

**ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF
SELECTED MEDICINAL PLANTS FROM LOW LAND NEPAL**

A DISSERTATION

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LAXMI BHATTA

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RECOMMENDATION

This is to certify that that the dissertation work entitled “**Antibacterial and Antioxidant Properties of Selected Medicinal Plants from Low Land Nepal**” submitted by Ms. Laxmi Bhatta has been carried out under our supervision. To the best of our knowledge, the result she acquired is not submitted or published for any other academic degree. So we recommend this dissertation to be accepted for the partial fulfillment of M.Sc. degree in Botany at institute of science and Technology, Tribhuvan University.

(Supervisor)

Prof. Dr. Krishna Kumar Shrestha
Former Head
Central Department of Botany
T.U. Kirtipur, Kathmandu

(Co- Supervisor)

Prof. Dr. Krishna Das Manandhar
Central Department of Biotechnology
T.U. Kirtipur, Kathmandu

Oct 06, 2015



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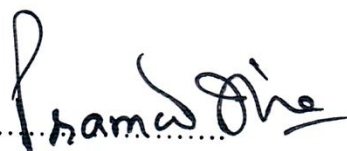
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LETTER OF APPROVAL

The M.Sc. dissertation entitled “**Antibacterial and Antioxidant Properties of Selected Medicinal Plants from Low Land Nepal**” submitted by Ms. Laxmi Bhatta, has been accepted for the partial fulfillment of requirements for master of Science in Botany (Plant Systematics and Biodiversity).

EXAMINATION COMMITTEE



(Head of the Department)

Prof. Pramod Kumar Jha
Central Dept. of Botany
T.U., Kirtipur, Kathmandu



(Supervisor)

Prof. Krishna Kumar Shrestha
Central Dept. of Botany
T.U., Kirtipur, Kathmandu



(Co- Supervisor)

Prof. Krishna Das Manandhar
Central Dept. of Biotechnology
T.U., Kirtipur, Kathmandu





(External Examiner)

Prof. Dr. Rajani Malla
Central Dept. of Biotechnology
T.U., Kirtipur, Kathmandu



(Internal Examiner)

Prof. Dr. Sangeeta Rajbhandary
Central Dept. of Botany
T.U., Kirtipur, Kathmandu

October 6, 2015

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Kathmandu, Nepal

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ACRONYMS

ATCC	American Type Culture Collection
BHA	Butylated Hydroxyl Anisole
CAT	Catalase
CFU	Colony Forming Unit
DMSO	Dimethylsulsooxide
DNA	Deoxyribonucleic acid
DPPH	2,2- diphenyl -1-picrylhydrazyl
F-C	Folin- ciocalteu
GAE	Gallic Acid Equivalent
GPx	Glutathione peroxidase
GRx	Glutathione reductase
IC ₅₀	Inhibitory concentration 50
KHz	Kilohertz
MHA	Muller hinton agar
MW	Molecular weight
NB	Nutrient Broth
ROS	Reactive Oxygen Species
RSA	Radical Scavenging Activity
TPC	Total Polyphenol content
WHO	World Health Organization
ZOI	Zone of Inhibition

Abstract

Medicinal plants, as source of remedies, are widely used as alternative therapeutic tool for the prevention or treatment of many diseases. The aim of the present study was to evaluate the antibacterial and antioxidant activity of the methanolic extract of selected medicinal plants of central Nepal. Twelve medicinal plants were studied on the basis of their ethnobotanical uses in the treatment of various ailments especially diarrhoea and dysentery. Antibacterial screening against four pathogenic microbes viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* was determined by agar well diffusion method and zone of inhibition was observed. Out of twelve plants tested for antimicrobial activity, eight plant species showed antibacterial activity by inhibiting one or more microorganisms. The methanolic extract of *Mangifera indica*, *Bombax ceiba*, *Bauhinia vahlii*, *Cassia fistula*, *Syzygium cumini*, showed significant inhibition against all the tested bacteria while, *Psidium guajava*, *Elephantopus scaber* and *Azadirachta indica* showed inhibition against few pathogens. But medicinal plants such as *Cassia occidentalis*, *Uraria lagopoides*, *Moringa oleifera* and *Costus speciosus* did not show antibacterial activity against selected bacteria. Total phenolic and flavonoid content were determined by Folin-Ciocalteu and aluminium chloride calorimetric method respectively. The antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and ascorbic acid was taken as standard. *Syzygium cumini*, *Bombax ceiba*, *Elephantopus scaber*, *Uraria lagopoides* and *Bauhinia vahlii* showed lower IC₅₀ value than standard ascorbic acid where as *Cassia occidentalis*, *Mangifera indica*, *Moringa oleifera*, *Azadirachta indica*, *Cassia fistula*, *Psidium guajava* and *Costus speciosus* showed higher value than standard ascorbic acid. *Moringa oleifera* showed the highest phenolic content whereas all the plant extracts showed comparable amount of Flavonoid content. The results of the present study suggest that the selected plants can be used as a source of antioxidants and antibacterial for pharmacological preparations as very well evidenced by the present work.

TABLE OF CONTENTS

RECOMMENDATION	
LETTER OF APPROVAL	
ACKNOWLEDGEMENTS	i
ACRONYMS	ii
ABSTRACT	iii
TABLE OF CONTENTS	iv
LIST OF FIGURES	v
LIST OF TABLES	vi
CHAPTER I: INTRODUCTION	1
1.1 Background	1
1.2 Medicinal plants in Nepal	2
1.3 Ethnobotany of Nepalese medicinal plants	3
1.4 Plants as source of Antimicrobials	4
1.5 Plants as source of Antioxidants	5
1.6 Research Objectives	7
1.7 Rationale	7
1.8 Limitation	7
CHAPTER II: LITERATURE REVIEW	8
2.1 Phytochemistry of selected plants	8
2.2 Ethnomedicinal uses of selected plants	10
2.3 Antimicrobial activity of selected plants	13
2.4 Antioxidant activity of selected plants	16
CHAPTER III: MATERIALS AND METHODS	20
3.1 Study Area	20
3.2 Research plan	21
3.3 Collection of plant materials	22

3.4 Short description of plants under study	22
3.5 Herbarium preparation and identification	25
3.6 Drying of samples	25
3.7 Grinding of samples	26
3.8 Extraction	26
3.9 Antibacterial Screening	26
3.10 Determination of Total Phenol content	28
3.11 Determination of Total Flavonoid content	29
3.12 Determination of Antioxidant activity	29
3.13 Statistical analysis	30
CHAPTER IV: RESULTS	31
4.1 Extraction	31
4.2 Antimicrobial Screening	31
4.3 Determination of Total Phenol Content	32
4.4 Determination of Total Flavonoid Content	34
4.5 Estimation of Antioxidant activity	35
4.6 Correlation of Phenolic Content and DPPH radical scavenging activity	36
CHAPTER V: DISCUSSION	37
5.1 Extraction	37
5.2 Antimicrobial activity	37
5.3 Total phenolic content determination	39
5.4 Total flavonoid content determination	40
5.5 Antioxidant activity	40
5.6 Relationship between total phenolic content and antioxidant activity	43
CHAPTER VI : CONCLUSION	44
References	46
Appendices	

List of Figures

Fig.1 Map showing study area

Fig.2 Flow chart showing the work plan of the research

Fig.3 Standard curve for estimation of total phenolic content

Fig.4 Estimation of total phenol content of crude methanolic extract

Fig. 5 Standard curve for estimation of total Flavonoid content

Fig.6 Estimation of total flavonoid content of crude methanolic content

Fig.7 The IC₅₀ values of the plant extract on DPPH

Fig. 8 Correlation between antioxidant activity and Phenolic content

List of Tables

Table 1 Name, collection sites and parts of plant selected

Table 2 Percentage yield and physical characteristics (color and consistency) of the crude methanolic extracts

Table 3 Antibacterial activity of crude methanolic extracts

CHAPTER I: INTRODUCTION

1.1 Background

Plants are vital component of the world's biodiversity and essential natural resource for human well-being. The plant kingdom represents an enormous reservoir of biologically active compound with various chemical structures and diseases preventing properties called phytochemicals (Peteros *et al.*, 2010). These phytochemicals, often secondary metabolites, which are present in small amount in plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins and many others (Chhetri *et al.*, 2008). A large proportion world population in the developing countries depends on traditional system of medicine for varieties of diseases. The type of plant and its medical applications depends on the region they are found and practice introduced by local/ indigenous people. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants to produce more cost effective remedies that are affordable to the population (Jayachandran *et al.*, 2010). Plant products have been parts of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, fruits, seeds, roots. Knowledge of the chemical constituents of plant is desirable because such information will be value for synthesis of complex chemical substances. Medicinal plants are the 'back bone' of traditional remedy. The traditional medicine related to treatment of both human and animal diseases with plant-derived preparations is considered a valuable knowledge. The potential of higher plants as sources for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller (Prabhu *et al.*, 2010).

Medicinal plants are defined as any plants which contain substances that can be used for the therapeutic purposes in one or more of its organ or substances which are precursors for the synthesis of useful drugs (Sofowora, 1982). Furthermore, Elujoba (1997) noted that a plant become a medicinal plant only when its biological activity has been ethnobotanically reported or scientifically established. It has been estimated that up to 90% of the population in developing countries rely on the use of medicinal plants to help meet their primary health care needs (WHO, 2002). As billions of people worldwide rely on medicinal plants for health, sustainability and conservation, it must be our first priority. We should do everything possible to preserve biodiversity of plant

ecosystems, especially in tropical rainforests (Mamedov, 2012). Understanding the relationship among medicinal plants used in traditional medicine systems can help identify plant materials with potential constituents applicable to modern medicine. All over the world research is now being directed into plants with traditional use as medicines, in the hope of finding new treatments for all manner of health problems.

From about 250,000 species of higher plants around the world, only 17% have been scholarly investigated for medical potential (Fransworth, 1992). Some example of medicinal plants that have been developed as source of lead molecule for the drug development are of species such as *Rauwolfia serpentina* for reserpine, *Atropa* for atropine, *Podophyllum* for podophyllotoxin, *Taxus* species for taxol, etc.

1.2 Medicinal plants of Nepal

Nepal lies on the central region of great Himalayan range located between Tibetan autonomous region of China in the north and India in the south east and west. Altitude varies from less than 60m in the low land of Terai in the south to the creast of the Himalaya reaching 8848m in the north. Himalayan region shows the highest richness for endemic species and medicinal herbs (Kunwar *et al.*, 2006) and plays fundamental role to fulfill the medicinal demand of nepalase society. Medicinal plants are considered as one of the economic resource of Nepal. Nepali people have been utilizing the plants and plants product since time immorial.

According to Malla and Shakya (1984-85), the distribution of medicinal plant is more (54% of the total medicinal plants in Nepal) in the subtropical belts, where as the alpine region have the comparatively less number (18%) of medicinal plant in Nepal. In the world, 10-18% of all plant species are used medicinally while in Nepal medicinal plants account about 20-28% of the expected 7000 species of flowering plant (Watanabe *et al.*, 2013).

The plant and plant resources for medicinal use are collected by local people and herbal healer from various habitats such as forest, scrubland, grassland and cultivated fields and use them as crude drugs. It is known that the way of administration to cure disease using a particular plant widely differs among the indigenous people and also healers, jhakris and amchies (Manandhar, 2002). Medicinal plants are accessible, affordable and culturally appropriate sources of primary health care for more than 80% of Asia's population. Poor

and marginalized, who cannot afford or access formal health care systems, are especially dependent on these culturally familiar, technically simple, financially affordable and generally effective traditional medicines.

However due to changing perception of the local people, commercialization and socio-economic transformation of all over the world, it has been observed that the indigenous knowledge on resource use has been degraded.

1.3 Ethnobotany of Nepalese Medicinal plants

The term ethnobotany was firstly coined by an American botanist, John W. Harsberger, in 1895 who defined it as the Study of "plants used by primitive and aboriginal people". The main aim of ethnobotany is to document the knowledge about plants that had come through generation, and use the knowledge for the benefit of the Society (Rajbhandari, 2001).

Nepal is a multiethnic and multilingual country. There are more than 60 different ethnic groups speaking about 75 languages in Nepal (Shrestha, 1998). The ethnic communities have significant customary knowledge on utilization of plant and plant parts and there is a long tradition of transferring this indigenous knowledge from generation to generation (Acharya and Acharya, 2009). Ethnobotany has taken its own way of development, depending on local traditions. In Nepal, ethnobotanical study started when Benarji (1955) published a paper on medicinal and food plants of Eastern Nepal and mentioned 13 species of which 9 were of medicinal value and 4 were noted for their food value (Manandhar, 2002). Ethnobotanical research acts a bridge between traditional knowledge in medical plants and their wider application in modern medicine.

There are many studies, related to ethnobotany published based on investigation in Nepal. "Ethnobotany of Nepal" published by Rajbhandari (2001) contains local name, botanical name, taxonomic description and ethnobotanic uses of 562 plant species. "Genetic heritage of medicinal and aromatic plants of Nepal Himalayas" by Joshi and Joshi (2001) covers ethnobotanical information about 372 medicinal plants of Nepal." Plants and Peoples of Nepal" by Manandhar (2002) contains botanical description and traditional uses of 1517 species of higher plants from 858 genera and 195 Families. "Handbook of Medicinal plants of Nepal" by Watanabe *et al.*, (2005 and 2013) covers the description, traditional uses, chemical constituents of 108 and 88 medicinal plants of Nepal respectively.

A large number of medicinal plants and associated indigenous knowledge on their uses still remain without proper documentation in Nepal (Chaudhary, 1998). The priority should be given to the documentation of indigenous knowledge and conservation of the existing species and inhabitants before some of these are eliminated from the area (Joshi *et al.*, 2003). In order to preserve traditional knowledge, studies in ethnobotany and ethnopharmacology are very important.

1.4 Plants as source of Antimicrobials

Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day (Parmar and Rawat, 2012). Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drug of plant origin. Antimicrobial properties of medicinal plants are being increasing as reported from different parts of the world. Antimicrobials therefore may have a significant clinical value in treatment of resistant microbial strain (Parmar and Rawat, 2012). It has been reported that the higher plants have shown to be a potential source for the new antimicrobial agents. It is concluded that medicinal plants can be used as antimicrobial agents in new drug for therapy of infectious diseases in human.

In recent years, the number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing in alarming rate. Antibiotics are one of the most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. The wide use of antibiotics in treatment of bacterial infections has led to the emergence and spread of resistant strains (Doughari and Manzara, 2008). Increasing development of drug resistance in human pathogens as well as the appearance of side effect of synthetic drugs need to develop new antimicrobial drugs from natural sources. This situation has forced to search new antimicrobial substances in various sources like medicinal plants.

The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phenolics and

polyphenolics are one of the largest groups of secondary metabolites that have exhibited antimicrobial activity (Cragg *et al.*, 1997). Most of the plant possesses one or more of the Medicinal properties, viz. antibacterial antifungal, antiviral, anthelmintic, anticancer, laxative and sedative, cardio tonic, diuretic, and others (Parajuli *et al.*, 1998).

1.5 Plants as source of Antioxidants

Free radicals are formed when cells use oxygen to generate energy. These by-products are generally reactive oxygen species (ROS) such as super oxide anion, hydroxyl radical and hydrogen peroxide that result from the cellular redox process. At low or moderate concentrations, ROS exert beneficial effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins, and DNA (Pham-Huy *et al.*, 2008). Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular and neurodegenerative diseases (Willcox *et al.*, 2004; Pham-Huy *et al.*, 2008). Therefore, to cure these complications the antioxidant therapy is only one alternative. Antioxidants may have an important role in prevention of these diseases by inhibiting or preventing the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress.

The antioxidants can be either endogenous or exogenous; the endogenous ones are naturally generated in the body, which include superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx) and exogenous ones are either dietary phytochemicals or other supplements which include phytoconstituents and phytonutrients such as Vitamin E, Vitamin C, Carotenoids, trace metal (Selenium, Manganese, zinc), Flavonoids, etc. (Benzie, 2003). Endogenous antioxidant is capable of scavenging the reactive oxygen species generated inside the cell by themselves under normal physiological condition and hence maintain the balance. But in critical conditions like diseased state or severe exposure to damaging environmental factor, the production of oxidative species is so high that the body can't defend it by itself and at that time there is need of some supplementary antioxidants.

Antioxidants may have an important role in prevention of diseases by inhibiting or preventing the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress, the most commonly used synthetic antioxidants are butylated

hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) and butylated hydroquinone. These synthetic antioxidants have possible activity as promoters of carcinogenesis. Therefore, there is a need for isolation and characterization of natural antioxidant having less or no side effects, for use in foods or medicinal materials in order to replace synthetic antioxidants (Basnet and Subba, 2014).

Natural products, especially plants are the sources of different classes of phytochemicals. Phenolics are aromatic compounds bearing one or more hydroxyl substituents. Plant phenolics include phenolic acids, flavonoids, tannins, stilbenes and lignins. Both flavonoids and phenolic acids are the most common polyphenols in our diet and distributed widely in fruits, vegetables, cereals and beverages (Kahkonen *et al.*, 1999).

Phenolic compounds are a large group of the secondary metabolites widespread in the plant kingdom which play a major role in the protection of oxidation processes. The antioxidant properties of phenolic compounds can act as free radical scavengers, hydrogen donors, metal chelators and singlet oxygen quenchers. Flavonoids represent the most common and widely distributed group of plant phenolics. Their common structure (C6-C3-C6) consists of two aromatic rings linked through a three carbon bridges that is usually an oxygenated heterocycle (C ring). The major flavonoid classes include anthocyanidins, chalcones, flavanols, flavanones, flavones, flavonol, and isoflavones. Flavonoids have been associated with possible role in the prevention of several chronic diseases involving oxidative stress as well as their protective effect against low-density lipoprotein (LDL) oxidation (Shanaz *et al.*, 2011).

1.6 Objectives

1.6.1 General objectives

To study and evaluate selected medicinal plants of Central Nepal; for their *in vitro* antibacterial and antioxidant activities.

1.6.2 Specific objectives

- To explore the medicinal value of selected plants.
- Extraction of phytochemicals using methanol solvent.
- Screening of the plant extracts for their antibacterial activity by using agar well diffusion method and antioxidant activity by using DPPH.
- Estimation of bioactive compounds like phenol and flavonoid.

1.7 Rationale

Nepal is a small country in the Himalayan mountain range, located between India and Tibet. The country has many rural parts where western pharmaceuticals or pre-packaged Indian ayurvedic medicines are not available. The use of traditional medicine is widespread in these rural areas, with much of the population relying on it. Although many plants have long been recognized and widely used in Nepalese traditional medicine, some are relatively unexplored and not arrived to mainstream medicine. It is quite necessary to carry out experiments to ensure their constituents, potency and safety. For pharmacologist this kind of studies forms the basis of drug development and discovery. Therefore, this research would be a preliminary step towards the isolation of active compounds which can further bring a new possibility in drug discovery.

1.8 Limitation

- Only one solvent (Methanol) was used for the study.
- Antioxidant activity was determined by only DPPH free radical scavenging assay.
- Folin-Ciocalteu and Aluminium chloride calorimetric method was used for phenol and Flavonoid content determination, which is not absolute measurement of phenol and Flavonoid content.
- All the experiment was carried out *in vitro* not *in vivo*.

CHAPTER II: LITERATURE REVIEW

Plants are the oldest source of pharmacologically active compounds and have provided humankind with many medically useful compounds for centuries. Medicinal plants have always fascinated all the people including researchers, Pharmacologists, and pharmaceutical companies. A large numbers of researcher and billions of dollars have been invested in the medicinal plant research under the name natural product research. Herbal medicine represents one of the most important fields of traditional medicine all over the world. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plant.

2.1 Phytochemistry of selected plants

Khan *et al.* (2010) studied that phytochemical analysis of *Azadiracta indica* leaves by using different solvent such as Petroleum ethar, chloroform, methanol show the presence of triterpenes, glycosides and fatty acids. Vinoth *et al.* (2012) studied that phytochemical analysis of *Azadiracta indica* leaves by using different solvent such as methanol, ethanol and acetone shows the presence of alkaloids, tannins, flavonoids, terpenoids, saponin.

Leave of *Bauhinia vahlii* contains betulinic acid, campesterol, Stigmasterol, β -sitosterol, quercetin, quercetin-3-glucoside, Kaempferol, agathisflavone and its derivatives (Hussain *et al.*, 1992).

Phytochemical investigation of the alcoholic and water extract of *Bombax ceiba* showed presence of alkaloids, flavonoids, glycosides, coumarins, proteins and aminoacids (Chakraborty *et al.*, 2010).

A wide range of chemical compounds including achrosin, aloe-emodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol, chrysoeriol etc. have been isolated from *Cassia occidentalis* (Yadav *et al.*, 2010).

Seed of *Cassia fistula* contains galactomannan comparising eight sugars including epimelibiose, mannobiose, galactomannobiose, mannotetraose, and mannohexaose. Pod pulp yields an anthraquione acid- fistulic acid. Stem bark contains tannins, lupeol, β -sitosterol and hexacosanol (Hussain *et al.*, 1992).

Rhizome of *Costus speciosus* used medically to contain the many of active compounds such as carbohydrates, alkaloids, saponins, flavonoids, phenols, glycosides, vitamin C and vitamin E and minerals like Zn, Cu, Se and Fe. It contains sterol, anthraquinones, steroids and sesquiterpens (Saraf, 2010).

The major phytochemical constitutes of the *Elephantopus scaber* are elephantopin, triterpenes, stigmasterol epifriedelinol and lupeol (Raj Kapoor, 2002). A new terpenoid was isolated from acetone extract of *Elephantopus scaber* (Kamalakaran et al., 2012).

Jayachandra, (2012) reported the presence of carbohydrates, aminoacids, tannins, saponins, phytosterols, terpenoids, phenols and flavonoids on the methanolic extract of *Syzygium cumini* bark. The leaves of *Syzygium cumini* were rich in flavonoids, alkaloids, glycosides, steroids, phenols, tannins and saponins. These phytochemicals confer antimicrobial activity on leaf extract (Gowri and Vasantha, 2010).

Mangifera indica leaves extract revealed the presence of steroids, flavonoid, reducing sugar and cardiac glycosides in the hexane extract; anthraquinone, tannin and reducing sugar in the ethyl acetate extracts and saponin, steroids, tannin, flavonoid, reducing sugars and cardiac glycosides in the methanolic extracts (Aiyelaagbe and Osamudiamen, 2009). The bark contains mangiferine and is astringent and employed against rheumatism and diphtheria in India (Ashok et al., 2014).

Moringine and Moringinine were the two alkaloids isolated from the stem bark of *Moringa oleifera* (Gilani et al., 1994). Karthika et al. (2013) reported presence of 28 compounds from the ethylacetate extract of *Moringa oleifera* leaves.

Psidium guajava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids. Guava fruit is higher in vitamin C than citrus (80 mg of vitamin C in 100 g of fruit) and contains appreciable amounts of vitamin A as well. Guava fruits are also a good source of pectin- a dietary fiber (Joseph, 2011). Guavas contain both carotenoids and polyphenols- the major classes of antioxidant pigments giving them relatively high potential antioxidant value among plant foods (Vyas et al., 2010).

Phytochemical studies on the ethanolic extracts of *Uraria lagopoides* revealed the presence of alkaloids, saponins, glycosides, steroids, tannins and flavonoids (Kumar and Nuthakki, 2014). Stem and leaf of the plant yield tannin (Manandhar, 2002).

A wide range of chemical compounds including achrosin, aloe-emodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol, chrysoeriol etc. have been isolated from *Cassia occidentalis* (Yadav *et al.*, 2010).

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2.2 Ethnomedicinal uses of selected plants

In Ayurvedic, Unani and folklore traditional medicine, different parts of *Azadirachta indica* were preferred in the treatment of a wide range of afflictions. Every part of the tree has been used as traditional medicine for household remedy against various human ailments, from antiquity (Asif, 2012). The extract from bark, leaves, fruits and root have been used to control leprosy, intestinal helminthiasis and respiratory disorders in children (Chattopadhyay *et al.*, 1993).

The leaves *Bauhinia vahlii* are demulcent and mucilaginous. A decoction of the leaf is given to treat diarrhea and dysentery. Leaves are used for fodder to make mats and containers for food stuffs sometimes for thatching, also for tobacco wrappers for smoking (Manandhar, 2002).

Medicinal usage of *Bombax ceiba* has been reported in the traditional systems of medicine such as Ayurveda, Siddha and Unani (Verma *et al.*, 2014). It has a place among the five scared plant of "Panchwati". Semal trees are useful in the treatment of diuretic, dysenteric, emetic, diarrhoeal, wounds, acne, skin blemish and pigmentation, Cold and cough. Juice of the plant is applied to treat headache, cut and wounds. Juice of the bark is given for cough, indigestion and stomachaches. A decoction of the bark is given for fever (Manandhar, 2002). Root of the young tree is useful in dysentery. Flower, bark and fruits are used in revitalizing sexual impotency (Watanabe *et al.*, 2005).

Cassia occidentalis is an annual or perennial ayurvedic plant which is used in several traditional medicines to cure various diseases. This weed has been known to possess antibacterial, antifungal, antidiabetic, anti-inflammatory, anticancerous, antimutagenic and hepatoprotective activity (Mohammed *et al.*, 2012). In Ayurveda their leaves, bark and

seeds were used in the treatment of diabetes, inflammation, ulcers and leprosy (Kathirvel and Sujatha, (2011). A paste of plant is applied for dropsy, rheumatism and snakebite (Manandhar, 2002).

Traditionally, *Cassia fistula* is used as an infusion, decoction, or powder, either alone or in combination with other medicinal plants (Ali, 2014). The whole plant is used to treat diarrhea; seeds are used to treat skin diseases, Flowers and fruits are used to treat skin diseases, fever, abdominal pain and leprosy (Perry, 1980). Fruit pulp called *Cassia* pulp is well- known laxative and purgative. Leaf juice is taken to cure skin disease. The pulp of basal part is used to treat diarrhea and that of apical region to treat constipation (Watanabe *et al.*, 2005).

The plant parts of *Costus speciosus* has many medicinal uses, juice of rhizome is applied to head for cooling and relief from headache, bruised leaves are applied in fever, decoction of stem is used in fever and dysentery, patients with high fever mostly utilize leaf infusion or decoction as a Sudorific or in a bath, sap from leaves, young stems are used against diarrhea (Srivastava *et al.*, 2011), cough, cuts, wounds, scabies, antidote for snake bite, jaundice, arthritis (Ariharan *et al.*, 2011) burning sensation, constipation, leprosy, skin diseases, asthma, bronchitis, inflammations, anemia.

The whole plant of *Elephantopus scaber* has been used to treat various diseases. Different countries have different type of practices on this plant for various diseases (Ho *et al.*, 2009). In Malaysia, decoction of *Elephantopus scaber* root has been used to accelerate contraction of abdominal area and prevent inflammation after childbirth (Ho *et al.*, 2009). In India, decoction of the whole plant has been used to treat gonorrhoea and colic pain (Hammer and Johns, 1993) while hot water extract of the root is applied for Filariasis. In Nepal, root decoction of *Elephantopus scaber* is widely used to treat diarrhea, dysentery (Manandhar, 2002). Also, the whole plant has been reported to be useful for curing insomnia, diabetes, bronchitis, viral or bacterial infection, leukemia, rheumatism, snake bite, diuresis, antipyresis, to eliminate bladder stones (Ho *et al.*, 2009).

In Nepal bark juice of *Mangifera indica* is heated and applied for rheumatism and to treat peptic ulcer. Juice of the leaves, is given for bloody dysentery. Ash of the leave is applied to burns for quick recovery. Gum mixed with mustard oil is applied for scabies, warts and other skin diseases. Juice of the bark is boiled in water which is used to provide relief from

Jaundice (Manandhar, 2002). The fruits have also been eaten with salt and honey as source of vitamin A and for the treatment of blood disorders. Infusion of the ground seeds have been used as remedy for diabetes (Doughari and Manzara, 2008).

In traditional medicine *Moringa oleifera* tree is known as a Miracle tree as almost every part of this tree possesses product useful for humans (Sayeed *et al.*, 2012). The leaves and pods are eaten. The plant is also reported to be medicinally important and almost all parts of the *Moringa oleifera* are considered to possess medicinal properties and are used in the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulant. The leaves mixed with chicken soup are used by Philippine women to enhance breast milk production (Chopra *et al.*, 1956). The root and bark are useful in treatment of heart complaints, eye disease, inflammation, dyspepsia and enlargement of spleen. The flowers are known to cure inflammation and muscle diseases. Seed oil is to be useful in the treatment of leprosy ulcers (Sayeed *et al.*, 2003). Leaves are purgative, the paste of leaves is applied to glandular swelling; and fruits have anthelmintic properties (Manandhar, 2002).

Psidium guajava leaves were used to treat diarrhea and stomach (Pandey and Shweta, 2012). The roots were also rich in tannin. Juice of the bark and unripe fruit is given for dysentery. Leaf buds are chewed to treat Fever and Headaches (Manandhar, 2002). The boiled water extract of guava, plant leaves and bark are used in medicinal preparations which are utilized as remedies for dysentery, diarrhea and upper respiratory tract infections in Florida the west Indies and parts of South America.

Almost all parts of the *Syzygium cumini* are used for various purposes. Ripe fruits are very juicy, almost odorless, with a pleasant, slightly bitter, astringent taste. The fruit pulp is used to make jams, jellies, juice, vinegar and puddings. Fruits are also used to make wine in vast quantities in the Philippines (Chaudhary and Mukhopadhyay, 2012). Bark is astringent to the bowels, good for sore throat, asthma, dysentery, diarrhea and ulcers. Seeds are good for diabetes (Watanabe *et al.*, 2013). Juice of the bark and leaf is taken in case of diarrhea and dysentery in Nepal (Manandhar, 2002).

In Nepal, especially in the central region the paste of *Uraria lagopoides* root is given to treat peptic ulcer and diarrhoea (Rajbhandari, 2001). Boiled juice of the leaf is taken for inflammation of the intestine and bladder. Fresh Flowers are expectorant. A paste of the

fruit is given for diarrhoea. A decoction of the fruit is vermifuge (Manandhar, 2002). Whole plant is used for the removal of swelling (Watanabe *et al.*, 2013).

2.3 Antimicrobial activity of selected plants

Antibacterial activity of *Azadirachta indica* leaves extracts of petroleum ether, acetone, benzene, chloroform studied against *Streptococcus pyogenes* and *Staphylococcus aureus*. The chloroform extract showed maximum activity than the other extracts (Gajanan, 2012). According to Panchal *et al.* (2013) *Azadirachta indica* leaves possessed good antibacterial activity confirming the great potential of bioactive compounds and is useful for rationalizing the use of this plant in primary health care. Sen and Batra, (2012) studied the antibacterial activity of ethanol, methanol, petroleum ether and Aqueous leaf extract of *Azadirachta indica* against different bacterial strain. Their finding conclude that all there extract were active against tested bacterial strain like *Escherichia coli*, *Streptococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa*.

Monohar *et al.* (2011) studied the aqueous and methanolic extract of *Bauhinia vahlii* against *Bacillus substilis*, *Salmonella typhi* and *Staphylococcus aureus*, and suggested that methanolic extract was having good antibacterial activity. Methanol extract of *Bauhinia vahlii* showed activity against *Escherichia coli* and *pseudomonas aeruginosa* (Sinha and Verma 2012).

The methanolic extracts of *Bombax ceiba* was highly active against *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi* and *Salmonella paratyphi* (Paudel and Gyawali, 2014). Mangiferin isolated from its leaf have shown antibacterial potential against *Listeria monocytogenes*, *Bacillus substilis*, *Streptococcus pyogenes*, *Salmonella typhi*, *Enterobacter cloacae* and *pseudomonas aeruginosa* (Jain and Verma 2012).

Sadiq *et al.* (2012) studied the ethanol and water extract of *Cassia occidentalis* leaf against *Staphylococcus aureus*, *pseudomonas aeruginosa*, *Escherichia coli*, *salmonella typhi*, *shigella* spp. their finding concluded that *Staphylococcus aureus* was resistant to both extract. Mohammed *et al.* (2012) observed that the *Escherichia coli* were sensitive to petroleum ether and ethanolic extract of leaves of *Cassia occidentalis* at concentration of 400 mg/ml. A report with *Cassia occidentalis* flower extract showed maximum inhibition

against *Klebsiella pneumonia* and no activity against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* (Daniyan *et al.*, 2011).

Hexane, chloroform, ethyl acetate, methanol and water extracts of *Cassia fistula* flowers showed significant zone of inhibition against “Gram-positive” bacteria, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Enterococcus faecalis* and one “Gram-negative” bacterium *Pseudomonas aeruginosa* (Duraipandiyan and Ignacimuthu, 2007). *Cassia fistula* exhibited significant antimicrobial activity and showed properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents (Kumar *et al.*, 2006). The antibacterial activities of the various parts of *Cassia fistula* were due to the presence of various secondary metabolites (Bhalodia *et al.*, 2011).

Antibacterial activity of *Costus speciosus* was studied by Ariharan *et al.* (2012), where high antibacterial activity of rhizome extract was seen against Gram positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram negative bacteria (*Escherchia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) which may be due to the presence of diosgenin, a precursor for the synthesis of steroidal hormones. This shows that the plant has disease resistance ability, which may be due to presence of phenolics and alkaloid substances. Antibacterial and Antifungal activity were observed by Duraipandiyan *et al.* (2012) in hexane, chloroform, ethyl acetate and methanol extracts. Among the tested extracts the hexane extract showed good activity against only Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*) and tested fungi.

The methanolic extract of *Elephantopus scaber* showed significant antibacterial activity against *Staphylococcus aureus*, *Bacillus substilis*, *Proteus vulgaris*, *Escherichia coli* and *Psedomonus aeruginosa* (Kumar *et al.*, 2004). Jenny *et al.* (2012) studied the antibacterial activity of aerial part of extract (petroleum ether, chloroform and methanol) of *Elephantopus scaber* against *Staphylococcus auerus*, *Salmonella paratyphi*, *Klebsiella pneumonia*, *Pseudmonus aeruginosa*, *Shigella sonnei*, *Salmonella typhimurium* and *Escherichia coli* and investigated that methanolic extract showed maximum antibacterial potential as compared to petroleum ether extract and chloroform extract. Kamalakannan *et.al.* (2012) studied the effect of Aqueous and Methanolic extract of *Elephantopus scaber* on pathogenic fungal strains *Aspergillus niger*, *Aspergillus falvus*, *Rhizous indicus* and

found that there was dose dependent increase in antifungal activity and maximum activity was observed with methanolic extract against *Mucor indicus* and minimum activity against *Rhizopus indicus*.

Joshua and Takudawa (2013) reported that Methanolic stem bark extracts of *Mangifera indica* showed significant antibacterial potency against the *Staphylococcus aureus* followed by ethyl acetate, water and hexane extract. Doughari and Manzara (2008) studied the antibacterial activity of acetone, aqueous and methanol extract of *Mangifera indica* against different bacterial strain. Their findings conclude that acetone extract exerted highest activity on bacterial strain tested compared to aqueous and methanol extract. The presence of phytoconstituents in the leaf extracts may be responsible for the antibacterial activity of the plant (Marjorie, 1999). Antibacterial activity of *Mangifera indica* was studied by Ashok *et al.* (2014) reported that ethyl acetate was active against *Klebsiella pneumoniae* followed by *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. Distilled water extract of *Mangifera indica* bark was not effective for the entire tested pathogens.

Antibacterial activity of leaf extract (Aqueous, hexane, ethanol and methanol) of *Moringa oleifera* was evaluated against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. The result revealed that the antibacterial activity of methanol extract showed higher antibacterial activity than other extract (Singh, 2013). Doughari *et al.* (2007) reported antibacterial activity from the aqueous, acetone and ethanolic extracts of the leaves of *Moringa oleifera*. Of the three solvents used, ethanolic extract of the plant demonstrated the highest activity, while the aqueous extract showed the least activity at 100 mg/ml.

Kalpana *et al.* (2013) reported antibacterial activity from the Petroleum ether, chloroform, ethanol and aqueous extract of leave of *Moringa oleifera*. Of the four solvents used, ethanolic extract of the plant demonstrated the highest activity, while the aqueous extract showed the least activity. Nepoleon *et al.* (2009) also reported *Enterobacter* spp, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* to be sensitive to ethanol, chloroform and aqueous extract of *Moringa olifera* leaf at concentration of 200 mg/1. The plant has been reported to possess antimicrobial properties and this explains the reason for its wide use in the treatment of human diseases (Anwar *et al.*, 2007).

According to Chaudhary *et al.* (2012) *Psidium guajava* leaf extract showed potential antibacterial agent for the bacteria causing gastrointestinal problem and can be used for such ailments. Pandey and Shweta, (2012) studied the antibacterial activities of ethanolic, methanolic, ethyl acetate and hot water extract from leaves, fruits and stems of *Psidium guajava* and investigated that stem showed best result in all extract. In several studies, guava showed significant antibacterial activity against common diarrhea causing bacteria such as *Staphylococcus*, *Shigella*, *Salmonella*, *Bacillus*, *Escherichia coli*, *Clostridium* and *Pseudomonas* (Joseph and Pariyar, 2011).

Gowri and Vasantha (2010) studied the Methanolic and Aqueous extract of leaves of *Syzygium cumini* against both gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*Salmonella enteritidis*, *Salmonella typhi*, *Salmonella typhi A*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Pseudomonas aeruginosa* and *Escherichia coli*) and observed that the both extract of leaf exhibited antibacterial activity against all the tested strain but methanolic extract was more potent than Aqueous extract. Prabhakaran *et al.* (2011) reported that ethanolic extract of leaves and aqueous extract of seeds of *Syzygium cumini* were found to have high antimicrobial activity against gram positive and gram negative bacteria.

In vitro antimicrobial activity of aqueous and ethanol extracts of *Uraria lagopoides* was screened against multi-drug resistant bacteria including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, *Bacillus cereus*. Ethanol extracts of *Uraria lagopoides* showed higher antimicrobial activity compared to aqueous plant extracts tested (Shivkumar *et al.*, 2012). Islam *et al.* (2012) studied the ethanol extract of *Uraria lagopoides* revealed a dose dependent but moderate antidiarrhoeal response. Antidiarrhoeal activity of ethanolic extract of *Uraria lagopoides* was confirmed by Kumar and Nuthakki (2014) which supports the traditional use of *Uraria lagopoides* in the treatment of diarrhoea.

2.4 Antioxidant activity of selected plants

Most of plants uses for medicinal purposes have been correlated to their possession of antioxidant activity (Sofidiya *et al.*, 2006).

Nahak and Sahu (2010) Suggested that different extract (Water, ethanol and methanol) of *Melia azedarach* leaves which contains highest amount of phenolic compounds exhibited

the greatest anti-oxidant activity in comparison to *Azadirachta indica*. The high scavenging property of *Melia* may be due to hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary components as a radical scavenger. Deka *et al.* (2013) reported that leaf extracts of *Azadirachta indica* was found to have a significant antioxidant property, which can be industrially exploited to produce many useful medicinal products.

The acetone extracts of *Bauhinia vahlii* leaves extract were found to have the maximum chelating ability (Sowndhararajan and Kang, 2012). The scavenging potential and metal chelating ability of the antioxidants are dependent upon their unique phenolic structure and the number of hydroxyl groups (Pazos *et al.*, 2005). Higher level of antioxidant activity is observed in the methanol extract of *Bauhinia vahlii* leaves when compared with other tested extracts (Sowndhararajan and Kang, 2012).

Methanolic extract of *Bombax ceiba* root showed a very good DPPH radical scavenging activity due the higher percentage of phenolic compounds and tannins (Antil *et al.*, 2013). Yu *et al.* (2011) assessed the antioxidant activities of water, 50% ethanol, 80% acetone extracts from flowers of *Bombax ceiba* on DPPH radical scavenging assay. All extracts showed remarkable antioxidant capacity compared with ascorbic/gallic acid.

Vadnere *et al.* (2011) reported that ethylacetate extract of whole plant of *Cassia occidentalis* possess significant antioxidant activity than benzene and methanol extract. Mehta *et al.* (2010) suggested that methanol extract of seeds have antioxidant potential and represent a potential source of medicine.

Nehete *et al.* (2010) tried to evaluate in vitro antioxidant activity of different extracts of *Costus speciosus* by DPPH radical scavenging activity, nitric oxide scavenging activity, ion chelating activity, hydroxyl radical scavenging activity and its correlation with total phenolic content. Among all the extracts analyzed, a significant phenolic content and antioxidant activity were found for benzene extract which predicted that the antioxidant activity may be due to the total phenolic content in the plant. *Costus speciosus* were investigated for their polyphenol content and antioxidant activity (Vijayalakshmi and Sarada, 2008).

Methanol extract of *Elephantopus scaber* showed medial antioxidant capacity with moderate electron donating activity and reducing capacity (Choi and Hwang, 2005).

Subramanian *et al.* (2014) reported that ethanol extract of *E. scaber* showed best antioxidant activity than other extract. The antioxidant and antihepatotoxic activities of methanolic extract of *Elephantopus scaber* root was probably due to free radical scavenging activity.

Though considerable work has been done with regard to antioxidants and polyphenols of mango, very few reports are available with regard to mango bark. The mango plant has been the focus of attention of many researchers searching for potent antioxidants (Kim *et al.*, 2010). The *in vitro* antimalarial activity of chloroform: methanol (1:1) extract of MI was evaluated. Antioxidant activity of *Mangifera indica* methanol leave extract was studied by Joon *et al.* (2013) reported that extract showed significant antioxidant activity due to presence of bioactive compounds. Kim *et al.* (2010) reported that mango peel, a major by-product obtained during the processing of mango product, exhibited good antioxidant activity and may serve as a potential source of phenolics with anticancer activity.

Shahid and Bhanger (2004) studied the Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves and they noted that season and agroclimatic locations have profound effect on the antioxidant activity of *Moringa oleifera* leaves, concluded that agroclimatic locations and seasons have profound effects on the AA of *Moringa oleifera* leaves. Antioxidant activity of samples from cold areas was relatively higher than those from temperate regions.

Joseph and Pariyar (2011) reported that Antioxidant activity of the *Psidium guajava* is due to the presence of polyphenol found in the leaves. Aqueous and ethanolic extracts of the leaves of *Psidium guajava* were determined by DPPH, Nitric oxide, and Superoxide radical Scavenging model. In all Models studied, the extract showed potent antioxidant activity (Rasappan *et al.*, 2012). According to Vyas *et al.* (2010) antioxidant activity of *Psidium guajava* leaf extract was found significant as compared to standard (Ascorbic acid). Mehta *et al.* (2010) reported that guava stem extracts showed effective potential source of natural antioxidants which might be helpful in preventing the progress of various oxidative stresses.

The invitro antioxidant activity of methanolic extract of *Syzygium cumini* bark was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, Hydrogen peroxide scavenging assay, and Ferric reducing antioxidant power (FRAP) assay. The

extract showed significant antioxidant activity in all antioxidant assays when compared to ascorbic acid (Jayachandra, 2012). Nair *et al.* (2013) studied the *invitro* antioxidant activity of seed and leaf extract of *Syzygium cumini* by DPPH radical Scavenging assay, Ferric reducing Antioxidant power (FRAP) assay and Nitic oxide radical Scavenging Assay and observed the extract showed significant antioxidant activity in all antioxidant assay compared to ascorbic acid.

The total phenolic and flavonoid content of the Methanolic extract of *Syzygium cumini* leaves was very high suggesting it to be a good antioxidant and it is recommended that this extract should be incorporated in daily diet as nutraceutical supplements (Belapurkar and Goyal, 2014).

Sivakumar *et al.* (2012) suggested that the ethanolic extract of *Uraria lagopoides* showed an increase antioxidant property compared to that of aqueous extract. According to Islam *et al.* (2012) ethanol extract of *Uraria lagopoides* produced significant antioxidant activity in comparison to the standard, Ascorbic acid.

CHAPTER III: MATERIALS AND METHODS

3.1 Study Area

The Plants freshly collected from Hetauda, is a Sub-Metropolitan City in Makwanpur District of the Narayani Zone in southern Nepal. It is also the administrative headquarters of the Makwanpur district which lies between 27°21' to 27°40'N latitude and 84°35' to 84°40'E longitude. The altitude of the district ranges from less than 160m to 2588m. The city has 84,671 inhabitants. The city is surrounded by three rivers, the Rapti to the west, the Samari to the north and the Karra to the south. It is one of the important industrial regions of Nepal.

Hetauda is situated in a unique geographical structure called Doon, which means that it has geography like a valley. It is surrounded by hills: the Mahabharata range in the north and the Siwalik range in the south. It covers an area of about 187.7 sq. km and is one of the cleanest cities in Nepal. Also, the main streets are full of green trees parallel to both of roads adding up to its elegance. The majority of people residing here are *Tamangs*, and besides this, other communities like *Gurung*, *tharu*, *Chhettri*, *Newar*, *Magar*, *Chepang*, *Rai* as well as *Brahmins*. The major forest species in the city is Sal (*Shorea robusta*). The city is all surrounded by beautiful Sal forest hill.

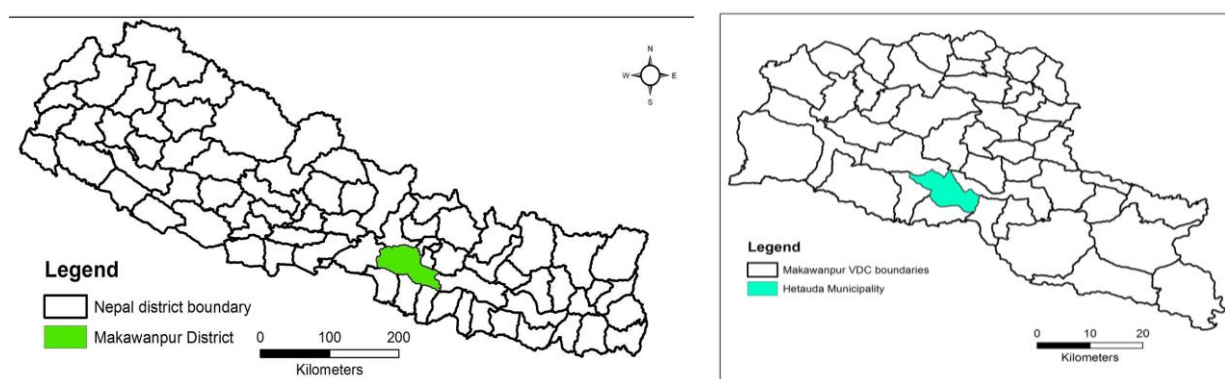


Figure 1 Map of Nepal, showing study area (Hetauda, Makwanpur)

3.2 Research plan

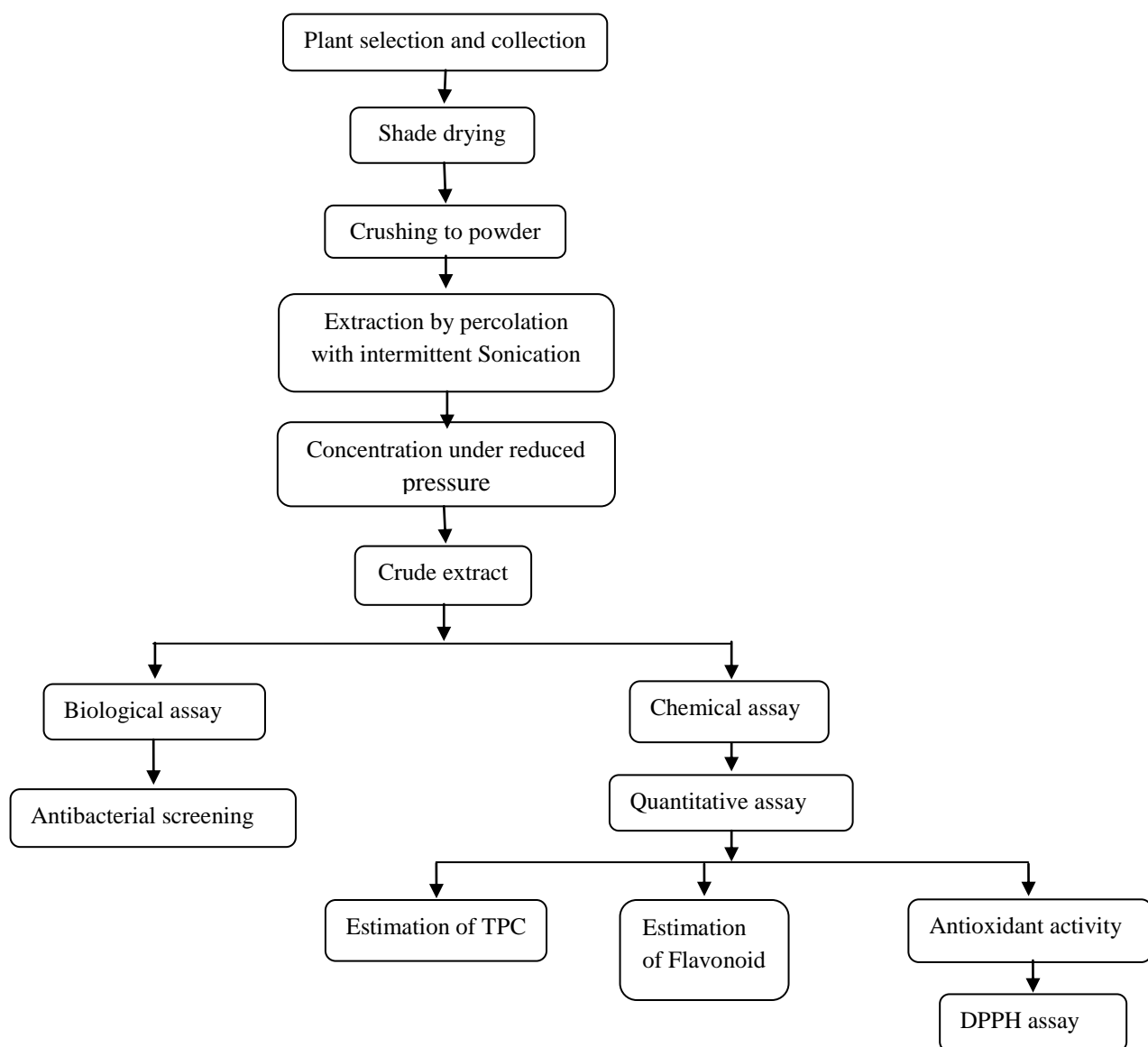


Figure 2 Flow chart showing the work plan of research

3.3 Collection of plant materials

In this study, plants were selected on the basis of their medicinal importance explored from literature review and knowledge of the local people of the area, especially which are commonly used against diarrhea and dysentery. All the plants were authenticated by Ethnobotanical society of Nepal team (ESoN).

Table 1 Name, collection sites and parts of plant selected

S. N.	Scientific name	Family	Vernacular name	Parts used
1	<i>Azadirachta indica</i> A. Juss	Meliaceae	Nim	Leave
2	<i>Bauhinia vahlii</i> Wight & Arn.	Fabaceae	Malu, Bhorla	Bark
3	<i>Bombax ceiba</i> L.	Malvaceae	Simal	Bark
4	<i>Cassia fistula</i> L.	Fabaceae	Rajbriksha	Bark
5	<i>Cassia occidentalis</i> L.	Fabaceae	Thulo tapre	Aerial
6	<i>Costus speciosus</i> (Koenig) Sm.	Zingiberaceae	Betlauri	Whole
7	<i>Elephantopus scaber</i> L.	Asteraceae	Mula pate	Whole
8	<i>Mangifera indica</i> L.	Anacardiaceae	Aanp	Bark
9	<i>Moringa oleifera</i> Lam.	Moringaceae	Sajyon	Twig
10	<i>Psidium guajava</i> L.	Myrtaceae	Amba	Leave
11	<i>Syzygium cumini</i> (L.) Skeels	Myrtaceae	Jamun	Bark
12	<i>Uraria lagopoides</i> (L.) Desv.	Fabaceae	Sano bhante	Whole

3.4 Short description of plant under study

Medicinal plants are the source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of country. For this study, the selected plants are as follows:

3.4.1 *Azadirachta indica* A. Juss. (MELIACEAE; Neem)

It is one of two species in the genus *Azadirachta*, native to India and Burma, growing in tropical and semi-tropical regions (Reddy *et al.*, 2013). It is a fast growing tree, average height 15-20 m but rarely to 35-40 m. Bark grayish to dark gray tubercled. Leaves compound, long, lanceolate, serrated and oblique at the base. Fruit one seeded drupe, greenish yellow when ripe. It is distributed throughout Nepal to about 900m, common in open places around the villages; also in Pakistan, India, Bangladesh, Myanmar, Thailand, and Indonesia (Manandhar, 2002).

3.4.2 *Bauhinia vahlii* Wight & Arn. (FABACEAE; Bhorla)

Bauhinia vahlii Wight & Arn. is a very large, usually evergreen, climber and is distributed in deciduous forests of India from Gujarat Southwards to Maharashtra and Northern Andhra Pradesh, commonly on hillsides and in forest valleys (Parrotta, 2001). It is an evergreen woody climber. Leaf with circinate tendrils, base cordate, apex lobed, obtuse, and Flower white. Fruit flat pod. Flower appears during the month of April to June and fruit formation takes place from the month of November to January. It is distributed throughout Nepal at 200-1500m, especially in *Shorea robusta* forest (Manandhar, 2002).

3.4.3 *Bombax ceiba* L. (MALVACEAE, previously in *Bombacaceae*; Simal)

Bombax ceiba is a tropical humid lowland species, often found near stream banks. It is a tall deciduous tree with straight butteressed trunk and wide spreading branches. Bark grey or brown covered with hard, sharp conical prickles. Leaves are large, palmate, leaflets 5-7 in numbers, long petiolated, lanceolate. Flowers are red, appearing at the end of the branches. The flower appears during the month of December to March and fruit formation takes place during the month of April to May. The plant is distributed in the subtropical belt of Nepal to about 1200m in open places (Manandhar, 2002).

3.4.4 *Cassia occidentalis* L. (FABACEAE; Thulo tapre)

It is an ayurvedic plant with important medical values and is native to south India (Vadnere *et al.*, 2011). It is an erect shrub about 1m high. Leaf stalked, ovate to oblong, acuminate, entire. Flower stalked yellow. Flower and fruits formation takes place from June to January. Distributed throughout Nepal to about 1500m in open area and on uncultivated land (Manandhar, 2002).

3.4.5 *Cassia fistula* L. (FABACEAE; Rajbrikchhya)

It is a medium sized ornamental tree of hight about 10-20 meters; with beautiful bunches of yellow flowers. Bark greenish grey. Leaf alternate, Pinnate, ovate to oblong, acuminate, entire. Flower formation occurs from May to July and fruit formation takes place from October to January. Distributed throughout Nepal to about 1500m in open, dry places or with other tree in the forest (Manandhar, 2002).

3.4.6 *Costus speciosus* (Koenig) Sm. (ZINZIBERACEAE; Betlauri)

Costus speciosus (Koenig) Sm. Zingiberaceae) is an erect herbaceous plant upto 2 m height with long lanceolate leaves and white fragrant flowers in terminal clusters. The plant flowers during the months of July and August and is distributed throughout Nepal from 400-700m (Manandhar, 2002).

3.4.7 *Elephantopus scaber* L. (ASTERACEAE: Mula pate)

Elephantopus scaber L. is a tropical perennial herb that grows in many Asian countries such as China, India, Vietnam, Malaysia etc (Kabeer and Pratapan, 2014). It is common wild weed that forms undergrowth in shady places. Leaves are mostly radial forming a rosette on the ground, ovate-oblong, Rounded or sub acute, serrate-dentate. Stem dichotomously branched and flowering heads are borne at the cluster at the end of the branches. The flowering heads are numerous, sessile and forming a terminal inflorescence, flower violet. Flowering season is from June to August. Distributed throughout Nepal to about 2000m, in open as well as shady places (Manandhar, 2002).

3.4.8 *Mangifera indica* L. (ANACARDIACEAE; Aap)

It is a large evergreen tree with rough, thick, dark grey bark. Leaves stalked, alternate, crowded at the ends of branches, ovate to lanceolate, entire, dark green, base acute. Flowers yellowish, fragrant, in terminal panicles. Fruit a drupe, ovoid. Flowering season starts from March to April and fruit formation takes place from June to July. Distributed throughout Nepal to about 1300m in open places; also in subtropical parts of the Himalaya, India, Sri Lanka and south east Asia (Manandhar, 2002).

3.4.9 *Moringa oleifera* Lam. (MORINGACEAE; Sajyon)

Moringa oleifera is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Busani *et al.*, 2012). It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses (Fahey 2005). It is about 10m high with corky bark. Leaves stalked, alternate, and elliptic to ovate, thin, and entire. Flower white in colour. It is distributed throughout Nepal to about 1100m (Manandhar, 2002).

3.4.10 *Psidium guajava* L. (MYRTACEAE; Amba)

Psidium guajava (Linn.) is a low evergreen tree or shrub 6 to 25 feet high, with wide spreading branches. It is native to and widely distributed in Mexico and Central America. Leave stalked, opposite, oblong- elliptic, apex pointed. Flower white. Fruit is small, pear shaped, reddish yellow when ripe. It is distributed throughout Nepal to about 1400m (Manandhar, 2002). It is the hardiest among tropical fruit trees and excels most of the other fruit crops in productivity and adaptability (Joseph and Pariyar, 2011).

3.4.11 *Syzigium cumini* (L.) Skeels (MYRTACEAE; Jamun)

It is a large, evergreen widely distributed forest tree of India, Sri Lanka, Malaysia and Australia which is also cultivated for its edible fruits (Belapurkar and Goyal, 2014). Leave opposite, lanceolate, elliptic or oblong. Flower whitish. Fruits drupes, ellipsoid or oblong, black with pinkish juicy pulp. The flower appears during the month of March to April and the fruit formation takes place during the month of May to July. It is distributed throughout Nepal to about 300 to 1200m (Manandhar, 2002).

3.4.12 *Uraria lagopoides* (L.) Desv. (FABACEAE; Sano bhante)

It is a perennial trailing herb, 20-27cm long. Leave 3 foliate or sometimes 1 foliate; leaflets ovate, broadly elliptic to ovate, apex obtuse. Flower bluish propagated by seeds. It is distributed throughout Nepal at 150-1700m in open, sandy soil also in India, china, Australia, and Polynesia (Mannandhar, 2002).

3.5 Herbarium preparation and identification

The herbarium specimens were prepared following the Standard herbarium techniques (Bridson and Forman, 1992). The plants were dried carefully and pressed. After mounting the herbarium on the herbarium sheets, they were cross checked with Herbarium at National Herbarium and plant Laboratories, Godavari, Lalitpur (KATH) and submitted to TUCH (Tribhuvan University Central Herbarium), Kirtipur.

3.6 Drying of samples

The collected samples were made into small pieces and allowed it to dry over 2-3 weeks as required inside the room.

3.7 Grinding of samples

The shade dried samples of each plant were grinded to fine powder with the help of electric grinder. The ground plant parts were preserved into zipper bag for the further analysis.

3.8 Extraction

The extraction technique followed was percolation with intermittent sonication. For this 16 gm of plant powder was taken in conical flask separately. Afterward 160 ml of methanol was poured into the conical flask such that the ratio of solvent in volume (ml) to the weight (gram) of plant material would be 10:1. Then resulting solution was allowed to stand for overnight at room temperature. Then the solution was subjected to intermittent sonication for 2 hours, i.e. continuous cycle of sonication at 30 kHz for 30 min, regularly for the next 3 days. On the next day it was filtered with Whatman no.1 filter paper (Whatman Ltd, Kent, and UK). The filtrate was then subjected to evaporation at reduced pressure in rotatoy vacuum evaporator (IKA RV 10). The condensed extract thus obtained was transferred to clean, dry and weighed glass vials and allowed to dry up at room temperature. Thus obtained extract was called crude extract of the respective plant samples and was calculated by using the following formula.

$$\text{Percentage Yield (\%)} = \frac{\text{Dry weight of Extract}}{\text{Dry weight of Plant material}} \times 100$$

The resulting dry extracts were then sealed and stored at 4°C until use.

3.9 Antibacterial screening

The antibacterial activity of the crude extract was studied by Agar well diffusion method (Perez and Bazevque, 1990). In this method fixed volume of extract solution are placed on the agar plate along with the positive and negative control. The zone of inhibition (Halozone) was measured. The zone of inhibition shows the antibacterial activity of the plant extract. Antimicrobial susceptibility test measures the ability of antibiotics or other antimicrobial agent to inhibit growth in vitro.

3.9.1 Microorganism

The test organisms were obtained from institute of medicine, Maharajgunj, Nepal and Central Department of Biotechnology, Tribhuvan University, Kathmandu, Nepal. Two

gram positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis*) and two gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853 and *Escherchia coli* ATCC 25922) were used for the antibacterial assay.

3.9.2 Culture of bacteria

After collecting the cultures, the test organisms were streaked on different nutrient agar plates and incubated on the incubator at 37°c for 24 hours so as to obtain pure and isolated colonies. Each distant colony was aseptically transferred to the Nutrient broth for suspension culture with the help of sterilized inoculating loop. The inoculated tubes were kept on incubator at 37°C for 12- 18 hours. The turbidity of the bacterial suspension was compared with 0.5 McFarland standards to obtain inoculums with desired suspension of bacteria i.e. $1.0-1.5 \times 10^8$ CFU per ml.

3.9.3 Preparation of extract

50mg/ml concentration of crude extracts were prepared by dissolving 100mg of extract in 2 ml of DMSO. The resulting solution was then stored at 4°c until use.

3.9.4 Preparation of media

3.9.4.1 Nutrient Agar and Muller Hinton agar

Nutrient agar (NA) and Muller Hinton agar (MHA) were prepared according to the manufactures (Hi- media) recommendations. The powder media (38gm/L for MHA and 28gm/L for NA) were added in distilled water in conical flask and boiled with continuous shaking to dissolve the powder and autoclaved at 121°C for 15 minutes. It was than cooled to about 50°C and 15-20 ml of it was poured into sterile petriplates aseptically and labeled properly. The thickness of the medium after solidification was approximately equals to 4mm.

3.9.4.2 Nutrient Broth

Nutrient broth (NB) was prepared as recommended by manufacturer (Hi- media). The powder (13gm/lit) was added in distilled water in conical flask and boiled with continuous shaking to dissolve the powder and autoclaved at 121°C for 15 minutes and it was than cooled and poured in sterilized test tubes.

3.9.4.3 Antibacterial screening via Agar well diffusion Technique

The crude methanolic extract of medicinal plant was screened for its antimicrobial activity. Zone of inhibitions against tested organism were determined by agar well diffusion technique.

At first, sterile and uniform thickness (4mm) of Muller Hinton Agar (MHA) plates were prepared. Plates were dried under laminar air flow for 30 minutes to remove excess moisture from the surface of the media.

Sterile cotton swab was dipped into the prepared inoculum. Excess inoculum in the swab was removed by pressing and rotating the swab against the upper side wall of the tube above the liquid culture level and seeded carefully all over the plate. Finally, the swab was passed round the edge of the agar plate. The inoculated plate was left for few minutes at room temperature with the lid closed.

With the help of sterile cork borer, six wells with diameter 4mm each were made in the inoculated MHA plates and labeled properly with permanent marker pen. Thirty microlitre of working solution of medicinal plants were filled into the well with the help of Micropipette. DMSO was taken as negative control while the Ampicillin at concentration of the 5mg/ml was taken as the positive control. Then the inoculated plates were incubated at 37°C in an incubator in upright position for 18-24 hours.

After 18-24 hours of incubation the plates were observed for zone of inhibition which is suggested by clear area around the well and result was noted. The zone of inhibition were measured with the help of ruler (ZOI also included the diameter of the well).

3.10 Determination of Total Phenol Content

The total phenolic content of the methanolic extracts were determined by Folin-Ciocalteu method (Roy *et al.*, 2011; Chang *et al.*, 2002) with slight modification. Briefly, 0.1 ml of extract (2.5mg/ml) was separately mixed with 1 ml Folin Ciocalteu phenol reagent (Fisher scientific, 1: 10 dilution with the distilled water) and 0.8ml of aqueous 1M Na₂CO₃ solution. The reaction mixture was allowed to stand for about 15 minutes at room temperature. After completion of the incubation the absorbance of the reaction mixture (Blue/violet solution) was measured at 765nm using the UV-Visible Spectrophotometer (Thermo fisher scientific, Genesystem- 10-5). For blank, 0.1 ml of absolute methanol was added instead of extract solution in above reaction mixture and gallic acid (Moly chem,

Mumbai, india) solution was used as standard in methanol and water (50:50 v/v) using the concentration ranging from 25-250 μ g/ml. Total phenolic content in the respect plant extract were then quantified by using standard calibration curve generated. The result was expressed in terms of milligram of gallic acid equivalent per gm of dry mass (mgGAE/g). For each extract, triplicates were performed to get more accurate result.

3.11 Determination of Total Flavonoid Content

The aluminium chloride calorimetric method was used for Flavonoid content determination (Chang *et al.*, 2002, Roy *et al.*, 2011) with slight modification. Briefly, each plant extract was dissolved in absolute methanol at the concentration of 10mg/ml. Then, 0.25 ml of each sample solution was separately mixed with the 0.75 ml of methanol, 0.05 ml of 1M potassium acetate (CH₃COOK) and 1.4 ml of distilled water. The resulting solution were shaken and allowed to stand at room temperature for 30 minutes. The absorbance of the reaction mixture (yellow coloured complex) was measured at 415nm using the UV-Visible spectrophotometer (Thermo Fisher Scientific, Genesystem-10-5). Quercetin was used as standard solution in methanol with the concentration ranging from 10-100 μ g/ml and blank was prepared by adding all the reagents except the plant sample. The total flavonoid content were then expressed in terms of quercetin equivalent per gram of dry mass i.e. milligram of quercetin per gram of dry weight of the plant. All the tests were carried in triplicates to get more accurate result.

3.12 Determination of Antioxidant activity

The free radical scavenging activity of samples and standard ascorbic acid solution in methanol was determined based on their ability to react with stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The method was carried out as described by Brand-Williams *et al.*, (1995) and Singh *et al.* (2002) with slight modification. Briefly, different concentration of plant extract (30-120 μ g/ml) and Ascorbic acid (30- 400 μ g/ml) were prepared in methanol on the clean and clear test tubes. The sample volume was taken 0.5ml. To this 0.5 ml of the 0.2mM DPPH solution was added. The solution on the test tubes were shaken well and incubated in dark for 30 minutes. Then after the absorbance of the solution in each tube were measured at 517nm using UV-Visible spectrophotometer. The control was prepared as above but without the plant extract or Ascorbic acid.

Methanol was taken to measure the baseline on the spectrophotometer (Thermo Fisher Scientific, genesystem- 10-5).

The free radical scavenging activity (RSA) of the plant samples were calculated in percentage by the formula:

$$\% \text{ Radical Scavenging activity (RSA)} = \frac{\text{abs. control} - \text{abs. sample}}{\text{Abs. control}} \times 100$$

IC50 value of each extract was calculated by using the following formula given below:

$$\text{IC50} = \text{EXP} [\text{LN} (\text{Conc} > 50\%) - (\text{Signal} > 50\% - 50) / (\text{signal} > 50\% - \text{signal} < 50\%) \times \text{LN} (\text{conc} > 50\%) / (\text{Conc} < 50\%)]$$

EXP: Exponential function, LN: Natural log function, both used in Microsoft excel 2007 software. Signal > 50%: RSA value just above 50%, Signal < 50%: RSA value just below 50%. Conc.> 50%: Concentration of extracts > 50%, Conc.< 50%: Concentration of extracts < 50%

3.13 Statistical analysis

All the experiments were performed in triplicates. Microsoft excel 2007 software was used for the calculation of phenol/flavonoid content, calculation of inhibitory concentration value (IC50) for antioxidant and for generation of graphs. Regression analysis was done using SPSS version 16.0.

CHAPTER IV: RESULTS

4.1 Extraction

In this study the parts of plants were selected on the basis of their reported uses in ethno medicine, especially based on diarrhoea and dysentery. Whole plants were recommended for some plant while in others Aerial parts, leaves and bark. Among the twelve selected plants the percentage yield of the extract varied from 2.00% to 15.63%. The highest yield was obtained with *Cassia fistula* whereas lowest yield was found in *Syzygium cumini*. The consistency of the extracts was sticky and powdery. The percentage yields and characteristics of the extracts obtained have been tabulated as below.

Table 2 Percentage yield and physical characteristics (Color and consistency) of the crude Methanolic extracts

Plant Sample	Dry weight taken (gm)	Weight of extract (gm)	Percentage yield (%)	Characteristics of extract	
				Colour	Consistency
<i>Azadirachta indica</i>	16	0.71	4.44	Dark green	Sticky
<i>Bauhinia vahlii</i>	16	0.99	6.19	Red	Powdery
<i>Bombax ceiba</i>	16	0.79	4.94	Red	Powdery
<i>Cassia fistula</i>	16	2.5	15.63	Red	Powdery
<i>Cassia occidentalis</i>	16	0.96	6	Red	Sticky
<i>Costus speciosus</i>	16	0.47	2.94	Dark green	Sticky
<i>Elephantopus scaber</i>	16	0.73	4.56	Dark green	Sticky
<i>Mangifera indica</i>	16	1.4	8.75	Red	Powdery
<i>Moringa oleifera</i>	16	0.33	2.06	Dark green	Sticky
<i>Psidium guajava</i>	16	1.46	9.13	Dark green	Powdery
<i>Syzygium cumini</i>	16	0.32	2	Red	Powdery
<i>Uria lagopoides</i>	16	0.78	4.88	Dark green	Sticky

4.2 Antimicrobial Screening

The methanolic extracts of twelve medicinal plants were tested against the four pathogenic microbes viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*. Out of twelve plants tested for antimicrobial activity, eight plant species showed antibacterial activity by inhibiting one or more microorganisms. Among the plants screened the methanolic extract of *Mangifera indica*, *Bombax ceiba*, *Bauhinia*

vahlia, *Cassia fistula*, and *Syzygium cumini*, showed significant inhibition of all tested bacteria followed by, *Psidium guajava*, *Elephantopus scaber* and *Azadirachta indica* against few pathogens. But *Cassia occidentalis*, *Uraria lagopoides*, *Moringa oleifera* and *Costus speciosus* did not show activity against tested bacteria.

Table 3 Antibacterial activity of the crude methanolic extract

Plants	Zone of inhibition in mm (With diameter of well = 4mm)			
Bacterial strain	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. subtilis</i>
ATCC no.	25922	27853	25923	
Ampicillin	24	4	46	15
<i>Azadirachta indica</i>	8	4	4	9
<i>Bauhinia vahlii</i>	12	11	11	13
<i>Bombax ceiba</i>	11	11	13	12
<i>Cassia fistula</i>	12	12	11	14
<i>Cassia occidentalis</i>	4	4	4	4
<i>Costus speciosus</i>	4	4	4	4
<i>Elephantopus scaber</i>	9	4	14	4
<i>Mangifera indica</i>	10	12	10	13
<i>Moringa oleifera</i>	4	4	4	4
<i>Psidium guajava</i>	11	4	7	8
<i>Syzygium cumini</i>	18	8	8	10
<i>Uraria lagopoides</i>	4	4	4	4
DMSO	4	4	4	4

4.3 Determination of total phenol contents

The total phenolic content of the crude methanolic extracts were determined in terms of gallic acid equivalent (mg of GAE/gm of dry weight of extract) by using the Calibration curve of gallic acid (25-250µg/ml, $Y = 0.06x + 0.215$, $R^2 = 0.97$). The total phenolic content varied from 10.83 ± 0.16 to 34.6 ± 0.13 mg GAE/g dry weight. *Moringa oleifera* (34.6 ± 0.13) was found to contain high phenolic contents while *Azadirachta indica* (10.83 ± 0.16) showed lowest phenolic content. Total phenolic content of standard and all the extracts are shown in figure 3 and 4 respectively.

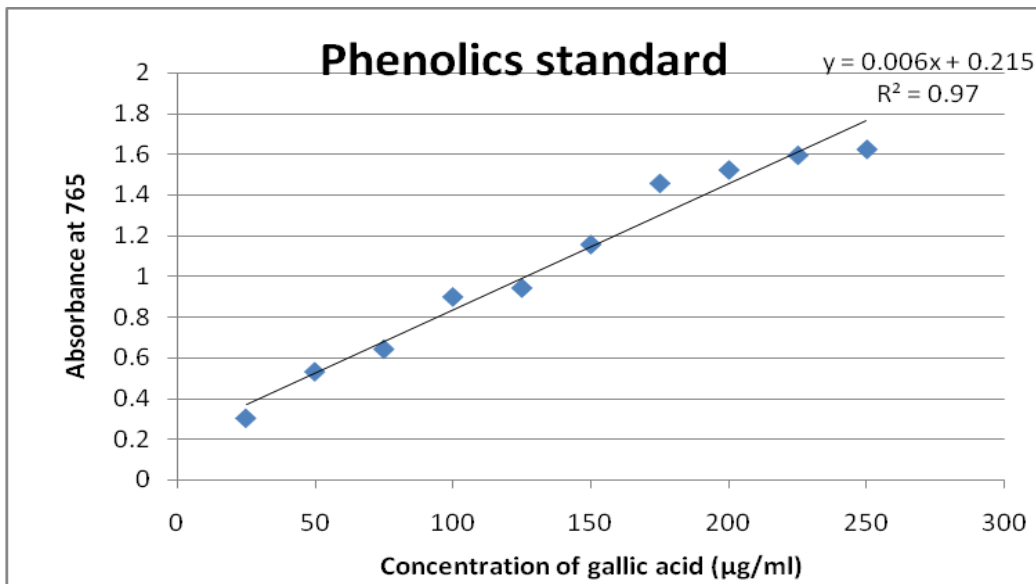


Figure.3 Standard curve for estimation of total phenol content

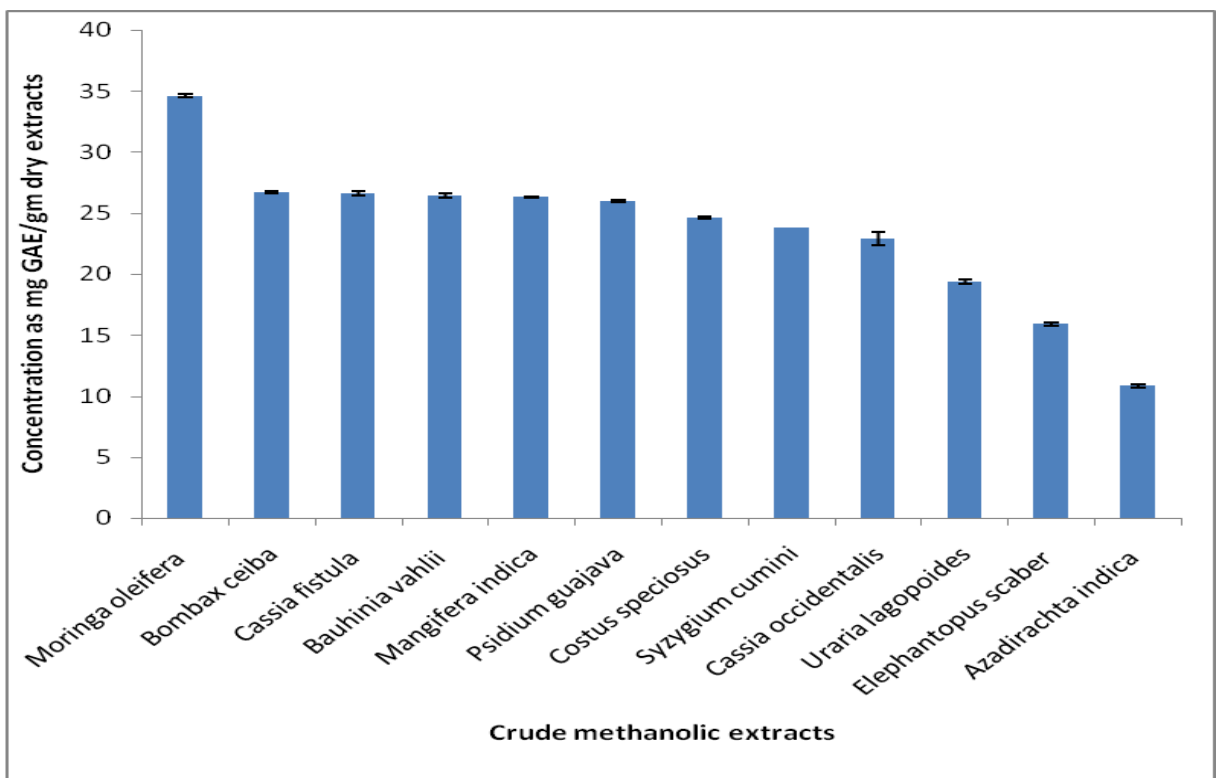


Figure 4 Estimation of total phenol content of crude methanolic extracts

The error bar represent the standard deviation(S.D.) from mean value

4.4 Determination of total Flavonoid content

The total flavonoid content of the methanolic extracts was determined in terms of mg Quercetin equivalent per gram of dry mass. The Calibration curve was generated by using Quercetin (10-100) with the line of equation $Y=0.011x- 0.064$, $R^2=0.989$ and using this equation, the flavonoid content of the plant were estimated. Among the plant selected for study the flavonoid content showed variation from 5.23 ± 0.50 to 8.13 ± 0.16 mg quercetin equivalent per gram or dry mass. *Cassia occidentalis* (8.13 ± 0.16) showed highest Flavonoid content whereas *Cassia fistula* (5.23 ± 0.50) showed lowest flavonoid content but remaining extracts showed comparable amount of flavonoid content. Total flavonoid content of standard and all the extracts are shown in figure 5 and 6 respectively.

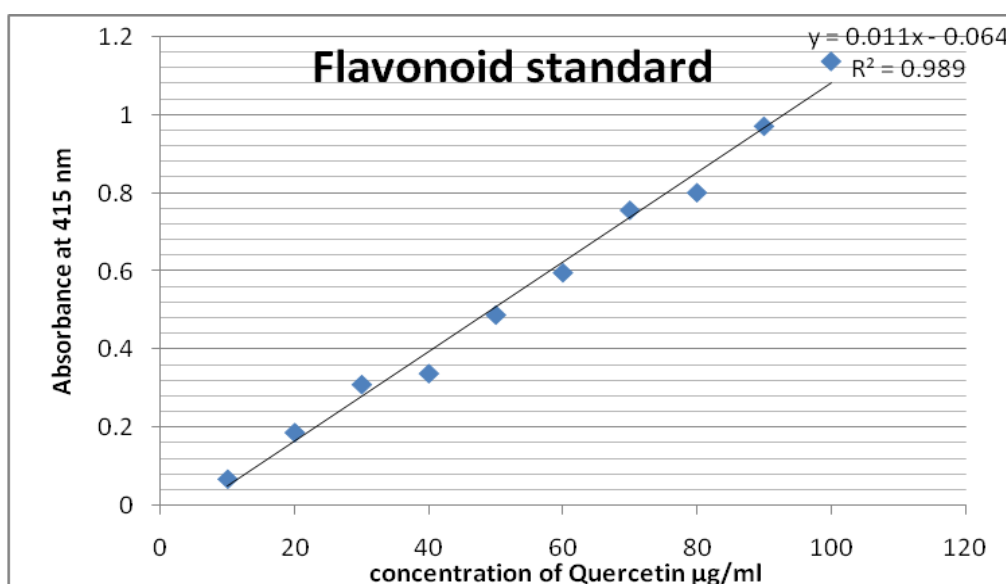


Figure 5 Standard curve for estimation of total flavonoid content

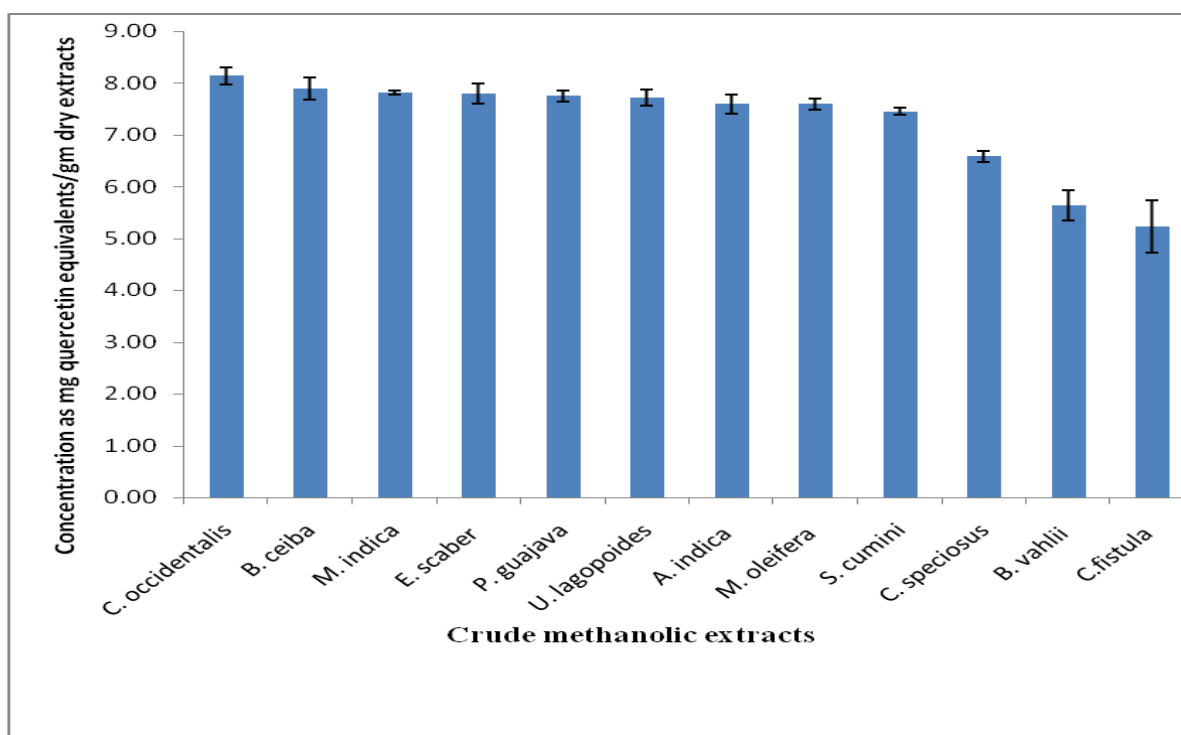


Figure 6 Estimation of total flavonoid content of crude methanolic extracts

*The error bar represents the standard deviation(S.D) from mean values.

4.5 Estimation of Antioxidant activity

The hydrogen donating or free radical Scavenging ability of extracts was determined by using 2,2-Diphenyl-1-picryl hydrazyl radical (DPPH). The absorbance of the resulting solution was measured at 517nm and the percentage of radical scavenging activity (RSA) was calculated. Thus from the obtained Value IC_{50} value was calculated. The antioxidant activity of the plant extract was found to be concentration dependent and their antioxidant activity was compared with standard ascorbic acid, which was found to be 48.66 μ g/ml. Among the plant samples *Syzygium cumini* showed lowest IC_{50} value while the *Cassia occidentalis* showed highest IC_{50} value. The IC_{50} value of each plant extract and standard ascorbic acid has been given in table *Syzygium cumini*, *Bombax ceiba*, *Elephantopus scaber*, *Uraria lagopoides* and *Bauhinia vahlii* showed lower IC_{50} value than standard ascorbic acid where as *Cassia occidentalis*, *Mangifera indica*, *Moringa oleifera*, *Azadirachta indica*, *Cassia fistula*, *Psidium guajava* and *Costus speciosus* showed higher value than standard ascorbic acid.

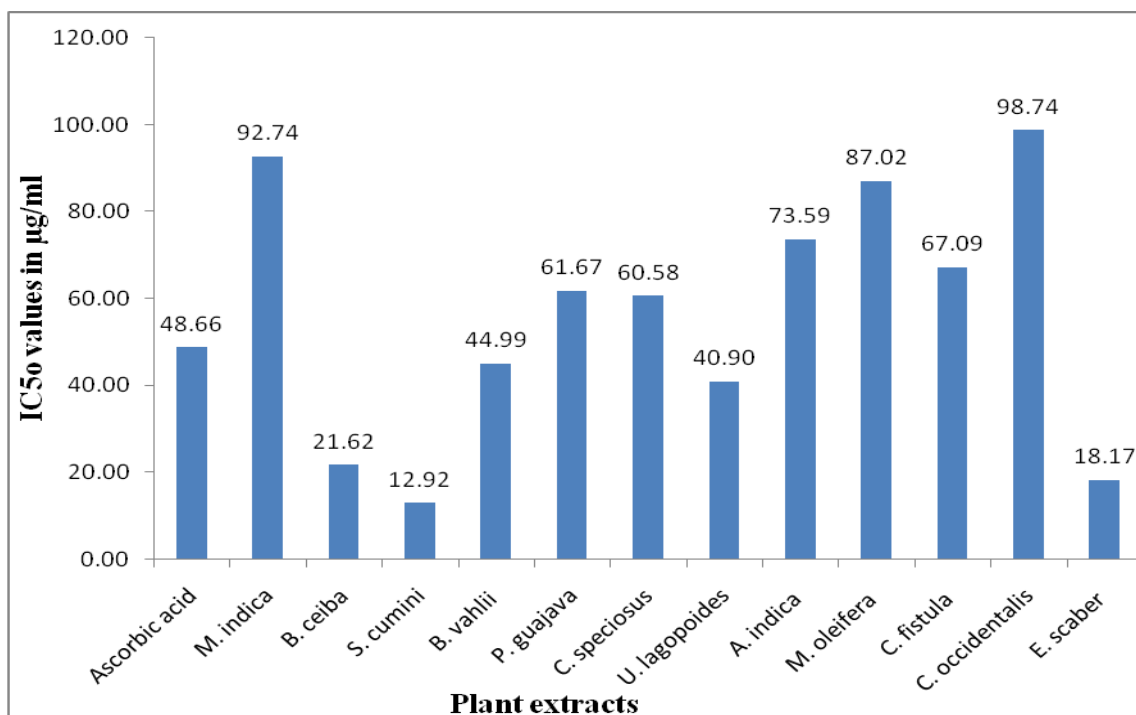


Figure 7 The IC₅₀ values of the plant extract on DPPH. (Lower value indicate more powerful antioxidant capacity)

4.6. Correlation between antioxidant and phenolic content

From the study it was found that phenolic content present in medicinal plants is not correlated with antioxidant activity. Regression analysis between values of phenolic content estimated in each medicinal plant of the study and antioxidant activity shows that very poor correlation between them ($R = 0.023$, $p = 0.633$)

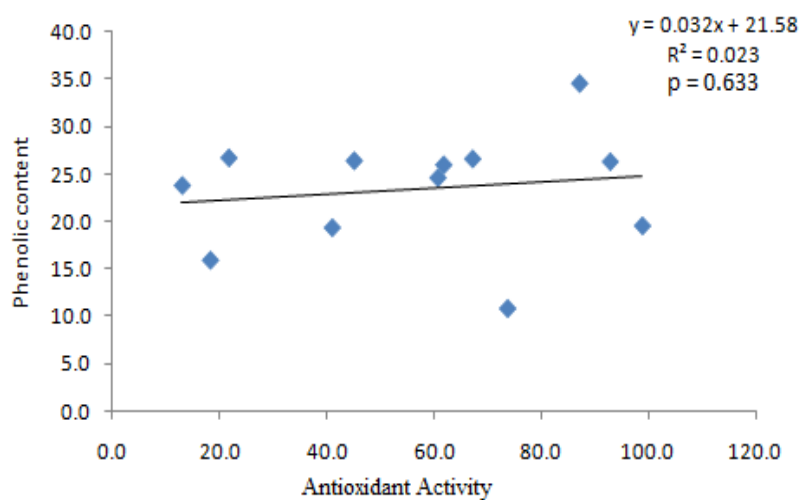


Figure 8 Correlation between Antioxidant activity and phenolic content

CHAPTER V: DISCUSSION

5.1 Extraction

The premier step to utilize the biologically active compound from plant resources are extraction, pharmacological screening, isolation, and characterization of bioactive compounds, toxicological evaluation and clinical evaluation (Sasidharan *et al.*, 2011).

Extraction is the crucial first step in the analysis of medicinal plants, because it involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. The extract thus obtained, contains complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoides and lignans (Handa *et al.*, 2008). The basic parameters influencing the quality of extracts are, plant part used as starting material, Solvent used for extraction and extraction procedure (Ncube *et al.*, 2008). Solvent diffuse into the solid plant tissues and solubilize compounds of similar polarity (Green, 2004). Properties of a good solvent in plant extraction include low toxicity, ease of evaporation at low heat, promotion of rapid physiological absorption of the extract, preservative action and inability to cause the extract to complex or dissociate (Hughes 2002). Extraction method was completed by means of sonication. Present work was carried out using methanol as solvent because it is polar than water and ethanol and believed to extract all the active constituents from the plant material. Another advantage of using methanol as extracting solvent is that it evaporates easily resulting in quick concentration of the extracts. The variation in quantity and secondary metabolite composition of the extract in different extraction method depends on, nature of solvent, time and temperature of the extraction, purity of solvent and Polarity.

5.2 Antimicrobial activity

Antimicrobial screening of the plant extracts was determined by agar well diffusion method by measuring zone of inhibition against the test microorganism. The antibacterial substance present in the plant material diffuse in the agar media and kill or inhibit the growth of the bacteria and thus zone of inhibition occurs around the disc in the agar surface. By measuring the zone of inhibition we can evaluate the potency of antimicrobial drugs present in the plant extract.

The diameter of zone of inhibition produced depends upon the various extrinsic and intrinsic parameters. Extrinsic parameter like pH of the medium, period and temperature of incubation, volume of the well, concentration of the plant extracts and size of the inoculums can be fixed and standardized during the experiment, hence no error results due to extrinsic factors. However, intrinsic factors such as nature of the medicinal plants including its components, Solubility and diffusing property are predetermined. Due to variable infusibility, the antibacterial with high potency may not determine ZOI commensurate to its efficacy (Prasai *et al.*, 2004).

Among the plant screened the methanolic extract of *Mangifera indica*, *Bombax ceiba*, *Bauhinia vahlii*, *Cassia fistula*, *Syzygium cumini*, showed significant inhibition of all tested bacteria followed by *Elephantopus scaber*, *Psidium guajava* against few pathogens. But *Cassia occidentalis*, *Uraria lagopoides*, *moringa oleifera* and *Costus speciosus* did not show activity against tested bacteria. Majority of the plant extracts inhibited by *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* compared to *Pseudomonas aeruginosa*. Among the plant screened, *Syzygium cumini* (18mm) showed highest antibacterial activity against *E. coli*, *Cassia fistula* and *Elephantopus scaber* showed highest activity against *Bacillus subtilis*, *Bombax ceiba* showed highest activity against *S. aureus* and *Mangifera indica* and *Cassia fistula* showed highest activity against *Pseudomonas aeruginosa*.

The highest antibacterial activity of *Syzygium cumini* against *Escherichia coli* was not supported by earlier study. Belapurkar and Goyal, (2014) reported that methanol extract of leaf of *S. cumini* showed highest antibacterial activity against *Staphylococcus aureus* compared to *Escherichia coli*. Antibacterial activity of *Cassia fistula* against all the tested microorganism was also supported by Bhalodia *et al.* (2011) and Beena and joji (2013).

No activity of *Cassia occidentalis* against the entire tested microorganism was also supported by Sadiq *et al.* (2012). They reported that ethanol extract of *Cassia occidentalis* did not show activity against tested bacteria. Kalpana *et al.* (2013) reported that different leaf extract of *M. oleifera* showed activity against all the tested pathogens at 200-800mg/ml Conc. which was supported by our finding. Root extract of *Costus speciosus* showed activity against tested bacteria (Sulakshana *et al.*, 2013) which was contradictory to our finding. Choudhury *et al.* (2012) reported the antibacterial activity of *Psidium guajava* leaf against *P. aeruginosa*, which was not similar to our finding. Aqueous leaf

extract of *Azadirachta indica* showed antibacterial activity against *pseudomonas aeruginosa* and *S. aureus* (Reddy *et al.*, 2013; Gajanan, 2012) which was not supported by our finding, which might be due to the different solvent used. 'Lack of inhibition zone' doesnot mean that the plants have no medicinal use. It merely states that the present extracts were not active against the tested microbes in the study under the lab environment. In many cases the plant was used as medicine after adding with another plant species as additive, but in laboratory it was tested separately. There may be some chemical in one plant and they activated the chemical constituent of another plant and they could kill or inhibit the growth of causative bacteria synergistically. In our research, gram positive bacteria are found more resistant than gram negative bacteria. Negative control (DMSO) did not show any action against the tested bacteria signifying that it serves as a dilutant (Sudharameshwari and Radhika 2007). The plant extract showed lower antibacterial activity than the standard Antibiotic ampicillin. This may be due to the reason that active compounds may be present in insufficient quantities in the crude extracts to show activity with the dose level employed (Taylor *et al.*, 2001).

Various workers have already shown that gram positive bacteria are more susceptible towards plant extracts as compared to gram negative bacteria (Lin *et al.*, 1999, Parekh and Chanda, 2007). These differences may be attributed to the fact that the cellwall in gram positive bacteria is of single layer, whereas the gram negative cell wall is multilayered structure (Yao *et al.*, 1995). Microorganisms show variable sensitivity to chemical substance related to different resistant strains (Cetin and Gurler 1989). Ampicillin was taken as positive control for gram positive and gram negative bacteria because of its broad-spectrum antibiotic activity. .

5.3 Total phenolic content determination

Phenols, sometimes called phenolics, are one of the main secondary metabolites present in the plant kingdom. They are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity (Kähkönen MP *et al* 1999). They are essential for the growth and reproduction of plants, and are produced as a response for defending injured plant against pathogens. Phenols are able to scavenge reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness depends on the stability in different systems, as well as number and location of hydroxyl groups (Podsdek, 2007).

Among the extract under studied *Moringa oleifera* showed the highest phenolic content which was also supported by leaf extract of *Moringa oliefera* (Sreelata and Padma, 2009). They suggested that Mature leaf extract had highest phenolic content followed by tender extract. Higher phenolic content was also supported by seed oil extracts of *Moringa oleifera* (Ogbunugafor *et al.*, 2011). The phenolic content of *Azadirachta indica* reported by Deka *et al.* (2013) was higher than the phenolic content reported in this study. The neem tree from the different tropical parts of the world is reported to contain high level of polyphenolic compounds, but due to the wide range of geographical distribution, a large variety of morphological and biochemical characteristics have been reported. It has also been reported that phenolic contents of neem can be influenced by geographical locations and other abiotic factors (Ghimeray *et al.*, 2009).

5.4 Total flavonoid content determination

Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and they have been found *in vitro* to be effective antimicrobial substance against a wide array of microorganism. Their activity is probably due to their ability to complex with extracellular and soluble protein and to complex with bacterial cellwall (Marjorie, 1996). They also are effective antioxidant and show anticancer activity (Salah *et al.*, 1995).

Among the studied plant extracts, there is very less variation among the flavonoid content. As compared to phenolic data, the flavonoid content data was very poor. Low flavonoid content despite their richness in polyphenol content might be due to rich in polyphenols other than flavonoid such as tannins, phenolic acids, and other non flavonoid polyphenolics. The other reason may be that some of the plants exist as glycosides so that their hydroxyl group remains unavailable for reaction with $AlCl_3$.

5.5 Antioxidant activity

Free radical scavenging capacity was evaluated on the basis of the scavenging activity of DPPH by measuring the reduction of absorbance at 517 nm. DPPH radical is a commonly used substrate for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay (Bozin *et al.*, 2008). This assay is known to give reliable information concerning the antioxidant ability of the tested compounds (Huang *et al.*, 2005).

Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems (Halliwell, 1994). The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defense mechanisms.

Antioxidants are those substances which possess free radical chain reaction breaking properties. Antioxidants are both natural and synthetic compounds, able to scavenge free radicals and to inhibit oxidation processes (Hayat *et al.*, 2010). Many synthetic antioxidants such as Butylated Hydroxyl Anisole (BHA) and Butylated Hydroxyl Toluene (BHT) are very effective and are used for industrial processing but they possess some side effects and toxic properties to human health therefore, there is an increasing interest in natural antioxidants, e.g., polyphenols, present in medicinal and dietary plants (Silva *et al.*, 2005). Antioxidants play an important role in defending the body against free radicals damage. They work by preventing the formation of new free radical species, converting existing free radicals into less harmful molecules and preventing radical-chained reactions (Ismail *et al.*, 2010). The principle function of antioxidants is in delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and they may reduce oxidative damage to the human body (Ismail *et al.*, 2004).

DPPH is stable nitrogen centered free radical, violet in colour, containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The absorption maximum of a stable DPPH radical in methanol was at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progresses, which results in the scavenging of the radical by hydrogen donation (Bhalodia *et al.*, 2011) which is indicated by progressive fading of the colour from violet to yellow. The antioxidant activity of the samples was expressed as IC₅₀ (inhibitory concentration), which was defined as the concentration (in µg/ ml) of sample required to inhibit the formation of DPPH radicals by 50%. The antioxidant Potential is inversely proportional to IC₅₀. Lower the value of IC₅₀ at lower concentration higher the free radical scavenging activity. So, IC₅₀ < 50 µg/ml value means a high antioxidant capacity; 50µg/ml < IC₅₀ < 100µg/ml value means a moderate antioxidant capacity and IC₅₀ > 200µg/ml value means no antioxidant capacity (Gulcin *et al.*, 2012)

In this research *Syzygium cumini* (12.92µg/ml), *Elephantopus scaber* (18.17µg/ml), *Bombax ceiba* (21.62 µg/ml), *Uraria lagopoides* (40.90 µg/ml), and *Bauhinia vahlii* (44.99 µg/ml) showed lower IC₅₀ value than standard ascorbic acid (48.66 µg/ml). It means that these were high antioxidant value than standard ascorbic acid. *Psidium guajava* (61.67 µg/ml), *Costus speciosus* (60.58 µg/ml), *Cassia fistula* (67.09 µg/ml), *Azadirachta indica* (73.59 µg/ml), *Moringa oleifera* (87.02 µg/ml), *Mangifera indica* (92.74) and *Cassia occidentalis* (98.74µg/ml) shows higher value than ascorbic acid which proved that it contains lower value of antioxidant potential.

High antioxidant activity of *Syzygium cumini* was in accordance with previous study which has concluded that seed and leaf extract of *Syzygium cumini* showed significant activity when compared with ascorbic acid (Belapur and Goyal, 2014, Nair *et al.*, 2013). According to Subramanian *et al.* (2014) pet-ether extract of *Elephantopus scaber* showed good antioxidant activity than ascorbic acid, this was also similar to our research. High antioxidant activity of *Bombax ceiba* than ascorbic acid was not supported by Gandhare *et al.* (2010). They suggested that aqueous and ethanol bark extract of *Bombax ceiba* showed comparable amount of activity as that of standard ascorbic acid. It may be due to the type of solvent used for the extraction. *Uraria lagopoides* showed higher antioxidant activity than ascorbic acid was against earlier finding which has concluded that aerial part of *Uraria lagopoides* showed lower antioxidant activity than ascorbic acid this may be due to the chemical constituent present between aerial part and whole part. Sowndhararajan and Kang, (2013) suggested that methanol extract of *Bauhini vahlii* leaves showed strong antioxidant activity than ascorbic acid but in this research it was comparable with standard ascorbic acid which may be due to the chemical constituent present between leaf and bark. Lower antioxidant potential of *Psidium guajava* and *Mangifera indica* was against earlier finding (Vyas *et al.*, 2010, Jona *et al.*, 2013) they reported that methanol leaf extract of both *Psidium guajava* and *Mangifera indica* showed higher antioxidant activity than standard. According to Nehete *et al.*, (2010) *Costus speciosus* showed similar activity compared with ascorbic acid which was against our finding.

The antioxidant activity of *Cassia fistula* leaf methanol extract was quite comparable with that of ascorbic acid (Jose and Reddy, 2013) but in this research it was lower than ascorbic acid. Lower antioxidant activity of *Azadirachta indica* is also supported by Ghimerary *et al.* (2009). They investigated that methanol extract of leaf and bark showed lower activity

than standard ascorbic acid. Lower antioxidant activity of *Cassia occidentalis* was also supported by Vadnere *et al.* (2011) and Mehta *et al.* (2010). They reported that *Cassia occidentalis* whole plant and seed showed lower antioxidant value than standard ascorbic acid. Many factors play important role in the antioxidant value but in this study main reason may be the physiological state of the plant during collection and parts of plant selected for study.

Among all the plant tested, *Syzygium cumini* has highest value of radical scavenging activity 12.92µg/ml, *Elephantopus scaber* was second highest 18.17µg/ml, which may be due to the presence of chemical constituents like tannins, reducing sugars and protein present in the extract. the strong antioxidant activity of these plants indicate the possible use of these plants in several chronic disorders like, atherosclerosis, diabetes, cardiac diseases, alzheimer's disease, parkinsonism and many more.

5.6 Relationship between total phenolic content and antioxidant activity

Some authors (Cai *et al.*, 2004, Djeridane *et al.*, 2006, Katsube *et al.*, 2004) have demonstrated a linear correlation between the content of total phenolic compounds and antioxidant capacity while others show poor linear correlation between antioxidant activity and phenolic content.

The antioxidant activity of extracts could not be explained just on the basis of their phenolic content but also required their proper characterization. Furthermore, the extracts are very complex mixtures of many different compounds with distinct activities. Our study is in agreement with Sengul *et al.* (2009), Kaur and Mandal (2014), which reported no correlation between total phenolic content and antioxidant capacities of a number of medicinal plant extracts no correlation between total phenolic content and antioxidant capacity in our plant samples is possible due to the presence of some other phytochemicals such as ascorbic acid, tocopherol and pigments etc which also contribute to the total antioxidant capacity. Also, phenolic content determined according to the Folin-Ciocalteu method is not an absolute measurement of the amount of phenolic materials. Different types of phenolic compounds have different antioxidant activities, which is dependent on their structure. The extracts possibly contain different type of phenolic compounds, which have different antioxidant capacities.

CHAPTER VI: CONCLUSION

Plants are presently the sources of medicines for many people of different age in many countries of the world, where diseases are treated primarily with traditional medicines obtained from plants. In developing countries the availability of modern medicines is limited. So traditional medicine is still the mainstay of health care and most drugs come from plants. Although many plants have long been recognized and widely used in Nepalese traditional medicine, some are relatively unexplored and not arrived to mainstream medicine. Therefore, search of new drugs must continue and natural products may prove to be a source of safe and affordable medicine.

The present study reported the antibacterial activity, total phenolic and flavonoid contents and antioxidant activity of twelve less known medicinal plants of Nepal (*Mangifera indica*, *Moringa oleifera*, *psidium guajava*, *Elephantopus scaber*, *Cassia fistula*, *Cassia occidentalis*, *Uraria lagopoides*, *Bauhinia vahlii*, *Azadirachta indica*, *Bombax ceiba*, *Costus speciosus* and *Syzigium cumini*). Among all the selected plants, eight plant species showed antibacterial activity by inhibiting one or more tested microorganisms. Majority of the plants showed good antioxidant activity. *Moringa oleifera* (34.6 ± 0.13) showed highest phenolic content (although it didnot showed antibacterial activity), whereas *Azadirachta indica* (10.83 ± 0.16) showed lowest phenolic content. *Cassia occidentalis* (8.13 ± 0.16) showed highest flavonoid content whereas *Cassia fistula* (5.23 ± 0.50) showed lowest flavonoid content but remaining extracts showed comparable amount of flavonoid content.

In this study, considerable differences were found in the activities of the plants samples in antibacterial and antioxidant. Despite the differences, all plants were found valuable as medicinal plants to be comprehended for utilization in health remedy. It is evident that the flavonoid, phenols, alkaloids and saponin contents in the plants evaluates them strongly for the antibacterial activities observed.

This study reveals that tested plant materials have moderate to significant antioxidant activity and antibacterial activity. The result of the present study suggests that selected plants can be used as a source of antioxidants and antibacterial for pharmacological preparations which is very well evidenced by the present work.

The result of this study also supports the view that the flavonoid, phenol, antibacterial and antioxidant activity is dependent on the type of plant part selected and solvent used for extraction.

Recommendations

1. Medicinal plants of different part of Nepal might have bioactivity such as antibacterial, antioxidant, anticancer but unexplored and limited.
2. This study covers the small study area. Thus documentation and test should be broadened from eastern, central and western Nepal.
3. The research work can be further extended by taking several varieties of bacteria, also antifungal, and anticancer property can be tested.
4. Different solvent extracts gives different activity results, so different solvent can be used for extraction and their comparative studies can be done.
5. Moreover, same plant can be collected from different locations and altitudes and comparative studies can be done for the more effective result.
6. The comparative study will obviously make it easy to select the plant from the specific location for further processing of isolated compound as well as synthesis of pharmaceutical drugs.
7. The traditional knowledge is the primary information of national pharmacopoeia so this knowledge should be documented before it is lost.

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Appendices

Appendix I List of the Materials used for the study

Glasswares

Petriplates	Conical flask	Beaker	Glass Rods
Funnel	Measuring cylinder	Test tubes	Culture tubes

Appartus and Equipments

Electric grinder	Refrigerator	Hot air oven	Filter papers
Electric Balance	Incubator	Aluminium foils	Cotton Swabs
Zipper bags	Laminar Air Flow	Cotton Rolls	Forceps
Plant Cutter	Autoclave	Camera	Gloves
Tag Mask	Ratatory evaporator	Parafilm	Needle

Appendix II Preparation of Reagents

1. Preparation of 1M Na_2CO_3 - 100ml

10.599 gram of the Na_2CO_3 (Merk Specialities Pvt. Ltd, Mumbai, India) was carefully weighed and then dissolved in distilled water and the volume was adjusted to 100ml at the end.

2. Preparation of aluminium chloride (10%) - 100ml

10gm of the commercially supplied aluminium chloride (Merk. Specialities. Pvt. Ltd. Mumbai, india) was weighed and dissolved in water. Finally the volume was maintained to 100ml.

3. Preparation of 1M. Potassium acetate (CH_3COOK) - 100ml)

Weighed 9.814gm of the potassium acetate (Merk Specialities Pvt. Ltd., Mumbai, india) and dissolved in water. Finally the volume was maintained to 100ml by addition of water.

4. Preparation of 0.2mM DPPH Solution -100ml

100ml of 0.2mM solution of 1,1, diphenyl- 2 picrylhydrazyl (DPPH) was prepared by weighing 7.886mg of the DPPH and dissolving it in methanol and finally maintaining the volume to 100ml by addition of methanol.

5. Preparation of 0.5 Mcfarland standards - 100ml

100ml of 0.5 Mcfarland standards was prepared by mixing carefully analysed 99.5ml of sulphuric acid (1%) and 0.5ml of Barium chloride (1.175%).

Appendix III Short description of bacteria under study

1. Escherichia coli

Escherichia coli is a gram negative, non spore forming , aerobic or facultative anaerobes, non capsulated rod that is found in the intestine of healthy warm- blooded animals and humans. It was identified in 1955 by Theodor Escherich and is a member of family Enterobacteriaceae. Some of the E. coli strains are Harmless commensals of the intestinal tracts and others that are major pathogens of Human and animals. the pathogenic E. coli is divided into those strains causing disease inside the intestinal tract and others capable of extra intestinal sites (Kaper et al., 2004). The Virulent strain of E. coli causes urinary tract infection, gallbladder and wound infection, appendicitis, Diarrhoea and dysentery and meningitis especially in new borne child (Sausa, 2006).

2. Pseudomonas aeruginosa

Pseudomonas aeruginosa is a member of pseudomonaceae and is polarly flagellated, aerobic, gram negative rod. When grown in suitable media it produces the non fluorescent bluish pigment phycocynin. It is a common environmental organism and can be found in faeces, soil, water and sewage. The main route of infection is by exposure of susceptible tissue, notably wounds and mucous membrane to contaminated water or contamination of surgical instrument. It is a classic opportunist pathogen which innate resistant to many antibiotics and disinfectants. It may cause ear infection and corneal ulceration. This organism is the major cause of burn wound infection (Ratkai, 2011).

3. *Staphylococcus aureus*

Staphylococcus aureus a gram-positive, non-spore forming spherical bacterium that belongs to the *Staphylococcus* genus and usually arranged in grape like irregular clusters. It is wide spread in the environment but is found mainly on the skin and mucous membrane of animals. The growth and survival of *S. aureus* is dependent on a number of environmental factors such as temperature, pH, the presence of oxygen and composition of the food. *S. aureus* is the leading cause of nosocomial infections and is responsible for a wide range of human diseases, including endocarditis, food poisoning, toxic shock syndrome, septicemia, skin infections, soft tissue infections and bone infections, as well as bovine and ovine mastitis (El-jakee et al., 2008).

4. *Bacillus subtilis*

Bacillus subtilis is a gram positive, rod shaped, naturally occurring saprophytic bacterium that is commonly recovered from soil, water, air and decomposing plant material. There are two categories of *Bacillus subtilis* strains. Those that are applied to the foliage of plant, and those applied to the soil or transplant mix when seeding. It causes an array of infection from ear infection to meningitis and urinary tract infection to septicemia (Rudner, 1998).

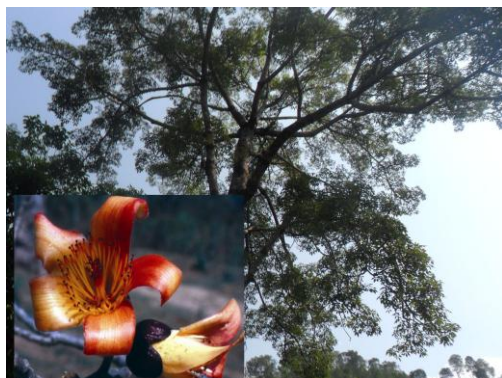
Appendix IV Photographs of plant selected for study



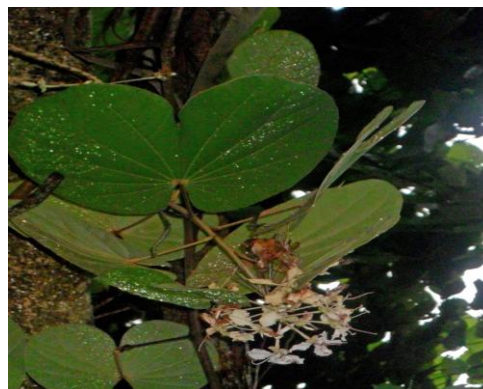
Elephantopus scaber



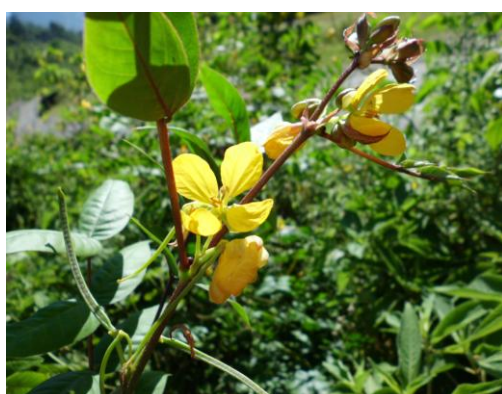
Mangifera indica



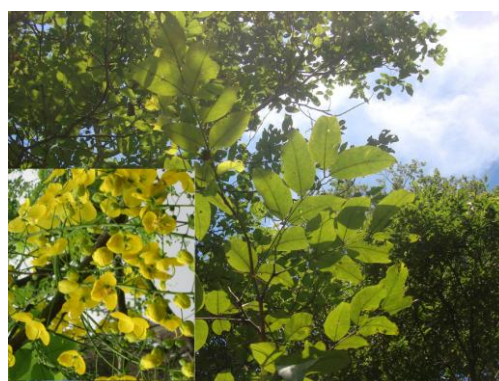
Bombax ceiba



Bauhinia vahlii



Cassia occidentalis



Cassia fistula



Uraria lagopoides



Azadirachta indica



Moringa oleifera



Psidium guajava



Syzygium cumini



Costus speciosus

Appendix V Equipments and lab work



Sonicator



Sample ready for filtration



Rotatory evaporator



Sample ready for Antioxidant

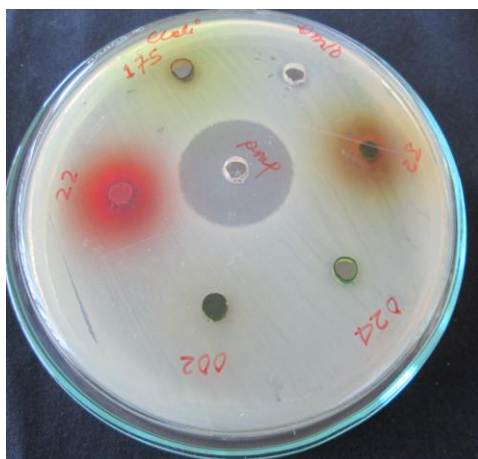


Spectrophotometer

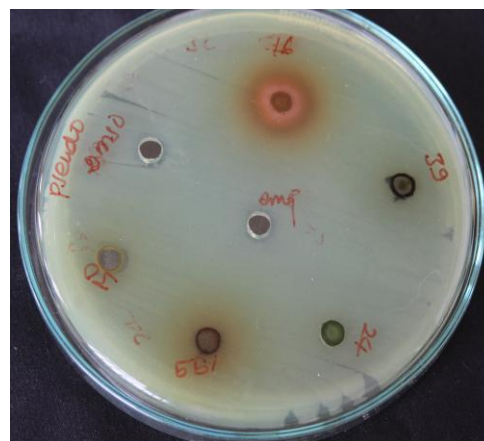


Working on laminar hood

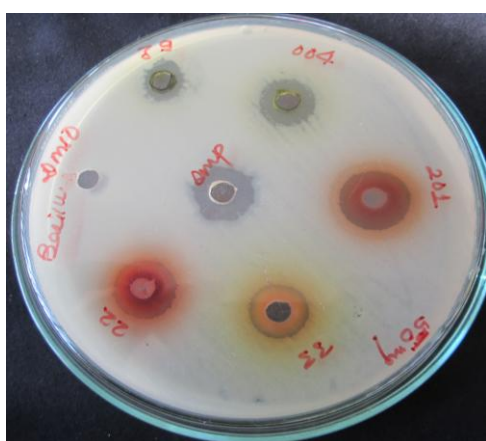
Appendix VI Antibacterial activity screening



Escherichia coli



Pseudomonas aeruginosa



Bacillus subtilis



Staphylococcus aureus

Fig. Antibacterial activity shown by plant samples: *Bombax ceiba* (22), *Uraria lagopoides* (175), *Psidium gaujava* (32), *Costus speciosus* (24), *Azadirachta indica* (39), *Cassia occidentalis* (199), *Moringa oleifera* (30), *Elephantopus scaber* (004), *Cassia fistula* (201), Ampicillin (Amp), Dimethyl sulphoxide (DMSO)