2017).

Table 11: Phytoconstituents identified from the hexane extract of *Aegle marmelos* barks (AMH)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	34.399	Tetradecanoic acid	0.27	Fatty acid
2	38.457	Methyl palmitate	0.69	Ester
3	39.439	Palmitic acid	10.48	Fatty acid
4	40.672	Octadecanal	0.57	Ketone
5	42.352	Methyl octadeca-9,12-dienoate	0.36	Ester
6	43.122	Methyl 16-methylheptadecanoate	0.40	Ester
7	43.280	Octadeca-9,12-dienoic acid	2.60	Fatty acid
8	43.411	Vaccenic acid	5.08	Fatty acid
9	43.716	Octadec-13-enoic acid	2.46	Fatty acid
10	43.967	Stearic acid	6.34	Fatty acid
11	48.146	Eicosanoic acid	0.60	Fatty acid
12	49.035	2-Hydroxycyclopentadecan-1-one	0.93	Ketone
13	50.628	3-Pentadecylphenol	4.59	Phenol
14	54.463	Tetracosane	6.61	Alkane
15	57.916	Heptacosane	9.78	Alkane
		Total	**51.76**	

As shown in Table 12, 70% methanolic extract of bark of *Antidesma acidum* constituted mainly aldehyde *viz.* 5-(hydroxymethyl)-2-furancarboxaldehyde accounting 28.17% of the total extract. 1,3-Dihydroxy-2-propanone (21.76%), flavonoid (10.15%) and sugar (13.36%) were also identified as major ingredients. 5-(Hydroxymethyl)-2-furancarbaldehyde exhibits antimicrobial activity (Jadhav et al., 2014).

Table 12: Phytoconstituents identified from 70% methanolic extract of *Antidesma acidum* (AAM)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	5.540	2-Furanmethanol	2.86	Alcohol
2	6.631	1,3-Dihydroxy-2-propanone	21.76	ketone
3	15.267	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	10.15	Flavonoid fraction
4	18.426	5-(Hydroxymethyl)-2-furancarboxaldehyde	28.17	Aldehyde
5	25.61	Sugar	13.36	Sugar
		Total	76.3	

As shown in Table 13, hexane extract of bark of *Antidesma acidum* constituted mainly fatty acids *viz*. 9,12-Octadecadienoic acid (Z,Z) (20.34%), n-hexadecanoic acid (16.91%), octadecanoic acid (5.30%), heptadecanoic acid (1.15%), hexadecanoic, methyl ester (0.7%) and eicosanoic acid (0.58%) accounting 44.98% of the total extract. The other major compound was stigmasterol (8.08%). Stigmasterol exhibits antiviral, anti-inflammatory and inhibit tumor promotion activities (Raman et al., 2012). 9,12-Octadecadienoic acid (Z,Z) exhibits hepatoprotective, antihistaminic, antieczemic, antiacne, antiarthritic, anticoronary, cancer preventive, anti-inflammatory and hypocholesterolemic (Duke, 1992; Alagammal et al., 2011).

Table 13: Phytoconstituents identified from the hexane extract of *Antidesma acidum* bark (AAH)

S. N.	R. T.	Compound name	Peak area%	Compound nature
1	4.471	Heptane, 2,4-dimethyl-	0.41	Alkane
2	38.436	Hexadecanoic, methyl ester	0.70	Fatty acid
3	39.401	n-Hexadecanoic acid	16.91	Fatty acid
4	41.649	Heptadecanoic acid	1.15	Fatty acid
5	43.394	9,12-Octadecadienoic acid, (Z,Z)-	20.34	Fatty acid
6	43.918	Octadecanoic acid	5.30	Fatty acid
7	48.108	Eicosanoic acid	0.58	Fatty acid
8	64.534	Stigmasterol	8.08	Steroid
		Total	53.47	

As shown in Table 14, the result of 70% methanolic extract of *Azadirachta indica* leaf constituted phenols (14.6%), fatty acids (15.09%), diterpenes (6.13%) and flavonoid fraction (2.72%). Phytol exhibits antimicrobial, anticancer, anti-inflammatory and diuretic activities (Duke, 1992).

Table 14: Phytoconstituents identified from 70% methanolic extract of *Azadirachta indica* leaf (AIM)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	15.234	3,5-Dihydro-6-methyl-2,3-dihydro-4H-pyran-4-one	2.72	Flavonoid fraction
2	21.109	2-Methoxy-4-vinylphenol	8.01	Phenol
3	22.364	2,6-dimethoxy phenol	6.05	Phenol
4	38.446	Hexadecanoic, methyl ester	5.55	Fatty acid
5	39.314	n-Hexadecanoic acid	9.54	Fatty acid
6	42.800	Phytol	6.13	Diterpene
		Total	38.00	

As indicated in Table 15, the hexane extract of *Azadirachta indica* constituted a number of fatty acids. Out of 13 compounds identified, six are fatty acids; two of

them are alcohols, two alkanes, one triterpene, one vitamin and one steroid. 2(4H)-Benzofuranone 5,6,7,7a tetrahydro-4,4,7a-trimethyl and vitamin E exhibit antimicrobial activity (Mujeeb et al., 2014). 9, 12-Octadecadienoic acid (Z,Z), 9,12,15-octadecatrien-1-ol, (Z,Z,Z) and oleic acid exhibit anti-inflammatory activity (Alagammal et al., 2011). Phytol exhibits antimicrobial, anticancer, anti-inflammatory and diuretic activities (Duke, 1992).

Table 15: Phytoconstituents identified from the hexane extract of *Azadirachta indica* leaf (AIH)

S. N.	R. T.	Compound name	Peak area%	Compound nature
1	27.743	2(4H)-Benzofuranone, 5,6,7,7α-tetrahydro-4,4,7α-trimethyl-	0.81	Triterpene
2	36.270	3,7,11,15-Tetramethyl 2-hexadecen-1-ol	3.32	Alcohol
3	39.352	n-Hexadecanoic acid	11.27	Fatty acid
4	40.078	Hexadecanoic acid, ethyl ester	0.43	Fatty acid
5	42.811	Phytol	2.71	Alcohol
6	43.220	9,12-Octadecadienoic acid, (Z,Z)-	1.10	Fatty acid
7	43.367	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	12.38	Fatty acid
8	43.651	Oleic acid	0.61	Fatty acid
9	43.891	Octadecanoic acid	1.29	Fatty acid
10	57.889	Nonacosane	5.83	Alkane
11	61.315	Eicosane	11.09	Alkane
12	61.921	Vitamin E	4.82	Vitamin
13	64.523	Stigmastane	8.16	Steroid
		Total	**63.82**	

As shown in Table 16, nine compounds were identified from the 70% methanolic extract of *Phanera vahlii.* The major constituent was pyridine accounting 35.98% of total content. As a flavonoid fragment, the extract constituted of 1.56% of 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, which was reported as an antimicrobial and anti-inflammatory ingredient (Kumar et al., 2010), anti-inflammatory and antiproliferative (Duke, 1992).

Table 16: Phytoconstituents identified from 70% methanolic extract of *Phanera vahlii* (PVM)

S. N.	R. T.	Compound Name	Peak area%	Compound Nature
1	3.074	Pyridine	35.98	Heterocycle
2	15.256	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	1.56	Flavonoid fraction
3	18.196	2,3-Dihydrobenzofuran	1.98	Benzofuran
4	22.397	2,6-Dihydroxy phenol	1.42	Phenol
5	31.671	2,4,6-Trihydroxybenzoic acid	0.20	Benzoic acid

6	31.922	1,3,5-Benzentriol	0.26	Alcohol
7	38.441	Hexadecanoic acid, methyl ester	1.36	Fatty acid
8	39.309	n-Hexadecanoic acid	1.70	Fatty acid
9	61.577	Silicic acid, diethyl bis(trimethylsilyl) ester	0.30	Silicic acid ester
		Total	44.76	

GC-MS analysis of the hexane extract of *Phanera vahlii* led to identify 33 compounds accounting 91.03% of the total constituents (Table 17). Out of 34 compounds, 19 linear alkylbenzene (42.31%), 6 alkanes (1.76%), three fatty acids (30.65%), one acid ester (11.37%), two phthalate ester (0.81%), two steroids (4.03%) and one flavonoid (0.1%) were identified. n-Hexadecanoic acid was reported as antioxidant and hypocholesterolemic (Kumar et al., 2010). 9,12-Octadecadienoic acid (Z,Z) was reported as hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, antiandrogenic, antiarthritic, anticoronary, insectifuge, cancer preventive anti-inflammatory and hypocholesterolemic (Alagammal et al., 2011). Stigmasterol was reported as antimicrobial, antioxidant and anti-inflammatory (Mujeeb et al., 2014).

Table 17: Phytoconstituents identified from the hexane extract of *Phanera vahlii* (PVH)

S. N.	R. T.	Compound name	Peak area%	Compound nature
1	23.902	Tetradecane	0.16	Alkane
2	26.974	Pentadecane	0.12	Alkane
3	28.054	Benzene, (1-butylhexyl)-	1.27	Linear alkylbenzene
4	28.294	Benzene,(1-propylheptyl)-	1.07	Linear alkylbenzene
5	28.807	Benzene,(1-ethyloctyl)-	1.06	Linear alkylbenzene
6	29.756	Diethyl phthalate	0.37	Phthalate ester
7	29.843	Benzene, (methylnonyl)-	1.47	Linear alkylbenzene
8	30.765	Benzene, (1-pentylhexyl)-	1.83	Linear alkylbenzene
9	30.863	Benzene, (1-butylheptyl)-	3.46	Linear alkylbenzene
10	31.131	Benzene, (1-propyloctyl)-	2.75	Linear alkylbenzene
11	31.676	Benzene, (1-ethylnonyl)-	2.49	Linear alkylbenzene
12	32.675	Benzene, (1-methyldecyl)-	3.21	Linear alkylbenzene
13	33.427	Benzene, (1-pentylheptyl)-	3.22	Linear alkylbenzene
14	33.547	Benzene, (1-butyloctyl)-	3.07	Linear alkylbenzene
15	33.858	Benzene, (1-propylnonyl)-	2.37	Linear alkylbenzene
16	34.404	Benzene, (1-ethyldecyl)-	2.22	Linear alkylbenzene
17	35.391	Benzene, (1-methylundecyl)-	2.51	Linear alkylbenzene
18	35.986	Benzene, (1-pentyloctyl)-	3.28	Linear alkylbenzene
19	36.155	Benzene, (1-butylnonyl)-	2.11	Linear alkylbenzene
20	36.455	Benzene, (1-propyldecyl)-	1.55	Linear alkylbenzene

21	37.017	Benzene, (1-ethylundecyl)-	1.69	Linear alkylbenzene
22	37.792	Tetradecane, 2, 6, 10-trimethyl	0.24	Alkane
23	37.972	Benzene, (1-methyldodecyl)-	1.68	Linear alkylbenzene
24	38.239	7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	0.10	Flavonoid
25	39.286	Butyl octyl phthalate	0.44	Ester
26	39.521	n-Hexadecanoic acid	10.84	Fatty acid
27	40.187	Estra-1,3,5(10)-trien-17β-ol	0.25	Alkane
28	42.478	Heneicosane	0.36	Alkane
29	43.400	9,12-Octadecadienoic acid, (Z,Z)-	19.81	Fatty acid
30	48.817	β-Sitostenone	1.35	Steroid
31	51.681	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	11.37	Aacid ester
32	54.452	Heptacosane	0.63	Alkane
33	64.637	Stigmasterol	2.68	Steroid
		Total	91.03	

As shown in Table 18, four compounds were identified from 70% methanolic extract of *Callicarpa macrophylla.* The major constituent was lupeol (76.69%). Lupeol was reported as having anti-inflammatory activity and as being non-toxic to normal cells could be used as a chemopreventive as well chemotherapeutic agent against skin cancer (Mohammad, 2009). Another compound, 2-methoxy-4-vinylphenol was reported as antioxidant and anti-inflammatory (Hameed et al., 2015) and antimicrobial (Al-Marzoqi et al., 2016).

Table 18: Phytoconstituents identified from 70% methanolic extract of *Callicarpa macrophylla* leaf (CMML)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	21.110	2-Methoxy-4-vinylphenol	2.01	Phenol
2	39.314	n-Hexadecanoic acid	1.36	Fatty acid
3	54.851	Lupeol	76.69	Triterpenoid
4	56.454	1-Undecane 3,6-dione,1-(2,6,6-trimethyl)1-cyclohexane-1-yl-	15.25	Ketone
		Total	95.31	

As indicated in Table 19, only one compound 1-undecane-3,6-dione,1-(2,6,6-trimethyl-1-cyclohexen-1-yl) could be identified.

Table 19: Phytoconstituents identified from the hexane extract of *Callicarpa macrophylla* leaf (CMHL)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	56.482	1-Undecane-3,6-dione,1-(2,6,6-trimethyl -1- cyclohexen-1-yl)-	90.04	Ketone

As shown in Table 20, nine compounds were identified from the 70% methanolic extract of *Callicarpa macrophylla* root accounting 60.74% of the total extract. 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one identified in the ectract was reported as an antimicrobial and anti-inflammatory ingredient (Kumar et al., 2010), and also anti-inflammatory and antiproliferative (Duke, 1992). 2-Methoxy-4-vinylphenol was reported as antioxidant and anti-inflammatory (Hameed et al., 2015). n-Hexadecanoic acid was reported as antioxidant and hypocholesterolemic (Kumar et al., 2010).

Table 20: Phytoconstituents identified from 70% methanolic extract of *Callicarpa macrophylla* root (CMMR)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	6.642	2-Propanone,1,3-dihydroxy-	6.21	Ketone
2	13.183	4,5-Diamino-2-hydroxypyrimidine	2.53	Pyrimidine
3	13.210	Pyrimidine-4,6-diol,5-methyl-	0.70	Pyrimidine
4	15.229	3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	2.56	Flavonoid fraction
5	21.098	2-Methoxy-4-vinylphenol	5.84	Phenol
6	22.359	Phenol 2,6-dimethoxy-	1.44	Phenol
7	25.495	1,3-Propanediol-2-ethyl-2(hydroxymethyl)-	32.08	Alcohol
8	29.292	n-Hexadecanoic acid	4.07	Fatty acid
9	51.643	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl)ester	5.31	Acid ester
		Total	60.74	

As shown in Table 21, nine compounds were identified from the hexane extract of *Cissampelos pareira*. Fatty acids were the main constituent accounting 41.63% of the total constituent.

Table 21: Phytoconstituents identified from the hexane extract of *Cissampelos pareira* (CPH)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	4.471	Heptane, 2,4-dimethyl-	1.50	Alkane
2	12.130	Decane, 3,7-dimethyl-	1.42	Alkane

3	38.436	Hexadecanoic acid, methyl ester	1.28	Fatty acid
4	39.330	n-Hexadecanoic acid	10.41	Fatty acid
5	42.320	9,12-Octadecanoic acid (Z,Z)-, methyl ester	1.70	Fatty acid
6	42.478	4,7,10-Hexaddecatrienoic acid, methyl ester	1.91	Fatty acid
7	43.225	9,12-Octadecadienoic acid, (Z,Z)-	24.53	Fatty acid
8	43.874	Octadecanoic acid	1.80	Fatty acid
9	52.467	2,4,7-trimethoxy-dibenz(a,c)cyclohexane	2.06	Aromatic hydrocarbon
		Total	46.61	

From the 70% methanolic extract of *Flemingia strobilifera*, five compounds were identified (Table 22). Phthalic andydride (36.62%), n-hexadecanoic acid (20.67%), 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (11.36%), 1-heptadecene (8.76%) and octadecanoic acid (5.28%) were the main constituents accounting 82.69% of the total constituents. 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one was reported to be antimicrobial agent (Kumar et al., 2010), anti-inflammatory and antiproliferative (Duke, 1992). Octadecanoic acid was reported to be antimicrobial (Mujeeb et al., 2014)

Table 22: Phytoconstituents identified from70 % methanolic extract of *Flemingia strobilifera* (FSM)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	15.234	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	11.36	Flavonoid fraction
2	21.033	Phthalic andydride	36.62	Anhydride
3	39.325	n-Hexadecanoic acid	20.67	Fatty acid
4	43.356	1-Heptadecene	8.76	Hydrocarbon
5	43.896	Octadecanoic acid	5.28	Fatty acid
		Total	82.69	

GC-MS analysis of the hexane extract of *Flemingia strobilifera* led to identify 27 compounds accounting 99.37% of the total constituents (Table 23). Out of 27 compounds, 19 hydrocarbons (60.74%), four fatty acids (13.18%), one acid ester (24.29%), one ester (0.55%), one alcohol (0.36%) and one ketone (0.25%) were identified. Octadecanoic acid was reported to be antimicrobial (Mujeeb et al., 2014). n-Hexadecanoic acid was reported to have antioxidant activity (Kumar et al., 2010). (Z,Z)-9,12-Octadecadienoic acid and oleic acid are cancer preventive and anti-inflammatory agents (Alagammal et al., 2011).

Table 23: Phytoconstituens identified from hexene extract of *Flemingia strobilifera* (FSH)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	26.962	4-(4-Methoxyphenyl)-2-butanone	0.25	Ketone
2	28.054	5-Phenylnonane	1.02	Hydrocarbon
3	28.299	4-Phenyldecane	0.88	Hydrocarbon
4	28.807	3-Phenyldecane	0.95	Hydrocarbon
5	29.843	2-Phenyldecane	1.43	Hydrocarbon
6	30.580	1,4α-Dimethyl-7-(propan-2-ylidene)decahydronaphthalen-1-ol; Juniper camphor	0.36	Alcohol
7	30.760	6-Phenylundecane	2.10	Hydrocarbon
8	30.858	5-Phenylundecane	4.42	Hydrocarbon
9	31.125	4-Phenylundecane	4.54	Hydrocarbon
10	31.671	3-Phenylundecane	3.27	Hydrocarbon
11	32.675	2-Phenylundecane	4.36	Hydrocarbon
12	33.422	6-Phenyldodecane	4.93	Hydrocarbon
13	33.547	5-Phenyldodecane	4.79	Hydrocarbon
14	33.853	4-Phenyldodecane	3.61	Hydrocarbon
15	34.404	3-Phenyldodecane	3.38	Hydrocarbon
16	35.391	2-Phenyldodecane	3.98	Hydrocarbon
17	35.986	6-Phenyltridecane	5.53	Hydrocarbon
18	36.150	5-Phenyltridecane	3.40	Hydrocarbon
19	36.455	4-Phenyltridecane	2.63	Hydrocarbon
20	37.012	3-Phenyltridecane	2.70	Hydrocarbon
21	37.977	2-Phenyltridecane	2.82	Hydrocarbon
22	39.286	Butyl octyl phthalate	0.55	Ester
23	39.423	n-Hexadecanoic acid	8.32	Fatty acid
24	43.247	9,12-Octadecadienoic acid, (Z,Z)-	0.68	Fatty acid
25	43.394	Oleic acid	2.01	Fatty acid
26	43.929	Octadecanoic acid	2.17	Fatty acid
27	51.67	2-(((2-ethylhexyl)oxy)carbonyl)benzoic acid	24.29	Acid ester
		Total	99.37	

The methanolic extract (70%) of *Lagerstroemia parviflora* bark constituted alkanes *viz*. tetradecane (16.54%) and hexadecane (28.75%) accounting 45.29% of the total extract (Table 24). The other constituents identified were hexadecanoic acid methyl ester (8.29%) and dibutyl phthalate (18.36%). Hexadecanoic acid, methyl ester was reported as antimicrobial (Rukshana et al., 2017).

Table 24: Phytoconstituents identified from the 70% methanolic extract of *Lagerstroemia parviflora* (LPM)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	23.886	Tetradecane	16.54	Alkane
2	29.865	Hexadecane	28.75	Alkane
3	35.260	Tetradecane, 26,10-trimethyl-	11.86	Alkane
4	38.430	Hexadecanoic acid, methyl ester	8.29	Fatty acid
5	39.270	Dibutyl phthalate	18.36	Ester
		Total	83.80	

GC-MS analysis of the hexane extract of *Lagerstroemia parviflora* led to identify 24 compounds accounting 96.31% of the total constituents (Table 25). Out of 24 compounds, 20 alkylbenzene (73.69%), three fatty acids (21.88%) and one alkane (0.74%) were identified.

Table 25: Phytoconstituents identified from the hexane extract of *Largestroemia parviflora* (LPH)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	4.471	2,4-Dimethyl heptane	0.74	Alkane
2	28.038	Benzene, (1-butylhexyl)-	2.56	Linear alkylbenzene
3	28.278	Benzene, (1-propylheptyl)-	2.17	Linear alkylbenzene
4	28.791	Benzene,(1-ethyloctyl)-	2.00	Linear alkylbenzene
5	29.734	Diethyl phthalate	0.29	Phthalate ester
6	29.833	Benzene, (methylnonyl)-	2.24	Linear alkylbenzene
7	30.744	Benzene, (1-pentylhexyl)-	2.63	Linear alkylbenzene
8	30.836	Benzene, (1-butylheptyl)-	5.83	Linear alkylbenzene
9	31.109	Benzene, (1-propyloctyl)-	5.20	Linear alkylbenzene
10	31.655	Benzene, (1-ethylnonyl)-	4.61	Linear alkylbenzene
11	32.658	Benzene, (1-methyldecyl)-	5.47	Linear alkylbenzene
12	33.411	Benzene, (1-pentylheptyl)-	5.53	Linear alkylbenzene
13	33.531	Benzene, (1-butyloctyl)-	5.41	Linear alkylbenzene
14	33.831	Benzene, (1-propylnolyl)-	4.20	Linear alkylbenzene
15	34.382	Benzene, (1-ethyldecyl)-	3.85	Linear alkylbenzene
16	35.370	Benzene, (1-methylundecyl)-	4.28	Linear alkylbenzene
17	35.959	Benzene, (1-pentyloctyl)-	5.83	Linear alkylbenzene
18	36.128	Benzene, (1-butylnolyl)-	3.51	Linear alkylbenzene
19	36.439	Benzene, (1-propyldecyl)-	2.71	Linear alkylbenzene
20	36.995	Benzene, (1-ethylundecyl)-	2.65	Linear alkylbenzene
21	37.961	Benzene, (1-methyldodecyl)-	2.72	Linear alkylbenzene
22	39.314	n-Hexadecanoic acid	2.43	Fatty acid
23	43.329	Oleic Acid	0.74	Fatty acid
24	51.638	1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	18.71	Fatty acid
		Total	96.31	

GC-MS analysis of the hexane extract of *Rhododendron arboreum* led to identify 16 compounds accounting 97.49% of the total constituents (Table 26). Stigmasterol exhibits antiviral, anti-inflammatory and inhibit tumor promotion activities (Raman et al., 2012). α-amyrin exhibits anti-diabetic, anti-inflammatory, anticancer and anti-arthritic activity (Raman et al., 2012). The mixture of α-amyrin and β-amyrin demonstrated immunostimulant as well as antidepressant activities (Nogueira et al., 2019).

Table 26: Phytoconstituents identified from the hexane extract of *Rhododendron arboreum* (RAH)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	16.369	3-Cyclohexen-1-ol, 4-methyl-1-1-(1-methylethyl)-, (R)-	4.06	Alcohol
2	20.651	Tridecane	0.96	Alkane
3	28.943	Dodecanoic acid	1.03	Fatty acid
4	38.217	(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	2.60	Alkene
5	38.430	Hexadecanoic acid, methyl ester	3.69	Fatty acid
6	39.358	n-Hexadecanoic acid	13.33	Fatty acid
7	42.320	9,12-Octadecadienoic acid, (E,E)-, methyl ester	1.09	Fatty acid
8	42.467	10-octadecenoic acid, methyl ester	1.49	Fatty acid
9	43.215	9,12-Octadecadienoic acid, (Z,Z)-	1.73	Fatty acid
10	43.340	10-Octadecenoic acid	8.01	Fatty acid
11	43.875	Octadecanoic acid	1.68	Fatty acid
12	50.557	α-Amyrin	1.23	Triterpene
13	51.267	Erucic acid	4.73	Fatty acid
14	56.651	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	5.99	Triterpene
15	61.888	Vitamin E	2.09	Vitamin
16	64.414	Stigmasterol	43.78	Steroid
		Total	97.49	

All together 18 compounds, accounting 94.09%, were identified in the 70% methanolic extract of the fruits of *Rhus chinensis* by GC-MS analysis (Table 27). The extract constituted abundance of anhydrides (52.38%) and fatty acids (39.09%). As a flavonoid fragment, the extract constituted of 0.32% of 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, which was reported as an antimicrobial and anti-inflammatory ingredient (Kumar et al., 2010). Hexadecanoic acid methyl ester and 3-pentadecylphenol possess antibacterial activity (Devakumar et al., 2017; Rukshana et al., 2017). Antioxidant activity of hexadecanoic acid ethyl ester and n-hexadecanoic

acid is known (Kumar et al., 2010; Alagammal et al., 2011). Linoleic acid and oleic acid are reported as anti-inflammatory, anti-androgenic, cancer preventive, etc (Alagammal et al., 2011). 5-(Hydroxymethyl) furan-2-carbaldehyde acts as an antimicrobial, antioxidant and anticancer agent, and inhibits the formation of sickled cells in the blood (Jadhav et al., 2014; Al-Marzoqi et al., 2016).

Table 27: Phytoconstituents identified from 70% methanolic extract of *Rhus chinensis* fruit (RCM)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	4.869	Furfural	0.43	Aldehyde
2	5.502	Maleic anhydride	18.16	Anhydride
3	8.224	3-Methylfuran-2,5-dione	17.37	Anhydride
4	10.815	Succinic anhydride	0.68	Anhydride
5	13.908	2,3,6,7-Tetrahydrooxepine-4-carboxylic acid, ethyl ester	0.88	Fatty acid
6	14.470	Butanedioic acid, monomethyl ester	1.13	Fatty acid
7	14.830	dl-Malic acid, dimethyl ester	25.84	Fatty acid
8	15.321	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	0.32	Flavonoid fraction
9	18.485	5-(Hydroxymethyl)furan-2-carbaldehyde	0.40	Aldehyde
10	21.066	Phthalic anhydride	16.17	Anhydride
11	26.117	Citric acid, trimethyl ester	0.79	Fatty acid
12	38.446	Hexadecanoic acid, methyl ester	3.60	Fatty acid
13	39.347	n-Hexadecanoic acid	3.79	Fatty acid
14	42.342	Methyl (9Z,11E)-octadeca-9,11-dienoate	1.10	Fatty acid
15	43.220	Linoleic acid	0.75	Fatty acid
16	43.351	Oleic acid	0.86	Fatty acid
17	43.891	Hexadecanoic acid, ethyl ester	0.35	Fatty acid
18	50.410	3-Pentadecylphenol	1.47	Phenol
		Total	94.09	

Phytoconstituents identified in the hexane extract of *Rhus chinensis* by GC-MS analysis are presented in Table 28. The hexane extract showed 16 compounds (accounting 94.68%) with a higher percentage of phenols (47.30%) and then fatty acids (25.25%) followed by hydrocarbons (16.90%). It has been reported that many fatty acids possess antibacterial and antifungal properties (Knapp & Melly, 1986). Antibacterial potentiality of hexadecanoic acid methyl ester, 1-heptatriacotanol and 3-pentadecylphenol that identified in the hexane extract of the *Rhus chinensis* fruits has been reported elsewhere (Devakumar et al., 2017; Rukshana et al., 2017).

Table 28: Phytoconstituents identified from hexane extract of *Rhus chinensis* fruit (RCH)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	38.446	Hexadecanoic acid, methyl ester	0.61	Fatty acid
2	39.445	n-Hexadecanoic acid	8.22	Fatty acid
3	40.661	Octadecanal	0.57	Aldehyde
4	42.347	8,11-Octadecadienoic acid, methyl ester	2.03	Fatty acid
5	43.411	Oleic acid	3.93	Fatty acid
6	43.716	cis-13-Octadecenoic acid	2.48	Fatty acid
7	43.973	Octadecanoic acid	5.86	Fatty acid
8	48.141	Eicosanoic acid	0.38	Fatty acid
9	50.454	(Z)-3-(Pentadec-8-en-1-yl)phenol	26.70	Phenol
10	50.623	3-Pentadecylphenol	4.67	Phenol
11	53.896	1-Heptatriacotanol	4.66	Alcohol
12	54.043	Butyl 6,9,12,15-octadecatetraenoate	1.74	Fatty acid
13	54.349	(Z)-3-(Heptadec-10-en-1-yl)phenol	15.93	Phenol
14	54.458	Heptacosane	6.55	Alkane
15	56.444	17-Pentatriacontene	0.53	Hydrocarbon
16	57.911	Octacosane	9.82	Alkane
		Total	94.68	

The 70% methanolic extract of *Stephania glandulifera* tuber constituted mainly alkaloids accounting 87.35% of the total extract (Table 29). The other constituents were fatty acids (0.5 %) and secondary amine (0.36%). The result of phytochemical screening also showed presence of alkaloid. The finding of high percent of alkaloid from *Stephania glandulifera* in this study is similar to the report of prvious study (Semwal et al., 2010).

Table 29: Phytoconstituents identified from the 70% methanolic extract of *Stephania glandulifera* (SGM)

S. N.	R. T.	Compound	Peak area %	Compound nature
1	23.281	Pyrrolidine-5-one, 2-[3-hydroxypropyl]-	0.36	Secondary amine
2	42.342	9, 12-Octadecadienoic acid (Z,Z)-, methyl ester	0.25	Fatty acid
3	42.483	9-Octadecenoic acid, methyl ester, (E)-	0.25	Fatty acid
4	50.732	3,4-Dihydroisoquinoline, 1-[3-methoxybenzyl]-6-methoxy-	1.69	Alkaloid
5	53.149	Spiro[2,5-cyclohexadiene-1, 7' (1' H)-cyclopent [ij]isoquinolin]-4-one,2',3',8'α-tetrahydro-5',6'-dimethoxy-1'-methyl-, (R)-	1.43	Alkaloid
6	53.863	Spiro[2,5-cyclohexadiene-1, 7' (1' H)-cyclopent [ij]isoquinolin]-4-one, 2', 3', 8'α-tetrahydro-5', 6--	23.90	Alkaloid

		dimethoxy-, (R)-		
7	60.344	6H-Dibenzo [a,g] quinolizine, 5,8,13,13α-tetrahydro-2,3,9,10-tetramethoxy-, (+/-)-	45.87	Alkaloid
8	61.85	6H-Dibenzo [a, g] quinolizine, 5, 8, 13, 13α-tetrahydro-2, 3,10,11-tetramethoxy-, (S)-	14.46	Alkaloid
		Total	88.21	

The result of GC-MS analysis of the hexane extract of *Stephania glandulifera* was shown in Table 30. In the hexane extract of *Stephania glandulifera*, nine compounds (accounting 90.70%) identified with a higher percentage of alkaloid (85.54%). Presence of high percentage of alkaloid supports the result of phytochemical screening. The other constituents were fatty acids (4.2%) and steroid (0.96%). It has been reported that many fatty acids possess antibacterial and antifungal properties (Knapp & Melly, 1986).

Table 30: Phytoconstituents identified from the hexane extract of *Stephania glandulifera* (SGH)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	39.385	n-Hexadecanoic acid	0.98	Fatty acid
2	42.336	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	0.39	Fatty acid
3	42.483	9-Oxtadecenoic acid, methyl ester, (E)-	0.34	Fatty acid
4	43.302	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	1.12	Fatty acid
5	43.438	cis-Vaccenic acid	1.37	Fatty acid
6	60.519	6H-Dibenzo[a,g]quinolizine, 5,8,13,13α-tetrahydro-2,3,9,10-tetramethoxy-, (+/-)-	66.28	Alkaloid
7	61.959	6H-Dibenzo[a,g]quinolizine, 5,8,13,13α-tetrahydro-2,3,9,10,11-tetramethoxy-, (S)-	18.24	Alkaloid
8	62.515	6H-Dibenzo[a,g]quinolizine-1-01,5,8,13,13α-tetrahydro-2,3,9,10,-tetramethoxy-, (S)-	1.02	Alkaloid
9	64.850	Stigmasta-5,7,22-trien-3-01, (3, β)-	0.96	Steroid
		Total	90.70	

The 70% methanolic extract of *Tectaria coadunata* rhizome constituted ketone (39.19%), aldehyde (14.62), flavonoid (6.47%), fatty acid (2.51%) and alcohol (1.97%) accounting 64.76% of the total extract (Table 31).

Table 31: Phytoconstituents identified from 70% methanolic extract of *Tectaria coadunata* (TCM)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	5.540	2-Furanmenthanol	1.97	Furfuryl alcohol

2	6.669	2-Propanone, 1,3-dihydroxy-	39.19	Ketone
3	15.245	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	6.47	Flavonoid fraction
4	18.442	2-Furancarboxaldyhyde, 5-(hydroxymethyl)-	14.62	Aldehyde
5	39.319	n-Hexadecanoic acid	2.51	Fatty acid
		Total	64.76	

GC-MS analysis of the hexane extract of *Tectaria coadunata* led to identify 24 compounds accounting 85.91% of the total constituents (Table 32). Out of 24 compounds, 19 alkylbenzene (48.53%), three fatty acids (15.93%), one alcohol (8.05) and one ester (13.4%) were identified. 1,2-Benzenedicarboxylic acid, disooctyl estser identified in the extract was previously reported as antimicrobial (Duke, 1992; Alagammal et al., 2011).

Table 32: Phytoconstituents identified from the hexane extract of *Tectaria coadunata* (TCH)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	28.032	Benzene, (1-butylhexyl)-	1.53	Linear alkyl benzene
2	28.272	Benzene, (1-propylheptyl)-	1.17	Linear alkyl benzene
3	28.785	Benzene, (1-ethyloctyl)-	1.23	Linear alkyl benzene
4	29.832	Benzene, (1-methylnonyl)-	1.67	Linear alkyl benzene
5	30.738	Benzene, (1-pentylhexyl)-	1.63	Linear alkyl benzene
6	30.836	Benzene, (1-butylheptyl)-	3.51	Linear alkyl benzene
7	31.104	Benzene, (1-propyloctyl)-	3.05	Linear alkyl benzene
8	31.654	Benzene, (1-ethylnonyl)-	3.06	Linear alkyl benzene
9	32.658	Benzene, (1-methyldecyl)-	3.47	Linear alkyl benzene
10	33.400	Benzene, (1-pentylheptyl)-	3.51	Linear alkyl benzene
11	33.531	Benzene, (1-butyloctyl)-	3.53	Linear alkyl benzene
12	33.831	Benzene, (1-propylnonyl)-	2.75	Linear alkyl benzene
13	34.382	Benzene, (1-ethyldecyl)-	2.50	Linear alkyl benzene
14	35.364	Benzene, (1-methylundecyl)-	2.80	Linear alkyl benzene
15	35.970	Benzene, (1-pentyloctyl)-	4.03	Linear alkyl benzene
16	36.128	Benzene, (1-butylnonyl)-	2.71	Linear alkyl benzene
17	36.259	3,7,11,15-tetramethyl-2-hexadecen-1-ol	8.05	Alcohol
18	36.433	Benzene, (1-propyldecyl)-	2.78	Linear alkyl benzene
19	36.995	Benzene, (1-ethylundecyl)-	1.76	Linear alkyl benzene
20	37.950	Benzene, (1-methyldodecyl)-	1.84	Linear alkyl benzene
21	39.330	n-Hexadecanoic acid	11.48	Fatty acid
22	43.198	9, 12-Octadecadienoic acid, (Z,Z)-	2.00	Fatty acid
23	43.329	Oleic acid	2.45	Fatty acid

24	51.637	1,2-Benzenedicarboxylic acid, disooctyl estser	13.40	Ester
		Total	85.91	

The 70% methanolic extract of *Thalictrum foliolosum* root constituted benzofuran (55.87%), berbine (15.05%) and flavonoid fraction (1.97%) accounting 72.89 % of the total extract (Table 33). 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one was reported to be antimicrobial agent (Kumar et al., 2010).

Table 33: Phytoconstituents identified from 70% methanolic extract of *Thalictrum foliolosum* (TFM)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	15.229	2,3-dihydro -3,5-dihydroxy-6-methyl- -4H-pyran-4-one,	1.97	Flavonoid fraction
2	59.657	3-[2-(2-Dimethylaminoethyl)-4,5-methylnedioxyphenyl]methylene-6,7-dimethoxy-3H-isobenzofuran-1-one	55.87	Benzofuran
3	63.650	Berbine, 13,13α-didehydro-9,10-dimethoxy-2,3-9methylenedioxy)-	15.05	Alkaloid
		Total	72.89	

GC-MS analysis of the hexane extract of *Thalictrum foliolosum* led to identify 7 compounds accounting 82.42% of the total constituents (Table 34). Out of 7 compounds, 5 fatty acids (78.97%), 1 phenol (1.06%) and 1 alcohol (2.39%) were identified.

Table 34: Phytoconstituents identified from the hexane extract of *Thalictrum foliolosum* (TFH)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	24.939	2H-1-Benzopyran-2-one	1.06	Phenol
2	39.036	9-Hexadecenoic acid	0.86	Fatty acid
3	39.385	n-Hexadecanoic acid	23.61	Fatty acid
4	42.085	1-hexadecanol	2.39	Alcohol
5	43.280	9,12-Octadecadienoic acid, (Z,Z)-	22.44	Fatty acid
6	43.411	cis-Vaccenic acid	27.95	Fatty acid
7	43.896	Octadecanoic acid	4.11	Fatty acid
		Total	82.42	

All together 16 compounds, accounting 72.92%, were identified in the 70% methanolic extract of the stem of *Tinospora sisnensis* by GC-MS analysis (Table 35). 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one was reported to be antimicrobial agent (Kumar et al., 2010). 2-Methoxy-4-vinylphenol exhibits antioxidant, antimicrobial and anti-inflammatory activities (Al-Marzoqi et al., 2016).

Table 35: Phytoconstituents identified from 70% methanolic extract of *Tinospora sinensis* stem (TSM)

S. N.	R. T.	Compound	Peak area %	Compound nature
1	15.229	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1.41	Flavonoid fraction
2	21.099	2-Methoxy-4-vinylphenol	3.17	Phenol
3	22.359	2,6-dimethoxy phenol	1.13	Phenol
4	23.859	Vanillin	2.11	Aldehyde
5	27.727	Dodecanoic acid, methyl ester	2.21	Fatty acid
6	32.697	2,6-dimethoxy-4-(propenyl)phenol	1.54	Phenol
7	33.662	4-(1E)-3-Hydroxy-1-propenyl -2-methoxyphenol	9.17	Phenol
8	36.985	1,2-Benzenedicarboxylic acid, diphenyl ester	1.39	Ester
9	38.430	Hexadecanoic acid, methyl ester	4.49	Fatty acid
10	39.319	n-Hexadecanoic acid	10.04	Fatty acid
11	42.331	9, 12-Octadecadienoic acid (Z,Z)-, methyl ester	4.28	Fatty acid
12	42.478	9-Octadecenoic acid-, methyl ester, (Z)-	5.56	Fatty acid
13	43.089	Octadecanoic acid, methyl ester	1.69	Fatty acid
14	43.215	9,12-Octadecadienoic acid, (Z,Z)-	6.51	Fatty acid
15	43.345	Oleic acid	15.50	Fatty acid
16	43.880	Octadecanoic acid	2.72	Fatty acid
		Total	72.92	

Phytoconstituents identified in the hexane extract of *Tinospora sinensis* by GC-MS analysis are presented in Table 36. Nineteen compounds (accounting 93%) were identified in the hexane extract with a higher percentage of steroids (42.23%) and then fatty acids (33.46%) followed by hydrocarbons (11.55%), triterpene (3.35%), vitamin (1.69%), ketone (0.46%) and alcohol (0.26%). Steroids such as stigmasterol are reported as antioxidant, antibacterial activity, anti-inflammatory, antiarthritic, antiasthma and diuretic (Tyagi & Agarwal, 2017). Sitosterol exhibits strong antifungal, antibacterial and anti-angiogenic activities (Raman et al., 2012). It has been reported that many fatty acids possess antibacterial and antifungal properties (Knapp & Melly, 1986). Vitamin E are reported as antioxidant, antimicrobial, analgesic, antidiabatic anti-inflammatory, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective and antispasmodic (Mujeeb et al., 2014).

Table 36: Phytoconstituents identified from the hexane extract of *Tinospora sinensis* stem (TSH)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	39.368	n-Hexadecanoic acid	10.75	Fatty acid
2	40.061	Hexadecanoic acid, ethyl ester	0.37	Fatty acid
3	42.080	9-Dexadecenoic acid	0.19	Fatty acid
4	42.320	10,13-Octadecadienoic acid, methyl ester	0.26	Fatty acid
5	42.467	6-Octadecenoic acid	0.29	Fatty acid
6	42.740	E-10,13,13-trimethyl-11-tetradecen-1-ol acetate	0.26	Alcohol
7	43.247	9,12-Octadecadienoic acid, (Z,Z)-	5.20	Fatty acid
8	43.389	cis-Vaccenic acid	12.29	Fatty acid
9	43.891	Octadecanoic acid	2.59	Fatty acid
10	43.967	(E)-9-Octadecenoic acid, ethyl ester	0.91	Fatty acid
11	47.961	beta-Sitosterol	32.63	Steroid
12	51.627	3',8,8'-Trimethoxy-3piperidyl-2, 2'-binaphthalene-1,1',4,4'-tetrone	0.46	Ketone
13	52.008	Docosanoic acid	0.61	Fatty acid
14	54.327	Ethyl isoallocholate	0.39	Steroid
15	61.342	17-Pentatriacontene	11.55	Hydrocarbon
16	61.883	Vitamin E	1.69	Vitamin
17	63.465	Betulin	3.35	Triterpene
18	63.579	Campesterol	4.21	Steroid
19	64.397	Stigmasterol	5.00	Steroid
		Total	93.00	

GC-MS analysis of the 70% methanolic extract of *Woodfordia fruticosa* led to identify seven compounds accounting 73.98% of the total constituents (Table 37). Out of seven compounds, two aldehydes (52.62%), two fatty acids (9.5%), one flavonoid fraction (9.1%), one anhydride (1.38%) and one phenol (1.38%) were identified. 5-hydroxymethyl, 2-furancarbaldehyde inhibits the formation of sickle cells in the blood , antimicrobial, preservative (Jadhav et al., 2014). As a flavonoid fragment, the extract constituted of 9.10 % of 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, which was reported as an antimicrobial and anti-inflammatory ingredient (Kumar et al., 2010), anti-inflammatory and antiproliferative (Duke, 1992).

Table 37: Phytoconstituents identified from 70% methanolic extract of *Woodfordia fruticosa* flower (WFM)

S. N.	R. T.	Compound name	Peak area %	Compound nature
1	4.847	Furfural	10.91	Aldehyde
2	8.208	2,5-Furadione, 3-methyl-	1.38	Anhydride

3	15.218	3,5-dihydroxy-6-methyl-2,3-dihydro -4H-pyran-4-one	9.10	Flavonoid fraction
4	18.398	5-(Hydroxymethyl), 2-furancarbaldehyde	41.71	Aldehyde
5	38.441	Hexadecanoic acid, methyl ester	1.51	Fatty acid
6	39.319	n-Hexadecanoic acid	7.99	Fatty acid
7	44.180	Phenol, 4,4-(1-methlethylidene)bis-	1.38	Phenol
		Total	73.98	

GC-MS analysis of the hexane extract of *Woodfordia fruticosa* led to identify 11 compounds accounting 91.55 % of the total constituents (Table 38). Out of 12 compounds, four fatty acids (37.93%), four alkanes (40.67%), two steroids (7.54%), one triterpene (1.09%) and one vitamin (4.32%) were identified. Squalene identified in the extract exhibits antibacterial, antioxidant, antitumour, cancer preventive, immunostimulant, chemo preventive, lipoxygenase-inhibitor, pesticide and diuretic activities (Alagammal et al., 2011).

Table 38: Phytoconstituents identified from the hexane extract of *Woodfordia fruticosa* flower (WFH)

S. N.	R. T.	Compound name	Peak area%	Compound nature
1	39.401	n-Hexadecanoic acid	13.93	Fatty acid
2	43.274	9,12-Octadecadienoic acid, (Z,Z)-	22.03	Fatty acid
3	43.907	Vaccenic acid	1.97	Fatty acid
4	50.748	Eicosane	4.22	Alkane
5	52.259	3β,4αl,5α-4,14-dimethyl-9,19-cycloergost-24(28)-en-3-ol	3.88	Steroid
6	54.436	Heptacosane	7.08	Alkane
7	56.193	Octacosane	1.78	Alkane
8	56.667	Squalene	1.09	Triterpene
9	57.895	Nonacosane	27.59	Alkane
10	61.910	Vitamin E	4.32	Vitamin
11	64.550	Stigmasterol	3.66	Steroid
		Total	91.55	

4.6 Antibacterial Susceptibility Assay

4.6.1 Antibacterial Susceptibility Assay of the Methanolic Extracts

The results of antibacterial susceptibility assay of the 70% methanolic extracts of the plant materials are presented in Table 39a. Antibacterial activities against *Shigella dysenteriae* are presented in table 39b. The methanolic extracts (70%) of the plant materials are abbreviated as AMM (*Aegle marmelos*), AAM (*Antidesma acidum*),

AIM (*Azadirachta indica*), PVM (*Phanera vahlii*), CMMR (*Callicarpa macrophylla*, root), CMML (*Callicarpa macrophylla*, leaf), CPM (*Cissampelos pareira*), FSM (*Flemingia strobilifera*), LPM (*Lagerstroemia parviflora*), RAM (*Rhododendron arboreum*), RCM (*Rhus chinensis*), SGM (*Stephania glandulifera*), TCM (*Tectaria coadunata*), TFM (*Thalictrum foliolosum*), TSM (*Tinospora sinensis*) and WFM (*Woodfordia fruticosa*).

Table 39a: Antibacterial activity of the 70% methanolic extracts

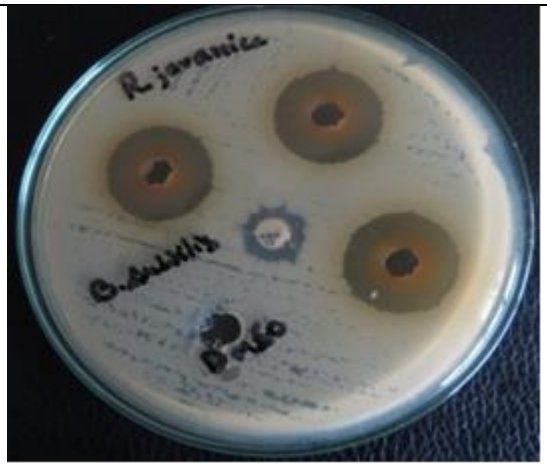
70% methanolic extract of the Plant species	Zone of inhibition (ZOI) ± Standard error mean (mm)						
	GRAM POSITIVE BACTERIA			GRAM NEGATIVE BACTERIA			
	S. aureus	*B. subtilis*	*E. faecalis*	*E. coli*	*S. typhi*	*K. pneumoniae*	*P. aeruginosa*
Aegle marmelos	12±0	9.4±0.6	12±0	-	10±1	10.3±0.33	11±0.43
Antidesma acidum	15±0.57	13±0.73	-	12.16±0.74	-	10±0	11±0.28
Azadirachta indica	-	12±0	12±0	-	9±1	9.5±0.86	9.6±0.23
Phanera vahlii	16±0.57	14.5±0.88	12±0	9.3±0.61	-	9.66±1	12±0.59
Callicarpa macrophylla root	-	9±0.57	9±0.99	-	-	-	11.3±0.23
Callicarpa macrophyla leaf	12.4±0.90	15.33±1.32	-	12±0.57	10±1	9±0.24	15±1
Cissampelos pareira	-	-	12±0	-	-	9±1	10.6±0.24
Flemingia strobilifera	15.66±0.33	13.33±0.88	11.66±0.33	16.5±0.67	12.66±0.33	11.66±0.33	11.8±0.33
Lagerstroemia parviflora	16.66±0.80	12±0	12±0	18±0.73	10±0	10±0	15±0
Rhododendron arboreum	16.33±0.50	16±0.44	-	15.33±0.55	-	-	11.6±0.46
Rhus chinensis	20.25±0.75	20.66±0.33	17.83±0.16	23±0.57	-	14.66±0.33	16.33±0.33
Stephania glandulifera	14.66±0.20	12.33±0.42	12±1	16±0.68		11.66±0.66	-
Tectaria coadunata	16.33±0.66	16±0	-	16.83±0.3	9±1	-	13±0
Thalictrum foliolosum	14.33±0.33	8±0	-	13.33±0.33	-	-	10.8±0.28
Tinosppora sinensis	-	11±0	-	8±0	-	-	12±0.48
Woodfordia fruticosa	19.16±0.40	14.66±0.33	18.66±0.66	18±0.73	10±1	13.5±0.67	16.4±0.24
Ampicillin	32.5±0.5	8.5±0.5	17.75±0.25	25±1	15.5±0.5	8.5±0.5	-
Gentamicin	16.75±0.25	15.5±0.5	18.5±0.5	17.5±0.5	12.66±0.33	11.33±0.88	14.66±0.33
DMSO	-	-	-	-	-	-	-

Table39b: Antibacterial activity of 70% methanolic extracts showing ZOI against *Shigella dysenteriae*

Rhus chinensis	*Flemingia fruticosa*	ampicillin	gentamicin	DMSO
14.66 ±• 0.33	10.33±0.33	23.75 • ± 0.25	18.66 • ± 0.66	-

As can be seen from Table 39a, the *Staphylococcus aureus* (ZOI = 32.5±0.5 mm) was found most susceptible to the standard drug ampicillin on the other hand, *Bacillus subtilis* (ZOI = 8.5±0.5 mm), *Klebsiella pneumoniae* (ZOI = 8.5±0.5mm) and *Pseudomonas aeruginosa* (no ZOI) were found resistant (Figure 10).

(a) against *Staphylococcus aureus*



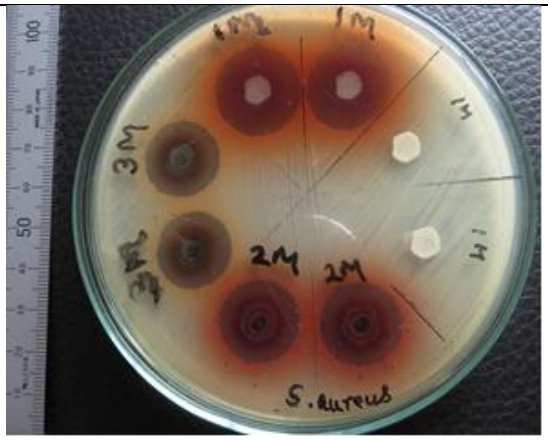
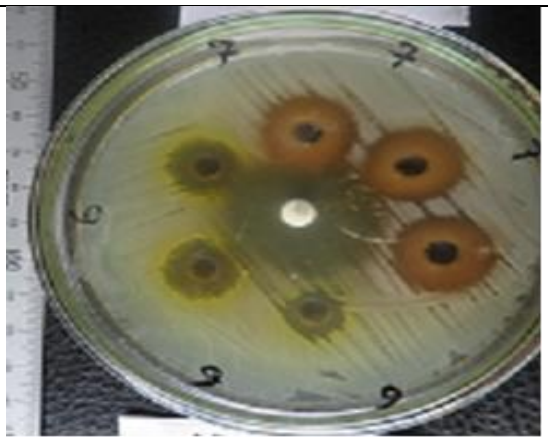
(b) against *Bacillus subtilis*

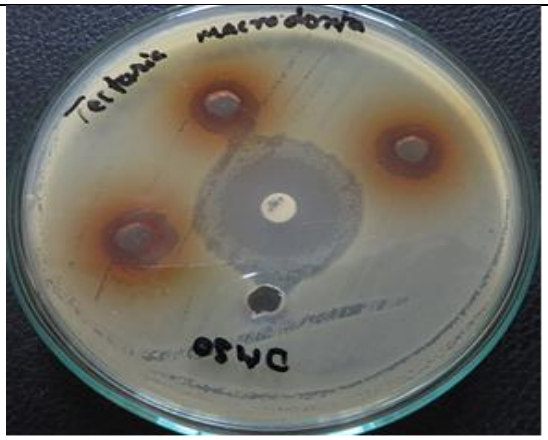
(c) against *Klebsiella pneumoniae*

(d) against *Pseudomonas aeruginosa*

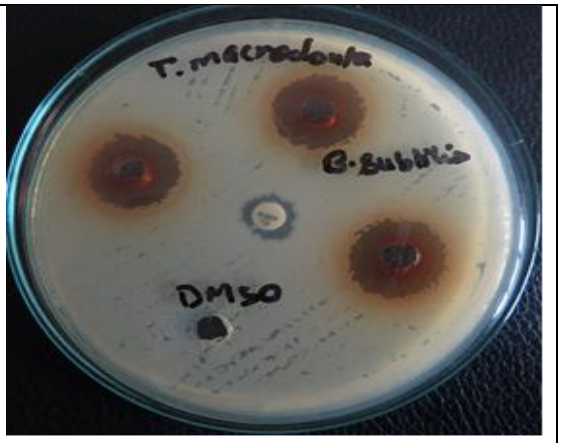
Figure 10: Antibacterial activity of standard antibiotic ampicillin showing susceptible against *Staphylococcus aureus* and resistance against *Bacillus subtilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Among the 16 plant extracts, AAM (Figure 11a), PVM (Figure 11a), CMML, FSM (Figure 11bc), LPM (Figure 11d), RAM (Figure 11a), RCM (Figure 11f), SGM, TCM (Figure 11ghi) and WFM (Figure 11e) showed larger zone of inhibitions (ZOI ≥ 15mm) against one or more than one strains of the tested bacteria (Table 39a).

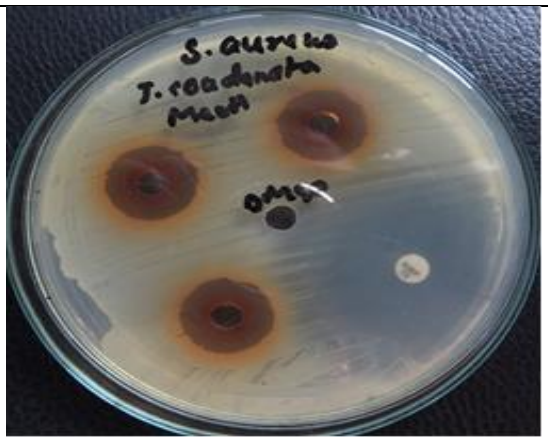
	
(a) 1M = RAM, 2M = PVM, 3M = AAM against *Staphylococcus aureus*	(b) FSM against *Bacillus subtilis*, ampicillin as positive control
	
(c) FSM against *Staphylococcus aureus*, ampicillin as positive control	(d) 15M = LPM, 18M = RCM, 18H = RCM against *Staphylococcus aureus*
	
(e) 6 = SGM, 7 = WFM against *Staphylococcus aureus*, ampicillin as positive control	(f) RCM against *Enterococcus faecalis*, gentamicin as positive control



(g) TCM against *Escherichia coli*, ampicillin as positive control



(h) TCM against *Bacillus subtilis*, ampicillin as positive control



(i) TCM against *Staphyloccus aureus*, ampicillin as positive control

(j) FSM against *Salmonella typhi*, ampicillin as positive control

Figure 11: Antibacterial assay showing the ZOI of the methanolic extract of the plant parts against the tested bacteria.

Interestingly, the result showed that CMML, FSM, LPM, RAM, RCM, TCM and WFM showed larger zone of inhibition (ZOI ≥ 15mm) against Gram –ve as well as Gram +ve bacteria. Out of 16 extracts, only extracts of AMM, AIM, CMML, FSM (Figure 16j), LPM, TCM and WFM have shown antimicrobial activity against *Salmonella typhi* (Gram –ve). All the extracts except SGM have shown antimicrobial activity against ampicillin resistant *Pseudomonas aeruginosa*.

AMM has shown antibacterial activity against all the tested bacteria except *Escherichia coli.* AMM showed same ZOI against *Klebsiella pneumoniae* as compared to the standards ampicillin and gentamicin. Interestingly, AMM was

effective against *Pseudomonas aeruginosa* as well as *Bacillus subtilis*, which were resistant to ampicillin. Poonkothai and Saravanan (2008) reported antibacterial activity of methanolic extract of bark of *Aegle marmalos* against *Staphylococcus aueus, Salmonella paratyphi, Bacillus spp., Escherichia coli, Klebsiella spp*. Nandkarni (1995) has reported that the barks of *Aegle marmelos* as antimicrobial. Taylor et al. (1996) had reported that methanol extract of root of *Aegle marmelos* showed no zone of inhibition *against Bacillus subtilis, Staphylococcus aureus*-methicillin sensitive, *Staphylococcus aureus*-methicillin resistant and *Pseudomonas aeruginoa*. Shakya et al. (2008) had reported that ethanolic extract of fruit of *Aegle marmalos* showed antibacterial activity against *Bacillus subtilis* but no activities against *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi*.

The antimicrobial activity shown by AMM may be due to phytochemicals having antimicrobial property. Phytoconstituents such as γ-Sitosterol and 2-Methoxy-4-vinylphenol present in AMM (Table 10) might be the cause of antibacterial activity of the extract,

AAM has shown antibacterial activity against all the tested bacteria, Gram +ve as well as Gram –ve bacteria, except *Enterococcus faecalis* and *Salmonella typhi*. Kiran et al. (2014) reported antibacterial activity of the aqueous extract of stems of *Antidesma acidum* against *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis*.

Antibacterial activity of AAM may be due to presence of high % of 5(Hydroxymethyl)-2- furancarboxaldehyde and 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one. (Table 12). *Antidesma acidum* is used for treatment of cough and fever as symptoms of pneumonia (lung infection) by the villagers, the antibacterial activity of its extract showed it is effective for *Klebsiella pneumoniae c*ausal agent of pneumonia.

The result of antimicrobial susceptibility assay of AIM revealed its antibacterial activity against *Bacillus subtilis, Enterococcus faecalis, Salmonella typhi, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. It did not show antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.* Maleki et al. (2017) have shown antibacterial activity of the methanolic extracts of *Azadirachta indica* against *Pseudomonas aeroginosa* and *Enterococcus faecalis*, and reported that the extract did not show antibacterial activity against *Escherichia coli* (Autade et al., 2015; Maleki et

al., 2017). Maleki et al. (2017) also reported that the ethyl acetate and ethanolic extracts show a better antibactrial activity than the methanolic extract against *Staphyloccus aureus*.

The antibacterial activity of AIM may be due to synergic effect of phytoconstituent present in the extract (Table 14).The antibacterial activity of AIM against *Enterococcus faecalis* and *Pseudomonas aeruginosa* (causal agents for UTI) support the use of *Azadirachta indica* for treatment of UTI by the Magars.

PVM showed antimicrobial activity against all the tested bacteria except *Salmonella typhi*. Dugasani et al. (2010) have also reported antimicrobial activity of *Phanera vahlii* against *Escherichia coli, Psedomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis* and *Bacillus subtilis*. Taylor et al. (1996) had reported that methanol extract of root of *Phanera vahlii* showed antimicrobial activities against *Bacillus subtilis, Staphylococcus aureus*-methicillin sensitive, *Staphylococcus aureus*-methicillin resistant and *Pseudomonas aeruginoa*.

The antibacterial activity of PVM may be due to 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (Table 16). The phytoconstituent and the antibacterial activity of PVM support the usefor trearment for diarrhea by the magars.

The result showed antibacterial activity of CMMR against *Bacillus subtilis, Enterococcus faecalis* and *Pseudomonas aeruginoa* whereas CMML showed antibacterial activity against all the tested bacteria except *Enterococcus faecalis.* Yadav et al. (2012) reported a moderate antibacterial activity of the ethanolic extract of the stems of *Callicarpa macrophylla* against *Staphyloccus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium,* and reported that the aqueous extrat is inactive against the all bacterial strains except *Salmonella typhimurium.* However, antibacterial activity of leaf and root extracts of *Callicarpa macrophylla* against these bacterial strains is not reported yet. Mona (2016) reported antibacterial activity of methanol extract of leaf against *Bacillus cereus* and also reported that the extract showed insignificant antibacterial activity against *Streptococcus pneumoniae* and *Shigella dysenteriae*.

The antibacterial activity may be due to presence of compound 2-methoxy-4-vinylphenol in CMML (Table 18) which was reported to be antimicrobial agent (Al-Marzoqi et al., 2016).

The phytochemical evaluation of CMML showed that lupeol was the major constituent (table 18). It has anti-inflammatory activity (Mohammad, 2009). It supports the use of *Callicarpa marophylla* leaf for inflammation on tongue by the Magars.

FSM apparently showed an equal antibacterial efficacy as gentamicin against *Salmonella typhi* (FSM, ZOI =12.66±0.33mm versus gentamycin, ZOI = 12.66±0.33 mm). The extract exhibited antibacterial activity against *Staphylococcus aureus* (ZOI = 15.66±0.33 mm), *Esherichia coli* (ZOI = 16.5±0.67 mm), *Shigella dysenteriae* (10.33±0.33) as well as *Enterococcus faecalis*. The extract also showed antibacterial activity against *Bacillus subtilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* which were found resistant to the standard antibiotic ampicillin. Madan et al. (2009) have reported antibacterial activity of some isoflavonoids isolated from the roots of *Flemingia strobilifera* against Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *pseudomonas aeruginosa*).

The antibacterial activity may be due to presence of 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (Table 22) which was reported to be antibacterial agent (Kumar et al., 2010), and Octadecanoic acid which was also reported to be antimicrobial (Mujeeb et al., 2014). The antibacterial activity of *Flemingia stobilifera* supports the use for the treatment of diarrhea as well as dysentery by the Magars.

LPM showed antibacterial activity against all the tested bacterial strains with a largest ZOI against *Escherichia coli* (ZOI = 18±0.73 mm). Mazumder et al. (2002) reported that benzene extract of *Lagerstroemia parviflora* leaves showed antibacterial activity against *Staphyloccus aureus, Bacillus subtilis, Klebsiella pneumoniae* and stronger potentiality against *Escherichia coli.* Similarly, Bobade et al. (2013) reported antibacterial activity of the ethyl acetate extract of *Lagerstroemia parviflora* leaf against *Escherichia coli, Staphyloccus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi.* However, antimicrobial activity of the bark extract has not been reported yet.

The antibacterial activity of LPM may be due to synergic effect of the phytoconstituents present in it (Table 24). The antibacterial activity of *Lagerstroemia parviflora* supports its use for diarrheal treatment by the Magars.

RAM showed antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Nisar et al. (2013) investigated antibacterial activity of the methanolic extract of the barks of *Rhododendron arboreum* and reported significant to low antibacterial activity in the order of *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. Shakya et al. (2008) reported antibacterial activity of 50% ethanol extract of whole plant of *Rhododendron arboreum* against *Bacillus subtilis* and *Esherichia coli* where as no antibacterial activity against *Salmonella typhi* and *Staphylococcus aureus*.

The antibacterial activities of RAM support the use of *Rhododendron arboreum* for treatment of diarrhea by the magars.

RCM extract showed strong antibacterial activity against resistant strain of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Bacillus subtilis*. This extract displayed similar antibacterial potentiality as standard antibiotic ampicillin used against *Enterococcus faecalis*. RCM was found more effective against *Staphylococcus aureus* than gentamicin. The growth of *Escherichia coli* and *Shigella dysenteriae* was also effectively inhibited (Nemkul et al., 2021). Yang et al. (2014) also reported antibacterial activity of *Rhus chinensis* against *Shigella dysenteriae*. It shows that the finding of this study is comparable to previous study. You et al. (2013) reported antibacterial activity of the ethanolic extract of *Rhus chinensis* leaf against methicillin-resistant *Staphylococcus aureus*. The extract constituted abundance of anhydrides (52.38%) and fatty acids (39.09%) and also presence of flavonoid fragment, 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (Table 27). The broad spectrum antibacterial activity may be due to synergic effect of the phytoconstituents.

SGM showed antibacterial activity against all the tested bacterial strain except *Salmonella typhi* and *Pseudomonas aeruginosa*. Adhikary et al. (2011) reported antibacterial activity of methanolic extract of the rhizomes against *Escherichia coli.* Shakya et al. (2008) reported antibacterial activity of ethanolic extract of root of *Stephania glandulifera* against *Bacillus subtilis* and *Escherichia coli* but no antibacterial activity against *Salmonella typhi* and *Staphylococcus aureus.* Shakya et al. (2008) also reported that ethanolic extract of leaf of *Stephania glandulifera* showed no antibacterial activity against these bacteria.

The phytochemical evaluation showed SGM constituted mainly alkaloids (Table 29). The broad spectrum antimicrobial activity of SGM may be due to presence of these alkaloids. It supports the use for the treatment of diarrhea.

TCM showed nearly equal potential against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. Parihar et al. (2010) also reported equal antibacterial potentiality of the aqueous extract of frond of *Tectaria coadunata* against *Staphylococcus aureus* and *Escherichia coli*.

The phytochemical screening as well as GC-MS evaluation showed absence of alkaloid in TCM. So the antibacterial activities of TCM may be due to presence of aldehyde, flavonoid and fatty acid (Table 31).

TFM showed antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.* Joshi and Sati (2017) investigated antibacterial activity of methanol, chloroform, hexane and aqueous extracts of the leaves of *Thalictrum foliolosum* and reported that methanol and hexane extracts showed highest activity against *Bacillus subtilis* while the aqueous extract had almost no inhibitory effect. Shakya et al. (2008) reported that 50% ethanoloc extract of whole plant of *Thalictrum foliolosum* showed antibacterial activity against *Bacillus subtilis, Escherichia coli, Salmonella typhi* but not against *Staphylococcus aureus*.

The antibacterial activity of TFM may be due to presence of 2,3-dihydro-3,5-Dihydroxy-6-methyl -4H-pyran-4-one (Table 33).

The results obtained by using TSM revealed a weak antibacterial activity against *Escherichia coli* and a moderate activity against *Bacillus subtilis* and *Psedomonas aeruginosa*, and no antibacterial activity against rest of the tested bacteria. Devi et al. (2014) reported methanolic extract of leaf, stem and flower of *Tinospora sinensis* had not shown antibacterial activity against Gram-positive and Gram-negative bacterial strains, but it was found effective against *Candida albicans*. Shakya et al. (2008) reported that 50% ethanolic extract of *Tinospora sinensis* stem showed antibacterial activity against *Bacillus subtilis* and *Escherichia coli* but not against *Salmonella typhi* and *Staphylococcus aureus*.

The antibacterial activities of TSM may be due to presence of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one and 2-Methoxy-4-vinylphenol (Table 35).

The result of antibacterial susceptibility assay of WFM showed larger ZOI against *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* than standard antibiotics. Methanolic extract of *Woodfordia fruticosa* showed antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Parekh & Chanda, 2007; Kumar et al., 2013).

The broad spectrum antibacterial activity of WFM may be due to presence of 5-(Hydroxymethyl), 2-furancarbaldehyde (Table 37) which was major constituent in WFM having antibacterial property. The another major antibacterial constituent present in WFM was 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one.

WFM, CMML, LPM, RCM and TCH showed larger zone of inhibition (ZOI ≥ 15mm) to *Pseudomonas aeruginosa* than the others. *Pseudomonas aeruginosa* frequently displays resistance to multiple antimicrobial agents (Carmeli et al., 1999). Savas et al. (2005) reported increasing resistant of *Peudomonas aeruginosa* to multiple antimicrobial agents including gentamicin. The result of this research showed susceptibility of *Pseudomonas aeruginosa* to gentamicin. The susceptibility to gentamicin has been reported as low as 49.8% in Greece to as high as 96.6% in United Kingdom (Van Landuyt, et. al., 1986). But the resistant rate is increased and reported to be 70.7% (Savas et al., 2005).

4.6.2 Antibacterial Susceptibility Assay of the Hexane Extracts

The results of antibacterial susceptibility assay of the hexane extracts have been presented in Table 40a. Antibacterial activities against *Shigella dysenteriae* are shown in table 40b. Sixteen hexane extracts of the plant materials are abbreviated as AMH (*Aegle marmelos*), AAH (*Antidesma acidum*), AIH (*Azadirachta indica*), PVH (*Phanera vahlii*), CMHR (*Callicarpa macrophylla*, root), CMHL (*Callicarpa macrophylla*, leaf), CPH (*Cissampelos pareira*), FSH (*Flemingia strobilifera*), LPH (*Lagerstroemia parviflora*), RAH (*Rhododendron arboretum*), RCH (*Rhus chinensis*), SGH (*Stephania glandulifera*), TCH (*Tectaria coadunate*), TFH (*Thalictrum foliolosum*), TSH (*Tinospora sinensis*) and WFH (*Woodfordia fruticosa*).

AAH showed antibacterial activity against *Bacillus subtilis, Enterococcus faecalis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The antibacterial activity may be due to presence of high percent of fatty acids in it.

AIH and RCH showed larger zone of inhibiton (ZOI ≥ 15mm) against Gram +ve as well as Gram –ve bacteria (Figure 12 a, b). Surprisingly, the result of AIH showed antibacterial activity to *Staphylococcus aureus* (ZOI = 15.66±0.66 mm) as well as to *Escherichia coli* (Figure 12 a, b). Gupta et al. (2013) reported antibacterial activity of ethanolic extract of leaves of *Azadirachta indica* against *Staphylococcus aureus* and *Escherichia coli*. AIH did not show antibacterial activity against *Pseudomonas aeruginosa* where as AIM showed antibacterial activity against *Pseudomonas aeruginosa*. Similarly Maleki et al. (2017) reported antibacterial activity of methanolic extract of *Azadirachta indica* against *Pseudomonas aeruginosa.*

The antibacterial activity of AIH may be due to presence of triterpene such as 2 (4H)-Benzofuranone, 5,6,7,7α-tetrahydro-4,4,7α-trimethyl-(o,81%), vitamin E (4.82%) and phytol (2.71%) (Table 15) which are reported as antibacterial compound (Duke, 1992; Mujeeb et al., 2014). The antibacterial activity could be due to synergic effect of these different compounds having antibacterial properties of the extract.

The result shows that the *Azadirachta indica* leaf extract of non polar solvent (hexane) showed good antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* than the extract of polar solvent (70% methanol).

The antibacterial activity of RCH may be due to presence of higher percentage of phenol (47.30%) and fatty acids (25.25%) (Table 28).

CMHL showed larger ZOI (ZOI = 15mm) against Gram +ve bacteria and TCH showed larger ZOI (ZOI = 15mm) against Gram –ve bacteria. Chemical evaluation of CMHL showed 90% of 1 -Undecane-3,6-dione,1-(2,6,6-trimethyl -1- cyclohexen-1-yl). The antibacterial activity of CMHL may be due to presence of this compound. Atibacterial activity of TCH may be due to presence of 1,2-Benzenedicarboxylic acid, disooctyl estser as major compound (13.40%) which was reported as antimicrobial (Duke, 1992; Alagammal et al., 2011).

Hexane extracts of some of the plant species displayed smaller zone of inhibition (ZOI <15mm) against *Escherichia coli* (Figure 12c). AIH, LPH and TSH showed no antibacterial activity against *Pseudomonas aeruginosa*, where as 70% methanolic extracts of these species showed antibacterial activities. Similarly FSH and RCH did not showed antibacterial activity against *Shigella dysenteriae* though FSM and RCM

did. SGH showed antibacterial activity against *Pseudomonas aeruginosa* though SGM did not.

The efficacy of different plant extracts depends on the presence of the antibacterial compounds in it. But not only that the efficacy also depens on the different bacterial species

Table 40a: Antibacterial activity of the hexane extracts

Hexane extract of the plant species	Zone of inhibition (ZOI)± Standard error mean (mm)						
	GRAM POSITIVE BACTERIA			GRAM NEGATIVE BACTERIA			
	S. aureus	*B. subtilis*	*E. faecalis*	*E. coli*	*S. typhi*	*K. pneumoniae*	***P. aeruginosa***
Aegle marmelos	-	12±0	-	8±0	-	10±0	10±0
Antidesma acidum	-	9±0	10±0	-	-	13±0	12.33±0.33
Azadirachta indica	15.66±0.66	14.5±0.28	-	15.66±0.66	-	10±0	-
Phanera vahlii	NA	11±0	-	10±0	-	-	NA
Callicarpa macrophylla root	9±0	12±0	10±0	-	-	11±0	10±0
Callicarpa macrophyla leaf	11.8±0.73	15±0	-	12.33±0.38	9±1	-	12±0
Cissampelos pareira	10.33±0.66	11±0	10±0	8±0	-	13±0	12±0
Flemingia strobilifera	11.25±0.47	13.3±0.33	-	11.66±0.33	-	-	12..33±0.66
Lagerstroemia parviflora	-	-	-	8±0	-	8±0	-
Rhododendron arboreum	-	-	9.5±0.5	-	-	14±057	10±0
Rhus chinensis	14±0.57	15.66±0.33	-	16±0.33	-	-	12.33±0.33
Stephania glandulifera	10.85±0.55	14±0	-	13.33±0.55	-	12±0	12±0
Tectaria coadunata	9±0	-	-	-	-	-	15±0
Thalictrum foliolosum	-	13±0	-	-	-	-	10.33±0.33
Tinosppora sinensis	-	9±1	-	10.5±0.5	9±1	-	-
Woodfordia fruticosa	10.66±0.76	12±0	11±0	12.8±0.48	11±0	11±0	13±0
Ampicillin	32.5±0.5	8.5±0.5	17.75±0.25	25±1	15.5±0.5	8.5±0.5	-
Gentamicin	16.75±0.25	15.5±0.5	18.5±0.5	17.5±0.5	12.66±0.33	11.33±0.88	14.66±0.33
DMSO	-	-	-	-	-	-	-

Table 40b: Antibacterial activity of hexane extracts showing ZOI against *Shigella dysenteriae*				
RCH	FSH	ampicillin	gentamicin	DMSO
-	-	23.75 • ± 0.25	18.66 • ± 0.66	-

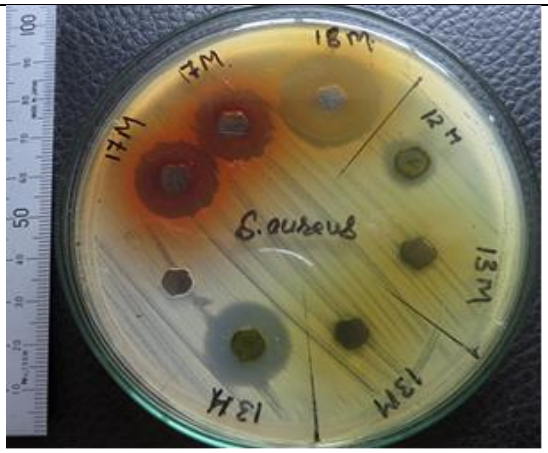
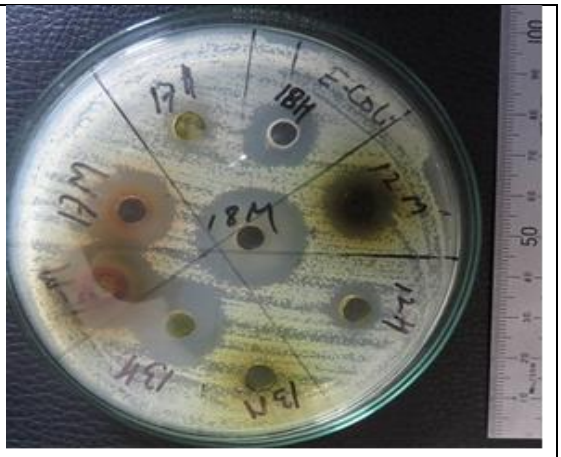
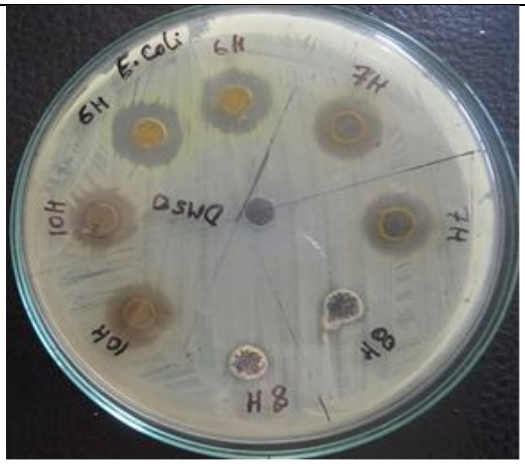
	
(a) 13H = AIH against *Staphylococcus aureus*	(b) 13H = AIH, 17H = TCH, 18H = RCH against *Escherichia coli*
	
(c) 6H = SGH, 7H = WFH, 8H = AMH, 10H = FSH against *Escherichia coli*	

Figure 12: Antibacterial assay showing the ZOI of the hexane extract of the plant parts against the tested bacteria.

The result of antibacterial susceptibility assay showed that the antibacterial activities depend on the phytoconstituent present in the extracts as well as solvent used for extraction and the plant parts used, not only that but also the tested bacteria.

4.6.3 MICs of the Methanolic Extracts

The results of MIC of 70% methanolic extracts are presented in Table 41a. Result against *Shigella dysentery* is presented in Table 41b.

Table 41a: MICs of the 70% methanolic extracts

70% methanolic extract of the plant species	Minimum inhibitory concentration (MIC) of the 70% methanolic extract (mg/mL)						
	GRAM POSITIVE BACTERIA			GRAM NEGATIVE BACTERIA			
	S. aureus	*B. subtilis*	*E. faecalis*	*E. coli*	*S. typhi*	*K. pneumoniae*	*P. aeruginosa*
Aegle marmelos	12.5	25	25	-	50	25	25
Antidesma acidum	12.5	25	-	12.5	-	12.5	6.25
Azadirachta indica	-	25	25	-	50	50	25
Phanera vahlii	25	25	6.25	12.5	-	25	12.5
Callicarpa macrophylla root	-	25	25	-	-	-	12.5
Callicarpa macrophyla leaf	25	25	-	6.25	25	25	12.5
Cissampelos pareira	-	-	6.25	-	-	50	25
Flemingia strobilifera	3.12	12.5	3.12	1.56	12.5	6.25	3.12
Lagerstroemia parviflora	12.5	25	25	6.25	25	50	6.25
Rhododendron arboreum	12.5	12.5	-	12.5	-	-	6.25
Rhus chinensis	3.12	25	25	25	-	6.25	12.5
Stephania glandulifera	12.5	25	25	6.25		12.5	-
Tectaria coadunata	6.25	6.25	-	1.56	50	-	25
Thalictrum foliolosum	12.5	>50	-	6.25	-	-	25
Tinosppora sinensis	-	25	-	>50	-	-	12.5
Woodfordia fruticosa	6.25	6.25	3.12	<1.56	25	50	<1.56

Table 41b: MICs of 70% methanolic extracts against *Shigella dysenteriae*				
Rhus chinensisCM	*Flemingia strobilifera*	ampicillin	gentamicin	DMSO
12.5±0	50±0	23.75 • ± 0.25	18.66 • ± 0.66	-

The result of minimum inhibition concentration (MIC) of AAM showed that the extract is more potential against *Pseudomonas aeruginosa* than other tested bacterial strains. The values of MIC varied from lowest <1.56 mg/mL to highest >50 mg/mL. The lowest MIC value was shown by WFM against *Escherichia coli* and *Pseudomonas aeruginosa*. The highest MIC value was shown by TSM against *Escherichia coli* and TFM against *Bacillus subtilis*. Likewise, the MIC value of WFM was 3.12 mg/mL against *Enterococcus faecalis* and 6.5 mg/mL against *Staphylococcus aureus* and *Bacillus subtilis.* It showed that WFM was potential antibacterial against Gram +ve as well as Gram –ve bacteria.

The result revealed that the MICs of FSM was 1.56 mg/mL against *Escherichia coli*; 3.12 mg/mL against *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*; 6.25 mg/mL against *Klebsiella pneumoniae.* The MIC was greater than 6.25 mg/mL against the remaining tested bacteria.

The result revealed that the MIC of RCM was 3.12 mg/mL against *Staphylococcus aureus* where as it was 6.25 mg/mL against *Klebsiella pneumoniae* and greater than 6.25 mg/mL against the remaining tested bacteria.

The result revealed that the MICs of TCM was 1.56 mg/mL against *Escherichia coli* where as 6.25 mg/mL against *Staphylococcus aureus* and *Bacillus subtilis*, and greater than 6.25 mg/mL against *Salmonella typhi* and *Pseudomonas aeruginosa*.

Among all 70% methanolic extract, WFM showed the strongest antibacterial potentiality (MIC <1.56 mg/mL) against *Escherichia coli* followed by FSM (1.56mg/mL) and TCM (1.56 mg/Ml) then LPM (6.25mg/mL), CMML (6.25mg/mL), SGM (6.25mg/mL) and TFM (6.25mg/mL); then AAM, PVM(12.5mg/mL) and RAM(12.5mg/mL) and RCM (25mg/mL).The magar use the plant species *Phanera vahlii, Flemingia strobilifera, Lagerstroemia parviflora, Rhododendron arboreum, Rhus chinensis, Stephania glandulifera, Tectaria coadunata* and *Woodfordia fruticosa* for the treatment of diarrhea.The efficacy of 70% methanolic extract of these plant species against *Escherichia coli* (causal agent of diarrhea) were in the order of WFM (MIC <1.56 mg/mL), FSM (1.56mg/mL), TCM (1.56 mg/Ml), LPM (6.25mg/mL), SGM (6.25mg/mL) PVM(12.5mg/mL) and RAM(12.5mg/mL) and RCM (25mg/mL).

The result showed that the most potential antibacterials against *Enterococcus faecalis* were WFM and FSM followed PVM, CPM, AMM, AIM, CMMR, LPM, RCM and SGM.

The results of MIC showed that the most potential antibacterial agents against *Staphyloccus aureus* were FSM and RCM, followed by TCM and WFM. The result of MICs showed that the most potential methanolic extracts against *Pseudomonas aeruginosa* were WFM, FSM followed by AAM, LPM and RAM. The MICs of the extracts might have bacteriostatic or bactericidal effect. The MIC of extract showing bacteriostatic effect works as antibacterial by stopping multiplication of the bacteria. The MICs showing bactericidal effect work as antibacterial agent by killing the bacteria.

4.6.4 MICs of the Hexane Extracts

The results of MICs of hexane extracts are presented in Table 42.

Table 42: MICs of the hexane extracts

Hexane extract of the plant species	Minimum inhibitory concentration (MIC) of the hexane extract (mg/mL)						
	GRAM POSITIVE BACTERIA			GRAM NEGATIVE BACTERIA			
	S. aureus	*B. subtilis*	*E. faecalis*	*E. coli*	*S. typhi*	*K. pneumoniae*	*P. aeruginosa*
Aegle marmelos	-	25	-	>50	-	25	25
Antidesma acidum	-		50		-	25	50
Azadirachta indica	3.12	25	-	12.5	-	25	-
Phanera vahlii	NA	25	-	25	-	-	NA
Callicarpa macrophylla root	25	12.5	25	-	-	25	25
Callicarpa macrophyla leaf	12.5	6.25	-	12.5	25	-	25
Cissampelos pareira	50	50	50	>50	-	50	25
Flemingia strobilifera	12.5	50	-	6.25	-	-	12.5
Lagerstroemia parviflora	-			>50			-
Rhododendron arboreum	-	-	50	-	-	12.5	25
Rhus chinensis	6.25	12.5	-	1.56	-	-	12.5
Stephania glandulifera	25	12.5		12.5	-	12.5	12.5
Tectaria coadunata	50	-	-	-	-	-	12.5
Thalictrum foliolosum		12.5		-		-	25
Tinospora chinensis	-	50	-	50	50	-	-
Woodfordia fruticosa	12.5	12.5	50	25	-	25	25

The minimum inhibition concentrations (MICs) of hexane extracts ranges from lowest 1.56 mg/mL to highest >50 mg/mL. The lowest MIC was shown by RCH and highest was shown by AMH, CPH and LPH against *Escherichia coli*. The MIC result showed that AIH exhibited the strongest antimicrobial potentiality with MIC value of 3.12 against *Staphyloccus aureus*.

Most Potential hexane extract against *Escherichia coli* was RCH (1.56mg/mL) followed by FSH (6.25mg/mL), AIH (12.5mg/mL), CMHL (12.5mg/mL), SGH (12.5mg/mL), PVH (25mg/mL) and WFH (25mg/mL). The efficacy of hexane extract of the plant used for treatment of diarrhea by the Magars were in the order of RCH (1.56mg/mL), FSH (6.25mg/mL), SGH (12.5mg/mL), PVH (25mg/mL) and WFH (25mg/mL).

The most potential hexane extract against *Staphylococcus aureus* was AIH followed by RCH, CMHL, FSH and WFH. More potential hexane extract against *Enterococcus faecalis* was CMHR. Comparatively more potential hexane extracts against *Klebsiella pneumoniae* were RAH and SGH.

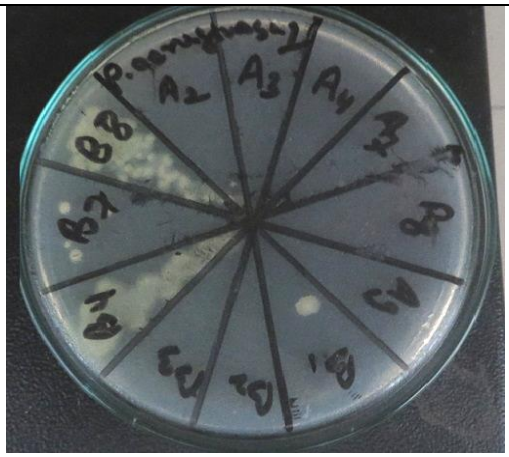
Similarly, more potential hexane extracts against *Pseudomonas aeruginosa* were FSH, RCH, SGH and TCH.

4.6.5 Minimum bactericidal concentration (MBC) of the Methanolic Extracts

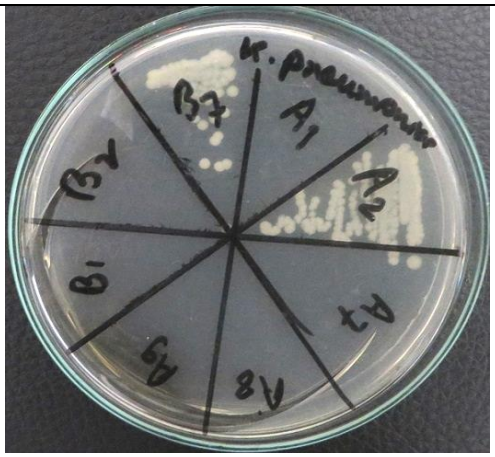
Results of MBCs of 70% methanol extracts were presented in Table 43a. Result of MBCs against *Shigella dysenteriae* is presented in table 43b.

The result of MBCs of AMM showed that MIC values of the AMM against all the tested bacteria were bacteriostatic and killed bacteria on increase in the concentration. The result of MBC showed that MIC value of AAM against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were bacteriostatic and killed bacteria on increase in concentration. Its MIC against *Pseudomonas aeruginosa* was 6.25 mg/mL in B4 well (i.e. location in the 96-well plate). MBC values were determined as no colony growth (or growth by 10-1%) by direct streaking the content of the well of MIC and higher concentratios than the MIC on sterile MHA plates. When content of B4, B3, B2 and B1 wells were striked on sterile MHA plate, MBC was found in the content of B3 well (12.5mg/Ml) (Figure 13a). But MICs against *Klebsiella pneumoniae* and *Bacillus subtilis* were

bactericidal. The MBC value of AAM against *Klebsiella pneumoniae* was 12.5 i.e. the content of A9 (Figure 13b).



(a) against *Pseudomonas aeruginosa*



(b) against *Klebsiella pneumoniae*

Figure 13: Determination of MBCs of AAM.

Table 43a: MBCs of the 70% methanolic extracts

70% methanolic extract of the plant species	Minimum bactericidal concentration (MBC) of the 70% methanolic extract (mg/mL)						
	GRAM POSITIVE BACTERIA			GRAM NEGATIVE BACTERIA			
	S. aureus	*B. subtilis*	*E. faecalis*	*E. coli*	*S. typhi*	*K. pneumoniae*	*P. aeruginosa*
Aegle marmelos	50	50	>50	-	>50	50	50
Antidesma acidum	25	25	-	25	-	12.5	12.5
Azadirachta indica	-	50	50	-	>50	50	50
Phanera vahlii	50	50	6.25	50		25	12.5
Callicarpa macrophylla root	-	50	25	-	-	-	50
Callicarpa macrophyla leaf	50	25	-	25	25	25	12.5
Cissampelos pareira	-	-	50	-	-	50	50
Flemingia strobilifera	6.25	12.5	3.12	6.25	25	6.25	6.25
Lagerstroemia parviflora	25	50	50	25	50	50	12.5
Rhododendron arboreum	12.5	25	-	50	-	-	25
Rhus chinensis	3.12	50	25	25	-	25	12.5
Stephania glandulifera	25	50	>50	25	-	50	-
Tectaria coadunata	50	50	-	25	50	-	50
Thalictrum foliolosum	50	>50	-	6.25	-	-	25
Tinospora chinensis	-	25	-	>50	-	-	50
Woodfordia fruticosa	25	12.5	3.12	6.25	50	50	< 1.56

Table 43b: MBCs of 70% methanolic extracts against *S. dysenteriae*

Rhus chinensis	*Flemingia strobilifera*	ampicillin	gentamicin	DMSO
25±0	50±0	23.75 • ± 0.25	18.66 • ± 0.66	-

IM showed bacteriostatic effect against *Bacillus subtilis, Enterococcus faecalis, Salmonella typhi* and *Pseudomonas aeruginosa* but bactericidal against *Klebsiella pneumoniae.*

The MBC result of PVM showed that MIC values against *Enterococcus faecalis, Klebsiella pneumonia* and *Pseudomonas aeruginosa* killed the bacteria but bacteriostatic against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli.*

The result of MBC showed that MIC value of CMMR against *Enterococcus faecalis* was bactericidal but against *Bacillus subtilis* and *Pseudomonas aeruginosa* was bacteriostatic. CMML was bactericidal against *Bacillus subtilis* (25mg/Ml), *Salmonella typhi* (25mg/mL), *Klebsiella pneumonia* (25mg/Ml) and *Pseudomonas aeruginosa* (12.5mg/Ml). The MIC of CMML was, contained in D3, i.e. 12.5 mg/mL, against *Pseudomonas aeruginosa* (Figure 14). CMML was bacterostatic against *Staphylococcus aureus* and *Escherichia coli.*

Figure 14: Determination of MBC of CMML

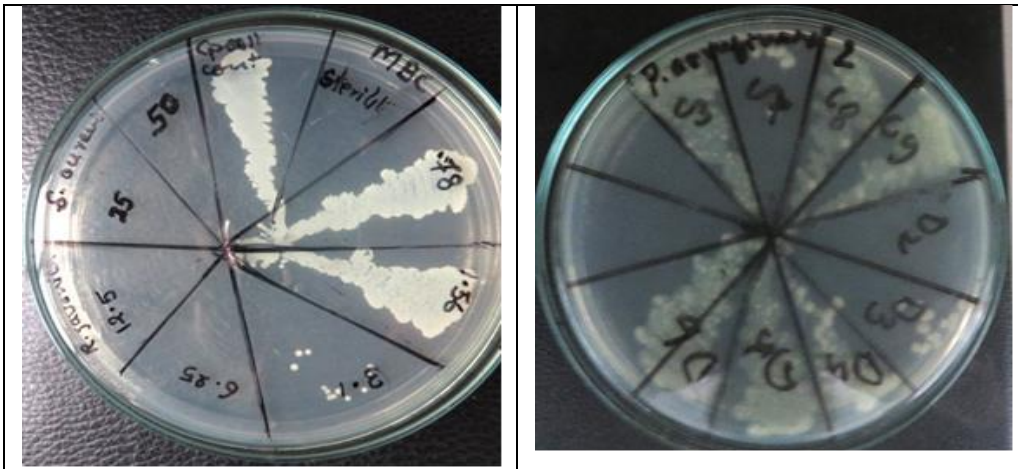
MIC value of CPM had bacteriostatic effect against *Enterococcus faecalis* and *Pseudomonas aeruginosa,* but bactericidal against *Klebsiella pneumoniae.*

The result of MBC showed that FSM showed bactericidal effect to *Bacillus subtilis, Enterococcus faecalis, Klebsiella pneumoniae* and *Shigella dysenteriae* at MIC values 12.5, 3.12, 6.25 and 50µg/mL. But, MICs against *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa* were bacteriostatic and kill the bacteria on increase of concentration.

The result of MBC showed that LPM had bacteriostatic effect against all the tested bacterial strains except *Klebsiella pneumoniae.* The extract inihibited only bacterial growth by stopping multiplication, but enabled to kill when the concentration was increased. The MIC value of the extract against *Klebsiella pneumoniae* was 50 µg/mL, and at that concentration the extract inhibits by killing the bacteria.

The result of MBC showed that the inhibitory effect of RAM was bactericidal against *Staphylococcus aureus* but it was bacteriostatic against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* and killed bacteria on increase of concentration.

The MBC value of RCM against *Stephylococcus aureus* was determined 3.12µg/mL (Figure 15a). The MIC was also 3.12 mg/mL. So the MIC showed bactericidal effect against *Staphylococcus aureus*. The MBC of RCM against *Pseudomonas aeruginosa* was determined to be 12.5 mg/mL (Figure 15b). MIC was also 12.5 mg/mL i.e. contain of D3. The MIC values of RCM were bactericidal against *Staphylococcus aureus, Pseudomonas aeruginosa Enterococcus faecalis* and *Escherichia coli* and bacteriostatic against *Bacillus subtilis, Klebsiella pneumoniae* and *Shigella dysenteriae.*



(a)MBC against *Staphylococcus aureus*

(b)MBC against *Pseudomonas aeruginosa*

Figure 15: Determnation of MBCs of RCM.

The result showed that SGM had bacteriostatic activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli* and *Klebsiella pneumoniae*. The extracts killed the bacterial strains on increase of the concentrations. TCM showed bacteriostatic activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* but bactericidal against *Salmonella typhi*.

The result of MBC of TFM showed that MICs against *Escherichia coli* and *Pseudomonas aeruginosa* were bactericidal in effect, whereas bacteriostatic against *Staphylococcus aureus* and *Bacillus subtilis*. MBC of TFM against *Escherichia coli* was 6.25 mg/mL i.e. contain of E4 (Figure 16).

Figure 16: Determination of MBC of TFM.

The result MBC of TSM showed that the extract was bactericidal against *Bacillus subtilis*, and bacteriostatic against *Escherichia coli* and *Pseudomonas aeruginosa*.

The result of MBC of WFM showed that the extract was bactericidal against *Enterococcus faecalis* and *Klebsiella pneumoniae*. The lowest MBC of methanol extract was <1.56 mg/mL against *Pseudomonas aeroginosa* shown by WFM.

The lowest value of MBC was 6.25 mg/mL against *Escherichia coli* and showed by FSM, TFM and WFM. At this concentration, CMML, LPM and SGM also inhibited the growth of *Escherichia coli* and killed *Escherichia coli* upon increasing of the concentration. It could be concluded that the most potential antibacterials against *Escherichia coli* were WFM, FSM and TFM. Though TCM inhibit growth of *Escherichia coli* at 1.56mg/mL (MIC), killed *Escherichia coli* at 25 mg/mL.

The communities of the study area use *Woodfordia fruticosa, Tectaria coadunata, Rhododendron arboreum, Phanera vahlii, Flemingia strobilifera, Rhus chinensis, Lagerstroemia parviflora* and *Stephania glandulifera* to treat diarrhea. This work proved that 70% methanolic extracts of these species exhibited antibacterial activity against *Escherichia coli*, causal bacteria of diarrhea (Nataro & Kaper, 1998), in support of the ethnobotanical knowledge. As the Magars used these plant species in traditional way in large quantity, they could be effective to treat diarrhea caused by *Escherichia coli*. The most efficient species for treatment of diarrhea according to MBCs were in order of WFM (6.25mg/mL), FSM (6.25mg/mL), LPM (25mg/mL), RCM (25mg/mL), SGM (25mg/mL), TCM (25mg/mL), PVM (50mg/mL) and RAM (50mg/mL)

The most potential antibacterials against *Staphylococcus aureus* were RCM and FSM followed by RAM.

The most potential antibacterials against *Pseudomonas aeruginosa* were WFM, followed by FSM, AAM PVM, CMML, LPM and RCM.

The result also revealed that the most potential antibacterials against *Enterococcus faecalis* were WFM, PVM and FSM. The RCM, CMMR, AIM, CPM and LPM also inhibited the growth and kill *Enterococcus faecalis* at higher concentration. *Enterococcus faecalis* is a causal bacterium for the urinary tract infection (UTI). Thus, the result of the research showed that these plants could be used to treat UTI. Hexane extract and 70% methanolic extract of *Cissampelos pareira* showed antibacterial activity against *Enterococcus faecalis*, supporting the tradition knowledge. The lowest value of MBC was 3.12 against *Enterococcus faecalis* shown by extract of *Flemingia strobilifera* and *Woodfordia fruticosa.*

MIC value of the 70% methanolic extract of *Aegle marmelos* bark against *Staphylococcus aureus* is 12.5mg/mL which is bactericostatic; it kills the bacteria at 50mg/mL. MIC value against *pseudomonas aeruginosa* is 25 mg/mL. It is also bacteriostatic and kills bacteria at 50mg/mL.MIC value against *Klebsiella pneumoniae* is 25 mg/mL and kills *Klebsiella pneumoniea* at 50mg/mL; against *Salmonella typhi* is 50 mg/mL and kills at >50mg/mL. Kothari et al. (2011) reported MIC value of methanolic extract of *Aegle marmelos* leaf against *Staphylococcus aureus, pseudomonas aeruginosa* and *Klebsiella pneumoniae* is

10mg/mL and MBC value of methanolic extract of *Aegle marmelos* leaf against *Staphylococcus aureus,* and *pseudomonas aeruginosa* is 10mg/mL, but MBC value against *Klebsiella pneumoniae* is 20mg/mL and MIC and MBC values against *Salmonella typhi* are 1.25 mg/mL and 2.5 mg/mL respectively. It shows that 70 % methanolic extract of *Aegle marmelos* bark has comparatively less antibacterial potentiality than leaf

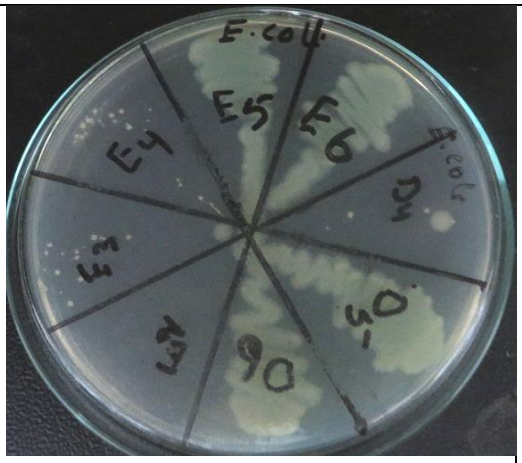
MIC values of the 70% methanolic extract of *Flemingia strobilifera* root against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* are 3.13, 3.12 and 1.56 mg/mL respectively. It shows that *Flemingia strobillifera* has significant antibacterial property. The antibacterial property mey be due to synergic effect of the phytochemical present in it (Nemkul et al., 2019). Madan et al. (2009) isolated four compounds belonging to isoflavones from root of *Flemingia strobilifera* and tested antibacterial activity against *Staphylococcus aureus, Pseudomonas* and *Esherichia coli* and MIC values were reportd against these bacteria were very low inμg/mL. These compounds isolated from root of *Flemingia strobilifera* have the most potential antimicrobial activity.

4.6.6 MBCs of the Hexane Extracts

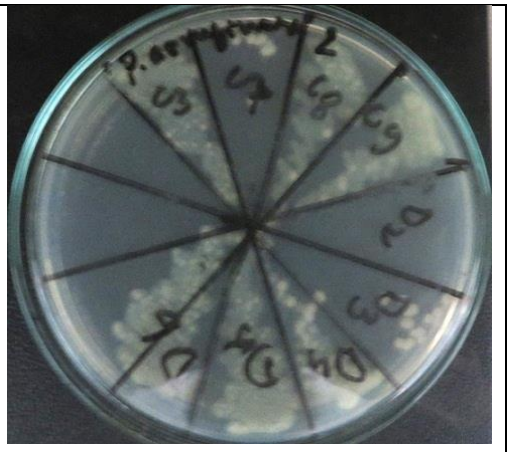
The results of MBCs of the hexane extracts are presented in Table 44. The result showed that the lowest MBC was 6.25 mg/mL against *Staphyloccus aureus* and *Escherichia coli* using AIH and RCH respectively amongst other hexane extracts of the plant species.

MIC value of AIH showed against *Staphylococcus aureus* is 3.12 mg/mL. This MIC value is bacteriostatic in effect against *Staphylococcus aureus* and kills the bacterial species at 6. 25mg/mL. But MIC showed bactericidal effect to *Klebsiella pneumoniae* and *Escherichia coli*. MBC of AIH against *Escherichia coli* was 12.5 mg/mL i.e. contain of E3 (Figure 17a), MIC was also found to be 12.5 mg/mL. The most efficient extracts according to MBCs of hexane extracts against *Escherichia coli* were in order of RCH (6.25 mg/mL), AIH (12.5mg/mL) and CMHL (12.5mg/mL).

The result showed that the MBC of RCH against *Pseudomonas aeruginosa* was 50 mg/mL (C_7) (Figure 17b). MIC of it was 12.5 mg/mL concentration, which was filled in C9 well.



(a) MBC of AIH against *Escherichia coli*



(b) MBC of RCH against *Pseudomonas aeruginosa*

Figure 17: Determination of MBC of the hexane extracts.

MIC values of hexane extract of *Azadirachta indica* against *Staphylococcus aureus* and *Esherichia coli* are 3.12 and 12.5 mg/mL respectively. MIC value against *Staphylococcus aureus* is bacteriostatic and kill the bacteria at 6.25mg/mL, but against *Escherichia coli* is bactericidal. The result shows that the *Azadirachta indica* leaf extract of non polar solvent (hexane) showed good antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* than the extract of polar solvent (70% methanol).

MIC values of hexane extract of *Callicarpa macrophylla* leaf against *Staphylococcus aureus* and *Echerichia coli* is 12.5mg/mL for each. This MIC value is bactericidal for these bacteria. Hexane extract of *Callicarpa macrophylla* leaf extract showed good antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* than 70% methanolicc extract.

MIC value of RCH against *Escherichia coli* is 1.56mg/mL. This MIC value is bacteriostatic and kills the bacteria at 6. 25mg/mL. But MIC value of RCM against *Escherichia coli* is 25 mg/mL. So hexane extract of *Rhus chinensis* fruit showed good antibacterial activity against *Echerichia coli* than methanolic extract.

The antibacteriall efficacy of the plant extract also depens on polarity of the solvent used for extraction. Antibacterial activity depends upon extraction procedure, type of plant parts used, solvents used for extraction and bacterial strain (Devi et al., 2014).

Table 44: MBCs of hexane extract

Haxene extract of the plant species	Minimum bactericidal concentration (MBC) of the hexane extract (mg/mL)						
	GRAM POSITIVE BACTERIA			GRAM NEGATIVE BACTERIA			
	S. aureus	*B. subtilis*	*E. faecalis*	*E.coli*	*S. typhi*	*K. pneumoniae*	*P. aeruginosa*
Aegle marmelos	-	50	-	>50	-	25	50
Antidesma acidum	-	>50	>50	-	-	>50	50
Azadirachta indica	6.25	50	-	12.5	-	25	-
Phanera vahlii	NA	50	-	NA	NA	-	NA
Callicarpa macrophylla root	25	12.5	50	-	-	25	NA
Callicarpa macrophyla leaf	12.5	>50	-	12.5	>50	-	NA
Cissampelos pareira	50	50	50	>50	>50	>50	NA
Flemingia strobilifera	25	50	-	25	-	-	25
Lagerstroemia parviflora	-	-	-	>50	-	>50	-
Rhododendron arboreum	-	-	50	-	-	50	NA
Rhus chinensis	50	25	-	6.25	-	-	50
Stephania glandulifera	25	50	-	25	-	12.5	NA
Tectaria coadunata	>50	-	-	-	-	-	>50
Thalictrum foliolosum	-	25	-	-	-	-	50
Tinospora sinensis	-	50	-	NA	>50	-	-
Woodfordia fruticosa	12.5	>50	50	NA	50	50	NA

4.7 Total Phenol Contents (TPC)

Upon addition of phenolic compounds, the yellowish colour of Folin Ciocalteu reagent got change into blue. This colour transformation was due to transfer of electrons in alkaline medium from phenolic compounds to phosphomolybedic/phosphotungstic acid complexes in Folin Ciocalteu reagent to form blue coloured complexes, $(PMoW_{11}O_{40})^{-4}$, which was determined spectrophotometrically at 760 nm (Bhandari & Rajbhandari, 2014).

The absorbances recorded at 760 nm wavelength for gallic acid solutions at different concentrations were presented in Table 45. A linear curve for standard gallic acid with the regression equation of y = 0.023 + 0.088 (R^2 = 0.996) was plotted from the average absorbance values using different concentrations of gallic acid (Figure 18).

Table 45: Absorbance of of gallic acid (GA) solution at 760 nm

Concentration of GA (µg/mL)	Average absorbance of GA
100	2.402
50	1.362667
25	0.6775
12.5	0.346
6.25	0.222
3.125	0.134
1.56	0.147667

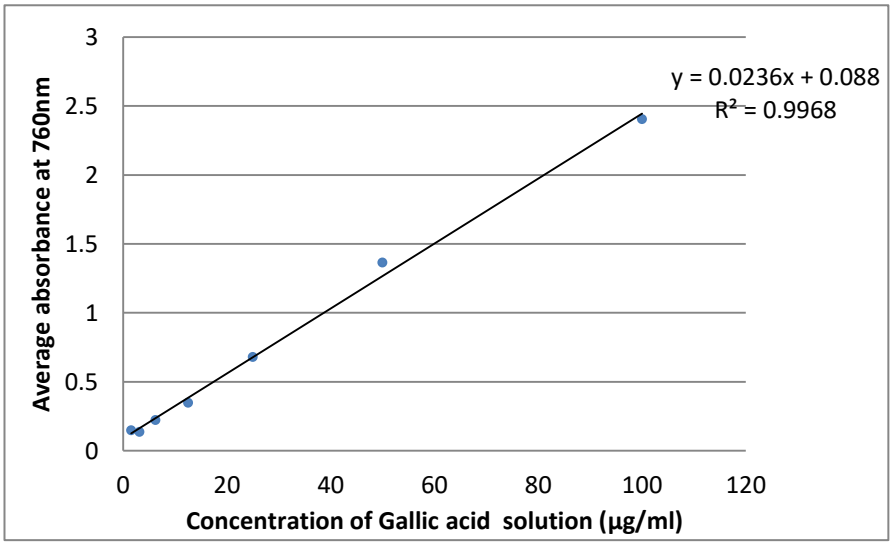


Figure 18: Standard gallic acid curve.

The absorbances recorded at 760 nm for the 70% methanolic extracts solutions at different concentrations in triplicate are presented in Annex II. Thus obtained absorbance values (y) were fit into the regression equation of gallic acid (y = 0.023x + 0.088) to obtain the phenolic concentration expressing in gallic acid equivalent (GAE) in dry weight (mg/g). Since the extract solutions prepared were 4 mg/10 mL concentration and therefore the use of 50 µL of the solution constituted of 20 µg mass. Thus calculated TPC values obtained are presented in Table 46.

Table 46: Total phenol content (TPC) of the 70% methanolic extracts

70% methanolic extracts of the Plant species	Average TPC ± SEM (mg GAE/g of dry extract)
Aegle marmelos	90.39±4.68
Antidesma acidum	126.38±5.86
Azadirachta indica	8.15±0.06
Phanera vahlii	246.15±3.01
Callicarpa macrophylla root	105.61±3.87
Callicarpa macrophyla leaf	132.24±0.32
Cissampelos pareira	54.20±1.08
Flemingia strobilifera	336.95±14.61
Lagerstroemia parviflora	291.24±1.20
Rhododendron arboreum	257.55±4.48
Rhus chinensis	123.52±1.29
Stephania glandulifera	66.35±1.61
Tectaria coadunata	188.98±3.31
Thalictrum foliolosum	65.78±3.44
Tinospora sinensis	50.55±2.69
Woodfordia fruticosa	258.40±6.26

The result showed that total phenol contain in the 16 different plant extracts of 15 ethno-medicinal plant species used had significant variation ranging from 8.15 to 336.95 mg of GAE/g of dry extract. FSM contained maximum amount of total phenol contain (336.95±14.61 mg of GAE/g of dry extract) followed by LPM, WFM, RAM, PVM, TCM, and so on (Table 45).

The total phenol contain barks of AMM was found to be 90.39±4.68 mg GAE/g dry extract. Wali et al. (2016) reported total phenol contain in the methanolic extracts of leaf, bark and fruit of *Aegle marmalos* and were 16.5, 14.2 and 10.6 mg GAE/g dry weight of sample, respectively. These data indicate that our sample constituted comparatively a higher amount of phenolics.

The total phenol contain of leaf of AIM was found to be 8.15±0.06 mg GAE/g dry extract. Shewale and Rathod (2018) reported total phenol contain on *Azadirachta indica* leaf varies with different solvent used for the extraction. They found that the maximum total phenolic extraction was obtained with methanol followed by ethylacetate, ethanol, butanol and water using Soxhlet extractions. They estimated total phenol contain in the methanolic extract of neem powder and was found 13.54 mg of GAE/g, and in the 70% ethanolic extract, it was 10.80 mg of GAE/g. The total phenolic amount was 43.57 mg GAE/g dry crude extract in the methanolic extract of leaf of *Azadirachta indica* (Al-Jadidi & Hossain, 2015). It indicates that our sample constituted comparatively low amount of phenolics.

The total phenol contain of roots of FSM was found to be 336.95±14.61 mg GAE/g of dry extract. Pizon et al. (2016) estimated total phenol contain of aqueous and 80% methanolic extract of leaves of *Flemingia strobilifera* by using Folin-Ciocalteu method and reported that total phenol contain of aqueous and 80% methanolic extracts were 102.98±0.003 and 12.49±0.02 mg GAE/g extract respectively. It indicates that our sample constituted high amount of total phenol contain in the roots of *Flemingia strobilifera*.

The total phenol contain of bark of of *Rhododendron arboreum* was found to be 257.55±4.48 mg GAE/g of dry extract. Bhandari and Rajbhandari (2014) reported total phenol contain of methanolic extract of the barks of *Rhododendron arboreum* was 240±00 mg GAE/g of dry extract and 330 mg GAE/g of dry extract in 50% methanolic extract. Our result of the total phenol contain data of *Rhododendron arboreum* is very nearly similar to that of Bhandari and Rajbhandari (2014). Painuli et al. (2018) estimated total phenolcontain of the methanolic extract of the leaves and it was reported to be 102.7±0.017 mg GAE/g of dry extract. It indicates thar total phenol contain is comparatively less in the leaf extract.

Total phenol contain of fruit of RCM was found to be 123.52±1.29 mg GAE/g dry extract. A few authors have reported total phenol contain values in different extracts of the fruits of *Rhus chinensis*. Fu et al. (2010) reported total phenol contain value of 17.2±1.49 mg GAE/g wet weight in the tetrahydrofuran extract; Sharma et al. (2015) reported 172.84±15.33 mg GAE/g extract in the methanolic extract; and Heirangkhongjam and Ngaseppam (2019) reported 7.25±0.03, 42.57±0.24, 28.37±0.46 and 35.76±1.71 mg GAE/g extract in the aqueous, acetone, ethanolic and

methanolic extracts, respectively. These data clearly indicate that the value of total phenol contain varies with the extractive solvents, and our sample constituted comparably a higher amount of phenolics.

4.8 Antioxidant Assays

Antioxidant activity of the extracts was determined by DPPH free radical scavenging and H_2O_2 scavenging methods.

4.8.1 DPPH Free Radical Scavenging Assay

The result of DPPH free radical scavenging activities was recorded in term of % inhibition as shown in Annex III. The absorbance values measured at wavelength 517 nm wavelength for the 70% methanolic extracts of the plant species at different concentrations are tabulated (Annex III). The values of absorbance recorded were used to calculate the percentage of inhibition of DPPH free radical by the corresponding extracts and are tabulated in Annex III (inhibition and absorbance at same table).

Linear curves were plotted taking different concentrations of the extracts used versus percentage of DPPH free radical inhibition. IC_{50}s of the samples were then calculated from the regression equations of respective linear curve (Annex III). DPPH free radical scavenging of gallic acid (GA) was taken as a positive control. The absorbance values measured at wavelength of 517 nm of the gallic acid solution at different concentrations are presented in Table 47. The inhibition percentage was calculated by using the absorbance values. The linear curve was ploted ($Y = 3.043 + 12.03$, $R^2 = 0.998$) (Figure 19) and IC_{50} of the gallic acid solution was calculated ($IC_{50} = 12.47$ µg/mL) from the linear curve. DPPH free radical scavenging range from 4 to 94% at 100 µg/mL concentration of the 70% methanolic extracts of different plant species and IC_{50} range from 21.59±0.26 to 1434.11±1.17 µg/mL (Table 48). Higher % inhibition indicates better scavenging activity.

Table 47: Absorbance and % of inhibition of different concentrations of gallic acid

Concentration of gallic acid	Average absorbance at 517 nm wavelength	Average % inhibition
188	0.192667	72.8054
94	0.193	72.74401
50	0.19166	72.98956
47	0.191667	72.98956

30	0.194333	72.49847
25	0.194	72.49847
20	0.194333	72.49847
10	0.351	43.64641
5	0.444555	26.45795
Control	0.588	
Blank	0.045	

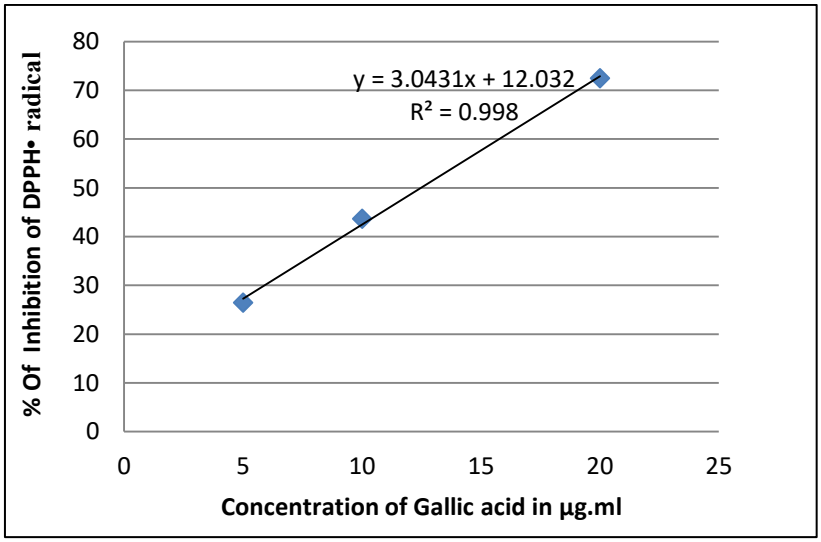


Figure 19: DPPH radical scavenging of gallic acid.

Higher IC_{50} value means weaker the DPPH free radical scavenging capacity. DPPH radical scavenging capacity by RAM, WFM, PVM and FSM was compared with that of gallic acid (Figure 20). DPPH free radical scavenging capacity of RAM and WFM was nearly similar to GA at 10µg/mL. But their DPPH radical scavenging capacity increased beyond the capacity of GA at 100µg/mL.

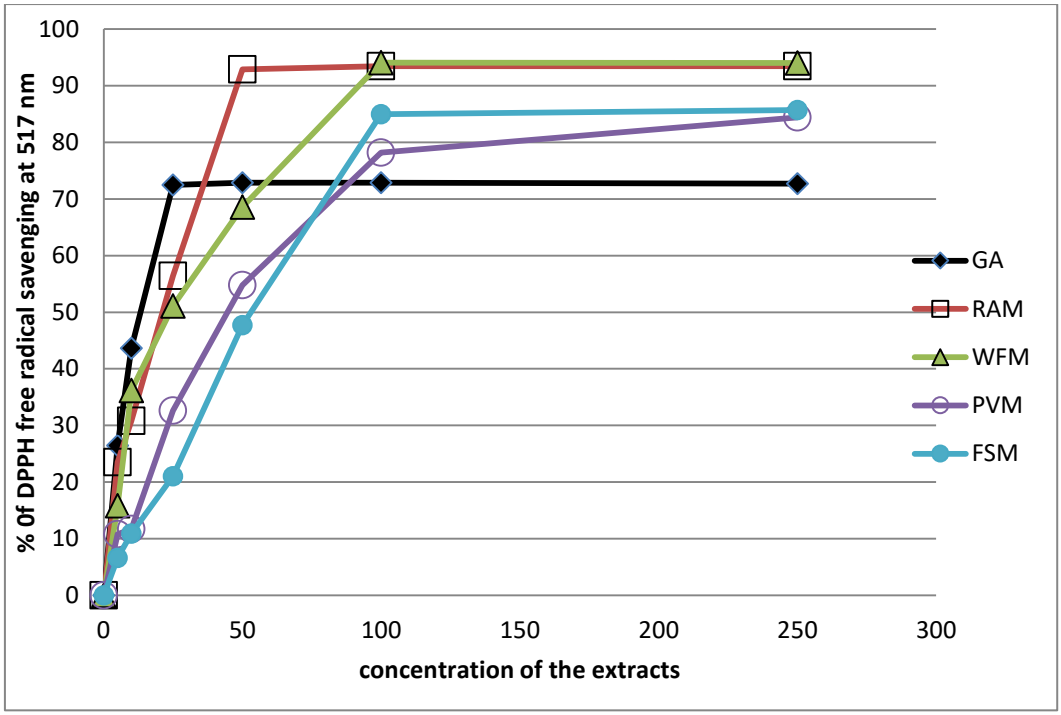


Figure 20: DPPH radical scavenging activity of the plant extracts compared to positive control gallic acid.

The values of IC_{50}s were high in the other extracts in comparison to the IC_{50} of GA (Table 48) but these extracts scavenged high % of DPPH radical at higher concentrations (Figure 21). This study showed that RAM and WFM possessed higher antioxidant capacity in term of DPPH free radical scavenging followed by FSM and PVM at 100 µg/mL concentration (Table 48).

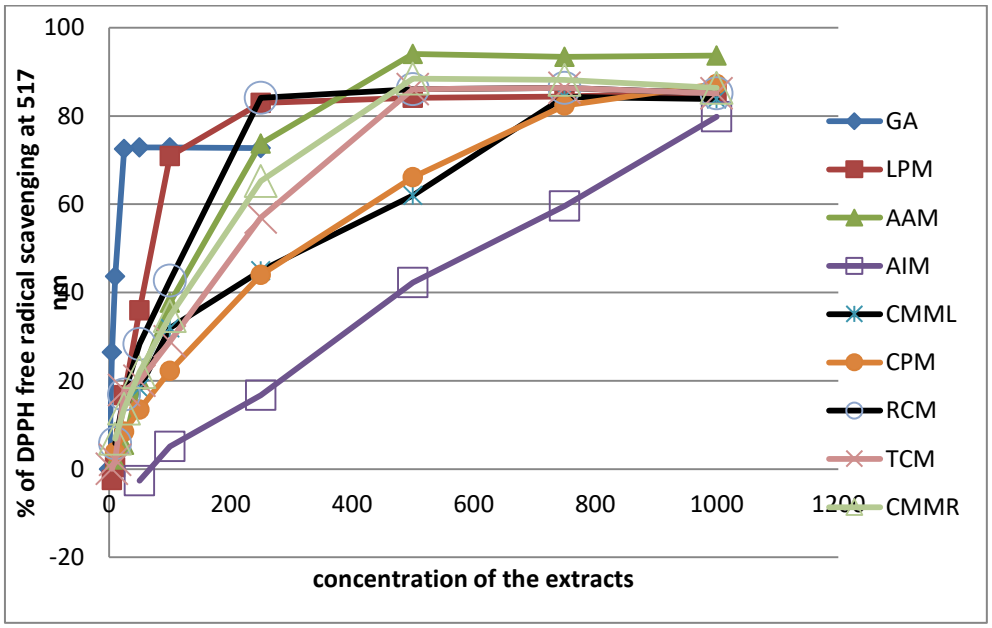


Figure 21: DPPH radical scavenging activity of the plant extracts.

Table 48: IC_{50} values and % of DPPH radical scavenging at 100µg/mL

Plant Extract	Average % of DPPH radical scavenging at 100 µg/mL	Maximum % DPPH radical scavenging	IC_{50} ± SEM
Aegle marmelos	8.08	87.13	961.53±0
Antidesma acidum	37.69	94	161.55±0.12
Azadirachta indica	5.10	79.8	619.00±0.25
Phanera vahlii	78.22	84.4	43.52±1.13
Callicarpa macrophylla root	34.67	88	216±1.37
Callicarpa macrophyla leaf	31.97	84	331.93±119
Cissampelos pareira	22.26	87	342.56±2.85
Flemingia strobilifera	85.00	86	56.69±0.72
Lagerstroemia parviflora	70.00	85	71.19±0
Rhododendron arboreum	93.00	93	21.59±0.26
Rhus chinensis	42.69	86	135.54±0.82
Stephania glandulifera	14.7	63	1045±5
Tectaria coadunata	28.94	86	208.50±8.17
Thalictrum foliolosum	12.40	77	1124.79±3.69
Tinospora sinensis	4.00	69	1434.11±1.17
Woodfordia fruticosa	94.00	94	33.68±0.52
GA			12.47±0

AMM displayed a maximum DPPH free radical scavenging capacity upto 87.13% (Table 48) at higher concentration. At 100µg/mL concentration it has scavenged DPPH radical only 8.08%. The result shows that the IC_{50} value of AMM is 961µg/mL Patra et al. (2015) reported a strong DPPH free radical scavenging activity of fermented fruit juice of *Aegle mormelos* with 81.1% scavenging at 15 µL concentration. They also indicated that the bark extract showed comparatively a low DPPH scavenging activity. Wali et al. (2016) reported DPPH free radical scavenging activity of the bark methanolic extract (IC_{50} = 268.7±8.5 µg/mL) and then extract of fruit (IC_{50} = 1032.2±7.03 µg/mL). It shows that our 70% methanolic extact of bark of *Aegle marmelos* has higher IC_{50} value than the bark metnanolic extract of Wali et al. (2016).

Our result shows that 70% methanolic extract bark of *Aegle marmelos* scavenge low percentage of DPPH radical at its lowere concentration but when the concentration of extract increases it scavenge DPPH radical more than 87%. Generally, the total polyphenols content in the sample is responsible for a high antioxidant activity. Compared to the polyphenols content, steroids were found to be major constituents in

the 70%. methanolic extract of *Aegle marmelos* barks in this study (Table 10). This could be a reason for exhibiting of a moderate antioxidant activity of the extract (Nemkul et al., 2018a).

The result of DPPH radical scavenging activity of CMML and CMMR was 31-35% at 100 µg/mL concentration. Jayaraman and Variyar (2015) reported the value of DPPH scavenging activity at a concentration of 2 mg/mL of the leaves of *Callicarpa macrophylla* was 40.11±0.005%. The DPPH free radical scavenging activity of the essential oil of leaves, fruits and seeds of *Callicarpa macrophylla* was observed between 55.67-62.63% (Chandra et al., 2017). It shows that our extract has scavenged higher percentage of DPPH radical. But our extract has scavenged comparatively lower percentage of DPPH radical than essential oil of leaves, fruit and seed.

FSM showed a good DPPH radical scavenging activity with IC_{50} vaue of 56.69±0.72 µg/mL. Madan et al. (2010) reported that the methanolic extract of roots of *Flemingia strobilifera* showed a very good DPPH radical scavenging activity with IC_{50} value of 11.4µg/mL and also reported that the methanolic extract of roots showed hydroxyl radical scavenging by p-NDA method with IC_{50} value of 378.33µg/mL. Pizon et al. (2016) evaluated antioxidant capacity of 80% methanolic extract of the leaves of *Flemingia strobilifera* by DPPH free radical scavenging method and reported that IC_{50} of it is 0.299 mg/mL. It indicates that methanol extract of root of *Flemingia strobilifera* is more potent antioxidant to scavenge DPPH than leaf.

IC_{50} of LPM in the DPPH assay was 71.19±00 µg/mL. Bobade et al. (2013) reported that DPPH free radical scavenging activity of *Lagerstroemia parviflora* methanolic leaf extract was weak showing IC_{50} value of 3482 µg/mL. It indicates that the barks of *Lagerstroemia parviflora* showed stronger antioxidant activity than its leaf using DPPH radical scavenging assay.

RAM showed a good DPPH radical scavenging with 92%. Painuli et al. (2018) investigated antioxidant activity of the aqueous and methanolic extracts of leaves and reported that methanol extract showed highest radical scavenging activity (91.67%). Our result was found comparable with it.

TCM scavenged DPPH radical by 28.94% at 100 µg/mL concentrations and IC_{50} was found to be 239.9±6.9 µg/mL. Poudyali and Singh (2019) reported antioxidant capacity of the methanolic extract of *Tectaria coadunata* rhizome with IC_{50} value of

30µg/mL with 53.26±0.33% inhibition of DPPH radical. Our extact showed comparatively low antioxidant capacity.

WFM showed 94% of DPPH radical scavenging activity at 100 µg/mL concentration. Grover et al. (2014) reported that flowers extracts scavenge 93.48±0.26% DPPH radical at 200 µg/mL concentration. It showed that our sample scavenge DPPH radical comparatively strongly.

Positive linear correlation obtained between TPC and DPPH free radical scavenging assays suggest that phenolic content could be used as an indicator of antioxidant properties of the examined plant species (Piluzza & Bullitta, 2011). Generally, total phenol content in sample is responsible for a high antioxidant capacity. Positive linear correlation between total phenolic content in the extracts and % of DPPH radical scavenging at 100 µg/mL concentration (correlation coefficient r = 0.92) at p <0.0001 (Figure 22) was observed at a 95% confidence level.

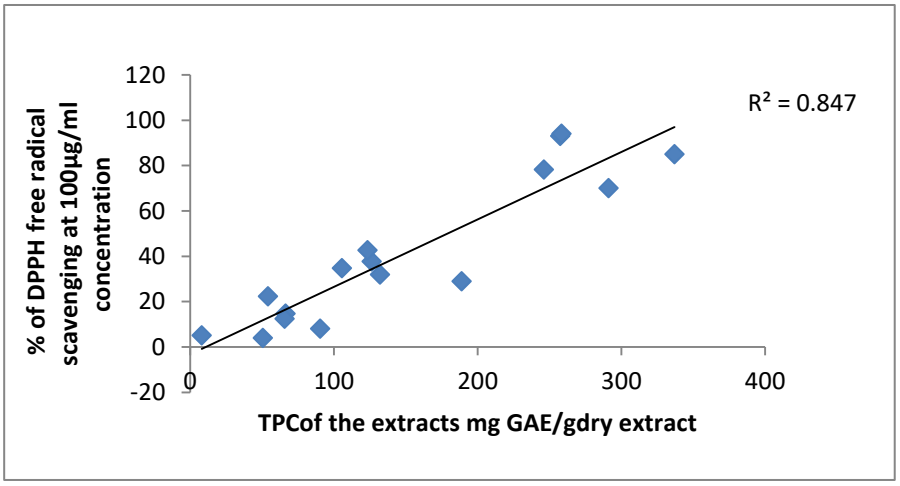


Figure 22: Linear correlation between TPC and % of DPPH free radical scavenging.

4.8.2 Hydrogen Peroxide Scavenging Assay

Percentage of hydrogen peroxide scavenging by the 70% methanolic extracts was presented in Table 49 and Figure 23. The % of H_2O_2 at 100 µg/mL scavenging ranged from 23.30±6.33 to 73.02±1.95% with minimum scavenging by SGM and maximum by WFM.

Table 49: Hydrogen peroxide scavenging activity

S. N.	Plant extracts	Average% of H_2O_2 scavenging ± SEM
1	*Aegle marmelos*	69.06±1.26
2	*Antidesma acidum*	57.18±2.60
3	*Azadirachta indica*	28.47±1.45
4	*Phanera vahlii*	66.69±0.66
5	*Callicarpa macrophylla* root	50.20±2.11
6	*Callicarpa macrophyla* leaf	42.51±4.41
7	*Cissampelos pareira*	48.55±3.18
8	*Flemingia strobilifera*	67.20±3.16
9	*Lagerstroemia parviflora*	NA
10	*Rhododendron arboreum*	67.69±0.91
11	*Rhus chinensis*	63.20±1.48
12	*Stephania glandulifera*	23.30±6.33
13	*Tectaria coadunata*	43.42±1.06
14	*Thalictrum foliolosum*	36.06±1.51
15	*Tinospora sinensis*	56.01±1.51
16	Woodfordia fruticosa	73.02±1.95

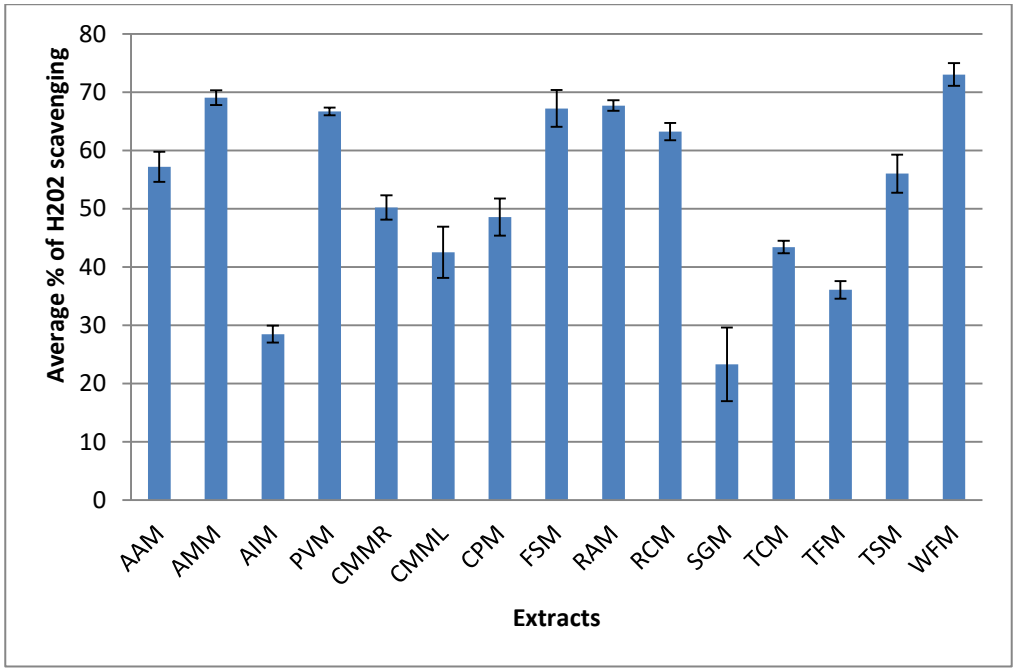


Figure 23: Hydrogen peroxide scavenging activity of the 70% methanolic extracts.

The result showed that AMM, PVM, FSM and RAM scavenged almost same % of H_2O_2. The result of this study showed that 70% methanolic extract of bark of *Aegle marmellos* scavenged higher percentage of H_2O_2 (69.06%) than DPPH radical

(8.08%) at 100µg/mL concentration. Patra et al. (2015) reported higher percentage of DPPH radical scavenged (81.1%) than H_2O_2 scavenged (34.45%) at 15 µL/mL concentration of the fermented fruit juice of *Aegle marmelos*. Contrary to the fermented fruit juice of *Aegle marmelos*, 70% methanolic extract of bark of *Aegle marmelos* higher percentage of H_2O_2 (69.06%) than DPPH radical (8.08%) at 100µg/mL concentration.

A positive linear correlation between TPC in the extracts and of % of H_2O_2 radical scavenging at 100 µg/mL concentration (correlation coefficient r = 0.68) at $p < 0.05$ (Figure 24) was observed at a 95% confidence level.

When compared in linear correlation model, the total phenol contain was more correlated with DPPH radical scavenging activity ($R^2 = 0.83$) than H_2O_2 scavenging activity of the studied ethnomedicinal plants ($R^2 = 0.46$). The results were statistically highly significance showing correlation between TPC and % of DPPH scavenging at $p < 0.0001$) and between TPC and % of H_2O_2 scavenging.

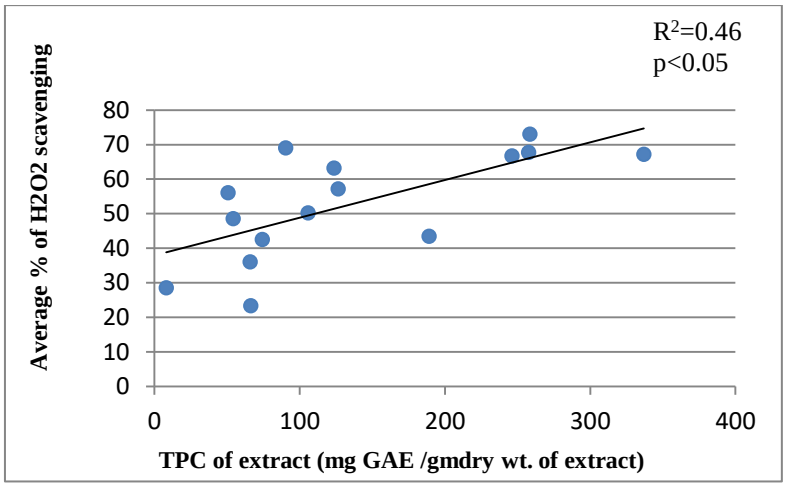


Figure 24: Linear correlation between TPC and % of DPPH free radical scavenging.

The phenolic compounds scavenge different percent of H_2O_2 and DPPH depending on many factors, such as the number of hydroxyl groups bonded to the aromatic ring, the site of bonding and mutual position of hydroxyls in the aromatic ring (Sroka & Cisowski, 2003). This could be a reason of the difference between the correlation of TPC with DPPH scavenging and H_2O_2 scavenging activities.

Hydrogen peroxide has positive role in energy production in *in vivo* system, phygocytosis, intercellular signal transfer, regulation of cell growth and the synthesis of important biological compounds (Packer et al., 2008). But H_2O_2 itself can be toxic

to the cells (Halliwell & Aruoma, 1991). Incubation of cells with H_2O_2 causes damage of deoxyribonucleic acid (DNA). Therefore, removal of H_2O_2 is obiviously biologically advantageous (Chance et al., 1979). Plant base H_2O_2 scavenger is important for human health. But study on it is rare.

This study find out that the 70% methanolic extract of *Aegle marmelos* bark is good scavenger of H_2O_2 than DPPH radical and also find out that the 70% methanolic extract of *Antidesma acidum* bark, *Azadirachta indica* leaf, *Callicarpa macrophylla* root, *Callicarpa macrophylla* leaf, *Cissampelos pareira*, *Rhus chinensis* fruit, *Stephania glandulifera* tuber, *Thalictrum foliolosum* root, *Tinospora sinensis* stem and *Tectaria coadunata* rhizome scavenged higher percentage of H_2O_2 than DPPH radical. This study also find out that the 70% methanolic extract of *Woodfordia fruticosa* flower, *Rhododendron arboretum* bark, *Flemingia strobilifera* root and *Phanera vahlii* root are good DPPH radical scavengers and also H_2O_2 scavenger.

CHAPTER 5

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Herbal remedies are deeply rooted in Nepali culture and also preferred for their accessibility and availability. It is uttermost important to undertake study of ethnomedicinal plants in Nepal. This study has been designed to verify herbal treatment of 15 species used by Magars of Nawalpur district. Herbal treatment were found to be basic treatment with total of 124 plant species and 66 special type of preparations reported by the informants in different ways than earlier researchers. *Ariopsis peltata* was first reported for bone fracture and muscular sprain. The documentation of such remedies will provide new frontiers for ethnopharmacology as well as drug discovery. Scientific verification of the ethnomedicinal data obtained is also important.

The result of antibacterial assay supports the use of *Phanera vahlii, Flemingia. strobilifera, Lagerstroemia parviflora, Rhododendron arboreum, Rhus chinensis, Stephania glandulifera, Tectaria coadunata* and *Woodfordia fruticosa*, for anti-diarrhea by the local villagers. Among these 8 species used for diarrhea the largest ZOI against *Escherichia coli* was given by 70% methanolic extract of *Rhus chinensis* followed by 70% methanolic extract of *Woodfordia fruticosa, Lagerstroemia parviflora, Tectaria coadunata, Flemingia strobilifera, Stephania glandulifera, Rhododendron arboreun* and *Phanera vahlii.* The most efficient species for treatment of diarrhea according to MBCs of 70% methanolic extracts were in order of 70 % methanolic extract of *Woodfordia fruticosa* (6.25mg/Ml), *Flemingia strobilifera* (6.25mg/Ml), *Lagerstroemia parviflora* (25mg/mL), RCM (25mg/mL), *Stephania glandulifera* (25mg/mL), TCM (25mg/mL), *Phanera vahlii* (50mg/Ml) and *Rhododendron arboreun* (50mg/Ml). The most efficient species for treatment of diarrhea according to MBCs of hexane extracts were in order of *Rhus chinensis* (6.25 mg/mL), *Flemingia strobilifera* (25 mg/mL) and *Stephania glandulifera* (25mg/mL). It shows that the efficacy depends on solvents used. But the villagers use the herbals in the form of decoction, juice and powder form in large quantity. The phytochemical tests of the extracts showed presence of phytochemicals responsible for antibacterial property in them.

The result of antibacterial activity supports the use of *Rhus chinensis* and *Flemingia. strobilifera* for the treatment of dysentery.

The ethnomedicinal data showed the local villagers used *Azadirachta indica, Cissampelos pareira,* and *Tinospora sinensis* for the treatment of UTI. The result of antibacterial assay of these three plant species showed antibacterial activity against UTI causing bacteria supporting the local use. But the antibacterial activity was weak to moderate and the extract showed bacteriostatic activity. The extract killed the bacteria only on high concentration. But the result of antibacterial assay showed that 70% methanolic extract of *Woodfordia fruticosa*, *Flemingia strobilifera* and *Phanera vahlii* have potential antibacterial activity against UTI causing bacteria such as *Escherichia coli, Enterococcus faecalis* and *Pseudomonas aeruginosa*. It can be concluded that it is better to use *Woodfordia fruticosa, Flemingia strobilifera* and *Phanera vahlii* for treatment of UTI. Presence of phytol in the extracts of *Azadirachta indica* (Table 14, 15) showed antimicrobial, anticancer, anti-inflammatory, and diuretic activities. Along with traditional use it could be used as anti-inflammatory and others.

The antibacterial assay showed that 70% methanolic and hexane extract of *Callicarpa macrophylla* leaf have potential antibacterial activity against *Salmonella typhi.* MIC value of 70% methanolic extract of *Callicarpa macrophylla* leaf (25mg/mL) against *Salmonella typhi* was bactericidal. The antibacterial assay of extracts of *Callicarpa. maccrophylla* support the ethnomedicinal data for the use in treatment of typhoid.

Magars in the study area use *Aegle marmelos* for cough and fever. The antibacterial activity of 70% methanolic extract of *Aegle marmelos* showed effective against all the tested bacteria except *Escherichia coli*. So it could be recommended for treatment of infection caused by these bacteria except *Escherichia coli*. The GC-MS analysis showed the presence of high amount of steroids (83.72% out of 88.87%). Among them γ-Sitosterol was 71.19%. γ-Sitosterol exhibits strong antifungal, antibacterial, antidiarrheal, antiviral and anti-angiogenic activities. Isolation of γ-Sitosterol from *Aegle marmelos* and *in vivo* animal tests are recommended for the discovery of new drug.

GC-MS analysis of *Antidesma acidum* showed presence of high amount of 9,12-Octadecadienoic acid (Z,Z), 5-(Hydroxymethyl)-2- furancarboxaldehyde and

Stigmasterol. Stigmasterol exhibits antiviral, anti-inflammatory and inhibit tumor promotion activities. Presence of stigmasterol supports the use of it for treatment of mumps by the magars. 9,12-Octadecadienoic acid (Z,Z) exhibits hepatoprotective, antihistaminic, antieczemic, antiacne, antiarthritic, anticoronary, cancer preventive, anti-inflammatory and hypocholesterolemic (Dr. Duke, 1992; Alagammal et al., 2011). Presence of high % of 9,12-Octadecadienoic acid (Z,Z) showed *Antidesma acidum* could be used for other diseases. Isolation of this compound and in vitro animal tests are recommended.

The antibacterial assay showed that extracts of *Thalictrum foliolosum* has potential antibacterial activity against *Escherichia coli*. It can be used for the treatment of diarrhea, though the local Magars used *Thalictrum foliolosum* for treatment of Rheumatic pain.

Lagerstroemia parviflora showed potential antibacterial activity against diarrhea and UTI causing bacteria. So it could be suggested that *Lagerstroemia parviflora* could be used for the treatment of UTI along with diarrhea.

The result showed that extracts of *Stephania glandulifera* has antibacterial activity against diarrhea and UTI causing bacteria. So *Stephania glandulifera* could be used for the treatment for them.

The result of DPPH free radical scavenging activity showed that 70% methanolic extract of *Rhododendron arboreum* was the most potential DPPH free radical scavenger amongst all. GCMS analysis reveals presence of high Stigmasterol, fatty acid and also presence of vitamins. It contain high amount of TPC and showed good DPPH radical scavenger and also hydrogen peroxide scavenger. Methanolic extract (70%) of *Rhododendron arboreum* was followed by 70% methanolic extract of *Woodfordia fruticosa, Bauhinia vahlii,* and *Flemingia strobilifera*. The result showed that along with treatment of infectious disease the plants could be used as antioxidant.

The result of hydrogen peroxide scavenging assay showed that the most potential hydrogen peroxide scavenger was 70% methanolic extract of *Woodfordia fruticosa* followed by *Aegle marmelos, Rhododendron arboreum, Flemingia strobilifera, Phanera vahlii, Rhus chinensis, Antidesma acidum,* and *Tinospora sinensis*.

The result showed that 70% methanolic extract of *Woodfordia fruticosa, Rhododendron arboreum, Flemingia strobilifera, Phanera vahlii,* were potential

antioxidant for scavenging of both hydrogen peroxide and DPPH radical. But 70% methanolic extract of *Aegle marmelos* and *Tinospora sinensis* were better antioxidant to scavenge hydrogen peroxide than DPPH free radical. GC-MS analysis showed that these extracts contained compounds 2-Methoxy-4-vinylphenol, n-Hexadecanoic acid, vitamin E, stigmasterol as well as Squalene which exhibit antioxidant activity.

The result of GC-MS analysis showed that 70% methanolic extract of *Callicarpa macrophylla* leaf contains maximum percentage of lupeol (76.69%) which has anti-inflammatory activity. This may consequently present new frontiers for the scientific community to investigate into their specific phytochemicals for the discovery of novel therapeutic compounds.

Many phytochemical researchers have neglected bioactivity screening related to ethnopharmacological uses. Thus, there is much additional work that can be carried out to identify phytochemicals associated with biological activities that support traditional uses of medicinal plants

5.2 Recommendations

The following recommendations are shaped from the present research:

a. Magars in the study area are living in remote hilly region without government facilitates hospital. So herbalist should be trained and make accessible of herbal treatment.
b. The plant extracts with potential antibacterial activity should be tested in *vitro* animal tests for anti-diarrheal and UTI.
c. *Woodfordia fruticosa, Flemingia strobilifera* and *Phanera vahlii* should be used for treatment of UTI along with local use of *Tinospora sinensis, Cissampelos pareira* and. *Azadirachta indica*. *In-vitro* animal test are required for these.
d. The result of GC-MS showed that CMML contained 76.65% of Lupeol out of 95.31% of total contain. Lupeol should be isolated from the leaf of *Callicarpa macrophylla* for commercialization.
e. Hexane extract of *Callicarpa macrophylla* contains 90.04% of 1-Undecane-3,6-dione,1-(2,6,6-trimethyl -1- cyclohexen-1-yl)- . The extract showed broad spectrum antibacterial activity. So isolation of the compound and *in vitro* as well as *in vivo* tests should be done to confirm its bioactivity.

Isolation and further search in phytoconstituents presents new frontiers for the scientific community to investigate into their specific phytochemicals for the discovery of novel therapeutic compounds.

CHAPTER 6

6 SUMMARY

6.1 Introduction

Magar is the largest indigenous ethnic communities in Nepal. The original homes of Magars are called Baramagarants (12 regions of the Magars) which include the hilly villages of Nawalpur district.

The hilly areas of Hupsekot and Bulingtar municipalities have no hospital facility. The people have to walk 1-3 hours to reach the health post and up to 6 hours walk or 3 hours driving by a jeep (due to mountainous road) to reach the hospital. People believe on local healers so they first visit healer and only after complication they go to market place to visit doctors. Sometimes the situation may be opposite. They visit doctor first for treatment of diseases. However, even after doing lots of laboratory tests, the disease cannot be diagnosed. Then they follow herbal treatment at their village place. Reciting "mantra" is a psychological treatment. This type of therapy is based on physical as well as psychological treatment. The villagers believe on the local familiar healers.

This study incorporates phytochemical analysis and antibacterial assay of the different extracts of plants that used in curing infectious disease and some non-infectious disease by Magars and would give scientific validation of the ethno-medicinal uses of the plant materials.

Medicinal properties of many plants are most likely due to their antioxidant content. Effectiveness of antioxidant is evaluated by either measuring the degree of oxidation inhibition or measuring the free radical scavenging ability. There are different *in vitro* methods to evaluate antioxidant activity such as Oxygen radical absorbance capacity (ORAC) method; 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity method; method of inhibition of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical cation; H_2O_2 scavenging, etc. The most important exogenous antioxidants are phenolic compounds, carotenoids, vitamin C and some minerals such as selenium and zinc.

6.2 Methods

Study sites were visited on January 2015, March 2015, April 2016, May 2016 and April 2017. Key informants were selected. In field survey, primary data were collected by (i) observation, (ii) interview, and (iii) questionnaires.

All the plant species were collected during transect walk with key informants through vegetation. The collected plants were pressed and dried using flame and were mounted on standard size (30 × 42 cm) herbarium sheets.

Laboratory testes along with evaluation of biologically active natural products present in ethnomedicinal plants would help in understanding their therapeutic actions of traditionally used. To select the important species used in traditional medicine quantitative ethnobotany *viz*. Factor for informant consensus (FIC) and fidelity level (FL) with number of mention were used.

The highly prioritized 15 ethno-medicinal plant species used by the Magar community were collected for laboratory experiments

Hexane and 70% methanol were successively used as extracting solvents. The extracts were concentrated using a rotary evaporator, vacuum dried at 37 °C and then stored in a refrigerator at 4 °C until further use

The test bacteria were *Pseudomonas aeruginosa*, *Staphylococcus aureus* , *Escherichia coli*, *Klebsiella pneumoniae, Enterococcus faecalis*, *Bacillus subtilis*, and *Salmonella enterica* subsp. *enterica serovar typhi*. These bacteria were sub-cultured in sterile MHA media

The inocula were prepared and standardized. Dried extracts were dissolved in DMSO to make 0.1 g/mL concentration for antimicrobial study. The antibacterial screening of these medicinal plant extracts were evaluated by using the agar well diffusion technique. The prepared inocula were swabbed on the sterile MHA plates. The wells were punched on the MHA plate. The wells were filled with 50 µL of the prepared plant extract. Ampicillin and gentamicin (Mast diagnostics) of 10 µg per disc were used as standard references. The plates were incubated at 37 °C for 18–24 hours following CLSI method. Broth dilution method was used to determine minimum inhibitory concentration (MIC). The standardized bacterial suspension was diluted to 1:100 and was vortexed. Each well containing the extract dilution and the growth control were filled with 50 µL of the diluted bacterial suspension. This results in the

final desired inoculum of 5×10^5 cfu mL^{-1}. After incubation for 18–24 hours at 37 °C, the MIC was taken as the lowest concentration of the antibacterial agent that inhibited visible growth of the tested bacteria as observed no turbidity with the unaided eye.

Minimum bactericidal concentration (MBC) values were then determined as no colony growth (or growth by 10-1%) by direct streaking the content of the wells inhibiting bacterial growth (MIC) and higher concentrations than the MIC value on sterile MHA plates.

Different phytochemical contents present in the hexane and 70% methanolic extracts were identified by the colour reactions with different reagents following the phytochemical screening method. Phytoconstituent present in the extracts were analyzed qualitatively and quantitatively by GC-MS analysis.

Estimation of total phenolic content was done using the Folin-Ciocalteu colorimetric method.

Total phenolic contents were calculated using the formula:

$$C = c\frac{V}{m}$$

Where, C = total phenolic content in mg GAE/g dry extract; c =concentration of gallic acid obtained from calibration curve in mg/mL; c = y/m where y = absorbance of extract solutions, m = gradient of calibration curve. V = volume of extract in mL, m = mass of extract in gram. Total phenolics content of the extract was expressed as mg gallic acid equivalents (GAE) per gram of extract in dry weight (mg/g).

Antioxidant assays were done by DPPH free radical scavenging and H_2O_2 scavenging methods.

The DPPH radical scavenging ability was calculated according to the following equation:

$$\text{DPPH radical scavenging rate (\%)} = 1 - \frac{\text{Absorbance (sample)} - \text{Absorbance (blank)}}{\text{Absorbance (control)} - \text{Absorbance (blank)}} \times 100$$

Each sample was analyzed in triplicates. The concentration of the sample at which 50% inhibition of $DPPH^{\bullet}$ radical was calculated as:

$$IC_{50} = \frac{(50 - c)}{m}$$

Where ‘c’ is intercept and ‘m’ is slop of linear curve describing dependence of percentage inhibition with concentration. IC_{50} is expressed in µg/mL.

Hydrogen peroxide scavenging activity was measured following the instructions of commercial kit (Radical catch; Hitachi Ltd., Tokyo, Japan)

Hydrogen scavenging activity was calculated following the equation below.

$$\text{Hydrogen peroxide scavenging activity (\%)} = \frac{\text{Luminescence (control)} - \text{Luminescence (sample)}}{\text{Luminescence (control)}} \times 100$$

6.3 Result

6.3.1 Ethnomedicine

Altogether 124 ethnomedicinal plant species belonging to 60 families are reported from the study area. The largest numbers of species were noted from Leguminosae family. The maximum number of the species are herb (44 species), followed by tree (43), shrub (28) and climber. The most frequently used plant part was leaf followed by bark and root.

Out of 124 medicinal plant species 66 species have been used in different ways than earlier researchers. Out of 66, medicinal use of seven species was not recorded as medicinal by Nepali scientists as per my knowledge. These seven species were *Acrocarpus fraxinifolius*, *Ariopsis peltata*, *Citrus paradise*, *Cryptolepis buchananii*, *Curcuma caesia*, *Opuntia monacantha* and *Zingiber montanum*. *Citrus paradise*, *Curcuma caesia* and *Zingiber montanum* are reported as medicine by other than Nepali scientist.

On the basis of Fidelity level with high mention *Flemingia strobilifera, Stephania glandulifera, Tectari coadunata, Rhus chinensis. Tinospora sinensis, Cissampelos pareira, Azadirachta indica, Rhododendron arboreum, Phanera vahlii, Woodfordia fruticosa, Callicarpa macrophylla, Antidesm acidum, Lagerstroemia parviflora* and *Aegle marmelos* were selected for laboratory tests. *Thalictrum foliolosum* was selected due to request of the local people.

6.3.2 Phytochemicals

Tannin and polyphenols were detected in all the extracts except 70% methanolic extract of *Azadirachta indica, Cissampelos pareira* and *Stephania glandulifera*.

Alkaloids were detected in 70% methanolic extract of *Aegle marmelos, Antides*ma *acidum, Phanera vahlii, Cissampelos pareira, Rhododendron arboreun, Stephania glandulifera, Thalictrum foliolosum* and *Tinospora sinensis*. Anthocyanosides was detected only in 70% methanolic extract of *Lagerstroemia parviflora* and *Tectaria coadunata.* Flavonoids were detected in 70% methanolic extract of *Azadirachta indica, Callicarpa macrophylla* leaf, *Rhus chinensis* and *Woodfordia fruticosa*. Saponins were detected in 5 extracts. Anthracenosides, cardiac glycosides, proteins and amino acids were not detected in the 70% methanolic extract of any of the species.

GC-MS analysis showed 70% methanolic extract of *Aegle marmelos* contained mainly steroids accounting 83.72% out of 88.87%. Hexane extract of *Aegle marmelos* contained mainly fatty acids. 70% methanolic extract of *Antidesma acidum* contained aldehyde, ketone, flavonoid and sugar. Hexane extract of *Antidesma acidum* contained mainly fatty acid and steroid. 70% methanolic extract of *Azadirachta indica* contained fatty acid, phenol and diterpene. Hexane extract of *Azadirachta indica* contained mainly fatty acid, vitamin and steroid. Hexane extract of *Phanera vahlii* contained mainly fatty acid, alkylbenzene and steroids. Methanolic extract (70%) of *Callicarpa macrophylla* leaf contained mainly triterpenoid where as hexane extract contained 90% of 1-Undecane-3,6-dione,1-(2,6,6-trimethyl -1- cyclohexen-1-yl)-. Methanolic extract (70%) of *Callicarpa macrophylla* root contained phenol, fatty acid etc. Hexane extract of *Cissampelos pareira* contained mainly fatty acid. Methanolic extract (70%) of *Flemingia strobilifera* contained flavonoid and fatty acid; whereas hexane extract mainly contained fatty acid. Methanolic extract (70%) of *Lagerstroemia parviflora* contained alkane and fatty acid whereas hexane extract contained alkylbenzene and fatty acids. Hexane extract of *Rhododendron arboreum* contained fatty acids, terpene, vitamin and steroid. Methanolic extract (70 %) of *Rhus chinensis* contained mainly fatty acids whereas hexane extract contained fatty acids and phenol. Methanolic extract (70%) of *Stephania glandulifera* mainly contained alkaloids whereas hexane extract contained fatty acid and alkaloids. Methanolic extract (70%) of *Tectaria coadunata* mainly contained ketone, aldehyde fatty acid. Whereas hexane extract mainly contained alkylbenzene and fatty acid. Methanolic extract (70%) of *Thalictrum foliolosum* mainly contained benzofuran and alkaloid where as hexane extract contained fatty acid. Methanollic extract (70%) of *Tinospora sinensis* mainly contained phenol and fatty acid whereas hexane extract mainly

contained fatty acid, steroid and triterpene. Methanolic extract (70%) of *Woodfordia fruticosa* mainly contained fatty acid, aldehyde and flavonoid whereas hexane extract mainly contained fatty acid, steroids vitamin and triterpene.

6.3.3 Antibacterial

Methanolic extract (70%) of *Rhus chinensis* showed largest ZOI against *Escherichia coli* followed by 70% methanolic extract of *Lagerstroemia parviflora* and *Woodfordia fruticosa* and so on. The value of MIC showed that the most potential antibacterial against *Escherichia coli* was 70% methanolic extract of *Woodfordia fruticosa* followed by 70% methanolic extract of *Tectaria coadunata*, *Flemingia strobilifera*, *Callicarpa macrophylla* leaf, *Lagerstroemia parviflora, Stephania glandulifera Thalictrum foliolosum* and so on. The value of MBC showed that 70% methanolic extract of *Woodfordia fruticosa, Thalictrum foliolosum* and *Flemingia strobilifera* killed *Escherichia coli* at 6.25 mg/mL.

The methanolic extract of *Woodfordia fruticosa* showed the largest ZOI against *Enterococcus faecalis* followed by *Rhus chinensis.* The value of MIC showed that the most potential antibacterial against *Enterococcus faecalis* was 70% methanolic extract of *Woodfordia fruticosa, Flemingia stobilifera* (3.12mg/mL) followed by 70% methanolic extract of *Cissampelos pareira* and *Phanera vahlii* and so on. The result showed that MIC value of *Woodfordia fruticosa, Flemingia strobilifera* and *Phanera vahlii* were bactericidal.

The methanolic extract (70%) of *Woodfordia fruticosa* and *Rhus chinensis* showed the largest ZOI against *Pseudomonas aeruginosa*. The value of MIC showed that the most potential antibacterial against *Pseudomonas aeruginosa* was *Woodfordia fruticosa* followed by *Flemingia stobilifera, Antidesma acidum, Lagerstroemia parviflora* and *Rhododendron arboretum.* Methanolic extract (70%) of *Rhus chinensis* and *Flemingia strobilifera* showed antibacterial activities against *Shigella dysenteriae* but MIC values of the extracts showed bacteriostatic effect.

The methanolic extract of *Rhus chinensis* showed the largest ZOI against *Staphylococcus aureus* then followed by *Woodfordia fruticosa*. The value of MIC showed that the most potential antibacterial against *Staphylococcus aureus* was 70% methanolic extract of *Rhus chinensis* followed by *Flemingia strobilifera*. MIC value of *Rhus chinensis* (3.12mg/ML) against *Staphylococcus aureus* was bactericidal

where as MIC value of *Flemingia strobilifera* was bacteriostatic. Methanolic extract (70%) of *Rhus chinensis* showed larger ZOI against *Klebsiella pneumoniae* followed by *Woodfordia fruticosa, Flemingia strobilifera* and *Stephania glandulifera*.

The MIC value of 70% methanolic extract of *Rhus chinensis* and *Flemingia strobilifera* was 6.25mg/mL where as *Woodfordia fruticosa* and *Stephania glandulifera* were 50mg/mL and 12.5mg/mL respectively. MIC value of *Flemingia strobilifera* was bactericidal against *Klebsiella pneumonia* where as MIC value of *Rhus chinensis* was bacterioststic. The result showed that MIC value of 70% methanolic extract of *Antidesma acidum* (12.5mg/ml) against *Klebsiella pneumoniae* was bacteridal.

MIC of 70% methanolic extract of *Flemingia strobilifera* was found 12.5 mg/mL and of *Callicarpa macrophylla* leaf was 25mg/mL against *Salmonella typhi*. The MIC values of *Flemingia strobilifera* was bacteriostatic and kill bacteria at 25mg/mL. MIC value of *Calllicarpa macrophylla* leaf against *Salmonella typhi* was bactericidal.

The antimicrobial activity of hexane extracts was comparatively low. Hexane extract of *Rhus chinensis* showed larger ZOI against *Escherichia coli* followed by *Azadirachta indica*. MIC of *Rhus chinensis* was1.56mg/mL and *Azadirachta indica* was 12.5mg/mL. MIC value of *Rhus chinensis* against *Escherichia coli* was bacteriostatic and kills the bacteria at 6.25mg/mL. MIC value of *Azadirachta indica* against *Escherichia coli* was bactericidal. Among the hexane extract *Azadirachta indica* showed larger ZOI followed by *Rhus chinensis* against *Staphylococcus aureus*. MIC of *Azadirachta indica* and *Rhus chinensis* were 3.12mg/mL and 6.25mg/mL. The MIC values were bacteriostatic.

6.3.4 Total Phenol Content (TPC)

The result showed that total phenol content in the 70% methanolic extract of 15 ethno-medicinal plant species used, had significant variation ranging from 8.15 to 336.95 mg of GAE/g of dry extract. *Flemingia stobilifera* contained maximum amount of TPC (336.95±14.61 mg of GAE/g of dry extract) followed by *Lagerstroemia parviflora, Woodfordia fruticosa, Rhododendron arboreum, Phanera vahlii* and *Tectaria coadunate*..

6.3.5 Antioxidant

DPPH free radical scavenging range from 4 to 94% at 100 µg/mL concentration of the 70% methanolic extracts of different plant species and IC_{50} range from 21.59±0.26 to 1434.11±1.17 µg/mL (Table 48). Higher % inhibition indicates better scavenging activity. On the basis of IC_{50} value the better DPPH free radical scavengers were *Rhododendron arboreum* followed by *Woodfordia fruticosa, Phanera vahlii, Flemingia strobilifera* and *Lagerstroemia parviflora*.

The result of hydrogen peroxide scavenging assay showed that the 70% methanolic extract of *Woodfordia fruticosa* was the best hydrogen peroxide scavenger followed by *Aegle marmelos, Rhododendron arboreum, Flemingia strobilifera, Phanera vahlii* and *Rhus chinensis.*

6.4 Conclusion

The most potential antibacterial against *Escherichia coli* were 70% methanolic extract of *Woodfordia fruticosa, Thalictrum foliolosum* and *Flemingia strobilifera*. The better extracts from the plants used for diarrhea by the Magars according to MBCs were in the order of *Woodfordia fruticosa* (6.25mg/Ml), *Flemingia sttrobilifera* (6.25mg/Ml), *Lagerstroemia parviflora* (25mg/mL), *Rhus chinensis* (25mg/mL), *Stephania glandulifera* (25mg/mL), *Tectaria coadunata* (25mg/mL), *Phanera vahlii* (50mg/Ml) and *Rhododenddron arboreum* (50mg/Ml). The most efficient hexane extracts for treatment of diarrhea according to MBCs were in order of *Rhus chinensis* (6.25 mg/mL), *Flemingia strobilifera* (25 mg/mL). The antibacterial assays support the use of these plants for treatment of diarrhea by the magars. The result of antibacterial activity supports the use of *Rhus chinensis* and *Flemingia strobilifera* for the treatment of dysentery.

The ethnomedicinal data showed the local villagers used *Azadichracta indica, Cissampelos pareira,* and *Tinospora sinensis* for the trearment of UTI. The result of antibacterial assay of these 3 plant species showed antibacterial activity against UTI causing bacteria supporting the local use. But the antibacterial activity was weak to moderate and the extract showed bacteriostatic activity. But the result of antibacterial assay showed that *Woodfordia fruticosa, Flemingia strobilifera* and *Phanera vahlii* have potential antibacterial activity against UTI causing bacteria such as *Escherichia coli, Enterococcus faecalis* and *Pseudomonas aeruginosa*. It can be concluded that

Woodfordia fruticosa, Flemingia strobilifera and *Phanera vahlii* could be used for treatment of UTI.

The most potential antibacterial against *Klebseilla pneumonia* was 70% methanolic extract of *Flemingia strobilifera* followed by *Antidesma acidum*.

The potential antibacterial against *Salmonella typhi* was 70% methanolic extract of *Flemingia strobilifera* followed by *Callicarpa macrophylla* leaf. MIC value of *Callicarpa macrophylla* leaf (25mg/mL) against *Salmonella typhi* was bactericidal. The antibacterial assay of *Callicarpa macrophylla* supports the ethnomedicinal data for the use in treatment of typhoid.

The most potential antibacterial against *Staphylococcus aureus* was70% methanolic extract of *Rhus chinensis* followed by *Flemingia stobilifera*. Among hexane extract *Azadirachta indica* was the most potential antibacterial against *Staphylococcus aureus*.

Hexane extract of all the plant species kill the bacteria at higher concentration only.

The result showed that the best DPPH free radical scavenger was extract of *Rhododendron arboreum* and best hydrogen peroxide scavenger was extract of *Woodfordia fruticosa*.

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