ANTIBACTERIAL AND ANTIOXIDANT PROPERTY OF SELECTED MEDICINAL PLANTS OF DAMAN VDC, MAKAWANPUR DISTRICT

A Dissertation Submitted to the

Central Department of Botany, Tribhuvan University

for the Partial Fulfillment of the Requirements of Masters of Science in Botany

Submitted by

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LETTER OF RECOMMENDATION

This is to certify that the dissertation work entitled **"Antibacterial and Antioxidant property of selected medicinal plants of Daman VDC, Makwanpur District"** submitted by Ms. Usha Adhikari has been carried out under our supervision. To the best of our knowledge, the entire work was based on the results of her fieldwork and lab work and has not been submitted for any other academic degree. Therefore, we recommend this dissertation to be accepted for the partial fulfillment of Masters of Science in Botany with special paper Plant Systematics and Biodiversity from Tribhuvan University, Kathmandu, Nepal.

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LETTER OF APPROVAL

This dissertation paper entitled **"Antibacterial and Antioxidant property of selected medicinal plants of Daman VDC, Makwanpur District"** submitted to the Central Department of Botany, Tribhuvan University by Usha Adhikari has been accepted for the partial fulfillment of requirements of Masters of Science in Botany.

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ABSTRACT

Altogether fifteen plants used to treat stomach troubles like indigestion, diarrhea and dysentery were selected for the study purpose. The medicinal plants were extracted in methanol by Percolation with intermittent Sonication method. *Bergenia ciliata* had the highest yield (20.03%) while *Cautleya spicata* had the lowest yield (2.90%). The methanol extracts of these plants were evaluated for antibacterial activity against medicinally important bacteria viz. *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* and also evaluated for the antioxidant activity. The *in vitro* antibacterial activity was performed by agar well diffusion method.

Among the 15 plants tested, in the present study, all plants except one (*Achyranthes bidentata*) showed activity against at least one bacterium. *Bergenia ciliata, Coriaria napalensis, Potentilla fulgens, Pyracantha crenulata, Pyrus pashia* and *Rubus ellipticus* inhibited all the tested bacteria at 50 µg/ml and 100 µg/ml. *Lindera neesiana* inhibited all the tested bacteria at 100 µg/ml only. *Callicarpa macrophylla* inhibited only 3 of the tested bacteria at 150 µg/ml while *Galium aparine* and *Uraria picta* inhibited Gram +ve bacteria only. *Adiantum philippense, Anemone vitifolia, Campanuma paillida* and *Cautleya spicata* inhibited *Bacillus subtilis* only. Most of the extracts showed activity against Gram +ve bacteria than Gram -ve bacteria.

Highest phenolic content was obtained for *Potentilla fulgens* (90.73 ± 0.71 mg GAE/g) while the lowest was shown by *Achyranthes bidentata* (2.21 ± 0.19 mg GAE/g). Similarly, highest content of flavonoid was obtained for *Adiantum philippense* (21.19 ± 0.28 mg QE/g dry plant) and *Cautleya spicata* (21.19±0.52 mg QE/g dry plant) and lowest for *Bergenia ciliata* (1.78 ± 0.11 mg QE/g dry plant).

Antioxidant activity of the methanolic extract was determined using DPPH method. Maximum IC_{50} value was obtained for *Achyranthes bidentata* i.e. 127.55 and minimum for *Bergenia ciliata* i.e. 40.56. Thus, *Bergenia ciliata* was considered to be the best antioxidant among the sampled plant samples.

LIST OF ABBREBIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
$AlCl_3$	Aluminium chloride
Bijdr.	*Bijdragen tot de Flora van Nederlandsch Indie*
B. subtilis	*Bacillus subtilis*
Bull. Dept. Med. Pl. Nepal	Bulletin of Department of Medicinal Plants of Nepal
CH_3COOH	Potassium acetate
DFO	District Forest Office
DMSO	Dimethyl sulfoxide
DNA	Deoxy-ribo nucleic acid
DPPH	1, 1 – diphenyl - 2 picryhydrazyl
E. coli	*Escherichia coli*
Fig.	Figure
Fl. Brit. Ind.	Flora of British India
Fl. E. Himal	*Flora of Eastern Himalaya*
Fn. Fl. Nep. Himal.	*Fauna and flora of Nepal Himalaya*
Gram –ve	Gram negative
Gram +ve	Gram positive
H_2O_2	Hydrogen peroxide
IC_{50}	Inhibition Concentration fifty
mg $GAEg^{-1}$	milligrams of the gallic acid equivalent per gram of dry mass
mg QEg^{-1}	milligram of quercetin equivalent per gram of the dry mass
MHA	Muller Hinton Agar

MRSA	Methicillin-resistant *S. aureu*s
NA	Nutrient Agar
NB	Nutrient Broth
OH	hydroxide
P. aeruginosa	*Pseudomonas aeruginosa*
ROS	Reactive Oxygen Species
RSA	Radical Scavenging Activity
S. aureus	*Staphylococcus aureus*
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
TUCH	Tribhuvan University Central Herbarium
VDC	Village Development Committee
vs.	versus
WHO	World Health Organization
wt.	Weight
ZOI	Zone of Inhibition

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CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 Ethnobotany

Ethnobotany is the study of the relationship between people and plants and most commonly refer to the study of how people of a particular culture and region make use of indigenous plants (Rajbhandari, 2001). Schultes (1962) defined ethnobotany as the study of relationship that exists between people of primitive societies and their plant environment.

Ethnobotany is a multidisciplinary science, embracing botany, ecology and anthropology. It is of great value to Ethnologist, Archaeologists, Anthropologists, Plant-geographers, Ethnobotanists, Botanists, Linguistics and ultimately to Pharmacologists and Phytochemists (Shah, 1981). The term "ethnobotany" was first coined by an American botanist John W. Harshberger in 1896, as the study of plants used by primitive and aboriginal people. In Nepal, ethnobotanical knowledge is the traditional knowledge acquired by experience and observation, which is communicated mainly verbally that has formed the basis of Nepalese ethnobotany.

The term has been redefined and the field has been much broadened by many workers. Martin (1995), a renowned, ethnobotanist has critically analyzed ethnobotany in his book "Ethnobotany: a methods manual".

Knowing the importance of plants, ethnobotanists started documenting about the various uses of plants. In Nepal, the first documented literature is Chandra Nighatu (Devkota, 1968), a herbal pharmacopoeia of medicinal value of plants. It contains good information on the ethnobotanical and medicinal uses of the Nepalese plants. The manuscript was initiated by the then Prime Minister Bir Shumsher Jung Bahadur Rana in the late 19^{th} century to use and develop traditional medicinal system based mainly on Ayurveda in Nepal but was completed during the regime of Chandra Shumsher. This is a handwritten herbal encyclopedia including about 840 colour

plates; 750 of plants and 90 of animals and over one thousand pages of their explanations (Rajbhandary and Ranjitkar, 2006).

Scientific documentation of plants started when Banerji (1955) published a paper on medicinal and food plants of eastern Nepal. In it, he mentioned 13 species, of which 9 were of medicinal value and 4 were noted for their food value. Singh (1960) recorded some 20 species of food plants, mainly ones sold by villagers in Kathmandu markets. Later on, further studied were done by Pandey (1964), Bhatta (1970), Sacherer (1979), Coburn (1984), Manandhar (1980), etc.

The main aim of ethnobotany is to document the knowledge about plants that had come through generations and use the knowledge for the benefit of the society. The main importance is that it brings to light numerous less known or unknown uses of plants, some of which have potential wider uses (Chaudhary, 1998). Historically, plants used in traditional medicine by the indigenous populations across the world have produced some of the most useful modern day pharmaceuticals.

1.1.2 Medicinal plants

The use of plant resources by humans has a long history. With the origin of mankind on the earth, people have been using plant resources for various purposes like food, fodder, fibre, medicines, condiments, dye and other useful materials. Among the different uses of plants, medicinal plants have served people throughout the world as the means of curing diseases since time immemorial (Chaudhary, 1998; Rajbhandari, 2001; Manandhar, 2002). Some of the oldest archaeological records in both the Old and New Worlds concern the medicinal uses of plants. Medicinal plants of the Indian subcontinent, including the Himalaya, have been explored since the Vedic period and compiled in different Ayurvedic texts dating back to the Vedic ages (ca. 2500 and 500 B.C.) (Ghimire *et al.*, 2008).

According to World Health Organization (WHO) report (2002), 70% of the world population use medicinal plants for curing diseases through their traditional practitioners. In Nepal, 75% of the population, especially in rural areas is getting health care by traditional practitioners, who prescribe herbal preparations (Hamayun *et al.*, 2006). Nepal, being an excellent repository of cultural heritage for diverse ethnic groups, has a long tradition of folk practices for utilization of wild plants

especially as medicinal plants (Manandhar, 2002). These ethnic groups use about 23% of the flowering plants for their medicinal properties (Shrestha *et al.*, 2000).

Most Nepalese live in very remote rural areas, far from the nearest health post or hospital. There is a shortage of medicines and health professionals, even those with very basic training. As a result, the rural people of Nepal continue to depend on local therapy for their health care, which is cheap, convenient and readily available. Furthermore, the villagers are acquainted with or related to the local healer. The healer's fee can be paid either in cash or crops and villagers may pay whenever they have the cash or crop (Manandhar, 2002).

1.1.3 Plants as a source of antimicrobials

Medicinal plants represent a rich source of antimicrobial agents (Abi Beaulah *et al.*, 2011). Medicinal plants are boon of nature to cure number of ailments of human beings. Practitioner of Ayurveda and Unani system of medicine regularly employ a large number of medicinal plants as antibiotic agents. In many parts of the world, medicinal plants were used against bacterial, fungal and viral infections (Perumal *et al.*, 2004). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structure and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious disease.

Another big concern is the development of resistance to antibiotic in current clinical use. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. The drug resistance bacterial and fungal pathogens have further complicated the treatment of infectious diseases in AIDS and cancer patients. Many efforts have been made to discover the new antimicrobial compounds from several of sources such as microorganisms, animals and plants. One of such sources is folk medicine. Systematic screening of such folk medicine may result in the discovery of novel effective compounds (Tomoko *et al.*, 2002). In contrary to the synthetic drugs, antimicrobial effects of plants origin is not associated with side effects and have massive therapeutic potential to heal many infectious diseases. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of phytomedicine to act against microbes (Iwu *et al.*, 1999).

Plants with possible antimicrobial effect should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The antimicrobial properties of plants have been investigated by a number of researchers' worldwide (Abi Beaulah *et al.*, 2011). But very limited work has been done in this field in Nepal (Panthi and Chaudhary, 2006). Among them, most work is related to ethnomedicinal plants.

Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities (Hamburger, 1991). The antimicrobial compounds produced by plants are active against plants and human pathogenic microorganisms (Mitscher, 1987). The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. It is expected that plants extracts showing target sites other than those used by antibiotics will be active against drug resistance microbial pathogens (Palombo, 2001). However, very little information is available on such activity of medicinal plants.

1.1.4 Plant as a source of antioxidants

Plants have an extraordinary ability to synthesize aromatic compounds which are usually phenols or their oxygen-substituted derivatives. The medicinally active plant compounds are usually their secondary metabolites like terpenoids, quinones, flavonoids, tannins, etc. that are responsible for protecting the plants from microorganisms, insects and other natural pests. In the recent past, there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally (Jain *et al*., 2006; Zafar, 2009; Hasan, 2014).

Biological and chemical research in Life Sciences evidenced that free radical and reactive oxygen species can be involved in a high number of diseases (Jain and Agarwal, 2008). Numerous physiological and biological processes in the human body may produce oxygen centered free radical and other reactive oxygen species and by-products. Overproduction of such free radical cause oxidative damage to biochemical leading to many chronic diseases (Erdemoglu, *et al*., 2006). Plants are important source for free radical scavenging molecules. Intake of natural antioxidant has been

associated with reduced risk of cancer, cardiovascular diseases, diabetes and other diseases associated ageing. Antioxidant is one of the most essential ingredient of today's menu/therapy because the antioxidant system protects the animal against reactive oxygen species (H_2O_2, superoxide, OH, singlet oxygen and nitrogen species) induced oxidative damage. Various synthetic antioxidants (BHT) are on the use, but they are suspected to be carcinogenic (Halliwell, 1994).

1.2 Rationale

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their uses in traditional medicine (Kumar *et al.*, 2012). Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct (Kumar *et al.*, 2008). Therefore, it is worthwhile to use modern science and technology tools for verifying therapeutic potential of medicinal plants as antioxidant as per international standards. Such information may be of potential value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals-induced tissue damage (Abi Beaulah *et al.*, 2011). Historically, plants used in traditional medicine by the indigenous populations across the world have produced some of the most useful modern day pharmaceuticals. The use of medicinal plants at home is not only cheaper from economic point of view, but also responsible for fewer side effects than that of chemical antibiotics.

1.3 Objectives

The major objective of the present research is the documentation of traditional knowledge of selected plants and to perform their antibacterial assay and quantitative analysis.

The specific objectives of this study are:

- To document the ethnobotanical uses of selected plants.
- To evaluate their antimicrobial and antioxidant property.
- To carry out quantitative estimation i.e. phenolic and flavonoid content estimation.
- To analyze the relationship between the biological activity and quantity of phytochemicals present.

1.4 Limitations

- Preliminary phytochemical screening of chemicals could not be performed.
- Minimum Inhibitory Concentration was not done.
- Only methanol extracts were used for the biological screening.
- Only Percolation with intermittent Sonication method was used for extraction.

CHAPTER 2

LITERATURE REVIEW

2.1 Ethnobotanical uses

Rajbhandari (2001) compiled 562 species of plants with introduction, historical background and ethnobotanical uses as used by various ethnic communities or tribes of Nepal in which all the selected plants are listed as ethnobotanically important except *Pyracantha crenulata* and *Galium aparine.*

Manandhar (2002) described more than 1500 species of plants from all 75 districts of Nepal with their introduction, uses and illustration describing all the selected plants as ethnobotanically important in local community.

Shah (2006) reported the ethnobotanical uses of *Achyranthes bidentata, Pyrus pashia* and *Rubus ellipticus* from Chhampi area of Lalitpur.

Dutta (2007) described the identification, classification, ethnic uses and cultivation of 141 plants in which *Achyranthes bidentata, Bergenia ciliata, Lindera neesiana, Potentilla fulgens, Pyrus pashia* and *Rubus ellipticus* has been reported.

Ghimire *et al.* (2008) reported the description, ecology and use values of *Bergenia ciliata, Lindera neesiana* and *Rubus ellipticus.*

Majupuria (2009) mentioned *Achyranthes bidentata* and *Rubus ellipticus* as religious and useful plants of Nepal and India among the 196 religious and medicinally important plants.

Malla and Chhetri (2009) while studying the indigenous knowledge of ethno-botanical plants of Kavrepalanchowk District reported that *Achyranthes bidentata* and *Bergenia ciliata* were locally used as medicine.

Pangeni (2009) also reported the ethnobotanical uses of *Anemone vitifolia, Bergenia ciliata* and *Rubus ellipticus* from Magar Community of Palpa District.

Poudel *et al.* (2010) reported the ethnomedicinal uses of *Pyrus pashia* and *Rubus ellipticus* from Argha VDC, Argakhanchi.

Thapa (2012) recorded the use of 75 species of medicinal plants belonging to 46 families and 72 genera for the treatment of 39 different ailments from Parbat in which *Achyranthes bidentata, Bergenia ciliata, Lindera neesiana, Potentilla fulgens* and *Rubus ellipticus* were mentioned as medicinally important.

Hasan *et al.* (2013) reported the medicinal uses of 76 species of Daman which included *Achyranthes bidentata, Bergenia ciliata* and *Rubus ellipticus*.

Prajapati (2013) listed 30 economically important pteridophytes from Daman and adjoining areas, out of which 5 were used for food value, 2 for fodder, 12 species had medicinal value and 3 had ornamental value. *Pteridium revolutum, Pteris wallichiana, Nephrolepis auricualata* and *Gleichenia gigantean* are preferred for preventing landslides. Rhizomes of *Drynaria mollis* and *Oleandra wallichii* is another major source of income for local people as huge amount of rhizome of the two species is exploited from the community forest and exported to different places.

Malla *et al.* (2014) collected the information of 61 plant species belonging to 59 genera and 43 families with their local name, parts used and ethnobotanical uses as prescribed by ethnic tribes Gurung, Magar and Majhi of Parbat district in which *Achyranthes bidentata, Lindera neesiana* and *Rubus ellipticus* were also included.

Shrestha and Shrestha (2061 B.S.) reported *Bergenia ciliata* and *Lindera neesiana* as the major Non-Timber Forest Products of Nepal with complete description including their ecology, phenology, use methods practiced in local community.

2.2 Antimicrobial Screening

Aqueous ethanolic extract of *Achyranthes bidentata* was subjected to *in vitro* antibacterial assay against human pathogenic *Escherichia coli, Salmonella typhi, Salmonella paratyphi, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* employing cup diffusion method. But, *Achyranthes bidentata* was found to be ineffective against all the tested organisms (Joshi *et al.*, 2011).

All the extracts of root, stem, leaves and flowers showed high sensitive to *Proteus, Escherichia coli, Bacillus subtilis, Salmonella typhi, Klebsiella pneumoniae*, moderate and less sensitivity to *Staphylococcus aureus* and *Pseudomonas* species (Devi *et al.*,

2007). Also, the plant has been tested for anticancer activity and was found to have significant anticancer activity at 200 µg/ml (Kota *et al.*, 2012).

All the ethanolic root extracts of *Anemone vitifolia* were found to possess antihelmintic activity when tested at different concentrations ranging from 10-100 mg/ml when tested with *Eudrilis euginea* (Sati *et al.*, 2011).

Essential oil of *Coriaria napalensis* is known to possess antimicrobial activity. Thus, essential oil could be a promising drug after improved formulation (Ahmad *et al.*, 2011).

Aqueous stem extract of *Callicarpa macrophylla* did not show any activity against the tested bacteria except *Salmonella typhimurium* at 200 µg/ml while the ethanolic stem extract showed moderate activity at 200 µg/ml (Yadav *et al.*, 2012).

Methanolic extract of the whole plant of *Adiantum philippense* showed maximum level of activity against *Pseudomonas aeruginosa* when tested against skin infections causing bacteria viz. *Staphylococcus aureus* subsp. *aureus, E. coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae* subsp. *pneumoniae* and *Serratia marcescens*. Acetone extract of the plant showed moderate antibacterial activity against *Staphylococcus aureus*. Petroleum ether and water extracts did not show any antibacterial activity against any of the tested organisms. None of the extracts showed any activity against *E. coli* (Thomas, 2013).

Joshi (2013) performed the antibacterial activity on aqueous and methanolic extract of selected medicinal plants including *Bergenia ciliata*. Both aqueous and methanolic extract showed positive result against *Salmonella typhii and Streptococcus pneumonia* but only aqueous extract showed activity against *Shigella flexneri* while only methanolic extract showed positive activity against *Klebsiella pneumonia* and *Vibrio cholera*.

Methanol extract showed significant antibacterial activity against Gram-positive and negative strains, as well as a strong antifungal activity. The maximum inhibition zone is found against *Staphylococcus aureu*s, *Micrococcus luteus* and methicillin-resistant *S. aureu*s (MRSA) (22, 17 and 18 mm) respectively. Against fungus, the inhibition zone ranged between 19 mm and 23 mm. Methanolic extract showed minimum inhibitory concentration value with 32.5 µg/ml against *S. aureu*s, 62.55 µg/ml against

MRSA, *Aspergillus flavus, Trichophyton mentagrophytes, Trichophyton rubrum* and 15 µg/ml against *Aspergillus niger* (Latha *et al.*, 2015).

2.3 Phytochemical Analysis

Qualitative phytochemical analysis of *Achyranthes bidentata* showed the presence of alkaloids, glycosides, terpenoids, steroids and reducing sugar (Joshi *et al.*, 2011).

Preliminary phytochemical analysis of ethanolic extracts of different parts viz. root, stem, leaf and flower showed the presence of alkaloids, flavonoids, protein, phenols and steroids while carbohydrates was present in all except in leaf extracts. Thiols were present in root and stem extracts only while tannins were present only in root extracts. Saponins, Glycosides and resins were absent in all extracts. Ethanolic extracts of *Achyranthes bidentata* root and flowers possessed significant *in vitro* lipid peroxidation inhibiting activities, which is possibly attributed to its free radical scavenging properties (Devi *et al.*, 2007).

The phenolic levels of *Rubus ellipticus* ranges from 21 to 225 mg/g of gallic acid depending upon the solvent used for extraction with highest level in methanolic extract and lowest in peteroleum ether extract. The flavonoid content ranged from 16 to 29 mg/g of rutin, highest content in methanol extract and lowest in chloroform extract (Vadivelan *et al.*, 2009).

Qualitative phytochemical analysis of bark of *Pyrus pashia* revealed the presence of flavonoids, phenolics, steroids, saponins, carbohydrates and tannins (Arya *et al.*, 2011).

N-hexane extract of *Bergenia ciliata* showed the presence of steroids only; chloroform extract showed the presence of steroids, terpenoids, anthraquinone, tannins and saponins; water extract showed the presence of steroids, terpenoids, flavonoids, anthraquinones, tannins and saponins while methanolic extract showed the presence of terpenoids, flavonoids, tannins and saponins but absence of alkaloids, phlobatanins, glycoside and reducing sugars (Uddin *et al.*, 2012).

Preliminary phytochemical screening of *Callicarpa macrophylla* revealed the presence of carbohydrates, steroids, saponins, flavonoids, glycosides and tannins, but absence of alkaloids, proteins and amino acids (Kumar *et al.*, 2012).

Phytochemical screening of *Adiantum philippense* showed the presence of phenolics, triterpenoids and flavonoids (Thomas, 2013).

The phenolic content of *Rubus ellipticus* was found to be 357.2 ± 3.6 mg of gallic acid equivalent/litre of wine and the flavonoid content was found to be 33.9 ± 0.40 mg of quercetin equivalent/ Litre of wine (Rana and Singh, 2013).

The phenolic content of bark of *Pyrus pashia* was found to be 162 ± 2.7 mg of gallic acid equivalent/litre of wine and the flavonoid content was found to be 15.9 ± 0.65 mg of quercetin equivalent/ Litre of wine. The herbal wines prepared from the fruits have poor market value but the wines showed promising antioxidant activity, total phenolic content and total flavonoid content (Rana and Singh, 2013).

Phytochemical screening of leaf, stem and root extracts of *Uraria picta* showed the presence of alkaloids, flavonoids, steroids, terpenoids, phenols and saponins in all parts; absence of tannins in stem and roots while absence of glycosides in roots (Saxena *et al*., 2014).

The total phenolic content was found to be 7.43 ± 0.371 mg/g extract (Pal *et al.*, 2013). Preliminary phytochemical screening of leaves and fruits of *Pyracantha crenulata* showed the presence of saponins, tannins, flavonoids, glycosides, carbohydrates, steroids, polyphenols, resin, but absence of alkaloids (Saklani and Chandra, 2014).

Preliminary phytochemical studies of aqueous extract of leaves, ethanolic extract of rhizome and butanol-hexane extract of root of *Bergenia ciliata* showed the presence of alkaloids, carbohydrates, cardiac glycosides, saponins, phenols, flavonoids, diterpenes and absence of phytosterols and proteins (Pokharel *et al.*, 2014).

Aerial part of *Galium aparine* contains anthraquinones, iridoids, alkanes, flavonoids, tannins, polyphenolic acids and vitamin C. Total polyphenolic content was found to be 2.40 ± 0.24 g GAE/100 g dry mass while flavonoid content was found to be 1.60 ± 0.53 g RE/100 g dry mass (Vlase *et al.*, 2014).

The phytochemical tests revealed the presence of phytocontituents significantly in methanol extract with high TPC and TFC (Latha *et al.*, 2015).

2.4 Antioxidant Activity

The strongest radical scavenging activity was found in methanol extract i.e IC_{50} value 12.2 ± 0.90 µg/ml while lowest radical scavenging activity in n-butanol extract i.e. IC_{50} value 49.5 ± 0.45 µg/ml against DPPH radical when compared the different antioxidant values in *Rubus ellipticus* (Vadivelan *et al.*, 2009).

Priya *et al.* (2010) assessed antioxidant activity of stem of *Achyranthes aspera* using DPPH radical scavenging assay. When compared between the methanolic and aqueous extract, high antioxidant potential was obtained for methanolic extract.

Free radical scavenging property of ethanolic extract was studied using different *in vitro* models viz. DPPH free radical scavenging assay, ABTS radical scavenging assay, 0-phenanthroline assay, lipid peroxidation assay, superoxide scavenging assay, total antioxidant and non-enzymatic haemoglobin glycosylation assay. The antioxidant activity was estimated by IC_{50} value. Significant antioxidant activity was concluded to be associated with presence of phenolic, flavonoid, sterol and terpene derivatives (Patel *et al*., 2011).

Antioxidant activity of selected medicinal plants of Nepal was determined by using DPPH free radical scavenging assay. The result showed that the IC_{50} value of selected plants were almost similar to that of ascorbic acid (Parajuli *et al*., 2012).

Kumar *et al.* (2012) evaluated the antioxidant activity of *Achyranthes aspera* using DPPH, OH radical scavenging and reducing power assays. The study found out that the plant had notable antioxidant activity which may be influenced by variations in plant type and growth, climate, season, temperature and soil conditions.

The n-hexane, chloroform and water extracts of the *Bergenia ciliata* showed moderated antioxidant property (Uddin *et al.*, 2012).

Machilus odoratissima was tested for its antioxidant and antibacterial activity. The methanolic extract exhibited high free radical scavenging activity when the experiment was carried out using scavenging activity of DPPH radical method (Subedi *et al*., 2012).

Antioxidant activity of methanolic extract of Swertia chirayita, determined by DPPH free radical scavenging assay, showed that the samples collected from different parts of Nepal showed significant antioxidant property (Bhattarai, 2014).

DPPH free radical scavenging assay of methanolic extract of *Bauhinia variegata* barks revealed the high antioxidant capacity compared to the standard ascorbic acid used (Sharma *et al.,* 2015).

Antioxidant activities of methanolic, hexane and aqueous extracts of leaves of *Adiantum caudatum* were analysed using reducing power, FRAP (Ferric Reducing Antioxidant Power), Phosphomolybdate and ABTS assays. The value obtained were in the order of methanolic > aqueous > hexanic (Ahmed *et al*., 2015).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

Daman VDC was selected for the study purpose. Daman VDC is located in Makawanpur district, Narayani zone of Central Development Region of Nepal. It lies in the north side of the district, perched on a hillock and one of the most beautiful village inhabitated by Chhetri (44%) and Tamang (39%) tribes with almost 83% of the total population of the VDC. Daman village is situated at an altitude of 2320 m and 100 km southwest of Kathmandu. The area of the VDC is 43.63 sq. km. The village provides a grand view of the Himalayas with Mount Everest in the east (Daman VDC office). The climate of the area is variable from tropical to temperate and remains rather cool with sometimes snowfall during the winter season.

Administratively, Daman VDC consists of nine wards. The total population of Daman VDC is 7053 which includes 3615 males (52%) and 3438 females (48%). The total number of households in the VDC is 1303 among which 38 households are landless. The average household size is 5.4. The literacy rate of Daman VDC is 38%. The main occupations of the villagers are agriculture (90%), 4% have private business and 3% are service holder (Daman VDC office).

Vegetation

Most (67%) of the area of Daman VDC is covered by forest which consists of tropical to temperate in nature. There are mainly three specific forest areas viz. community forest, national forest and private forest. Major forest types found in Makawanpur district are Sal forest, Terai hardwoods forest, Chirpine forest, Upper mountain hardwood, *Quercus* forest and Riverine forests.

According to altitudinal and climatic variations, mixed forest, both evergreen and coniferous as well as bushes and shrubs are found in the study area. The mixed forest mainly consists of *Alnus nepalensis* and *Pinus* species (Basnet, 2007). In the high hills and mountains areas, the predominant vegetation comprises mostly of grasses and valuable medicinal plants i.e. *Swertia*

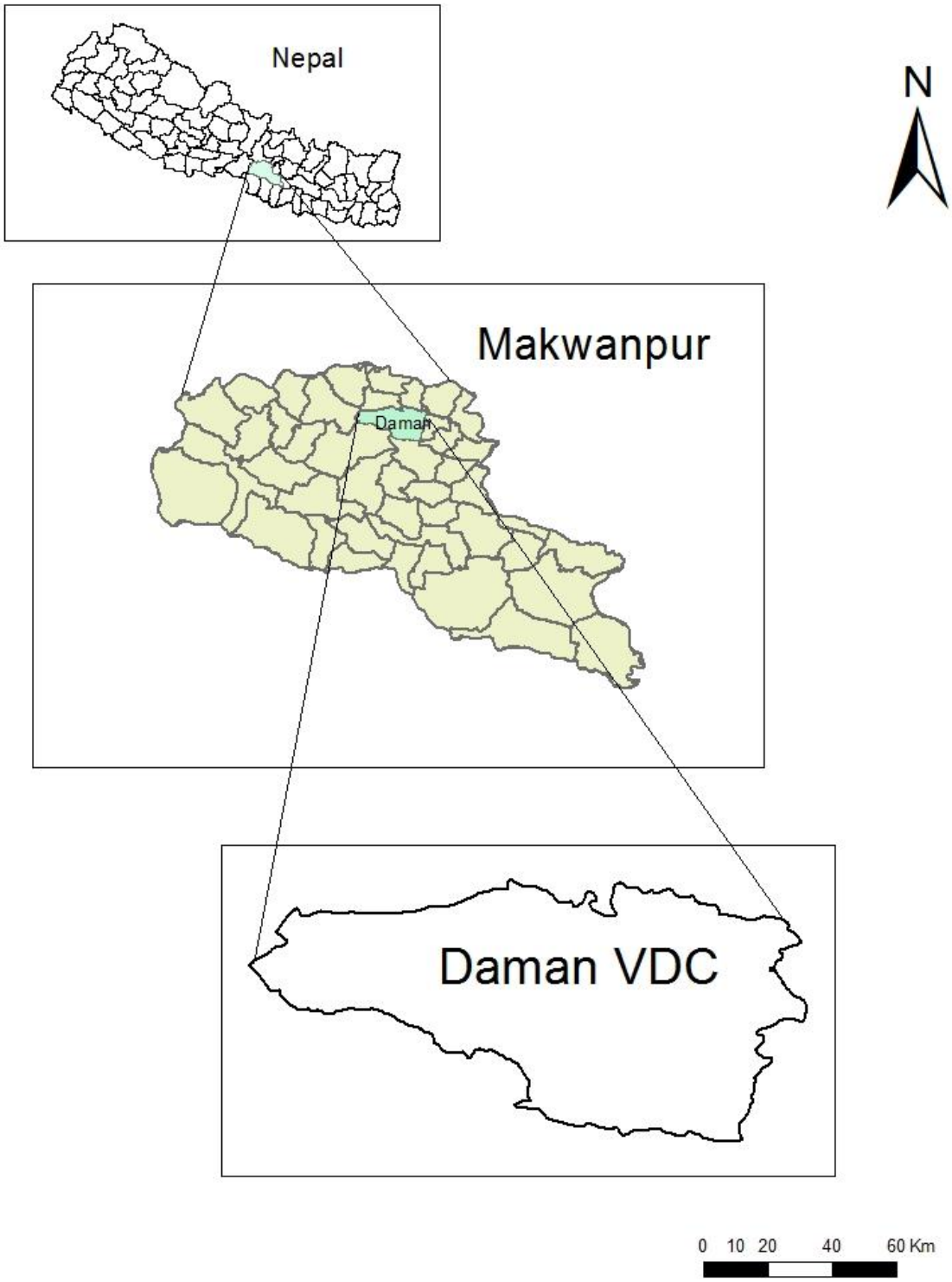


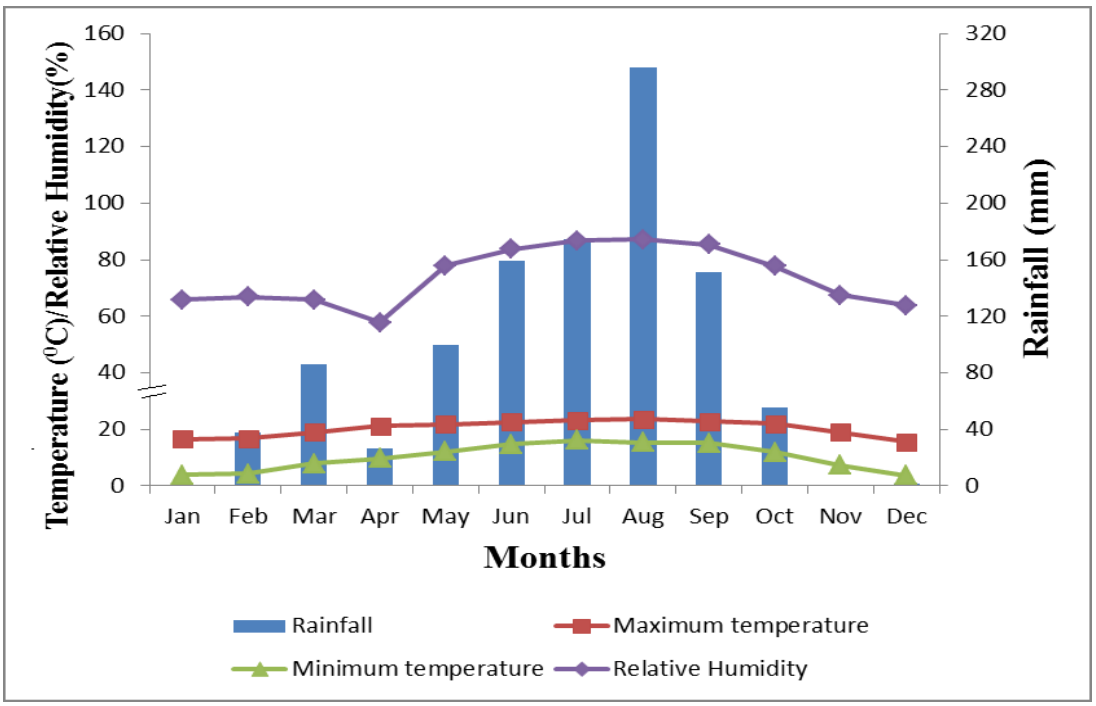
Fig.1 Map of Study Area

chiraita, Rubia manjith, Valeriana jatmansii, Taxus baccata, Bergenia ciliata, Astilbe rivularis, Acorus calamus, Nardostachys grandiflora, Zanthoxylum armatum, Berberis asiatica, Paris polyphylla, Aconitum sp., Parmelia sp., etc.

Climate and Seasons

Climatic variation in the district ranges from tropical to temperate. The northern side of the Mahabharat range is predominated by the temperate climate. Makawanpur district can be broadly divided into three seasons: cold, hot and rainy season. Cold season exists between December and February. The temperature in cold season falls down to 0.6^0C (avg) but in the up hills, particularly Daman and Simbhanjyang area, snowfall occurs. Hot season exist between March to June with the average temperature of 32.9^0C (DFO, 2003).

Fig. 2: Monthly Average of Maximium and Minimum Temperature, Rainfall and Humidity of Daman for the period of 2006-2010



Source: Department of Hydrology and Meteorology, 2015

Monsoon remains active, generally from June to September and is rainy during these months. With the approach of November, the temperature starts to fall and the rain is also stopped. Average rainfall is 1908.6 mm in Mahabharat range. Average humidity of the district is 73.5% (DFO, 2003).

3.2 Ethnobotanical Study

The present study has been carried out to document ethnobotanical knowledge and to evaluate antibacterial properties, quantitative analysis and antioxidant property of selected medicinal plants that are used to treat stomachaches, indigestion, diarrhoea and dysentery. Primary data regarding the use of plants were collected during fieldwork in the study area. The data collected from the field was further validated using secondary literatures. For ethnobotanical information and sample collection, following procedures were followed.

3.2.1 Field Visit

The field visit was conducted in 2013 and the research area was visited twice during the research period. The valuable information and plant samples were collected with the help of local people who had knowledge of medicinal plants and their uses. The people were kind and cooperative and the information was collected by group discussion.

3.2.2 Sample Collection, Herbarium Preparation and Identification

The information and voucher specimens were collected with the help of local people. The collected plant specimens were presented to interviewees to confirm their identity in terms of vernacular name and uses. The samples collected for laboratory testing were cleaned, in case of rhizome and root, and were chopped into small pieces. Then, the chopped pieces were allowed to dry in air-shaded area which after complete drying was collected in cotton bags for grinding.

For the herbarium preparation, the collected specimens were kept into a large polythene bag to keep specimens fresh. The collected species were tagged and the details were recorded accordingly. Blotters, newspapers, plant press was used to dry the plant specimens. The plant specimens were dried on the same day of collection. The specimens were dried by simple drying process just by properly pressing, regularly checking the specimens during drying and changing paper to obtain neat and clean herbarium. Once the specimens were dried, as described by Lawrence (1951), herbarium was prepared accordingly by attaching dried plant specimen to the herbarium sheet of standard size by pasting. The herbarium was also labelled with

complete details such as date of collection, tag number, scientific name, local name, family, locality, altitude, name of the collector and additional information. The voucher specimens will be deposited at Tribhuvan University Central Herbarium (TUCH), Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu.

Some plant species were identified in the field by team members. The voucher specimens were further identified and confirmed with the help of experts and standard literatures like *Flora of Kathmandu Valley, Flora of Langtang and Cross Section Vegetation Surv*ey (Central Zone), *Flora of Phulchoki and Godawari, Enumeration of the Vascular Plants of West Nepal* and *Flora of British India*. The distribution of the plants was noted with the help of *Annotated Checklist of Flowering Plants of Nepal* (Press *et al*., 2000).

3.3 Laboratory Analysis

The major equipment and materials used for laboratory testing are provided in Appendix-II. The test organisms used are *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis*. Their morphology, biochemical characters and pathogenicity are given in Appendix-I. They were received from Institute of Medicine, Maharajgunj and Central Department of Biotechnology, Tribhuvan University.

3.3.1 Extraction

The shade dried plant materials were crushed into powder in an electric grinder and subjected to extraction. The extraction technique followed was Percolation with intermittent Sonication. For this, 20 g of the dried plant materials were taken in glass bottles with lid, separately. Into the glass bottles, the methanol was poured in such a way that the ratio of the gram weight of the plant material to the volume of the solvent in ml would be 1:8. The solution was subjected to Sonication for one and a half hour each day for 3 days. Then, it was allowed to settle for a day. On the following day, the solution was filtered and the residue was collected for 2nd round of Sonication.

Methanolic extract was *in vacuo* concentrated at reduced pressure using rotatory vacuum evaporator and the solvent thus recovered was again poured in the vessel containing residue for second round of extraction. The condensed extract thus

obtained was transferred to clean, dry and weighed petri plates and allowed to dry in incubator at 37^0C. The petri plates were left for 3 days in incubator. On 4^{th} day, the extract was scratched using clean blades and kept in cryovials. The percentage yield of the extract thus obtained was calculated by using the formula:

$$Percentage\ yield(\%) = \left[\frac{Dry\ wt.of\ extract}{Dry\ wt.of\ plant\ material}\right] \times 100$$

The resulting dry extracts were then sealed and stored at 4^0C until use.

3.3.2 Extract Dilution

25 mg of crude extract of each sample was weighed and dissolved in 1 ml methanol. Thus prepared 25 mg/ml stock solution was used for quantification of the total phenolic and total flavonoids and also to quantify antioxidant activity.

150 mg of the crude extract of each sample was weighed and dissolved in 1 ml of Dimethyl sulfoxide (DMSO). This stock solution was used for antimicrobial screening and was stored at 4^0C until use.

3.3.3 Antimicrobial activity

3.3.3.1 Preparation of Culture media

Nutrient agar (NA)

About 28 grams of the powder (HiMedia Laboratories Pvt. Ltd., Mumbai, India) was carefully weighed and poured in conical flask containing distilled water. The contents were dissolved in water completely and the final volume was maintained to 1000 ml followed by boiling for uniform mixing. This media was sterilized in an autoclave at 15 lbs pressure at 121^0C for 15 minutes. The autoclave tape was used as an indicator for the completeness of sterilization. After this, the media was taken out of autoclave and cooled to about 45-50^0C and poured on sterilized and properly labelled Petri dishes. About 20 ml of the media was poured on each Petri dish of 9 cm diameter. The plates were then left for the solidification. The pouring process was carried out on the sterile cabinet.

Nutrient Broth (NB)

About 13 grams of NB powder (HiMedia Laboratories Pvt. Ltd., Mumbai, India) was carefully weighed and transferred to a conical flask. The content was dissolved in distilled water and final volume was maintained to 1000 ml. This media was transferred to the culture bottles and sterilized by autoclaving at 15 lbs pressure and 121^0C for 15 minutes. Autoclave tape was used for the indication of the completeness of the sterilization. Finally, this media was cooled in laminar air flow and was used for the suspension type of bacterial culture.

Muller Hinton Agar (MHA)

38 grams of MHA powder (HiMedia Laboratories Pvt. Ltd., Mumbai, India) was weighed and suspended in distilled water. The final volume was maintained to 1000 ml. The content was heated to boiling to dissolve the medium completely. The media was sterilized by autoclaving at 15 lbs pressure at 121^0C for 15 minutes. The media was allowed to cool to 40-50^0C and was mixed carefully before pouring. The media was poured on sterile Petri dishes under aseptic conditions for further purposes.

3.3.3.2 Preparation of the standard culture inoculums

The individual pure culture of bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* were streaked on the different nutrient agar plates. Those plates were incubated on the incubator at 37^0C for about 24 hours and pure and isolated colonies were obtained. Each distinct colony was aseptically transferred to the Nutrient Broth for the suspension culture with the help of the sterilized inoculating loop. The inoculated bottles were kept on the incubator at 37^0C for overnight. The turbidity of the bacterial suspension was adjusted at the 0.5 McFarland standards for the antibacterial test. These inoculums were used for the swapping of the plates to test the antibacterial effects of the plant extracts.

3.3.3.3 Transfer of the bacteria on the Petri dishes

The test plates for the antimicrobial activity were first labelled with date, name of bacteria, name of plant samples and the concentration of the plant sample to be added. The MHA plates were inoculated with the appropriate bacterial culture by a sterile

cotton swab. Seperate swab was used for each bacterium. The culture plates were allowed to dry for about 30 minutes.

3.3.4 Antibacterial Screening

The antibacterial test of the crude extracts was studied by Agar well diffusion technique (Abi Beaulah *et al*., 2011). In this method, fixed volume of the extract solutions are placed on equal sized wells bored on the pre-set agar plates along with the positive and negative controls. Then, the zone of inhibition marked as halozone was measured. The diameter of this clear zone shows the antibacterial efficacy of the extracts tested. This is the qualitative method of testing anti-bacterial efficacy.

Seven wells were prepared on the solid MHA media with the help of the sterile cork borer of 4 mm diameter. Different concentrations of the plant extracts were prepared in the DMSO. With the help of the sterile pipette, 20 µl of the each individual plant extract were poured in the above prepared well. Pure DMSO was taken as negative control while ampicillin of 10 mg/ml concentration was taken as the positive control. The plates were incubated on the microbial incubator overnight at 37^0C and the zone of inhibition was observed and noted for individual plant extract of individual bacteria for different concentration for further analysis. All the experiments were performed in triplicate.

3.3.5 Quantitative phytochemical analysis

3.3.5.1 Total phenolic content determination

The total phenolic content of the samples were determined using the Folin-Ciocalteu phenol reagent (Ainsworth and Gillespie, 2007) with slight modification. 0.1 ml of each plant extract (2.5 mg/ml) was separately mixed with the 1 ml of Folin-Ciocalteu phenol reagent (1:10 dilution with the distilled water) and was shaken properly. After 2-3 minutes, 0.8 ml of aqueous 1M Na_2CO_3 solution was added. Then, the reaction mixture was allowed to stand for about 15 minutes and the absorbance of the reactants were measured at 765 nm using the UV-visible spectrophotometer (Thermo Fisher Scientific, Genesystem-10-5). The calibration curve was obtained using the solution of Gallic acid as standard in methanol and water (50:50 v/v) using the concentration ranging from 25-250 µg/ml. Based on this standard graph, the concentration of each

samples were calculated. The total phenol content was expressed in terms of the milligrams of the Gallic acid equivalent per gram of dry mass (mg GAE/g). For each sample, three replicates were performed for the reproducibility of results.

3.3.5.2 Total flavonoid content determination

The total flavonoid content in the plant extracts were estimated using the Aluminium Chloride ($AlCl_3$) colorimetric method (Chang *et al*., 2002; Roy *et al*., 2011) with slight modification. 0.25 ml of extract (10 mg/ml) was separately mixed with the 0.75 ml of Methanol, 0.05 ml of the 10% Aluminium chloride, 0.05 ml of the 1M Potassium acetate (CH_3COOH) and 1.4 ml of the distilled water. The absorbance of the mixture was measured at 415 nm using the UV-visible spectrophotometer (Thermo Fisher Scientific, Genesystem-10-5). The calibration curve was obtained with the help of the quercetin standard solutions in methanol with the concentration ranging from the 10-100 µg/ml. The total flavonoid content was expressed in terms of the milligram of quercetin equivalent per gram of the dry mass (mg QE/g). For each experiment, three replicates were used for the accuracy and reproducibility of results.

3.3.6 Antioxidant activity

3.3.6.1 Preparation of the 0.2 mM DPPH solution

1, 1-diphenyl-2 picryhydrazyl (DPPH) has the molecular weight of 394.32 g/mol. Thus, 100 ml of 0.2 mM solution of DPPH was prepared weighing the 7.886 mg of the DPPH and dissolving it in methanol and finally maintaining the volume to 100 ml.

3.3.6.2 Measurement of DPPH free radical scavenging activity

The antioxidant activity of extracts of different plant samples and standard (Ascorbic acid) was assessed on the basis of the free radical scavenging effect of the stable DPPH- free radical scavenging activity following the protocol of Singh *et al.* (2002) with slight modification. Different concentration of plant extract (30-120 µg/ml) and ascorbic acid (30-420 µg/ml) were prepared in methanol on the clean and dry test tubes. 0.5 ml of the sample volumes was taken. To this sample, 0.5 ml of the 0.2 mM DPPH solution was added. The tubes were shaken vigorously for the uniform mixing. These tubes were allowed to stand for half an hour in dark. The control was prepared

as above but without the plant extract or ascorbic acid. The absorbance of the mixture was taken on spectrophotometer at 517 nm.

$$\%\ radical\ scavenging\ activity = \left[\frac{Control\ abs - Sample\ abs}{Control\ abs}\right] \times 100$$

Standard graph was plotted taking the concentration on the X-axis and percentage scavenging activity on the Y-axis. Based on this graph, IC_{50} value of each plant extract was calculated based on the formula:

$$IC_{50} = EXP\left[LN(Conc.> 50\%) - \frac{pi > 50\% - 50}{pi > 50\% - pi < 50\%} \times LN\frac{conc.> 50\%}{conc.< 50\%}\right]$$

EXP: Exponential function; LN: Natural log function; pi > 50%: RSA value just above 50%; pi < 50%: RSA value just below 50%; Conc.> 50%: Concentration of extracts > 50%; Conc.< 50%: Concentration of extracts < 50%.

The IC_{50} is the concentration of an inhibitor where the response is reduced by half. The IC_{50} value of the different species was compared. The plant sample having the lowest IC_{50} is considered to have the best antioxidant property.

3.4 Statistical Analysis

All the experiments were performed in triplicates for each sample. All the data from the experiment were entry into Excel Program. The data was then transferred in the R Software Program. Mean ± SEM for Total Phenolic content and Total Flavonoid content was calculated using R software.

CHAPTER 4

RESULT

4.1 Ethnobotanical uses

Altogether, 152 species of ethno-medicinal plants were recorded from the study area belonging to 74 families and 135 genera. The list of the ethno-medicinal plants has been kept in Appendix V. Among them, only 15 plants were selected for the laboratory analysis based on the information that they are used to treat stomach disorders like stomachaches, indigestion, diarrhoea and dysetery. The detail description and uses of the selected plants are described below:

1. ***Achyranthes bidentata*** **Blume**, Bijdr. 545 (1825) - Fl. Brit. Ind. **4**:730 (1885) - Kitamura in Fn. Fl. Nep. Himal. **1**:121 (1955) - Hara in Fl. E. Himal.76 (1966) - Bull. Dept. Med. Pl. Nepal **11**:577 (1986).

Family: Amaranthaceae

Local name: Datiwan

English name: Hill Chaff Flower

Description: Erect herb about 1 m high, branches pubescent. Leaves stalked 3.5 - 18.5 cm long, 1.5-9 cm wide, elliptic, entire, acuminate, pubescent, base obtuse or acute. Flowers greenish in axillary and terminal spikes. Fruits a utricle, oblong, enclosed in the hardened perianth.

Flowering: June-October

Fruiting: July-December.

Distribution: Central and Eastern Nepal at 1200-3000 m in moist, shady places; Bhutan, China, India, Indonesia, Korea, Laos, Malaysia, Myanmar, New Guinea, Philippines, Russia, Sikkim, Thailand, Vietnam.

Medicine: Root juice is applied in treatment of toothaches, indigestion and is also considered good for asthma.

Other: Twigs are used as toothbrush during Teej festival.

Specimen: Nayagaun, Daman-5, Makawanpur, 2079 m, 23.6.2013, $27^0$36.011'N/$85^0$05.005'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK-232 (TUCH).

2. *Adiantum philippense* L., H. Ito in Fl. E. Himal. 459 (1966) - Banerji in Candollea **27** (2): 269 (1972) - Bull. Dept. Med. Pl. Nepal **11**: 577 (1986).

Adiantum lunulatum Burman fil., Bedd., Handb. Ferns Brit. Ind. 82 (1883).

Family: Pteridaceae

Local name: Kani unyun

Description: Tufted fern. Fronds 8-25 cm long, 3-5.5 cm wide, pinnate, pinnae alternate, lunate. Sori brown, along the edge of the frond, protected by the reflexed margin.

Spores: June-July.

Distribution: Throughout Nepal to about 2000 m in rock crevices, on moist walls and along stream banks; also in India, Sri Lanka and Myanmar.

Medicine: Juice of the rhizome is given in cases of fever, dysentery and glandular swelling.

Specimen: Nayagaun, Daman-5, Makawanpur, 1537 m, 23.6.2013, $27^0$37.01'N/$85^0$05.06'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK-110 (TUCH).

3. *Anemone vitifolia* Buch. - Ham. ex DC., Fl. Brit. Ind. **1**: 8 (1872) - Tamura et Kitamura in Kihara, Fn. Fl. Nep. Himal. **1**: 125 (1955) - Hara, Fl. East. Himal. : 87 (1966) - Bull. Dept. Med. Pl. Nepal **6**: 37 (1973) - Bull. Dept. Med. Pl. Nepal **11**: 122 (1986).

Family: Ranunculaceae

Local name: Dhanero

Description: Perennial herb about 50 cm high. Lower leaves long-stalked, five lobed, orbiculate, serrate, white tomentose beneath, upper leaves short-stalked. Flowers white with pink markings under the petals. Fruit an achene.

Flowering: August-September.

Fruiting: October-November.

Distribution: Throughout Nepal at 1600-3000 m, common along waysides in open, moist places; also in Afghanistan, northern India, western China and northern Myanmar.

Medicine: Root paste is applied in treating scabies. Root juice used in case of dysentery.

Specimen: Simbhanjyang, Daman-5, Makawanpur, 2260 m, 23.6.2013, $27^0$35.006'N/$85^0$04.015'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK-167 (TUCH).

4. *Bergenia ciliata* (Haw.) Sternb., Bull. Dept. Med. Pl. Nepal **6**: 37 (1997) -Bull. Dept. Med. Pl. Nepal **11**: 299 (1986).

Saxifraga ligulata Wall. var. *ciliata* (Royle) C. B. Cl. in Hook.f., Fl. Brit. Ind. **2**: 398 (1878).

Family: Saxifragaceae

Local name: Pakhanbed

Description: Herb with thick rootstocks. Leaves stalked, 3.5-16.5 cm long, 3-12 cm wide, suborbiculate, entire, fringed with short, stiff hairs. Flowers pink.

Flowering: March-July.

Distribution: Throughout Nepal at 1300-3000 m in moist, rocky places; also in Afghanistan, northern India, Bhutan, northern Tibet, western China and northern Myanmar.

Medicine: Juice or powder of the whole plant is taken to treat urinary trouble. This juice is given for cough and colds. Powdered rhizome is used to treat dysentery.

Other uses: Flowers are boiled and pickled.

Specimen: Simbhangyanj, Daman-5, Makawanpur, 2191 m, 24.06.2013, $27^0$36.014'N/$85^0$05.016'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK-64 (TUCH).

5. *Callicarpa macrophylla* Vahl, Fl. Brit. Ind. **4**: 568 (1885) - Kitamura in Fn. l. Nep. Himal. 1: 208 (1955) - Bull. Dept. Med. Pl. Nepal **11**: 540 (1986).

Family: Lamiaceae

Local name: Dahikaulo, Guyenlo

Description: Shrub about 3 m high with straggling branches. Leaves stalked, 10-25 cm long, 3.5-10 cm wide, oblong to lanceolate, acuminate, crenate, soft

pubescent above, thickly cottony tomentose beneath. Flowers small, pinkish, in a dense compound cyme. Fruit a drupe, white, spongy, succulent when fully ripe.

Flowering: May-November.

Fruiting: November-January.

Distribution: Throughout Nepal to about 1500 m in moist places; also in northern India, Bhutan, southern China and Indo-China.

Medicine: Root paste is taken to treat fever. Juice of the root is given for indigestion. Decoction of the leaves is given in cases of diarrhoea and dysentery.

Other uses: Ripe fruits are eaten fresh and are sweet. Wood serves as fuel. Leaves are gathered for fodder.

Specimen: Nayagaun, Daman-5, Makawanpur, 1138 m, 24.06.2013, $27^0$37.013'N/$85^0$05.06'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK-181 (TUCH).

6. *Campanula pallida* Wall.

Campanula colorata Wall.in Roxb., Fl. Brit. Ind. **3**: 440 (1881) - Kitamura in Fn. Fl. Nep. Himal., **1**: 239 (1955) - Hara in Fl. E. Himal. 326 (1966) - Bull. Dept. Med. Pl. Nepal **6**: 142 (1973) - Bull. Dept. Med. Pl. Nepal **11**: 433 (1986).

Family: Campanulaceae

Local name: Ganobuti, Majari

Description: Hispid herb about 50 cm high. Leaves short-stalked, alternate, oblong to elliptic, acute, serrate. Flowers bluish or purplish, solitary, axillary, in terminal panicles. Fruit a capsule, hemispheric, hairy.

Flowering: March-July.

Fruiting: July-November.

Distribution: Throughout Nepal at 700-4500 m, generally in moist rock crevices or on walls; also in Afghanistan, southern Tibet, India, western China and Indo-China.

Medicine: Root is used in cases of diarrhoea and dysentery.

Specimen: Simbhanjyang, Daman-5, Makawanpur, 2260 m, 23.6.2013, $27^0$35.006'N/$85^0$04.015'E Rajbhandary, S., Tiwari, N.N., Shrestha, I.,

Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK-168 (TUCH).

7. *Cautleya spicata* (Sm.) Baker in Baker in Fl. Brit. Ind. **6**: 209 (1890) - Hara in Fl. E. Himal. 421 (1966) - Bull. Dept. Med. Pl. Nepal **11**: 682 (1986).

Family: Zingiberaceae

Local name: Pani saro

Description: Herb about 50 cm high. Leaves short-stalked, elliptic, acuminate. Flowers yellow in short-stalked spikes.

Flowering: August-September.

Distribution: Central and Eastern Nepal at 1000-2600 m in moist, shady places; also in northern India.

Medicine: Juice of the rhizome is used for stomach disorders.

Other uses: Stem pith is eaten as a vegetable.

Specimen: Simbhanjyang, Daman-5, Makawanpur, 2270 m, 23.6.2013, $27^0$35.006'N/$85^0$04.015'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK-220 (TUCH).

8. *Coriaria napalensis* Wall., Fl. Brit. Ind. **2**: 44 (1876) - Kitamura in Fn. Fl. Nep. Himal **1**: 172 (1955) - Bull. Dept. Med. Pl. Nepal **6**: 76 (1973) - Bull. Dept. Med. Pl. Nepal **11**: 235 (1986).

Family: Coriaraiaceae

Local name: Machaino

Description: Trees about 5 m high. Leaves subsessile, opposite, 2-10 cm long, 1.5-4 cm wide, oblong to ovate or lanceolate, short-pointed, entire, three-veined, smooth. Flowers sessile, reddish. Fruit black when ripe.

Flowering: February-March.

Fruiting: May-June.

Distribution: Throughout Nepal at 1000-2800 m in open or shady places; also in northern India, Bhutan, western China and northern Myanmar.

Medicine: Juice of the bark is given in cases of stomachache.

Other uses: Ripe fruits are eaten fresh. Branches are used for making baskets.

Specimen: Nayagaun, Daman-5, Makawanpur, 1537 m, 23.6.2013, $27^0$37.01'N/$85^0$05.06'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK-171 (TUCH).

9. *Galium aparine* L., Kitamura in Fn. Fl. Nepal Himal. **1**: 229 (1955) - Bull. Dept. Med. Pl. Nepal **6**: 122 (1997) - Bull. Dept. Med. Pl. Nepal **11**: 361 (1986).

Family: Rubiaceae

Local name: Kangre jhar

Description: Rambling or climbing herb, stems prickly along the angles. Leaves sessile, five to eight in a whorl, 0.3-1.8 cm long, 0.2-0.5 cm wide, elliptic, tip spiny, midrib and margin minutely prickly. Flowers small, white. Fruit covered with hooked bristles.

Flowering and Fruiting: July – August.

Distribution: Central and Western Nepal at 2700-3600 m in open places along rocky tracks; also in western Asia, Europe and North America.

Medicine: Plant juice is applied to cuts and wounds. It is also used for indigestion.

Specimen: Nayagaun, Daman-5, Makawanpur, 2079 m, 23.06.2013, $27^0$36.011'N/$85^0$05.005'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK-62 (TUCH).

10. *Lindera neesiana* (Wall. ex Nees) Kurz, Fl. Brit. Ind. **5**: 186 (1886) - Bull. Dept. Med. Pl. Nepal **11**: 601 (1986).

Family: Lauraceae

Local name: Siltimur

Description: Tree about 4 m high. Leaves stalked, 2.5-19 cm long, 1.5-9 cm wide, ovate, glabrous. Flowers yellow. Fruit globose.

Flowering: October-November.

Fruiting: March- June.

Distribution: Central and Eastern Nepal at 700-2600 m in openings along ravines in forests; also in northeastern India, Bhutan and Myanmar.

Medicine: Fruits are chewed in cases of diarrhoea and toothache. Oil from the seed is applied for boils and scabies.

Specimen: Nayagaun, Daman-5, Makawanpur, 2079 m, 23.06.2013, $27^0$36.011'N/$85^0$05.005'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK-261(TUCH).

11. *Potentilla fulgens* Wall. ex Hook., Fl. Brit. Ind. **2**: 349 (1878)- Kitamura in Fn. Fl. Nep. Himal. **1**: 152 (1955) - Bull. Dept. Med. Pl. Nepal **11**: 284 (1986).

Family: Rosaceae

Local name: Bajradanti

Description: Herb about 50 cm high. Leaves stalked, odd-pinnate, leaflets numerous, 0.5-5.5 cm long, 0.5-3 cm wide, alternately large and small, diminishing in size from the uppermost downward, ovate, silky tomentose beneath. Flowers yellow.

Flowering: July-August.

Fruiting: September-November.

Distribution: Throughout Nepal at 1700-3800 m in open, moist places; also in northern India and Bhutan.

Medicine: Plant juice is used to treat stomachaches, cough and colds. It is also taken to treat peptic ulcer.

Specimen: Nayagaun, Daman-5, Makawanpur, 2432 m, 23.06.2013, $27^0$35.001'N/$85^0$04.015'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK- 65 (TUCH).

12. *Pyracantha crenulata* (D. Don) M. Roemer, Kitamura in Fn. Fl. Nep. Himal. **1**: 155 (1955) - Bull. Dept. Med. Pl. Nepal **11**: 288 (1986).

Family: Rosaceae

Local name: Ghangaru

Description: Spiny evergreen shrub about 3 m high. Leaves stalked, crowded on short lateral branches, 1.5-5.5 cm long, 0.8-1.8 cm wide, oblong to ovate, narrowed toward the base, crenate, smooth. Flowers stalked, white. Fruit globose, red.

Flowering: November-December.

Distribution: Throughout Nepal at 1000-2500 m on open hillsides among other shrubs; also in northern India, Bhutan, Tibet, China and Myanmar.

Medicine: Powdered dry fruit is taken for bloody dysentery.

Other uses: Ripe fruits are eaten fresh. Branches are used as walking sticks.

Specimen: Nayagaun, Daman-5, Makawanpur, 1537 m, 23.6.2013, $27^0$37.01'N/$85^0$05.06'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK-192 (TUCH).

13. *Pyrus pashia* Buch. - Ham. ex D. Don, Fl. Brit. Ind. **2**: 374 (1878) - Kitamura in Fn. Fl. Nep. Himal. **1**: 155 (1955) - Bull. Dept. Med. Pl. Nepal **6**: 92 (1997) - Bull. Dept. Med. Pl. Nepal **11**: 289 (1986).

Family: Rosaceae

Local name: Mayal

Description: Deciduous tree about 8 m high. Leaves stalked, 3-7 cm long, 1.5-3 cm wide, ovate to lanceolate, acuminate, crenate, glabrous, shiny, often woolly beneath on young plants. Flowers stalked, white. Fruit globose, covered with white dots.

Flowering: March- April.

Fruiting: September-October.

Distribution: Throughout Nepal at 700-2600 m in open, rocky places; also in northern India, Bhutan, western China and Myanmar.

Medicine: Ripe fruit juice is put in the eye of animals to treat conjunctivitis. The juice is also given to treat diarrhoea.

Other uses: Ripe fruits are edible. Leaves and twigs are lopped for fodder.

Specimen: Nayagaun, Daman-5, Makawanpur, 1981 m, 23.062013, $27^0$37.01'N/$85^0$05.001'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L. EK-59 (TUCH).

14. *Rubus ellipticus* Sm. Fl. Brit. Ind. **2**: 336 (1878) - Bull. Dept. Med. Pl. Nepal **11**: 292 (1986).

Family: Rosaceae

Local name: Ainselu

Description: Straggling shrub about 5 m high, with rusty brown bristles. Leaves stalked, pinnately trifoliate, leaflets short- stalked, 1.5-9 cm long, 1-7 cm wide, terminal leaflet largest, elliptic to ovate, serrate, hoary pubescent beneath, apex rounded. Flowers white in dense axillary and terminal panicles. Fruit an aggregation of drupelets, yellow.

Flowering: December-March

Fruiting: May-July.

Distribution: Throughout Nepal at 1600-2300 m on open slopes; also in northern India, Sri Lanka, eastern and western China, Myanmar and the Philippines.

Medicine: Juice of the root, about 6 teaspoons four times a day, is given for fever, diarrhoea and dysentery. A paste of the root is applied to wounds. The root and young shoot are considered good for colic. Leaf buds mixed with leaves of *Centella asiatica* and *Cynodon dactylon* is given for peptic ulcer.

Other uses: Ripe fruits are eaten fresh. These fruits are sold in local markets.

Specimen: Nayagaun, Daman-5, Makawanpur, 2200 m, 23.6.2013, $27^0$35.006'N/$85^0$04.015'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK-162 (TUCH).

15. *Uraria picta* (Jacq.) Desv.ex DC. Bull. Dept. Med. Pl. Nepal **12**: 292 (1996).

Family: Fabaceae

Local name: Bhatte jhar

Description: Perennial undershrub, 20-180 cm tall, branches pubescent, lower leaf 1-3-foliate, upper leaf 5-9 foliate, leaves broad, ovate-lanceolate, acute, mucronate. Flower purplish, pink or bluish.

Distribution: Throughout Nepal at 400-1500 m in grassy slopes; also in Tropical Africa, India, China, Malaysia and Australia.

Flowering: June-September.

Medicine: Decoction of whole plant is used in cough, chills and fever. The juice of root is also used to treat stomach pain.

Specimen: Nayagaun, Daman-5, Makawanpur, 1138 m, 24.06.2013, $27^0$37.013'N/$85^0$05.06'E Rajbhandary, S., Tiwari, N.N., Shrestha, I., Thapa, K.P., Poudel, M.R., Subedi, P., Bista, S.R., Adhikari, U. and Bhatta, L., EK-140 (TUCH).

4.2 Extraction

The parts of plants were selected according to the use value as reported by the local people. Whole plant, rhizome, stem, root, fruits or leaves were collected for the laboratory study. The yield percentage and characteristics of the extracts obtained has been tabulated below (Table 1).

Table 1: Percentage yield and physical characteristics of the crude methanolic extracts

S.N.	Botanical Name of Plants used	Parts used	% yield of extract	Characteristic of extract	
				Colour	Consistency
1	*Achyranthes bidentata*	Whole plant	3.9	Green	Sticky
2	*Adiantum philippense*	Whole plant	3.4	Greenish	
3	*Anemone vitifolia*	Whole plant	7.4	Greenish brown	Sticky, scratched as layer
4	*Bergenia ciliata*	Rhizome	20.3	Brown shiny	Sticky
5	*Callicarpa macrophylla*	Aerial part	7.85	Straw brown	Powdery
6	*Campanula pallida*	Whole plant	4.55	Light green	Sticky
7	*Cautleya spicata*	Rhizome	2.9	Light green	Sticky
8	*Coriaria napalensis*	Bark	12.65	Green shiny	Powdery
9	*Galium aparine*	Whole plant	6.1	Light green	Sticky
10	*Lindera neesiana*	Fruit	8.05	Brownish green	Sticky
11	*Potentilla fulgens*	Root	17.05	Reddish shiny	Powdery
12	*Pyracantha crenulata*	Fruit	12.9	Dark brown	Crystallic, powder
13	*Pyrus pashia*	Fruit	4.7	Brownish shiny	Very sticky, scratched as a layer
14	*Rubus ellipticus*	Root	11.35	Light brown	Powdery
15	*Uraria picta*	Whole plant	4.7	Light green	Sticky

Upon extraction, the percentage yield of extract varied from 2.9% to 20.3%. The maximum yield was obtained for *Berginia ciliata* and minimum for *Cautleya spicata*. The texture and consistency of the resulting extract also showed some variations. Most of them were sticky in nature, while few powdery.

4.3 Antimicrobial Screening

In this study, 15 selected plants were screened for antibacterial property against four strains of bacteria causing stomach disorders like stomachache, diarrhoea, dysentery, indigestion, etc. The bacteria used are:

Gram negative: *Pseudomonas aeruginosa* (ATCC no. 27853)

Escherichia coli (ATCC no. 25922)

Gram positive: *Staphylococcus aureus* (ATCC no. 25923)

Bacillus subtilis

Among the 15 species screened, variation has been seen on the zone of inhibition depending upon the species and the different concentration of extracts used.

The zone of inhibition was measured using scale for all the bacteria and the results were expressed on mm including 4 mm diameter of well and are tabulated in the following tables (Tables 2 and 3).

The table shows the activity of different plant extracts against different bacteria. Increased zone of inhibition was observed with increase in concentration of the plant extracts. Most of the extracts showed activity against Gram +ve bacteria i.e. *Staphylococcus aureus* and *Bacillus subtilis*. Among the 15 plants extracts tested, 7 samples showed activity at lower concentrations (12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml) while the other 8 plant samples did not show any activity at lower concentrations. Those samples which did not show any activity up to 100 mg/ml were further tested at higher concentration i.e. 150 mg/ml.

At 12.5 mg/ml, among the 7 samples that showed activity, *Rubus ellipticus* showed activity against *Pseudomonas aeruginosa* and both Gram +ve bacteria i.e. *Staphylococcus aureus* and *Bacillus subtilis*. Five showed activity against Gram +ve bacteria only while *Pyracantha crenulata* showed activity against *Bacillus subtilis* only.

Table 2: Antibacterial activity of methanolic extracts at different concentrations

Botanical name of plants used	**Concentration (mg/ml)**	**Zone of inhibition(mm) with well of 4 mm diameter**			
		P. aeruginosa	***E. coli***	***S. aureus***	***B. subtilis***
Bergenia ciliata	12.5	-	-	6	9
	25	-	-	8	10
	50	7	6	10	10
	100	8	8	12	13
Coriaria napalensis	12.5	-	-	9	11
	25	-	-	11	11
	50	8	8	14	12
	100	10	12	**16**	15
Lindera neesiana	12.5	-	-	**5**	6
	25	-	-	**5**	7
	50	**5**	-	6	9
	100	6	**5**	8	9
Potentilla fulgens	12.5	-	-	7	8
	25	-	-	8	9
	50	10	8	12	12
	100	12	12	**16**	14
Pyracantha crenulata	12.5	-	-	-	9
	25	6	-	10	11
	50	8	8	12	11
	100	12	10	13	13
Pyrus pashia	12.5	-	-	**5**	**5**
	25	-	-	6	**5**
	50	**5**	**5**	6	6
	100	6	6	8	6
Rubus ellipticus	12.5	7	-	7	9
	25	10	-	12	9
	50	12	6	14	10
	100	14	8	16	12
Control					
Ampicillin	10	6	24	40	12

At 25 mg/ml, *Rubus ellipticus* and *Pyracantha crenulata* showed activity against *Pseudomonas aeruginosa* and both Gram +ve bacteria while the other 5 showed activity against Gram +ve bacteria only.

At 50 mg/ml, 6 samples showed activity against all the tested organisms while *Lindera neesiana* showed activity against all bacteria except *Escherichia coli*.

At 100 mg/ml, all 7 samples showed activity against all the tested orgainsms.

Table 3: Antibacterial activity of methanolic extracts at 150 mg/ml

Botanical name of plants used	**Concentration (mg/ml)**	**Zone of inhibition(mm) with well of 4 mm diameter**			
		P. aeruginosa	***E. coli***	***S. aureus***	***B. subtilis***
Achyranthes bidentata	150	-	-	-	-
Adiantum philippense	150	-	-	-	8
Anemone vitifolia	150	-	-	-	6
Callicarpa macrophylla	150	-	6	**5**	10
Campanula pallida	150	-	-	-	6
Cautleya spicata	150	-	-	-	7
Galium aparine	150	-	-	8	6
Uraria picta	150	-	-	12	5
Control					
Ampicillin	10	6	24	40	12

Among the eight plants which did not show any activity at concentrations at and below 100 mg/ml, 7 of them showed activity at 150 mg/ml but *Achyranthes bidentata* did not show any activity at all. *Callicarpa macrophylla* showed activity against *Pseudomonas aeruginosa* and both Gram +ve bacteria. *Galium aparine* and *Uraria picta* showed activity against Gram +ve bacteria only. *Anemone vitifolia*, *Campanula pallida* and *Cautleya spicata* showed activity against *Bacillus subtilis* only.

Among all the plants screened for antibacterial property, highest zone of inhibition was observed for *Coriaria napalensis*, *Potentilla fulgens* and *Rubus ellipticus* i.e. 16 mm at 100 mg/ml. Lowest zone of inhibitions were observed for *Lindera neesiana*,

Pyrus pashia, *Anemone vitifolia* and *Uraria picta* at different concentrations and against different bacteria.

4.4 Total phenolic content determination

Phenolic compounds exhibit a wide range of biological and physiological properties due to their ability to act as antioxidants, free radical scavengers and chelators of divalent cations (Sharma *et al.*, 2014). The total phenolic content of the methanolic extracts were calculated by using calibration curve (Fig. 3) obtained from the Gallic acid solutions ranging from 25 µg/ml to 250 µg/ml. Based on the equation obtained, total phenolic content was calculated and expressed in mg GAE/g. The phenolic content is shown in Fig. 4.

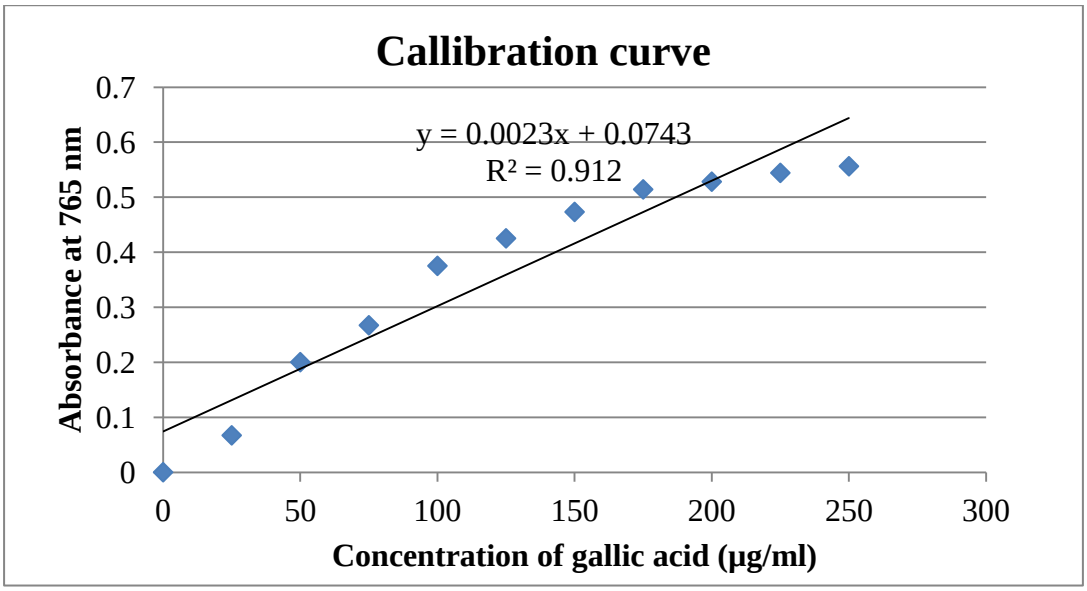


Fig. 3: Standard curve for calibration of total phenolic content

The highest phenolic content was obtained for *Potentilla fulgens* i.e. 90.73 ± 0.71 mg GAE/g while the lowest was shown by *Achyranthes bidentata* (2.21 ± 0.19 mg GAE/g).

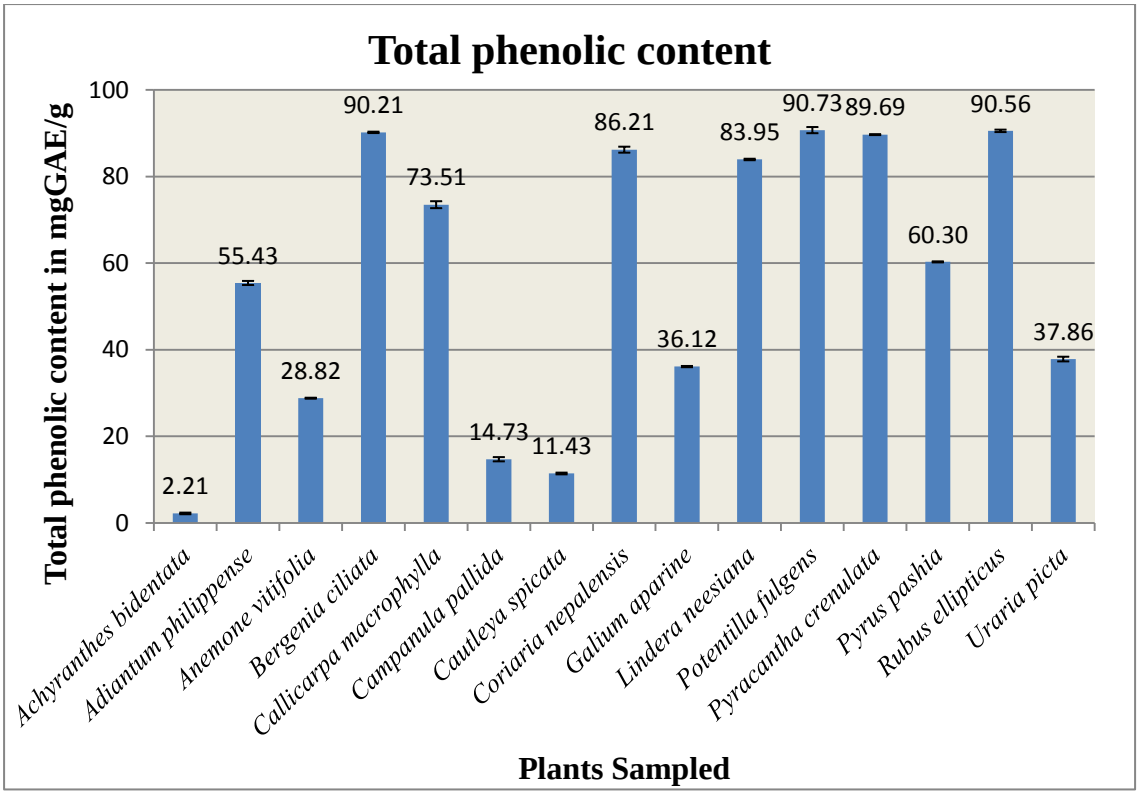


Fig. 4: Total phenolic content present in methanolic extract of the plant samples

4.5 Total flavonoid content determination

The standard graph for the estimation of total flavonoid content was obtained by using standard solutions of Quercetin ranging from 10 µg/ml to 100 µg/ml. The total flavonoid content for the plant samples were calculated by using the equation obtained from graph (Fig. 5) and then expressed in mg QE/g dry plant.

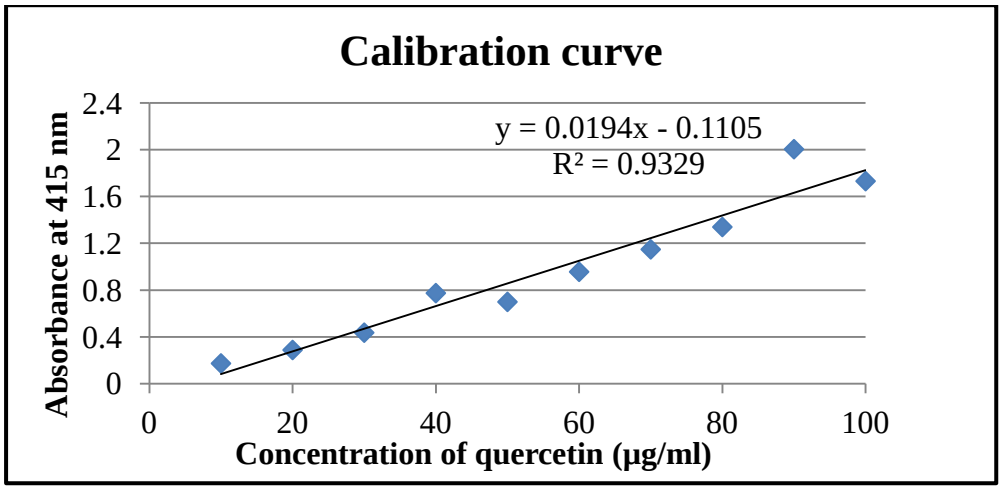


Fig. 5: Standard curve for calibration of total flavonoid content

Highest amount of flavonoid was obtained for *Adiantum philippense* (21.19 ± 0.28 mg QE/g dry plant) and *Cautleya spicata* (21.19 ± 0.52 mg QE/g dry plant) while the lowest amount of flavonoid was determined for *Bergenia ciliata* (1.78 ± 0.11 mg QE/g dry plant). The flavonoid content obtained for different samples is shown in Fig. 6.

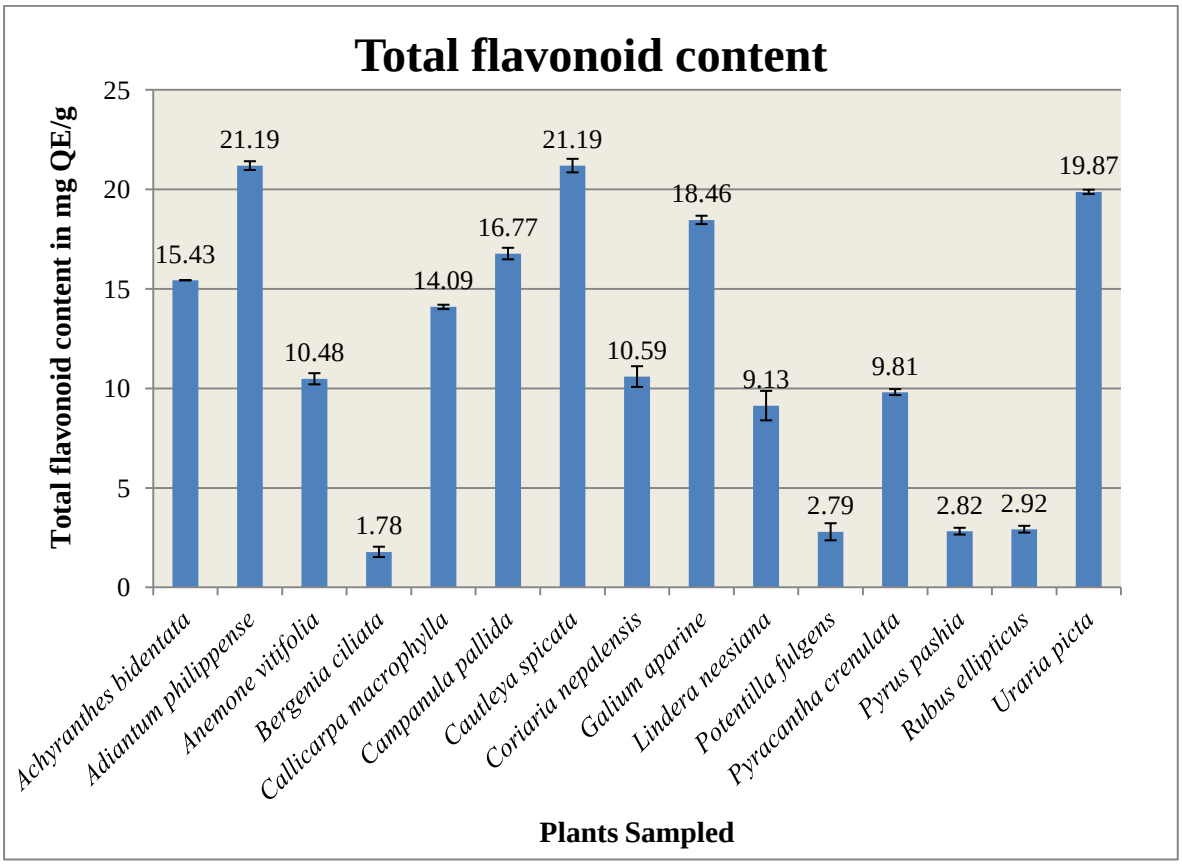


Fig.6: Total flavonoid content present in methanolic extract of different plant samples

4.6 Antioxidant activity of the plant extract

Oxidation reactions are necessary part of life, unfortunately they can also be damaging because of the production of reactive oxygen species (ROS). ROS are by-products of basic metabolic processes, immune reaction against pathogens, air pollution, tobacco smoke, herbicides, and pesticides. In biological systems, phenolic compounds and flavonoids are associated with scavenging ROS. *In vitro* condition DPPH is considered as a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Abi Beaulah *et al.*, 2011).

Thus, antioxidant activity of the different methanolic plant extracts were determined using the solution of DPPH (0.2 mM) and taking ascorbic acid as the pure antioxidant reference compound (Fig. 7). DPPH test provides simplified version to detect the antioxidant properties of various molecules present in the extracts. A DPPH solution is decolourized when the odd electron becomes paired off in the presence of a free radical scavenger. The colour becomes light yellow from deep violet. There was gradual increase in % radical scavenging with the increase in concentration of the extracts which is shown in Figures 8, 9 and 10.

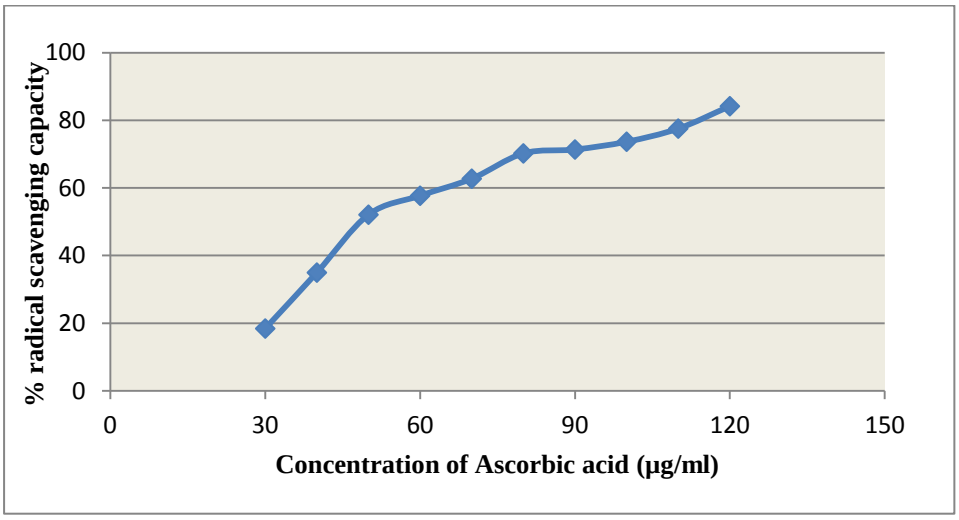


Fig. 7: Standard graph for Ascorbic acid

IC_{50} value of each plant extract was calculated and is shown in Fig. 11. The IC_{50} value for ascorbic acid was found to be 48.66. Maximum IC_{50} value was found for *Achyranthes bidentata* i.e. 127.55 and minimum for *Bergenia ciliata* i.e. 40.56. The samples with lower IC_{50} value are considered as the best antioxidants and vice versa. Thus, *Bergenia ciliata* can be considered as the best antioxidant among the sampled species.

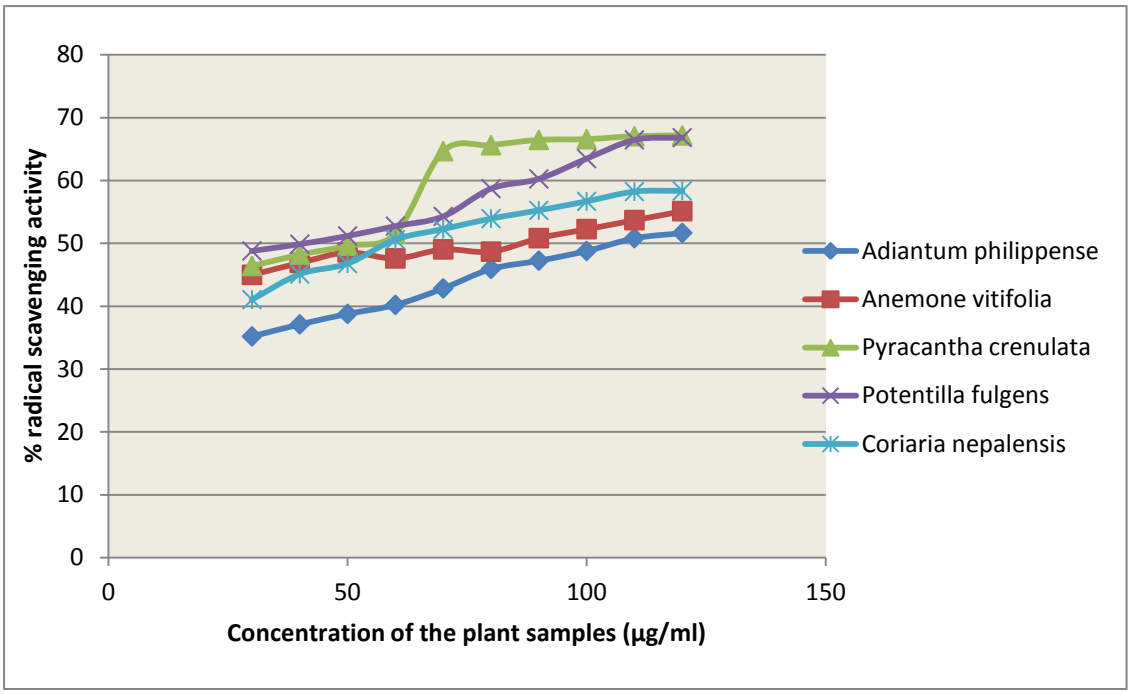


Fig.8: Concentration vs. % radical scavenging activity of plant samples

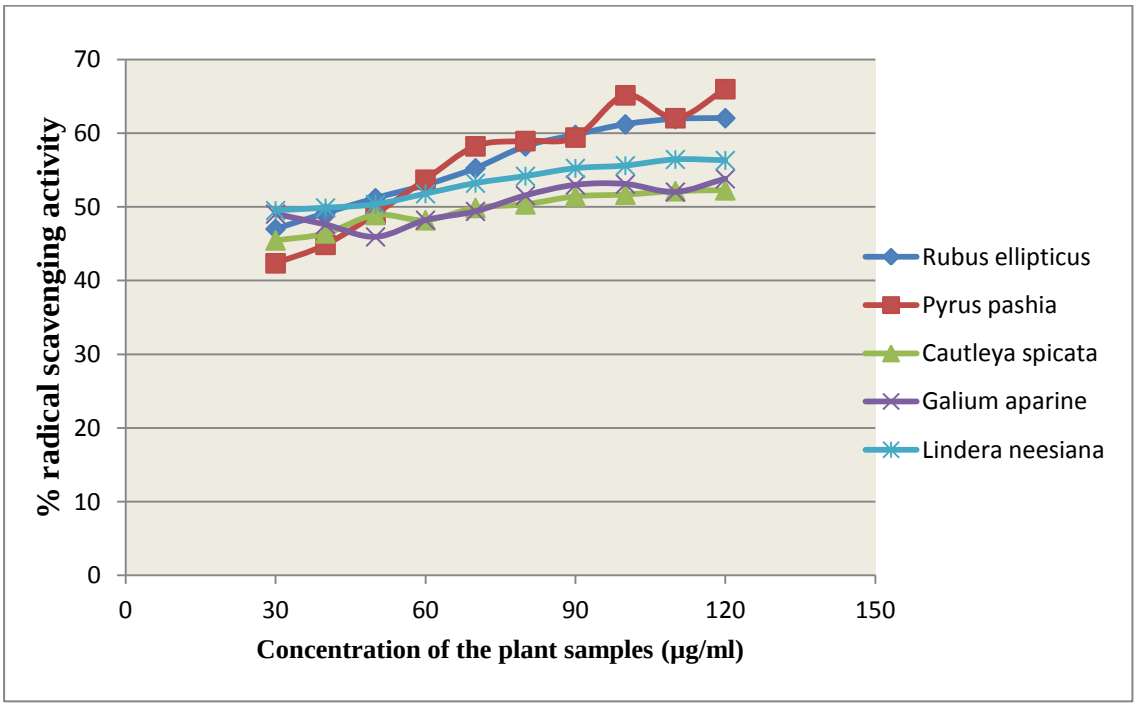


Fig. 9: Concentration vs. % radical scavenging activity of plant samples

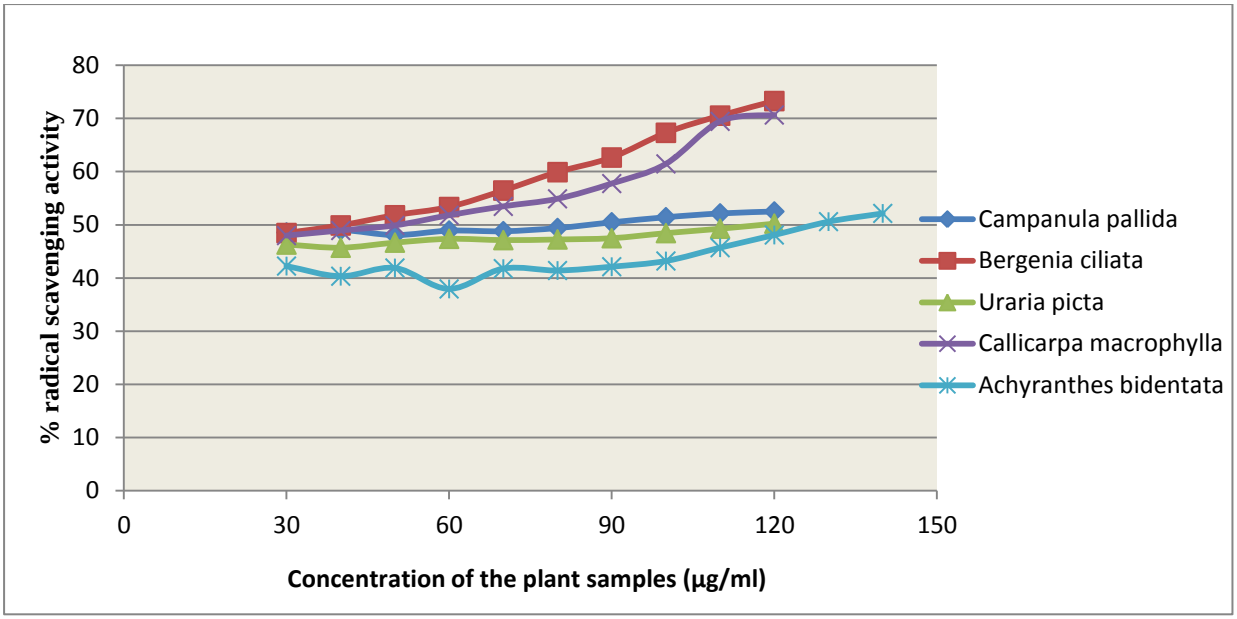


Fig.10: Concentration vs. % radical scavenging activity of plant samples

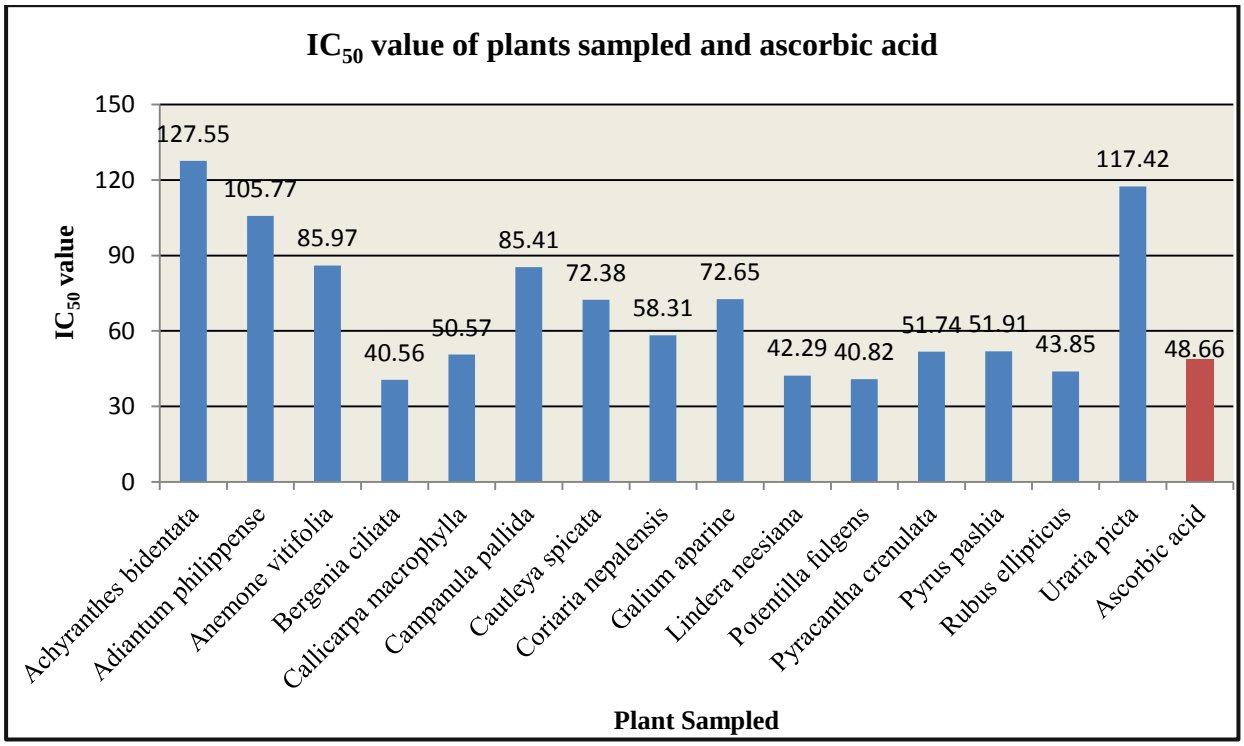


Fig.11: IC_{50} value of standard (Ascorbic acid) along with the other samples

4.7 Correlation Analysis

Correlation analysis between biological activities and phytochemical constituents is given below:

Table 4: Correlation analysis

S.N.	*In vitro* assay	Pearson's r value
1.	DPPH (IC_{50}) and TPC	-0.393
2.	DPPH (IC_{50}) and TFC	0.092
3.	TPC and TFC	-0.694**

**Correlation is significant at the 0.01 level.

Correlation analysis shows that there is no significant correlation between IC_{50} value and TPC or TFC. However, negative correlation is shown between IC_{50} value and TPC while positive correlation is shown between IC_{50} value and TFC. There is negative significant correlation between TPC and TFC at 0.01 level of significance.

CHAPTER 5

DISCUSSION

5.1 Ethnobotanical uses

The ethnobotanical uses of selected plants from study area are similar to that of the previous studies. Furthermore, some of them have various uses than those mentioned in the study area. *Achyranthes bidentata* is also used for pyorrhea, kidney and liver disorders, whooping cough, cholera and rheumatic pain (Manandhar, 2002; Dutta, 2007; Hasan *et al.*, 2013). *Adiantum philippense* is further used for dysentery, diseases of blood, ulcers, burning sensations, epilepsy, leprosy and other skin diseases (Paul *et al.*, 2012; Thomson, 2013). Similarly, *Anemone vitifolia* is used for headache, eye problems, infertility and toothache (Rajbhandari, 2001). *Bergenia ciliata* is used in cases of diarrhoea, fever, boils, eye diseases, rheumatic pains, urinary troubles and in removing stones from body (Dutta, 2007; Ghimire, 2008). *Callicarpa macrophylla* is also used to treat boils on tongues and gastric troubles besides the uses mentioned in the study area (Rajbhandari, 2001; Manadhar, 2002). *Lindera neesiana* is used in cases of stomach complaints, nausea, cough and cold, fever and cholera. *Potentilla fulgens*, besides the uses mentioned in the study area, are used to treat toothaches, throat infection and painful urination (Rajbhandari 2001; Dutta, 2007). *Rubus ellipticus* is prescribed for indigestion, cough and cold, and food poisoning (Rajbhandari, 2001; Majupuria, 2009). This shows that the plant taken for the study is not only used in the study area but highly used in other places and for other disorders.

5.2 Yield of plant extract

Extraction is the crucial first step in the analysis of medicinal plants as it is essential to extract the desired chemical components from the plant materials for further separation and characterization. Percentage yield of extract depends upon the parts of the plants used, nature of solvent used and method of extraction employed. The traditional healers use primarily water as solvents. But a number of researchers found that plant extracts prepared with alcohol i.e. methanol and ethanol as solvent provided more consistent antimicrobial activity and other biological activities (Allero & Afolagan, 2006; Parekh and Chandra, 2007). In this study, extraction method

followed is Percolation with intermittent Sonication. Among the plants used for the study, maximum percentage yield was obtained for *Bergenia ciliata* in which hard rhizome were used for extraction and minimum for *Cautleya spicata* in which fleshy plant parts were used for extraction. Percentage extraction is lowest in plants where the whole plant or fruits were used for extraction as in *Achyranthes bidentata, Adiantum philippense, Campanula pallida, Pyrus pashia* and *Uraria picta* and is higher for the bark, rhizomes and roots as in *Bergenia ciliata, Coriaria napalensis, Potentilla fulgens, Pyracantha crenulata* and *Rubus ellipticus*.

5.3 Antibacterial Activity

Plants are rich sources of important phytoconstituents which has been proved from various previous studies. Each constituent has its own effect against microorganisms; tannins and flavonoids were known to possess antimicrobial potential against bacteria and fungi (Adegoke *et al*., 2009).

Methanolic extract of *Achyranthes bidentata* did not show any activity against any of the tested bacteria even up to 150 mg/ml which is similar to the result obtained by Joshi *et al.* (2011) but the result obtained by Devi *et al.* (2007) is somehow different. According to Devi *et al.* (2007), ethanolic extracts showed activity against the five bacteria at 100 µg and 200 µg but less sensitive than the control used i.e. Tetracycline. *Adiantum philippense* showed activity against the Gram +ve bacteria, *Bacillus subtilis* only according to the work performed which does not go completely with the result obtained by Thomson (2013). According to Thomson, methanolic extract showed activity even against *Pseudomonas aeruginosa* and was less sensitive to *Staphylococcus aureus*. No activity was observed against *E. coli*, which is similar to the present study.

Anemone vitifolia also showed activity against *Bacillus subtilis* only at 150 µg/ml. High sensitivity of *Bergenia ciliata* against the bacteria at greater concentrations and low sensitivity at lower concentrations is similar to the study performed by Pokharel *et al.* (2014). *Callicarpa macrophylla* showed low sensitivity against the tested bacteria similar to the result shown by Yadav *et al.* (2012). *Galium aparine* showed very less activity against Gram +ve bacteria only which is supported by the result shown by Vlsae *et al.* (2014) where the ethanolic extract did not show any activity.

Saklani and Chandra (2014) described the moderate antibacterial activity of *Pyracantha crenulata* which is proved from this study as well. Latha *et al.* (2015) explains that methanolic extract of *Rubus ellipticus* showed a significant antibacterial activity which supports the result of this study.

The maximum Zone of Inhibition (ZOI) obtained by *Coriaria napalensis*, *Potentilla fulgens* and *Rubus ellipticus* against *Staphylococcus aureus* shows that these plants are highly significant in controlling the disease caused by the organism. No activity shown by 8 plant sample extracts at 100 µg/ml and very low activity at higher concentration is due to their less sensitivity to act against the diseases caused by these bacteria.

In classifying the antibacterial activity as Gram +ve or Gram -ve bacteria, it would generally be expected that a much greater number would be active against Gram +ve than Gram-ve bacteria (McCutcheon *et al.*, 1992), which is similar to this study as more extracts showed activity against Gram +ve bacteria even at low concentrations compared to Gram –ve bacteria. Among the plants screened for antibacterial property, all plants except *Achyranthes bidentata* showed activity at either lower to higher or at higher concentrations only. Among the others, all showed activity against *B. subtilis*, a Gram +ve bacteria. Besides, 10 samples showed activity against *S. aureus*, a pyrogenic bacterium known to play a significant role in invasive skin diseases including superficial and deep follicular lesion and food poisoning while 8 showed activity against *E. coli* and 7 against *P. aeruginosa*.

5.4 Total phenolic and flavonoid content determination

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways in plants. Phenolics are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. Plant phenolic constitutes as the major groups of compounds acting as primary antioxidants or free terminators. Phenolic compounds are commonly found in both edible and non-edible plants and have been reported to have multiple biological effects, including antioxidant activity. Phenolics are able to scavenge reactive oxygen species due to their electron donating

properties (Jothy *et al.*, 2011). The phenolic contents for different plants sampled differed from 2.21 ± 0.19 to 90.73 ± 0.71 mg GAE/g.

Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic oxidative enzymes and anti-inflammatory action (Kumar *et al.*, 2012). The flavonoid content ranged from 1.78 ± 011 to 21.19 mg QE/g dry plant.

The greater flavonoid content was obtained *for Adiantum philippense, Cautleya spicata, Galium aparine* and *Uraria picta* which did not show significant antibacterial activity.

The phenolic and flavonoids present in the plants are natural antioxidants and also have proved to antimicrobial activity. Flavonoids, the major group of phenolic compounds present in the plants, are natural antioxidants and also have proved to antimicrobial activity. They are important for prevention of diseases associated with oxidative damage of the membrane, proteins and DNA.

5.5 Antioxidant Activity

DPPH is a stable free radical, due to the delocalization of the spare electron on whole molecule. Thus, DPPH does not dimerize. The delocalization of the electron determines the purple colour with an absorbance band with a maximum 517. When DPPH reacts with the hydrogen donors, the reduced form (molecular) DPPH is generated, accompanied by the disappearance of the purple colour and appearance of the yellow colour. Therefore, absorbance reduction depends linearly on the antioxidant concentration (Kumar *et al.*, 2012; Thaipong *et al.*, 2006). Antioxidant property can be inferred on the basis of % radical scavenging activity (RSA) and IC_{50} value. Antioxidant activity DPPH inhibition of the plant extract is expressed as % inhibition of stable radical or inhibition concentration fifty (IC_{50}) in reference to a standard compound. The plant with higher % RSA has the lower IC_{50}. The plant extract with lowest IC_{50} value is considered having better antioxidant properties (Gulcin *et al.*, 2012). Antioxidant property of plants might be due to their phenolic compounds (Kumar *et al.*, 2012).

Among the selected plants, *Bergenia ciliata* (40.56) and *Potentilla fulgens* (40.82) have comparatively low IC_{50} value, thus have high antioxidant capacity among the selected plants. *Rubus ellipticus* and *Lindera neesiana* also have high antioxidant capacity (IC_{50} value $\leq$ 50 µg/ml). The antioxidant capacity obtained by George *et al*. (2013) for *Rubus ellipticus* is 12.2 ± 0.90 which is very less compared to the study which may be due to the difference in habitat, process of drying and the process of extraction. These plants having high antioxidant capacity can thus be useful in treating the diseases caused by oxidative damage leading to different chronic diseases like cancer, cardiovascular diseases, etc. *Pyracantha crenulata, Coriaraia napalensis, Pyrus pashia* and *Callicarpa macrophylla* have moderate antioxidant capacity (50µg/ml < IC_{50} value $\leq$ 100 µg/ml) while *Anemone vitifolia, Campanula pallida, Cautleya spicata* and *Galium aparine* have relatively less moderate antioxidant capacity. Similarly, *Achyranthes bidentata, Adiantum philippense* and *Uraria picta* have very less antioxidant capacity (IC_{50} value > 100 µg/ml).

5.6 Correlation Analysis

Correlation between IC_{50} and TPC or TFC showed negative correlation, which is similar to the findings of Farasat *et al*. (2014) and Bhattarai (2014). But they showed poor relation which may be due to the fact that other compounds might also be responsible for the antioxidant activity along with phenols relation (Javanmardi *et al*., 2003). Very poor correlation between IC_{50} and flavonoid indicates that flavonoid has a negligible role in the antioxidant activity. The antioxidant activity may be due to the presence of sterol and terpene derivatives besides phenolics and flavonoids (Patel *et al*., 2011).

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The present study concludes that most medicinal plants used in traditional way to treat against bacterial diseases have antibacterial properties. The antibacterial activity may be due to the presence of one or more compounds present in them which is shown by various previous studies. Percolation with intermittent sonicaton method was used to extract the samples. The medicinal plants were extracted in methanol by Percolation with intermittent Sonication method. *Bergenia ciliata* had the highest yield (20.03%) while *Cautleya spicata* had the lowest yield (2.90%).

Altogether fifteen plants used to treat stomach disorder, indigestion, diarrhoea and dysentery were selected for the study purpose. The methanol extracts of these plants were screened for their antibacterial activity against two Gram +ve bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram –ve bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). Among them, Gram +ve bacteria were found to be more sensitive than the Gram –ve bacteria. The *in vitro* antibacterial activity was performed by agar well diffusion method.

Among the 15 plants tested, in the present study, all plants except one (*Achyranthes bidentata*) showed activity against at least one bacterium. *Bergenia ciliata, Coriaria napalensis, Potentilla fulgens, Pyracantha crenulata, Pyrus pashia* and *Rubus ellipticus* inhibited all the tested bacteria at 50 µg/ml and 100 µg/ml. *Lindera neesiana* inhibited all the tested bacteria at 100 µg/ml only. *Callicarpa macrophylla* inhibited only 3 of the tested bacteria at 150 µg/ml while *Galium aparine* and *Uraria picta* inhibited Gram +ve bacteria only. *Adiantum philippense, Anemone vitifolia, Campanula pallida* and *Cautleya spicata* inhibited *Bacillus subtilis* only. Most of the extracts showed activity against Gram +ve bacteria than Gram -ve bacteria.

Quantitative estimation of compounds (total phenolic and flavonoid content estimation) and their antioxidant activity were also evaluated. Four plant species (*Bergenia ciliata, Lindera neesiana, Potentilla fulgens and Rubus ellipticus*) were found to have high antioxidant capacity, eight species (*Anemone vitifolia, Callicarpa*

macrophylla, Campanula pallida, Cautleya spicata, Coriaraia napalensis, Galium aparine, Pyracantha crenulata and *Pyrus pashia*) were found to have moderate antioxidant capacity and remaining three species (*Achyranthes bidentata, Adiantum philippense* and *Uraria picta*) were found to have comparatively less antioxidant capacity.

Highest phenolic content was obtained for *Potentilla fulgens* (90.73 ± 0.71 mg GAE/g) while the lowest was shown by *Achyranthes bidentata* (2.21 ± 0.19 mg GAE/g). Similarly, highest content of flavonoid was obtained for *Adiantum philippense* (21.19 ± 0.28 mg QE/g dry plant) and *Cautleya spicata* (21.19 ± 0.52 mg QE/g dry plant) and lowest for *Bergenia ciliata* (1.78 ± 0.11 mg QE/g dry plant).

Antioxidant activity of the methanolic extract was determined using DPPH method. Maximum IC_{50} value was obtained for *Achyranthes bidentata* i.e. 127.55 and minimum for *Bergenia ciliata* i.e. 40.56. Thus, *Bergenia ciliata* was considered to be the best antioxidant among the sampled plant samples.

6.2 Recommendation

The present work is a preliminary study of antibacterial and antioxidant of selected medicinal plants in *in vitro* condition. Due to time constraints, limited work has been done in this research. From this study, following points can be recommended:

- Only methanolic extract of medicinal plants have been used in this experiment. Further research should be done using other solvent of various polarities which will extract the other active compounds present in plants.
- The plants *Bergenia ciliata, Lindera neesiana, Potentilla fulgens* and *Rubus ellipticus* showed the broad spectrum antibacterial and high antioxidant activity. These plants can be further subjected to isolation of the therapeutic antibacterial compounds and carry out further pharmacological evaluation.
- The plants which showed remarkable activity towards *in vitro* test should be further carried out towards *in vivo* test.
- Qualitative phytochemical screening could be done to find out the various secondary metabolites found in the plants.

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*Original not seen.

Appendix I

Short description of bacteria involved in the present study and their pathogenicity

1. Pseudomonas aeruginosa

a. Morphology and Biochemical Characters

It is a gram negative, motile, non-spore forming, non-fermentative, aerobic rod-shaped. It is widely distributed in nature. It grows readily on minimal media. Cetrimide agar and Pseudomonas isolation agar are best selective media for *Pseudomonas aeruginosa*. Its optimum temperature for growth is 37^0C and it is able to grow at temperatures as high as 42^0C. *P. aeruginosa* may produce three colony types (Todar, 2008). *P. aeruginosa* strains produce two types of soluble pigments, the fluorescent pigment pyoverdin and the blue pigment pyocyanin. The latter is produced abundantly in media of low-iron content and functions in iron metabolism in the bacterium.

b. Pathogenicity

It is an opportunistic pathogen, meaning that it exploits some break in the host defenses to initiate an infection. It may cause ear infections and is the major cause of malignant otitis media (Collee *et al.*, 1996). It also causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systematic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed (Todar, 2008).

2. Escherichia coli

a. Morphology and Biochemical Characters

It is facultative anaerobic gram-negative rods. Physiologically, it is versatile and well-adapted to its characteristic habitats. It can grow in media with glucose as the sole

organic constituent. Wild type *E. coli* has no growth factor requirements and metabolically it can transform glucose into all of the macromolecular components that make up the cell. The bacterium can grow in the presence or absence of O_2. Under anaerobic conditions, it will grow by means of fermentation, producing characteristic "mixed acid and gas" as end products. However, it can also grow by means of anaerobic respiration, since it is able to utilize NO_3, NO_2 or fumarate as final electron acceptors for respiratory electron transport processes (Todar, 2008). Most of them are lactose fermenter and produce green metallic sheen on EMB agar. Their optimal growth temperature is 36-37^{0}C.

b.Pathogenicity

Pathogenic strains of *E. coli* are responsible for three types of infections in humans: urinary tract infections (UTI), neonatal meningitis and intestinal diseases (gastroenteritis). The diseases caused (or not caused) by particular strain of *E. coli* depend on distribution and expression of an array of virulence determinants, including adhesions, invasions, toxins and abilities to withstand host defense (Todar, 2008). On the basis of their pathogenicity, they are divided into four groups viz. enteritoxigenic *E. coli* (ETEC) strains cause an acute watery diarrhoea, enteroinvasive strains of *E. coli* (EIEC) can cause blood and mucus in stool, verocytotoxin producing, also termed enterohaemorrhagic *E.coli* (VTEC/EHEC) cause hemorrhagic colitis and enteropathogenic *E. coli* (EPEC) which is of minor importance (Collee *et al.*, 1996).

3. Staphylococcus aureus

a. Morphology and Biochemical Characters

It is a gram positive, spherical bacteria that occur in microscopic clusters resembling grapes. On nutrient agar, at 37^{0}C, it forms colonies 1-3 mm in diameter with smooth, low convex, opaque and of butyrous consistency within 18-24 hours. It forms a fairly large yellow colony on rich medium. It is often hemolytic on blood agar. The bacteria are catalase-positive and oxidase-negative. It can grow at a temperature of 15 to 45^{0}C and at

NaCl concentrations as high as 15 percent. Nearly, all strains of *S.aureus* produce the enzyme coagulase (Todar, 2008).

b. Pathogenicity

It causes localized infection when enter through break in skin. It causes pyogenic infections including folliculitis, impetigo, furuncles, carbuncles, breast abscess, post-operative wound infections, cellulites, pyomyositis, osteomyelitis, septic arthritis, bronchopneumonia, lungs abscess, etc. It also causes boils, secondary infections, septicemia, pneumonia, meningitis, acute endocarditis, conjunctivitis, toxic shock syndrome and more commonly food poisoning (Collee *et al.*, 1996).

4. Bacillus subtilis

a. Morphology and Biochemical Characters

It is a gram positive, rod shaped bacteria that grows aerobically on nutrient agar and forms resistant endospores. Spores are ellipsoidal, not bulging sporangium, centrally located and heat resistant. It is common saprophyte found as contaminants in foods, clinical specimens and laboratory culture. It is facultative thermophile, capable of growth over the range 12-55^{0}C. It can grow well on ordinary media, forming large colonies that are circular or irregular, grey yellow, granular and difficult to emulsify. They hydrolyze gelatin (Colee *et al.*, 1996).

b. Pathogenicity

It is less commonly found opportunistic pathogen. It sometimes causes food poisoning (Colee *et al.*, 1996).

Appendix II

List of materials used for the study

Glassware

Screw capped bottles	Funnels	Petriplates
Round bottom flask	Test tubes	Measuring cylinder

Apparatus and Equipment

Electric grinder	Micropippete	Refrigerator
Incubator	Hot air oven	Electric balance
Filter papers	Vortex	Aluminum foils
Cotton swabs	Inoculating loops	Polythene bags
Cotton rolls	Sticker	Wash bottle
Autoclave	Sonicator	Camera
Notebook/Markers/Pencil		

Media for culture, chemicals and reagents

Nutrient Broth (NB)	Ampicillin	Methanol
Nutrient Agar (NA)	Quercetin	Ascorbic acid
Muller Hinton Agar (MHA)	Gallic acid	Ammonium chloride
Folin-Ciocalteu phenol reagent	Sodium bicarbonate solution	Potassium acetate
DPPH (1,1-diphenyl-2 picryhydrazyl)		

Appendix III

Preparation of Reagents

1. Preparation of 1M Na_2CO_3-100 ml

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

2. Preparation of the Folin-Ciocalteu phenol reagent (1:10 dilution)

6 ml of the commercially supplied Folin-Ciocalteu phenol reagent (Sigma-Aldrich Co.3050, Spruce Street, St. Louis) was taken and mixed with 54 ml of the distilled water to prepare 60 ml of 1:10 dilution of Folin-Ciocalteu phenol reagent.

3. Preparation of Aluminium Chloride (10%) - 100ml

10 gram of the commercially supplied aluminium chloride (MerkSpecialities Pvt. Ltd., Mumbai, India) was weighed and dissolved in water, finally maintaining the volume to 100 ml.

4. Preparation of Potassium Acetate -100ml

After weighing 9.814 gram of Potassium actetae (MerkSpecialities Pvt. Ltd., Mumbai, India), it was dissolved in distilled water maintain the volume to 100ml.

Appendix IV

Data for calibration of Gallic acid

Concentration of gallic acid	Absorbance at 765 nm
0	0
25	0.067
50	0.2
75	0.267
100	0.375
125	0.425
150	0.473
175	0.514
200	0.528
225	0.544
250	0.556

Data for calibration of Ascorbic acid

Ascorbic acid((µg/ml)	% radical scavenging capacity
30	18.417
40	34.964
50	52.086
60	57.698
70	62.734
80	70.216
90	71.367
100	73.669
110	77.554
120	84.173

Data for calibration of Quercetin

Conc(µg/ml)	Absorbance
10	0.172
20	0.287
30	0.435
40	0.773
50	0.698
60	0.955
70	1.147
80	1.338
90	2.003
100	1.73

APPENDIX V

List of plant species with their ethnomedicinal uses documented from Daman VDC of Makwanpur District

Scientific name	Family	Nepali name	Life forms	Parts used	Preparataion	Medicinal use
Achyranthes bidentata Blume	Amaranthaceae	Datiwan	H:Wil	Wp, R, S	Juice, raw	Toothache, indigestion, asthma, pyorrhea
Aconitum ferox Wall. ex Ser.	Ranunculaceae	Bikh	H:Wil	R	Paste	Uric acid, rheumatism
Aconitum spicatum (Bruhl).Stapf.	Ranunculaceae	Bishjara	H:Wil	R	Juice/ paste	Poison, joint pain , fever
Aconogonum molle (D.Don) H.Hara	Polygonaceae	Thotne	H:Wil	L, Wp		Diarrhoea, dysentery
Adiantum capillus-veneris L.	Pteridaceae	Pakhaale Uniu	H: Wil	L, Wp	Paste	Headache, cough, boils, irregular mensturation
Adiantum philippense L.	Pteridaceae	Kaane uniu	H: Wil	Rh		Fever, diarhea, dysentery, glandular swellings
Ageratina adenophora (Spreng.) R.M.King & H.Rob.	Asteraceae	Banmaaraa	H:Wil	L	Juice	Haemostatic, antiseptic
Ageratum conyzoides L.	Asteraceae	Ganmane Ghaans	H:Wil	L	Juice	Cuts, wounds
Alangium chinense (Lour.) Harms	Alangiaceae	Timil	Tr:Wil	R	Paste	Setting of dislocated bones
Allium wallichii Kunth	Amaryllidaceae	Ban lasun	H:Wil	R, L	Raw, Paste	Cough cold, altitude sickness
Alnus nepalensis D. Don.	Betulaceae	Utis	Tr:Wil	Br, Wp	Powder	Body pain, chronic fever

Alternanthera sessilis (L.) R.Br. ex DC.	Amaranthaceae	Bhiringi Jhaar	H:Wil	Wp	Juice	White discharge in urine, scabies, cuts and wounds, bloody dysentery
Anaphalis contorta (D.Don.) Hook.f.	Asteraceae	Buki phul	H:Wil	Fl, L	Juice	Cough and cold, chest pain, inner bleeding
Anemone vitifolia Buch.-Ham. ex DC.	Ranunculaceae	Mauro mulo	H:Wil	Wp		Scabies and Dysentery
Arisaema tortuosum (Wall.) Schott	Araceae	Sarpa makai	H:Wil	Tu		Worm infestation
Artemisia dubia Wall.ex Besser	Asteraceae	Titepati	H:Wil	R	Decoction	Headache, fever, diarrhoea
Artemisia indica Willd.	Asteraceae	Titepati	H:Wil	L, S	Juice	Antihelmintic, diarrhoea, dysentery, cough
Asparagus racemosus Willd.	Asparagaceae	Kurilo	Cl:Wil	Tu	Paste	Diuretic, tonic, diarrhoea, dysentery, fever
Astilbe rivularis Buch.-Ham.ex D. Don	Saxifragaceae	Budho Okhati	H:Wil	R	Paste	Excessive post-partum bleeding, postnatal diarrhoea and dysentery
Berberis aristata DC.	Berberidaceae	Chutro	Sh:Wil	S	Paste	Malarial fever, Diarrhoea, Jaundice
Bergenia ciliata (Haw.) Sternb.	Saxifragaceae	Paashanabed	H:Wil	Rh	Juice	Urinary disorder, dysentery, cough
Betula alnoides Buch.-Ham. ex D. Don	Betulaceae	Lekh Painyu	Tr:Wil	Fr, S, L		Cuts, burns, Dislocated bones
Persicaria amplexicaulis (D.Don) Ronse Decr.	Polygonaceae	Ratnaulo	H:Wil	Wp	Paste	Cut and wounds
Boehmeria	Urticaceae	Kamle	Sh:Wil	R	Paste	Diarrhoea of cattle(1)

macrophylla Hornem.						
Boehmeria rugulosa Wedd.	Urticaceae	Daar	Tr:Wil	Br	Paste	Sprained parts, cuts and wounds
Boerhavia diffusa L.	Nyctaginaceae	Punarnava	H:Wil	Wp	Juice	Wounds, fever, stomach troubles
Buddleja asiatica Lour.	Loganiaceae	Bhimsenpati	Sh:Wil	L, Fl		Skin disease
Butea buteiformis (Voigt) Grierson & Long	Fabaceae	Bhujetro	H:Wil	Sd	Powder	Antihelminthic
Callicarpa macrophylla Vahl	Lamiaceae	Dahikamlo	Tr:Wil	R, Br, F		Fever, Indigestion, diarrhoea, dysentery
Campanula pallida Wall.	Campanulaceae	Ganobuti	H:Wil	R		Diarrhoea, dysentery
Cannabis sativa L.	Cannabaceae	Gaanjaa	H:Wil	Sd	Paste	Diarrhoea, dysentery
Castanopsis indica (Roxb. ex Lindl.) A.DC.	Fagaceae	Katus	Tr:Wil	L, Tw, F		Indigection, diarrhoea
Cautleya spicata (Sm.) Baker	Zingiberaceae	Pani saro	H:Wil	Tu		Stomach disorder
Centella asiatica (L.) Urb.	Apiaceae	Ghod Taapre	H:Wil	Wp, L	Juice, paste	Dysentery, coolant, diuretic, cuts and wounds, snakebite, skin diseases
Cheilanthes dalhousiae Hook.	Pteridaceae	Raanisinkaa	H: Wil	Wp	Paste	Cuts, wounds
Chlorophytum nepalense (Lindl.) Baker	Asparagaceae	Ban supari	H:Wil	R		Gout
Cinnamomum tamala (Buch.-Ham.) T.Nees &	Lauraceae	Tajpat	Tr:Wil	L	Juice	Colic pain, diarrhoea

Eberm.						
Cipadessa baccifera (Roth) Miq.	Meliaceae	Paireti	Sh:Wil	R	Squeezed	Bowel complaints, bleeding and swelling of gums
Cissampelos pareira L.	Menispermaceae	Baatule Laharaa	Tr:Wil	R	Paste	Stomachache, diuretic, applied on dislocated bones
Clematis grewiiflora DC.	Ranunculaceae	Bhere kuro	Sh:Wil	F, Sh		Diarrhoea, dysentery, toothache, cuts and wounds
Colebrookea oppositifolia Sm.	Lamiaceae	Dhusure	Sh:Wil	L		Antihelmintic to animals, antiseptic, dysentery
Coriaria napalensis Wall.	Coriariaceae	Machaino	Tr:Wil	Br		Stomachache
Crassocephalum crepidioides (Benth.) S. Moore	Asteraceae	Anikaale Jhaar	H:Wil	R	Paste	Diarrhoea, cuts, wounds
Crotalaria alata Buch.-Ham.ex D. Don	Fabaceae	Singesinge	Sh:Wil	Wp	Juice	Malarial fever, nocturnal discharge
Curcuma angustifolia Roxb.	Zingiberaceae	Kachur	H:Wil	Rh	Paste	Food-poisoning, boilsapplied on dislocated bones
Cuscuta reflexa Roxb.	Convolvulaceae	Akashbeli	H:Wil	Wp	Juice, paste	Jaundice, headache, rheumatism, stomachache
Cynodon dactylon (L.) Pers.	Poaceae	Dubo	H:Wil	L	Juice	Indigestion
Cynoglossum glochidiatum Wall.ex Benth	Boraginaceae		H:Wil	R	Paste	Cuts and wounds, burns, vomiting in infants
Daphniphyllum himalense (Benth.) Müll. Arg.	Daphniphyllaceae	Rakchan	Sh:Wil	L		Boils
Debregeasia	Urticaceae	Tushaare	Sh:Wil	L	Juice	Scabies

longifolia (Burm.f.) Wedd.						
Dendrobium macraei Lindl.	Orchidaceae	Jibonti	Sh:Wil	R	Powder	Colic pain, urinary trouble, astringent, expectorant
Dennstaedtia appendiculata (Wall. ex Hook.) J. Sm	Dennstaedtiaceae	Raunne	H: Wil	Fo		Cuts and wounds
Desmodium confertum DC.	Fabaceae	Bhatte	Sh:Wil	R	Juice	Gastric troubles
Desmodium multiflorum DC.	Fabaceae	Bhatte	Sh:Wil	R	Powder	Acidity
Desmostachya bipinnata (L.) Stapf.	Poaceae	Kush	H:Wil	Wp	Raw, Juice	Cooling, aphrodisiac, diuretic, asthma, jaundice, biliousness
Dicentra macrocapnos Prain	Papaveraceae	Jogi lahara	Cl:Wil	A		Cuts and wound
Didymocarpus aromaticus Wall.ex D. Don	Gesneriaceae	Pakhanbhetta	H:Wil	Wp		Blood urine in cattles
Dioscorea bulbifera L.	Dioscoreaceae	Githa	Cl:Wil	Fr		Worms and germs
Dioscorea deltoidea Wall. ex Griseb.	Dioscoreaceae	Bhyakur	Cl:Wil	Rh		Roundworm, constipation
Drymaria cordata (L.) Willd. ex Schult.	Caryophyllaceae	Abijaal	H:Wil	R	Juice	Headache, diarrhoea, dysentery, indigestion, Fever
Dryopteris chrysocoma (Christ) C. Chr.	Dryopteridaceae	Neuro	H: Wil	L		Cuts and wounds
Dryopteris filix-mas	Dryopteridaceae	Unyu	H: Wil	Rh	Juice	Antihelmintic(2)

(L.) Schott						
Elatostema sessile J.R.Forst. & G.Forst.	Urticaceae	Gagleto	H:Wil	Wp		Stomachache, Wound
Equisetum diffusum D.Don	Equisetaceae	Aankhle jhaar	H: Wil	Wp		Burns, scabies, fever, Sprain, bone dislocation
Euphorbia parviflora L.	Euphorbiaceae	Dudhi	H:Wil	Wp		Wounds and boils
Ficus auriculata Lour.	Moraceae	Nemaro, Timala	Tr:Wil	L		Diarrhoea, Dysentery
Ficus neriifolia Sm.	Moraceae	Dudhilo	Tr:Wil	Br		Boils on tongue
Ficus religiosa L.	Moraceae	Pipal	Tr:Wil	Br, L, Fl, La	Juice, Paste	Astrngent, gonorrhoea, scabies, diarrhoea, dysentery
Fimbristylis squarrosa Vahl	Cyperaceae	Jire Jhaar	H:Wil	Wp	Ash	Wounds, Throat aches
Flemingia strobilifera (L.) W.T.Aiton	Fabaceae	Bhatwasi	Sh:Wil	R	Paste	Diarrhoea, dysentery, scabies
Fraxinus floribunda Wall.	Oleaceae	Lakuri	Tr:Wil	S		Stomach disorders of sheep
Galium aparine L.	Rubiaceae	Charchare	H:Wil	Wp		Cuts and wounds, diarrhoea, dysentery
Galium asperifolium Wall.	Rubiaceae	Chitu	H:Wil	Wp	Paste	Urinal problems, wounds, boils
Gaultheria fragrantissima Wall.	Ericaceae	Dhasingare	Sh:Wil	L	Oil	Stimulant, carminative, hookworms, rheumatism
Gaultheria nummularioides D. Don	Ericaceae	Kaaligedi	Sh:Wil	L	Juice	Diuretic, Painful urination
Geranium nepalense Sweet	Geraniaceae	Gurije	H:Wil	R	Paste	Renal disease, cuts and wounds
Gerbera maxima	Asteraceae	Jhula	H:Wil	R		Worm infestation, Muscular swelling

(D.Don) Beauv.						
Girardinia diversifolia (Link) Friis	Urticaceae	Allo/Allo sisnu	H:Wil	Wp, L, R	Juice, Paste, Ash	Eczema, worm killer, gastric, headache, fever
Hedyotis scandens Roxb.	Rubiaceae	Baakhri Laharaa	Sh:Wil	R	Paste	Indigestion, gout
Heracleum candicans Wall.ex DC.	Apiaceae	Tokar	Sh:Wil	Sd	Powder	Cough, Cold, Diarrhoea, dysentery
Hydrocotyle nepalensis Hook.	Apiaceae	Hattipaile	Sh:Wil	L		Cough, fever, boils
Hypericum uralum Buch.-Ham. ex D. Don	Hypericaceae	Khareti	Sh:Wil	Fr		Cuts
Indigofera atropurpurea Buch.-Ham. ex Hornem.	Fabaceae	Saakino	Sh:Wil	A		Diarrhoea , dysentery
Inula cappa (Buch.-Ham. ex D. Don) DC.	Asteraceae	Gai tihare	Sh:Wil	Wp		Fever, menstrual disorder, peptic ulcer
Juglans regia L.	Juglandaceae	Okhar	Tr:Wil	Br	Raw	Antihelmintic, headache
Justicia adhatoda L.	Acanthaceae	Asuro	Sh:Wil	L	Decoction	Antipyretic
Lannea coromandelica (Houtt.)Merr.	Anacardiaceae	Hallunde	Tr:Wil	Br		Ulcer
Lepisorus bicolor (Takeda) Ching	Polypodiaceae		H: Wil	Rh		Sweeling, sprain
Lepisorus morrisonensis (Hayata) H. Itô	Polypodiaceae	Dhule Uniu	H: Wil	Rh	Paste	Sprained parts, Bone fracture

Leptodermis lanceolata Wall.	Rubiaceae	Bhuin champa	Sh:Wil	L		Cuts and wounds
Ligustrum confusum Decne.	Oleaceae	Kanike	Sh:Wil	Wp		Toothache
Lindera neesiana (Wall. ex Nees) Kurz	Lauraceae	Siltimur	Tr:Wil	Fr		Diarrhoea, toothache, boils, scabies
Phyla nodiflora (L.) Greene	Verbenaceae	Phuli jhar	H:Wil	Wp	Fever	
Litsea cubeba (Lour.) Pers.	Lauraceae	Siltimur	Tr:Wil	R, Br, F	Powder, Raw	Pain
Lobelia pyramidalis Wall.	Campanulaceae	Eklebir	H:Wil	L, Fl	Juice	Expectorant, asthma, chronic bronchitis, vomiting
Lycopodium clavatum L.	Lycopodiaceae	Nagbeli	H: Wil	Sp	Powder	Burns, headache
Lygodium japonicum (Thunb.) Sw.	Lygodiaceae	Janai Laharaa	H: Wil	P	Paste	Boils, Scabies, Jointache
Lyonia ovalifolia (Wall.) Drude	Ericaceae	Angeri	Sh:Wil	L	Juice	Boils, pimples, skin diseases, antidote for dogbite
Mahonia napaulensis DC.	Berberidaceae	Jamaneman dro	Tr:Wil	Br, F	Raw, Juice	Dysentery, diarrhoea, diuretic
Micromeria biflora (Buch.-Ham.ex D.Don) Benth.	Lamiaceae	Pinaase Jhaar	H:Wil	Wp	Juice	Toothaches, Nosebleed, sinuisitis
Mimosa himalayana Gamble	Fabaceae	Areli	Sh:Wil	R	Paste	Sprain
Myrica esculenta Buch.-Ham. ex D.Don	Myricaceae	Kaphal	Tr:Wil	Br, F, S	Crushed, Raw	Headache, sprains, diarrhoea, dysentery, wounds
Nephrolepis	Nephrolepidaceae	Paani	H: Wil	R	Juice	Indigestion, fever, cough, cold

cordifolia (L.) C. Presl		Amalaa				
Ocimum basilicum L.	Lamiaceae	Babri/Bantulasi	H:Cul	Sd	Powder	Chest pain
Osbeckia nepalensis Hook. f.	Melastomataceae	Arbal	Sh:Wil	Wp	Juice	Indigestion, Typhoid, cuts and wounds
Osbeckia stellata Buch.-Ham. ex Ker Gawl.	Melastomataceae	Laal angeri	H/Sh: Wil	Wp	Juice	Scabies, diarrhoea, dysentery
Osyris lanceolata Hochst. & Steud.	Santalaceae	Nundhiki	Sh:Wil	L	Extract	Bone fracture
Oxalis corniculata L.	Oxalidaceae	Chari Amilo	H:Wil	L	Juice	Fever, dysentery, nose bleeding, muscular swellings, ringworm, dysentery
Paris polyphylla Sm.	Melanthiaceae	Satuwaa	H:Wil	R	Paste	Diarrhoea, dysentery, cuts and wounds
Phyllanthus emblica L.	Phyllanthaceae	Aala/Amala	Tr:Wil	Br, L, F	Juice, Dried fruit	Dysentery, acidity, astringent, diarrhoea, constipation
Phytolacca acinosa Roxb.	Phytolaccaceae	Jaringo	H:Wil	Wp	Raw	Narcotic
Pinus roxburghii Sarg.	Pinaceae	Khote sallo	Tr:Wil	L, S		Cut and wounds, gastric troubles
Piper peepuloides Roxb.	Piperaceae	Pipala	Cl:Wil	Wp		Cough and cold
Pogostemon glaber Benth.	Lamiaceae	Rudilo	H:Wil	R	Juice	Indigestion, fever, headache
Potentilla fulgens Wall. ex Hook.	Rosaceae	Bajradanti	H:Wil	R		Stomach trouble, peptic ulcer, cough, cold
Pouzolzia zeylanica (L.) Benn.	Urticaceae	Chiple ghans	H:Wil	L		Boils, Dysentery, fever, toothaches
Pteridium revolutum	Dennstaedtiaceae	Ainu	H: Wil	Rh		Eye swelling, burning of eye

(Blume) Nakai						
Pteris wallichiana J. Agardh	Pteridaceae	Dalumo	H: Wil	Fo		Cuts, indigestion in animals
Pyracantha crenulata (D.Don) M. Roemer	Rosaceae	Ghangaru	Tr:Wil	Fr		Bloody dysentery
Pyrrosia mollis (Kunze) Ching	Polypodiaceae		H: Wil	Rh	Decoction	Tonic to women after delivery
Pyrus pashia Buch.-Ham. ex D.Don	Rosaceae	Mayal	Tr:Wil	Sd		Diarrhoea, Dysentery, Conjunctivitis
Quercus glauca Thunb.	Fagaceae	Banjh	Tr:Wil	Br		Dysentery
Raphanus raphanistrum L.	Brassicaceae	Bonmula	H:Wil	L	Raw, Cooked	Rheumatism
Rhododendron arboreum Sm.	Ericaceae	Lali gurans	Sh:Wil	Br, Fl	Paste, Raw	Headache, profuse diarrhoea, cough, choking of fish bone
Rhus javanica L.	Simaroubaceae	Bhakimlo	Tr:Wil	L, Fr		Swellings wounds, stomachache
Rorippa nasturtium-aquaticum (L.) Hayek	Brassicaceae	Simsaag	H:Wil	L		Fever, indigestion
Rubia manjith Roxb.ex Fleming	Rubiaceae	Majitho	H:Wil	R	Paste	Scabies, skin diseases
Rubus ellipticus Sm.	Rosaceae	Ainselu	Sh:Wil	F		Fever, diarrhoea, dysentery, wounds, peptic ulcer
Rubus paniculatus Sm.	Rosaceae	Dudhilo lahara	Cl:Wil	S		Scabies, sprains
Saccharum spontaneum L.	Poaceae	Kasa	H:Wil	R	Paste	Inflammations, cold, dysuria
Saurauia napaulensis DC.	Saurauiaceae	Gogan	Tr:Wil	F	Sqeezed	Cough, Cold
Scutellaria discolor	Lamiaceae	Daampaate	H:Wil	Wp	Paste	Fever, indigestion, wounds

Colebr.						
Selinum wallichianum (DC.) Raizada & H.O. Saxena	Apiaceae	Bhutkesh	H:Wil	Wp	Juice	Cough, cold, fever
Senecio cappa Buch.-Ham. ex. D. Don	Asteraceae	Rana veng (Tam)	Sh:Wil	R		Fever, boils
Sida acuta Burm.f.	Malvaceae	Balu jhaar	H/Sh: Wil	L	Paste	fever, boils, indigestion
Sigesbeckia orientalis L.	Asteraceae	Dudhe Jhaar	H:Wil	R	Paste	Indigestion, wounds
Solanum aculeatissimum Jacq.	Solanaceae	Kantakaari	Sh:Wil	Sd	Smoke	Swelling of gums and toothaches, headache
Solanum nigrum L.	Solanaceae	Kaalo Bihin	H:Wil	F	Chewed	Dysentery, fever, wounds, inflammation of urinary bladder
Sphenomeris chinensis (L.) Maxon	Dennstaedtiaceae		H: Wil	Wp		Swelling, sprains
Stellaria monosperma Buch.-Ham.ex D.Don	Caryophyllaceae	Jethimodhu	H:Wil	R, S	Juice	Stimulant, astringent, tonic
Stephania glabra (Roxb.) Miers	Menispermaceae	Gurgo gano	Cl:Wil	Rh		Asthma, Dysentery
Stephania glandulifera Miers	Menispermaceae	Baatulpaate	Cl:Wil	Tu	Peeled	Sprains, muscular swellings, diarrhoea in cattle
Swertia angustifolia Buch.-Ham. ex D. Don	Gentianaceae	Chiraito	H:Wil	L		Fever

Swertia chirayita (Roxb.ex Fleming)Karsten	Gentianaceae	Chiraito	H:Wil	Wp	Juice	Fever, Headache, Skin diseases
Tectaria macrodonta (Fee) C. Chr.	Dryopteridaceae	Kaali Nyuro	H: Wil	Rh	Decoction	Bloody dysentery, diarrhoea, throatache, cuts, wounds
Thysanolaena maxima (Roxb.) Kuntze	Poaceae	Amreso	H:Wil	Wp	Paste	Boils
Trichosanthes wallichiana (Ser.) Wight	Cucurbitaceae	Indrayani	Cl:Wil	Fr		Fever
Uraria picta (Jacq.)Desv.ex DC	Fabaceae	Sano bhatte	H:Wil	Wp		Boils, diarrhoea, dysentery, peptic ulcer
Urtica dioica L.	Urticaceae	Sisno	H:Wil	R, S, L	Paste, Juice	Dog bites, cuts and wounds, fever, boils
Viburnum cylindricum Buch.-Ham. ex D. Don	Sambucaceae	Ghare ghure	Tr:Wil	L		Itchy skin
Viscum album L.	Loranthaceae	Hadchur	Sh:Wil	Wp	Paste	Dislocated bones and wounds of cattle
Zanthoxylum armatum DC.	Rutaceae	Timur	Sh/Tr: Wil	Fr	Decoction	Constipation, antihelmintic, Cold, Abdominal pain, indigestion, fish poison, toothache, cholera, leech repellant, wound

Life forms: H: Herb; Sh: Shrub; Tr: Tree; Cl: Climber; Wil: Wild; Cul: Cultivated

Parts Used: Wp: Whole plant; R: Root; Rh: Rhizome; L: Leaves; S: Stem; Fr: Fruit; F: Flower; Sd: Seed; B: Bud; Tu: Tuber; Br: Bark; Sp: Spore; Fo: Frond; A: Aerial part; Tw: Twig

Photo plate I

Achyranthes bidentata

Galium aparine

Adiantum philippense

Anemone vitifolia

Bergenia ciliata

Callicarpa macrophylla

Campanula pallida

Photo plate II

Cautleya spicata

Coriaria napalensis

Lindera neesiana

Potentilla fulgens

Pyracantha crenulata

Pyrus pashia

Rubus ellipticus

Uraria picta

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Photo Plate III

Photo 1: Collection of materials in the field

Photo 2: Herbarium preparation

Photo 3: Air drying of samples after collection

Photo 4: Sonication of the samples for extraction

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Photo plate IV

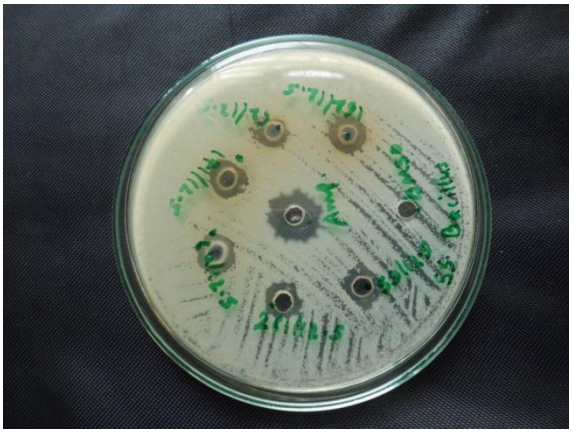

Photo 5: ZOI of samples against *B. subtilis* at 12.5mg/ml

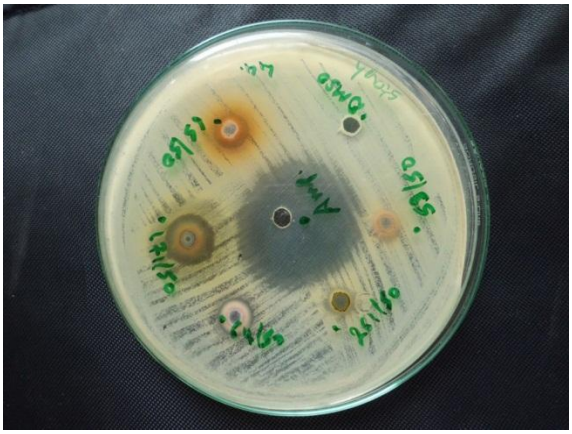

Photo 6: ZOI of samples against *S. aureus* at 50 mg/ml

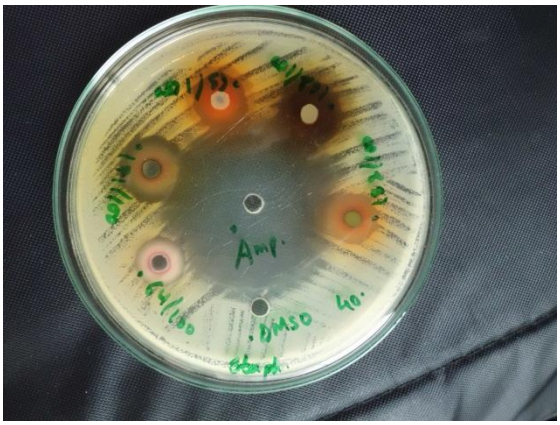

Photo 7: ZOI of samples against S. aureus at 100mg/ml

Photo 8: ZOI of samples against S. aureus at 25 mg/ml