# CHAPTER-I

# INTRODUCTION

## 1.1 General Background

Nature has been the source of medicinal agents for thousands of years and impressive numbers of modern drugs have been isolated from natural sources, many based on their use on traditional medicine (Nair *et al.* 2005). The widespread use of herbal remedies and health care preparations such as those described in ancient texts like Vedas and Bible have been traced to the occurrence of natural products with medicinal property. In fact, plants produce a diverse range of bioactive molecules making them rich source of different medicine. In recent time, attention has been reverted back to plants as a source of therapeutic agents due to the presence of their medicinal value. These include mainly the reduced cost, relative lower incident of other adverse effects compared to modern conventional pharmaceuticals and their easily availability (Adomi 2008). The number of such plant is very large. It is difficult to ascertain just how many of earth's estimated 250,000 species of higher plants are used in traditional medicines but one estimate puts it somewhere between 35,000 and 70,000 (Baral and Kurmi 2006).

The discovery of bacteria in 1683 by Van Leuwenhoek helped mankind to understand the infectious pathogens and approximately develop antiseptic and antibiotic protocol in the following years. By the beginning of the $20^{th}$ century, Paul Ehrlich proposed the principle of chemotherapy and his work including Structure-Activity Relationships significantly contributed for shaping synthetic protocols and helped in later discoveries of antibacterial drugs (Dax 1997). After the discovery of microorganisms as the causative agents for many infections and septic diseases of human beings and animals, more interest has been given in plant substances which were toxic to those microorganisms.

The history of medicine and medicinal plants in Nepal can be traced back to the Vedic period, where Nepal-Himalaya was mentioned as a sacred heaven of potent medicinal and aromatic plants (Baral and Kurmi 2006). The earliest mention of the medicinal use of plant in Hindu culture is found in "Rig-Veda", which has said to have been written between 4500 B.C. and 1600 B.C., is supposed to be the oldest repository of human knowledge. Good information on the ethnobotanical and medicinal uses of the

Nepalese plants can be found in the “Chandra Nighantu”, an herbal pharmacopoeia of medicinal value of plants in the 19th century (Malla 1999). Even today plant materials continue to play major role in primary health care as therapeutic remedies in many parts of countries. This is due to lack of western doctors and medications, and the expenses associated with such treatments (Manandhar 2002). It is estimated that various communities in Nepal use approximately 1000 species of wild plants in traditional medicinal practice (Chaudhary 1998). Nepal has a natural gift of over 7000 species of vascular plants among them 1463 species of medicinal plants have been reported, representing about 20% of the total flora (Tiwari 1999).

### 1.1.1 Antibacterial Activity of Plants

Medicinal plants are boon of nature to cure a 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 world medicinal plants were used against bacterial, fungal and viral infections (Perumal *et al.* 2004). Many efforts have been made to discover the new antimicrobial compounds from various kinds of sources such as microorganisms, animals and plants. One of such source 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, antimicrobials of plant origin are not associated with side effects and have massive therapeutic potential to heal many infectious diseases. The potential for developing antimicrobial compounds 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 effects of plant extracts on bacteria have been studied by large number of researcher in different parts of world. 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.

### 1.1.2 Development of Antibacterial Resistance

Many antibacterial drugs were developed in the late 1940s following the first report of resistance in *Staphylococcus aureus* (1941) and in *Mycobacterium tuberculosis* (1940) (Dax 1997). However, by the end of 1980s most major infectious diseases in

developed world were almost eradicated and half the major pharmaceutical companies in Japan and USA stopped their antibacterial drug development programs (Williams *et al*. 1996). After that period, drug-resistant pathogens were on the rise worldwide (Levy 1998). A *Streptococcus* that causes nosocomial infections showed innate resistance to drugs including Cephalosporin, Clindamycin and Aminoglycoside (Dax 1997, Levy 1998). The bacterium (*Staphylococcus aureus*) has now developed multidrug resistant strains and threatens to put an end to successful chemotherapy (Mitscher *et al.* 1999). The emergence of drug resistant bacterial strains limits the effectiveness of current drugs. This is due to the misuse and over prescription of the drugs (Leggadrio 1995). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by the pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio 1996; Scazzocchio *et al.* 2001). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies (Sieradski *et al.* 1999). Thus, there is a need to develop new antibiotics which is a global challenge preoccupying research institutions, pharmaceutical companies and academic institutions (Latha and Kannabiran 2006). However, the past record of rapid, widespread emergence of resistance to newly introduced antibiotics indicates that even new antibiotics are expected to have a short life (Coates *et al.* 2002). This situation coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections is now one of the serious medical problems (Marchese and Shito 2001).

The mode of bacterial resistance is either intrinsic (maintained on the bacterial chromosome) or acquired through chromosomal mutation, plasmid transformation, transposition, transduction, and conjugation from other species (Dax 1997, Lipsitch 2001). The fundamental mechanisms of resistance is generally observable in bacteria include inactivation or degradation of antibacterial drugs by enzymatic action, decreasing or changing of membrane permeability of bacterial cell wall to antibiotics, the alteration of the bacterial proteins that are microbial targets, and less often, autotrophic or metabolic by-pass (Dax 1997).

## 1.2 Objectives

### 1.2.1 General objective

The overall aim of the study was to access the antibacterial activities of few medicinal plants of Nepal which are used by people for ethno-medicinal purpose.

### 1.2.2 Specific objectives

- To screen the antibacterial activity of crude methanol and aqueous extract of selected medicinal plants.
- To evaluate the antibacterial property of those medicinal plants.

## 1.3 Rationale of the study

The different medicinal plants are being used by tribal people to treat different bacterial disease and other diseases. These plants are also being used by people of rural area of Nepal. The people of rural areas suffering from various bacterial disease like diarrhea, dysentery, jaundice, pneumonia etc. and they mainly depend on primary health care such as treatment provided by traditional healers by using medicinal plants. Due to lack of modern facilities of hospital, doctors, allopathic medicine and economic problems, rural people depend on traditional healers. These plants may contain some antibacterial effects. Therefore, to study whether the plants contain antibacterial activity or not, it should be tested by scientific method. In addition to this, the antibiotic resistance has become a global concern today. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years. The effects of compounds found in medicinal plant on bacteria have been studied by large number of researchers in the different parts of world. In Nepal few plants are screened for antimicrobial activities but very limited work has been done to evaluate the antimicrobial activity. The study of antimicrobial compounds on plants is the basis to prepare the antimicrobial compound to be used in the allopathic system of medication. The need of today is to evaluate the different plants to investigate different components which can fight against the bacteria possessing multiple resistances.

# CHAPTER-II

# LITERATURE REVIEW

## 2.1 Plants and plant products used in medicine

Herbal medicinal practice plays an important role in the primary healthcare system in most developing countries. WHO (2002) estimated that 80% of the population living in rural areas use or depend on herbal medicine for their health care. Herbal medicines are defined as any preparation containing one or more active herbal substances or herbal extractives. For majority of these preparations, the active principles or compounds are unknown. Among the first priorities designed by WHO in its strategy for traditional medicine, the study of plants for external use with antiseptic and wound healing promoting activity are emphasized (Akerele 1984).

In the last few decades, there has been exponential growth in the field of herbal medicine. It is getting popularize in developing and developed countries owing to its natural origin and lesser side effects. In olden times, *Vaidyas* used to treat patients on individual basis, and prepared drugs according to the requirement of the patients. But the scene has been changed now; herbal medicines are being manufactured on a large scale in mechanical units, where manufacturers are facing many problems such as availability of good quality of raw material, authentication of raw material, availability of standards, proper standardization methodology of drugs and formulations, quality control parameters (Ali *et al.* 2005, Agrawal, 2005). Many medicines including strychnine, aspirin, vincristine, and taxol are of herbal origin. About one quarter of the present prescription drugs dispensed by community pharmacies in the United States contain at least one active principle originally derived from plant materials (Farmsworth and Morris 1976).

Since then large number of works had been done to test the antibacterial activities of plants throughout the world (Taylor *et al*. 1995, Dagmar *et al*. 2003, Parekh and Chanda 2006, Chehregani *et al.* 2007, Adomi 2008). Much of the work has been done on the ethnomedicinal plants (Kelmanson *et al.* 2000, Parajuli *et al*. 2001, Barbour *et al.* 2011, and Mahato and Chaudhary 2005).

## 2.2 Work on antimicrobial activity

Shakya (1982) performed the preliminary antimicrobial activities of 45 indigenous medicinal plants by disk diffusion method on the dried extract of petroleum ether (40-60), 95% alcohol and sterile water. The test organisms were *Staphylococcus aureus, Agrobacterium tumefaciens, Bacillus subtilis, Salmonella typhi, Escherichia coli, Shigella dysenteriae, Candida albicans, Saccharomyces cerevisiae* and *Candida neoformans.* Some plant species showed weak and moderate activities on both bacteria and fungi, while some extract showed encouraging activities.

Shrestha and Sharma (1988) observed the antimicrobial activities of some essential oil viz. *Mentha arvensis, Acorus calamus, Zanthoxylum oxyphyllum* and turpentine oil against some fungi and bacteria. The extent of efficiency of the essential oil was studied at two different growth stages of filamentous fungi and non-filamentous fungi, gram-positive (*Staphylococcus* sp. and *Streptococcus* sp.) and gram-negative bacteria (*E. coli* and *Pseudomonas* sp.) by minimum inhibitory concentration (MIC) techniques and spore germination test. Turpentine oil exhibited strong activities against tested bacteria.

Taylor *et al.* (1995) studied the *in vitro* screening of selected medicinal plants of Nepal for their antimicrobial activities. Duplicate assays were conducted with and without exposure to UV-A radiation to test for light-activated or light-enhanced activity. Methanolic extract of all twenty one medicinal plants showed activity against two bacterial species, and twenty showed activity against at least two fungi. Six extracts were active only when exposed to UV-A radiation, and the antibiotic or antifungal effect of 14 extracts was enhanced upon exposure to light.

Devkota *et al.* (1999) studied the antibiotic properties of some lichen species. Lichens were tested with both strains of bacteria as gram-positive (*Bacillus subtilis*) and gram-negative (*Escherichia coli*). The different chemical constituents present in the tested lichens were capable of inhibit the growth of gram-positive bacteria but did not inhibit the growth of gram-negative bacteria. The chemical constituents of these lichens which inhibited the growth were also discussed.

Thomas *et al.* (1999) studied *in vitro* antimicrobial study of 21 plant species against multi-resistant bacteria isolates including gram-positive and gram-negative strains

found species specific response to microorganisms. He reported maximum antibacterial activity of *Adhatoda vasica, Cardiospermum holicacabum, Euphorbia hirta, Murraya koenigii, Oldenlandia corymbosa* and *Phyllanthus niruri.*

Devkota *et al.* (2000) performed antimicrobial activity on nine medicinal plants as *Glycyrrihiza globra, Azadirachta indica, Swertia chirayita, Acorus calamus, Withania somnifera, Terminalia chebula, Barberis asiatica, Paranassia nubicola* and *Curcuma angustifolia* used as medicine in village area of Nepal. These were extracted in ethanol by soxhlet extraction and extract was tasted against *Pseudomonas aeruginosa, Staphylococcus aurous, Escherichia coli, Vibrio cholera, Salmenella typhii, Shigella dysenteriae a*nd *Shigella flexneri.*

Kelmanson *et al.* (2000) studied the antimicrobial activity of Zulu medicinal plants. Extracts of 14 plants used in traditional Zulu medicine for treatment of an infectious nature were screened for antibacterial activities. Most of the activity detected was against gram-positive bacteria. Tuber extracts of *Dioscorea sylvatica* had activity against gram-negative *Escherichia coli* and extracts from *Dioscorea dregeond, Cheilanthes viridis* and *Veronia colorata* were active against *Pseudomonas aeruginosa*.

Samy and Ignacimuthu (2000) studied the antibacterial activity of 30 Indian folklore medicinal plants used by tribal healers to treat infections by using disc diffusion method against *Bacillus subtilis, Escherichia coli, Klebsiella aerogenes* and *Staphylococcus aureus*. Twenty plant species showed activity against one or more species of bacteria used in this assay. Among them the leaf extract of *Cassia occidentalis* and *Cassia auriculata* exhibited significant broad spectrum activity against *Bacillus subtilis* and *Staphylococcus aureus.*

Sairam *et al.* (2002) evaluated the anti-diarrheal activity of seed extracts of *Mangifera indica*. The *in vitro* antimicrobial activity of Methanolic (MMI) and aqueous (AMI) extracts showed variable results, while AMI significantly inhibit the growth of *Streptococcus aureus* and *Proteus vulgaris.* Both MMI and AMI did not show any significant effect on growth of *Escherichia coli* and *Klebsiella* sp. The results illustrate that the extracts of *Mangifera indica* have significant anti-diarrheal activity.
Dagmar *et al.* (2003) studied the antimicrobial activity of crude ethanolic extracts of

10 medicinal plants used in traditional Chinese medicine. The plants were tested against five species of microorganisms: *Bacillus cereus, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida albicans*. Among the ten plants tested, five showed antimicrobial activity against one or more species of microorganisms. The most active antimicrobial plants were *Chelidonium majus, Sanguisorba officinalis*, and *Tussilago farfara*.

Bonjar (2004) studied traditional medicinal plants used by Iranian people and showed antibacterial activity against eleven different strains of bacteria along with *Bacillus aureus, Bacillus pumilus, Escherichia coli, Pseudominas aeruginosa* and *Staphylococcus aureus*. The species as *Trachyspermum ammi, Lawsonia inermis* and *Cuminum cyminum* were active against seven different strains of bacteria. *Cuscuta epithymum, Mentha longifolia, Malva sylvestris, Smilax china* were active against only one strain of bacteria.

Mahato and Chaudhary (2005) documented 25 plant species of Palpa district, Nepal for their ethnomedicinal uses and screened for their antibacterial activity. The disk diffusion method was used to test the antibacterial activity against four strains of bacteria *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. Out of the 25 plant species, the extracts of 13 species (52%) showed positive response against at least one of the tested bacteria, while the extracts of 11 species (44%) showed positive response against at least two bacteria. Similarly the extracts of 10 species (40%) showed positive response against three bacteria and nine species (36%) showed positive response against all of the four tested bacteria. However the extracts from 12 plant species showed no such antibacterial activity against any of the four strains of tested bacteria. They constitute about 48% of the total tested plant species.

Nair *et al.* (2005) screened nine plants for potential antibacterial activity by agar disc diffusion and agar ditch diffusion method. In evaluating antibacterial activity both aqueous and organic solvents were used. The plants screened were *Sapindus emarginatus, Hibiscus rosa-sinensis, Mirabilis jalapa, Rheo discolor, Nyctanthes arbortristis, Colocasia esculenta, Gracilaria corticata* and *Dictyota* sp. The test organisms employed were *Pseudomonas testosterone, Staphylococcus epidermidis, Klebsiella pneumoniae, Bacillus subtilis, Proterus morganii* and *Micrococcus flavus.*

*Pseudomonas testosterone* and *Klebsiella pneumoniae* were the most resistant bacterial strains. *Sapindus emarginatus* showed strong activity against the tested bacterial strains.

Adwan *et al.* (2006) studied the antibacterial effects of single and combined plant extracts of water, ethanol and methanol for two nutraceuticals utilized in Palestine were studied against multiple drug resistances *Pseudomonas aeruginosa* using agar well diffusion method. These plants were *Rhus coriaria* and *Thymus vulgaris*. Combination of these extracts showed an additive action against this pathogen.

Panthi and Chaudhary (2006) tested eighteen plant species used in folklore medicine in west Nepal for their antibacterial activity by the disk diffusion method. The bacteria employed were gram-positive *(Staphylococcus aureus)* and gram-negative *(Escherichia coli, Pseudomonas aeruginosa* and *Shigella boydii).* Extracts of eight plants showed encouraging result against three strains of bacteria, while other showed activity against one or two strains.

Raghavendra *et al.* (2006) tested *Oxalis corniculata* for antibacterial activity against three important pathovars of *Xanthomonas* and 14 human pathogenic bacteria. Different solvents were used viz. petroleum, ether, benzene, chloroform, methanol and ethanol. Among five solvent tested, methanol and ethanol extracts showed significant antibacterial activity when compared with K-cycline and Bact-805 for plant pathogens, Gentamicin and Streptomycin for human pathogens. Phytochemical analysis of the leaf material revealed that the antibacterial activity of the plant material is because of the presence of phenolic compounds.

Chanda and Nair (2007) screened ten medicinal plants for antibacterial activity by using both agar disc diffusion and agar well diffusion method against bacterial strains *Pseudomonas aeruginosa, Proteus mirabilis, Starhylococcus aureus, Bacillus cereus, Alcaligenes faecalis* and *Salmonella typhimurium*. The ethanol extract were more potent than aqueous extracts of all plants. The ethanol extracts of *Emblica offacinalis* showed strong activity against all tested bacteria and *Commiphora wightii, Hibiscus cannabinus, Anethum gravelon, Ficus religiosa, Ficus benghalensis, Ficus tisela, Mentha arvensis* and *Mimusops elengi* showed moderate activity against bacterial strain.

Parekh and Chanda (2007) screened thirty-four medicinal plants for potential antibacterial activity against six bacterial strains belonging to Enterobacteriaceae, viz. *Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris*, and *Salmonella typhimurium.* Antibacterial activity of aqueous and alcoholic extracts was tested by the agar disc diffusion and agar well diffusion methods. The ethanol/methanol extracts were more active than aqueous extracts for all the plants studied. The most susceptible bacterium was *K. pneumonia*e, while the most resistant bacteria were *S. typhimurium* and *E. col*i. From the screening experiment, *Woodfordia fruticosa* showed best antibacterial activity.

Uma Devi *et al.* (2007) evaluate the antibacterial efficiency of *Achyranthes bidentata* against seven different bacterial strains. *Escherichia coli, Bacillus subtilis, Proteus vulgaris, Salmonella typhi and Klebsiella pneumoniae.* All the extract of root, stem, leaves and flowers showed high sensitive to *Proteus vulgaris, Escherichia coli, Bacillus subtilis, Salmonella typhii and Klebsiella pneumonia* but moderate and less sensitive to *Staphylococcus aureus* and *Pseudomonas* species.

Adomi (2008) carried out the screening of leaves of three Nigerian medicinal plants *Alstonia boonei, Morinda lucida* and *Petiveria alliacea,* and latex of *A. boonei* for antibacterial activity. In evaluating antibacterial activity, both aqueous and ethanol extracts of the plants were used. Agar well diffusion method was used to determine the antibacterial activity of the plants. Among the bacteria screened, *Pseudomonas aeruginosa* was the most resistant bacteria strain, while *Flavobacterium* sp., the most susceptible one. *M. lucida* extract was active against all the tested bacteria. The latex of *A. boonei* was not active against any of the bacteria tested.

Mahesh and Satish (2008) studied the Methanol leaf extracts of *Acacia molotica, Sida cardifolia, Tinospora cardifolia, Withania somnifera* and *Ziziphus mauritiana* showed significant antibacterial activity against *Bacillus subtilis, E. coli, Pseudomonas fiuorescens, Staphylococcus aureus.* Rootbar extract of *Acacia molotica* and *Sida cardifolia* leaf extracts showed highest antibacterial activity against *Bacillus subtilis.* Root and leaf extracts of *Sida cardifolia* recorded significant activity against all the tested bacteria.

Shakya *et al.* (2008) studied the alcoholic extract of 215 medicinal plants for their

antimicrobial properties, among these 144 plant species were found to be moderately active against seven microorganisms and 20 species showed encouragingly active against six microorganisms. *Rheum australe* showed meditatively active against five microorganisms. The plants *Andrographis paniculata*, *Berbaris aristata*, *Campylandra aurntica*, *Rheum australe,* and *Cinnamomum tamala* showed antimicrobial activity against four microbes. These plants may have effective broad spectrum antimicrobial phyto-compounds. Only *Compylandra aurntica* showed encouraging activity against fungi.

Rajendran and Ramkrishnan (2009) studied the antibacterial activity of aqueous and methanol extracts of some medicinal plants were screened against bacteria including *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli*. The methanol extracts of *Withania somnifera* showed the highest antibacterial activity as compared to other plant extract tasted. The MIC of methanol extract of *Withania somnifera* was in the range of 50 to 100 mg/ml.

Barbour *et al.* (2011) tested the *in vitro* antimicrobial efficiency of 39 water and methanol extract each derived from the different parts of 27 indigenous wild plant species that have been commonly employed in Lebanese folk medicine. The antibacterial efficiency was determined using the single disc diffusion method, with 10 and 20µl load extract volume per disc. Alcoholic extracts were more effective than aqueous extracts and the activity also differ with difference in their ecological habitat. The change to find antimicrobial activity was apparent in methanol rather than water extracts of the same indigenous plants of Lebanon.

# CHAPTER-III

# MATERIALS AND METHODS

## 3.1 Sample Material Preparation

### 3.1.1 Selection of Medicinal Plants

Twenty two different medicinal plants were selected on the basis of the plant materials used in different bacterial diseases like-diarrhea, dysentery, pneumonia, cholera, typhoid, cut and wounds etc. The plants were only selected if the same plant is referred in at least three different literatures. The description and uses of these plants are provided in Appendix-B.

### 3.1.2 Plant Identification

The plants were identified and authentication was done with the help of literature and comparing the herbarium specimens deposited on TUCH.

### 3.1.3 Collection, drying and packaging of plant materials

Fresh plant or plant parts were collected from different places of Nepal (Kathmandu, Lalitpur, Kaski, Rupandhei, Rasuwa, Darchula, Kanchanpur and Syanjya) during August and September 2008. Fresh plant materials were washed with the help of tap water and allowed for air dry for few days. Hard parts like stems, roots, and barks were chopped into small pieces. These plant materials were spread under shade on the blotting paper till they become completely dry. The completely dried samples of each plant parts were ground separately into fine powder with the help of electric grinder. The completely dry powder samples were stored in zipper plastic bags until laboratory analysis. Exposure to sunlight was avoided to prevent the loss of active components.

## 3.2 Antibacterial Test

Inhibition of bacterial growth (*in-vitro*) was tested by using the paper disc diffusion method (Bauer *et al*. 1966, Parekh and Chanda 2007) with some modifications considering the access and availability of equipments and chemicals.

### 3.2.1 Preparation of Extract

Two grams of ground material was soaked in 25ml of methanol for 24 hours and filtered using standard filter paper (Whatsman no. 1). The residue was soaked again with 25mL fresh methanol and filtered after 24 hours. Same process was repeated once again. The extract after treating with 75ml (25ml x3 times) methanol was then filtered. The filtrate was transferred into beakers and allowed to evaporate until completely dry. Once dry, the extract was re-suspended in 2ml of methanol. The concentration of the final extract was 1g material/1ml.

### 3.2.2 Collection of Test Organisms

The microbial strains employed were identified strains that were obtained from Sahid Sukraraj Hospital, Teku. The studied strains include seven different types of bacteria, two gram-positive *(Streptococcus pneumoniae* and *Staphylococcus aureus*) and five gram-negative (*Escherichia coli, Klebsiella pneumonia, Vibrio cholera, Shigella flexneri,* and *Salmonella typhi*). They were taken on slants and later cultured on petri plates having nutrient agar. The description of bacteria and their pathogenicity are given in Appendix-C.

### 3.2.3 Preparation of the Test Discs

Sterile test discs were prepared by dipping and saturating sterilized filter paper discs in plant extract. Same sized filter paper discs (6mm diameter), made by cutting the Whatsman no. 1 filter paper with the help of punching machine, and absorbed the same volume of extract. For negative control methanol paper discs were used, prepared by dipping the disc into the methanol.

### 3.2.4 Preparation of Culture Media

**3.2.4.1 Nutrient Agar:** Nutrient agar was prepared with the help of manufactures (Hi-media) recommendations. Twenty eight gram of nutrient agar was weighed and dissolved in distilled water to make final volume of 1000ml. It was sterilized by autoclaving the media inside the round bottomed flask at 15lb pressure and 121ºC for 15 minutes. It was then cooled to 50ºC. About 20ml of media was poured to sterilized petri plates aseptically and labeled properly. For the slant preparation, the required

amount of media was poured in appropriate sized screw capped bottle, autoclaved and cooled in tilted position to make slant.

**3.2.4.2 Nutrient Broth:** Nutrient broth was also prepared with the help of manufactures (Hi-media) recommendations. Thirteen gram of powder was weighed and dissolved in distilled water to make final volume of 1000ml. It was sterilized by autoclaving at 15lb pressure and 121ºC for 15 minutes inside the conical flask. It was cooled and 10ml of it was poured inside the suitable sized screw capped bottle and again sterilized.

### 3.2.5 Preparation of Standard Culture Inoculums

Three to five colonies of similar appearance of the organism to be tested were aseptically touched with the help of inoculating loop from primary culture plate. It was transferred to a tube containing 10ml sterile liquid media of nutrient broth. The tube was incubated overnight inside the incubator at 37ºC.

### 3.2.6 Transfer of Bacteria on Petri Plates

The agar plates for the assay were prepared by labeling them with the date, the name of bacteria and the name code of the discs. The inoculums of bacteria were transferred into petri dish containing solid nutrient media of agar using sterile swab. The sterile cotton swab was dipped into a well mixed saline test culture and removed excess inoculums by pressing the saturated swab against the inner wall of the culture tube. The swab was used to spread the bacteria on the media in a confluent lawn. It was done by rotating the petri plates at 90º and continuing the spread of bacteria. One swab was used for one species of bacteria. The culture plates were allowed to dry for five minutes.

### 3.2.7 Placing Test Discs

Dried test discs were transferred on bacterial lawn under aseptic conditions using flame-sterilized forceps each time. Each disc was placed gently on the agar surface on equidistance and patted with the forceps to ensure the disc adhere to the surface of agar. The petri plates were incubated in an inverted position for 24 hours at 37ºC.

### 3.2.8 Observation of Result

After 24 hour of incubation at 37ºC, results were recorded as the presence or absence of inhibition zones. Resulting zones of inhibition were observed and recorded as “+” and “-”. The diameter of zone of inhibition (ZOI) produced by plant extract on particular bacteria was also measured with the help of millimeter ruler. The inhibitory zone around test paper discs indicates absence of bacterial growth and that was recorded as positive and absence of zone as negative. Tests were repeated three times to insure the reliability of the results. Mean value of positive (+) and negative (-) standard deviation of three determinations were calculated.

# CHAPTER-IV

# RESULTS

## 4.1 Screening of Medicinal Plants for Antibacterial Activity

It is revealed that all the tested medicinal plants were effective against the employed bacteria (Table 1). Among twenty two medicinal plants tested, in the present study, all plants show activity against at least one bacteria in aqueous solution (Table 1) and at least two bacteria in methanolic solution (Table 2). *Rubus nepalensis* and *Rumex nepalensis* inhibited all the tested bacteria in aqueous solution. Similarly, *Epilobium roseum* inhibited all the tested bacteria in methanolic solution. The medicinal plants like *Murraya koenigii* and *Smilex aspara* were effective against 85.71% of tested bacteria in aqueous solution and the plants like *Astilbe rivularis, Gaultheria fragrantissima, Rubia manjith, Rumex nepalensis,* and *Murraya koenigii* were effective against 85.71% of tested bacteria in methanol solution. Four plants such as *Epilobium roseum, Rubia manjith, Justicia adhatoda* and *Aloe vera* inhibit the growth of 71.43% of tested bacteria in aqueous solution and six plants *Rubus nepalensis, Lobelia pyramidalis, Meconopsis horridula, Smilex aspara, Justicia adhatoda* and *Aloe vera* were effective against 71.43% of screened bacteria in methanol solutions (Table 3). Similarly, only two plants such as *A. rivularis* and *Paris polyphyla* plants inhibited the growth of 57.14% of tested bacteria in methanol extract and two plants such as *Digitalis purpurea* and *Berginia ciliata* inhibited growth of 57.14% of tested bacteria in methanol extract. Likewise, seven plants *Dioscorea bulbifera, Gaultheria fragrantissima, Daphne bholua, Digitalis purpurea, Meconopsis horridula* and *Bergenia ciliata* inhibit the growth of 42.86% of tested bacteria in aqueous solution and four plants such as *Dioscorea bulbifera, Stellaria vestita, Mahonia nepalensis* and *Taxus baccata* were effective against 42.86% of screened bacteria in methanol solution. Remaining two plants *Vetiveria zizanoides, Stellaria vestita* and *Taxus baccata* inhibited growth of 28.57% of the screened bacteria in aqueous extracts. *Vetiveria zizanoides, Daphne bholua* and *Paris polyphyla* inhibit the 28.57% of tested bacteria in methanol extract (Table 3). Similarly, *Mahonia nepalensis* inhibit single bacteria in aqueous extracts (Table 1).

**Table 1:** Antibacterial properties of aqueous extracts of different medicinal plants against tested bacteria.

| S.N. | Name of Plants | Sa | St | Ec | Kp | Vc | Sp | Sf |
|---|---|---|---|---|---|---|---|---|
| 1 | *Aloe vera* | + | + | + | + | - | - | + |
| 2 | *Astilbe rivularis* | - | + | - | + | + | - | + |
| 3 | *Bergenia ciliata* | - | + | - | - | - | + | + |
| 4 | *Cuscuta reflexa* | + | + | + | + | - | - | + |
| 5 | *Daphne bholua* | - | + | + | - | - | + | - |
| 6 | *Digitalis purpurea* | + | - | - | + | - | - | + |
| 7 | *Dioscorea bulbifera* | + | + | - | - | - | - | + |
| 8 | *Epilobium roseum* | + | + | - | + | + | - | + |
| 9 | *Gaultheria fragrantissima* | + | + | - | - | - | - | + |
| 10 | *Justicia adhatoda* | + | + | - | + | - | + | + |
| 11 | *Lobelia pyramidalis* | - | - | - | - | + | + | + |
| 12 | *Mahonia nepalensis* | - | - | - | - | - | - | + |
| 13 | *Meconopsis horidula* | - | + | - | - | - | + | + |
| 14 | *Murraya koenigii* | + | + | - | + | + | + | + |
| 15 | *Paris polyphyla* | + | + | - | + | + | - | - |
| 16 | *Rubia manjith* | + | - | + | + | - | + | + |
| 17 | *Rubus nepalensis* | + | + | + | + | + | + | + |
| 18 | *Rumex nepalensis* | + | + | + | + | + | + | + |
| 19 | *Smilex aspara* | + | + | - | + | + | + | + |
| 20 | *Stellaria vestita* | + | - | - | - | - | - | + |
| 21 | *Taxus baccata* | - | + | - | + | - | - | - |
| 22 | *Vetiveria zizanoides* | + | - | - | + | - | - | - |

All the plants show positive result for positive and negative result for negative control. Abbreviations: Sa – *Staphylococcus aureus*, St – *Salmonella typhi*, Ec – *Escherichia coli*, Kp - *Klebsiella pneumonia*, Vc - *Vibrio cholera*, Sp - *Streptococcus pneumonia*, Sf - *Shigella flexneri.*

**Table 2:** Antibacterial properties of methanolic extracts of different medicinal plants against tested bacteria.

| S.N. | Name of Plants | Sa | St | Ec | Kp | Vc | Sp | Sf |
|---|---|---|---|---|---|---|---|---|
| 1 | *Aloe vera* | + | + | + | + | - | - | + |
| 2 | *Astilbe rivularis* | + | + | - | + | + | + | + |
| 3 | *Bergenia ciliata* | - | + | - | + | + | - | + |
| 4 | *Cuscuta reflexa* | + | + | + | + | - | - | + |
| 5 | *Daphne bholua* | - | + | - | - | + | - | - |
| 6 | *Digitalis purpurea* | + | - | - | + | + | - | + |
| 7 | *Dioscorea bulbifera* | - | + | - | - | + | + | - |
| 8 | *Epilobium roseum* | + | + | + | + | + | + | + |
| 9 | *Gaultheria fragrantissima* | + | + | - | + | + | + | + |
| 10 | *Justicia adhatoda* | + | + | - | + | + | + | - |
| 11 | *Lobelia pyramidalis* | - | + | - | + | + | + | + |
| 12 | *Mahonia nepalensis* | - | - | - | + | - | + | + |
| 13 | *Meconopsis horidula* | - | + | + | + | - | + | + |
| 14 | *Murraya koenigii* | + | + | - | + | + | + | + |
| 15 | *Paris polyphyla* | + | - | - | + | - | - | - |
| 16 | *Rubia manjith* | + | + | + | + | - | + | + |
| 17 | *Rubus nepalensis* | + | + | - | + | + | - | + |
| 18 | *Rumex nepalensis* | + | + | + | + | + | - | + |
| 19 | *Smilex aspara* | + | + | - | + | + | - | + |
| 20 | *Stellaria vestita* | + | - | - | - | + | - | + |
| 21 | *Taxus baccata* | - | + | + | - | - | - | + |
| 22 | *Vetiveria zizanoides* | - | + | - | + | - | - | - |

All the plants show positive result for positive and negative result for negative control. Abbreviations: Sa – *Staphylococcus aureus,* St – *Salmonella typhi,* Ec – *Escherichia coli,* Kp - *Klebsiella pneumonia,* Vc - *Vibrio cholera,* Sp - *Streptococcus pneumonia,* Sf - *Shigella flexneri.*

### 4.2 Evaluation of Antibacterial Activity of Medicinal Plants

Among twenty two medicinal plants, three plants such as *Rubus nepalensis, Rumex nepalensis* and *Epolobium roseum* shows antibacterial activity against all seven tested bacteria. Similarly, six medicinal plants such as *Rubia manjith, Murraya koenigii, Astilbe rivularis, Gaultheria fragrantissima, Smilex aspara* and *Justicia adhatoda* inhibited the growth of six bacteria out of seven tasted bacteria. *Dioscorea bulbifera, Lobelia pyramidalis, Aloe vera, Meconopsis horidula, Cuscuta reflexa* and *Bergenia ciliate* inhibited the growth of five bacteria among seven tested bacteria. Likewise, four medicinal plants such as *Daphne bholua, Digitalis purpurea, Paris polyphyla* and *Taxus baccata* inhibited growth of four bacteria out of seven bacteria tasted. Finally, three medicinal plants such as *Vetiveria zizanoides, Stellaria vestita* and

*Mahonia nepalensis* showed antibacterial activity against three bacteria out of seven bacteria tested.

Two medicinal plants viz. *Rubus nepalensis*, and *Rumex nepalensis* had shown broad spectrum activity for aqueous extract and only one medicinal plants viz. *Epilobium roseum* had shown broad spectrum activity for methanolic extract. All these plants inhibited the growth of all seven tasted bacteria. Similarly *Epilobium roseum, Rubia manjith, Cuscuta reflexa, Justicia adhatoda* and *Aloe vera* inhibited the growth of five bacteria out of all tasted bacteria in aqueous solutions. Likewise, *Astilbe rivularis, Gaultheria fragrantissima, Rubia manjith, Rumex nepalensis* and *Murraya koenigii* inhibited six bacteria against all tested bacteria in Methanolic extract. Similarly, *Rubus nepalensis, Lobelia pyramidalis, Meconopsis horidula Smilex aspara, Cuscuta reflexa, Justicia adhatoda* and *Aloe vera* inhibits growth of five bacteria against all tested bacteria.

*Cuscuta reflexa* showed the *strongest* ZOI (1.6cm) against two tested bacteria like *Escherichia coli* and *Staphylococcus aureus* in aqueous solutions. *Cuscuta reflexa* also showed the strongest ZOI (2.0cm) against *Staphylococcus aureus* in methanolic solution.

The screened medicinal plants like *Dioscorea bulbifera* and *Smilex aspara* showed weakest ZOI (0.3cm) against *Staphylococcus aureus*, and *Vibrio cholera* for aqueous solutions and single species like *Daphne bholua* showed weakest ZOI (0.3cm) towards *Vibrio cholera* for methanolic extract.

### 4.3 Evaluation of Susceptibility of tested bacteria

*Escherichia coli* showed resistance towards aqueous extract of sixteen medicinal plants out of twenty two medicinal plants. Similarly *Vibrio cholera* showed resistance towards aqueous extract of fourteen medicinal plants, *Streptococcus pneumonia* showed resistance towards aqueous extract of twelve medicinal plants, *Klebsiella pneumonia* showed resistance towards aqueous extract of eight medicinal plants, *Staphylococcus aureus* showed resistance towards aqueous extract of seven medicinal plants, *Salmonella typhi* showed resistance towards aqueous extract of six medicinal plants and *Shigella flexneri* showed resistance towards aqueous extract of only four medicinal plants out of twenty two medicinal plants.

*Escherichia coli* showed resistance towards methanolic extract of fifteen medicinal plants out of twenty two medicinal plants. Similarly, *Streptococcus pneumonia* showed resistance towards methanolic extract of twelve medicinal plants, *Vibrio cholera* and *Staphylococcus aureus* showed resistance towards methanolic extract of eight medicinal plants, *Shigella flexneri* showed resistance towards methanolic extract of five medicinal plants and *Salmonella typhi* and *Klebsiella pneumonia* showed resistance towards methanolic extract of only four medicinal plants out of twenty two medicinal plants.

The growth of *Staphylococcus aureus* was inhibited by fifteen medicinal plants and maximum inhibition was observed with *Cuscuta reflexa* as 1.6cm zone of inhibition (ZOI) followed by *Rumex nepalensis* and *Stellaria vestita* (0.9cm), *Epilobium roseum, Rubus nepalensis, Paris polyphyla* and *Justicia adhatoda* (0.8cm). In contrast, lowest inhibition was observed with *Dioscorea bulbifera* with ZOI of 0.3cm. Similarly *Staphylococcus aureus* was inhibited by methanolic extract of fourteen plants. The maximum inhibition of *Staphylococcus aureus* was observed with *Cuscuta reflexa* as 2.0cm zone of inhibition (ZOI) followed by *Epilobium roseum* (1.4cm), *Paris polyphyla*(1.2cm), *Gaultheria fragrantissima*, *Digitalis purpurea* and *Aloe vera* (1.0cm). In contrast, lowest inhibition was observed with *Rumex nepalensis* with zone of inhibition (ZOI) of 0.5cm.

The growth of *Salmonella typhi* was inhibited by aqueous extract of sixteen medicinal plants and maximum inhibition was observed with *Murraya koenigii* as 1.1cm zone of

**Table 3**: Number of microorganisms inhibited by tested medicinal plants.

| S.N. | Name of Plants | No. of Bacteria inhibited in aqueous extract | % of Bacteria inhibited in aqueous extract | No. of Bacteria inhibited in methanol extract | % of Bacteria inhibited in methanol extract | Total No. of Bacteria inhibited | % of Bacteria inhibited |
|---|---|---|---|---|---|---|---|
| 1 | Aloe vera | 5 | 71.43 | 5 | 71.43 | 5 | 71.43 |
| 2 | Astilbe rivularis | 4 | 57.14 | 6 | 85.71 | 6 | 85.71 |
| 3 | Bergenia ciliate | 3 | 42.86 | 4 | 57.14 | 5 | 71.43 |
| 4 | Cuscuta reflexa | 5 | 71.43 | 5 | 71.43 | 5 | 71.43 |
| 5 | Daphne bholua | 3 | 42.86 | 2 | 28.57 | 4 | 57.14 |
| 6 | Digitalis purpurea | 3 | 71.43 | 4 | 57.14 | 4 | 57.14 |
| 7 | Dioscorea bulbifera | 3 | 42.86 | 3 | 42.86 | 5 | 71.43 |
| 8 | Epilobium roseum | 5 | 71.43 | 7 | 100 | 7 | 100 |
| 9 | Gaultheria fragrantissima | 3 | 42.86 | 6 | 85.71 | 6 | 85.71 |
| 10 | Justicia adhatoda | 5 | 71.43 | 5 | 71.43 | 6 | 85.71 |
| 11 | Lobelia pyramidalis | 3 | 42.86 | 5 | 71.43 | 5 | 71.43 |
| 12 | Maconopsis horidula | 3 | 42.86 | 5 | 71.43 | 5 | 71.43 |
| 13 | Mahonia nepalensis | 1 | 14.29 | 3 | 42.86 | 3 | 42.86 |
| 14 | Murraya koenigii | 6 | 85.71 | 6 | 85.71 | 6 | 85.71 |
| 15 | Paris polyphyla | 4 | 57.14 | 2 | 28.57 | 4 | 57.14 |
| 16 | Rubia manjith | 5 | 85.71 | 6 | 85.71 | 6 | 85.71 |
| 17 | Rubus nepalensis | 7 | 100 | 5 | 71.43 | 7 | 100 |
| 18 | Rumex nepalensis | 7 | 100 | 6 | 85.71 | 7 | 100 |
| 19 | Smilex aspara | 6 | 85.71 | 5 | 71.43 | 6 | 85.71 |
| 20 | Stellaria vestita | 2 | 28.57 | 3 | 42.86 | 3 | 42.86 |
| 21 | Taxus baccata | 2 | 28.57 | 3 | 42.86 | 4 | 57.14 |
| 22 | Vetiveria zizanoides | 2 | 28.57 | 2 | 28.57 | 3 | 42.86 |

**Table 4**: Mean zone of inhibition (ZOI) shown by different medicinal plants against tested bacteria (Mean ± S.D.)

| | | SA | | ST | | EC | | KP | | VC | | SP | | SF | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SN** | ***Name of Species*** | **A** | **M** | **A** | **M** | **A** | **M** | **A** | **M** | **A** | **M** | **A** | **M** | **A** | **M** |
| 1 | *Astilbe rivularis* | 0.00 | 0.9± 0.1 | 0.6±0.1 | 0.9±0.2 | 0 | 0.00 | 0.7±0.2 | 1.2±0.2 | 0.3±0.1 | 1.0±0.2 | 0.00 | 1.0±0.2 | 0.6±0.1 | 1.3±0.2 |
| 2 | *Aloe vera* | 0.7±0.1 | 1.0±0.0 | 0.9±0.2 | 1.7±0.1 | 0.8±0.1 | 0.6±0.0 | 0.6±0.2 | 0.5±0.2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.9±0.1 | 1.2±0.1 |
| 3 | *Bergenia ciliata* | 0.00 | 0.00 | 0.8±0.0 | 0.6±0.1 | 0 | 0.00 | 0.00 | 0.6±0.1 | 0.00 | 0.9±0.1 | 0.5±0.1 | 0.00 | 0.9±0.1 | 1.5±0.1 |
| 4 | *Cuscuta reflexa* | 1.6±0.2 | 2.0±0.1 | 0.7±0.1 | 0.9±0.2 | 1.6±0.2 | 1.9±0.2 | 0.9±0.2 | 0.7±0.2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.6±0.0 | 0.7±0.1 |
| 5 | *Daphne bholua* | 0.00 | 0.00 | 0.6±0.2 | 0.5±0.2 | 0.4±0.2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.3±0.0 | 0.6±0.1 | 0.00 | 0.00 | 0.00 |
| 6 | *Digitalis purpurea* | 0.6±0.1 | 1.0±0.1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.7±0.2 | 0.7±0.1 | 0.00 | 0.8±0.1 | 0.00 | 0.00 | 0.7±0.0 | 0.7±0.1 |
| 7 | *Dioscorea bulbifera* | 0.3±0.1 | 0.00 | 0.7±0.1 | 0.8±0.2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.6±0.0 | 0.00 | 0.6±0.1 | 1.0±0.4 | 0.00 |
| 8 | *Epilobium roseum* | 0.8±0.2 | 1.4±0.1 | 1.0±0.2 | 1.1±0.3 | 0.00 | 1.3±0.2 | 0.7±0.1 | 0.9±0.2 | 0.4±0.0 | 1.0±0.2 | 0.00 | 1.0±0.1 | 0.9±0.1 | 1.9±0.3 |
| 9 | *Gaultheria fragrantissima* | 0.4±0.2 | 1.0±0.2 | 0.7±0.2 | 0.9±0.2 | 0.00 | 0.00 | 0.00 | 0.8±0.2 | 0.00 | 0.9±0.1 | 0.00 | 0.7±0.2 | 0.8±0.2 | 1.1±0.1 |
| 10 | *Justicia adhatoda* | 0.8±0.1 | 0.7±0.2 | 0.6±0.2 | 0.5±0.1 | 0.00 | 0.00 | 0.7±0.2 | 1.2±0.2 | 0.00 | 0.8±0.2 | 1.0±0.2 | 1.2±0.2 | 0.5±0.0 | 0.00 |
| 11 | *Lobelia pyramidalis* | 0.00 | 0.00 | 0.00 | 0.9±0.2 | 0.00 | 0.00 | 0.00 | 1.0±0.1 | 0.7±0.2 | 0.8±0.1 | 0.7±0.2 | 0.7±0.2 | 0.7±0.1 | 1.0±0.1 |
| 12 | *Maconopsis horidula* | 0.00 | 0.00 | 0.8±0.2 | 0.7±0.1 | 0.00 | 0.7±0.2 | 0.00 | 0.8±0.1 | 0.00 | 0.00 | 0.8±0.1 | 0.9±0.1 | 0.9±0.2 | 0.6±0.1 |
| 13 | *Mahonia nepalensis* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.9±0.2 | 0.00 | 0.00 | 0.00 | 0.6±0.1 | 0.8±0.1 | 0.7±0.1 |
| 14 | *Murraya koenigii* | 0.7±0.1 | 0.9±0.2 | 1.1±0.2 | 1.4±0.2 | 0.00 | 0.00 | 0.7±0.1 | 1.5±0.1 | 0.9±0.0 | 0.7±0.0 | 0.6±0.2 | 0.7±0.1 | 0.7±0.2 | 0.8±0.2 |
| 15 | *Paris polyphyla* | 0.8±0.1 | 1.2±0.1 | 0.5±0.2 | 0.00 | 0.00 | 0.00 | 0.8±0.2 | 0.9±0.1 | 0.6±0.1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16 | *Rubia manjith* | 0.6±0.2 | 0.6±0.1 | 0.00 | 0.6±0.1 | 0.7±0.2 | 0.7±0.2 | 0.8±0.1 | 0.8±0.2 | 0.00 | 0.00 | 0.8±0.1 | 0.7±0.2 | 0.9±0.0 | 0. 7±0.2 |
| 17 | *Rubus nepalensis* | 0.8±0.2 | 0.7±0.1 | 0.8±0.2 | 0.8±0.2 | 0.7±0.2 | 0.00 | 1.0±0.2 | 0.7±0.1 | 0.9±0.2 | 0.8±0.0 | 0.5±0.0 | 0.00 | 0.8±0.1 | 0.9±0.2 |
| 18 | *Rumex nepalensis* | 0.9±0.2 | 0.5±0.2 | 0.6±0.2 | 0.7±0.2 | 1.0±0.2 | 0.9±0.2 | 0.9±0.2 | 0.8±0.1 | 1.0±0.2 | 0.8±0.2 | 0.7±0.1 | 0.00 | 1.1±0.1 | 0.9±0.2 |
| 19 | *smilex aspara* | 0.7±0.2 | 0.6±0.2 | 0.7±0.2 | 0.6±0.0 | 0.00 | 0.00 | 0.9±0.0 | 0.7±0.2 | 0.8±0.1 | 0.6±0.1 | 0.3±0.1 | 0.00 | 0.8±0.1 | 0.7±0.2 |
| 20 | *Stellaria vestita* | 0.9±0.2 | 0.7±0.0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.7±0.1 | 0.00 | 0.00 | 1.1±0.1 | 0.9±0.2 |
| 21 | *Taxus baccata* | 0.00 | 0.00 | 0.4±0.2 | 1.0±0.2 | 0.00 | 0.7±0.2 | 0.8±0.1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.0±0.1 |
| 22 | *Vetiveria zizanoides* | 0.4±0.1 | 0.00 | 0.00 | 1.0±0.0 | 0.00 | 0.00 | 0.7±0.2 | 0.7±0.1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Abbreviations: A- Aqueous, M- Methanolic, SA – *Staphylococcus aureus*, ST – *Salmonella typhi*, EC – *Escherichia coli*, KP-*Klebsiella pneumonia*, VC-*Vibrio cholera*, SP-*Streptococcus pneumonia*, SF-*Shigella flexneri*.

inhibition followed by *Epilobium roseum* (1.0cm), *Aloe vera* (0.9cm), *Rubus nepalensis, Maconopsis horidula* and *Bergenia ciliate* (0.8cm). In contrast, lowest inhibition was observed with *Taxus baccata* with ZOI of 0.4cm. Similarly, *Salmonella typhi* was inhibited by methanolic extract of eighteen medicinal plants. The maximum inhibition was observed with *Aloe vera* as 1.7cm zone of inhibition followed by *Murraya koenigii* (1.4cm), *Epilobium roseum* (1.1cm), *Vetiveria zizanoides*, and *Taxus baccata* (1.0cm). In contrast, lowest inhibition was observed with *Justicia adhatoda* and *Daphne bholua* with ZOI of 0.5cm.

The growth of *Escherichia coli* was inhibited by aqueous extract of six medicinal plants and maximum inhibition was observed with *Cuscuta reflexa* as 1.6cm zone of inhibition followed by *Rumex nepalensis* (1.0), *Aloe vera* (0.8cm), *Rubia manjith* and *Rubus nepalensis* (0.7cm). On the other hand, lowest inhibition was observed with *Daphne bholua* with ZOI of 0.4cm. Similarly, *Escherichia coli* was inhibited by methanolic extract of seven medicinal plants. The maximum inhibition was observed with *Cuscuta reflexa* as 1.9cm zone of inhibition followed by *Epilobium roseum* (1.3cm), *Rumex nepalensis* (0.9cm). Similarly, the lowest inhibition was observed with *Aloe vera* with ZOI of 0.6cm.

*Klebsiella pneumonia* was inhibited by aqueous extract of fourteen medicinal plants and maximum inhibition was observed with *Rubus nepalensis* with 1.0cm zone of inhibition followed by *Rumex nepalensis, smilex aspara* and *Cuscuta reflexa* (0.9), *Rubia manjith, Paris polyphyla* and *Taxus baccata* (0.8cm), *Astilbe rivularis, Vetiveria zizanoides, Epilobium roseum, Digitalis purpurea, Murraya koenigii* and *Justicia adhatoda* (0.7cm). On the other hand, lowest inhibition was observed with *Aloe vera* with ZOI of 0.6cm. Similarly, *Klebsiella pneumonia* was inhibited by methanolic extract of eighteen medicinal plants. The maximum inhibition was observed with *Murraya koenigii* as 1.5cm zone of inhibition followed by *Astilbe rivularis* and *Justicia adhatoda* (1.2cm), *Lobelia pyramidalis* (1.0cm). Similarly, the lowest inhibition was observed with *Aloe vera* with ZOI of 0.5cm.

The growth of *Vibrio cholera* was inhibited by aqueous extract of eight medicinal plants and maximum inhibition was observed with *Rumex nepalensis* with 1.0cm zone of inhibition followed by *Murraya koenigii* (0.9), *smilex aspara* (0.8cm). In contrast, lowest inhibition was observed with *Astilbe rivularis* with ZOI of 0.3cm. Similarly,

*Vibrio cholera* was inhibited by methanolic extract of fourteen medicinal plants. The maximum inhibition was observed with *Astilbe rivularis* and *Epilobium roseum* with 1.0cm zone of inhibition followed by *Gaultheria fragrantissima* and *Bergenia ciliate* (0.9cm). Similarly, the lowest inhibition was observed with *Daphne bholua* with ZOI of 0.3cm.

*Streptococcus pneumonia* was inhibited by aqueous extract of ten medicinal plants and maximum inhibition was observed with *Justicia adhatoda* as 1.0cm zone of inhibition followed by *Rubia manjith* and *Meconopsis horidula* (0.8cm). Besides that, lowest inhibition was observed with *smilex aspara* with ZOI of 0.3cm. In addition, *Streptococcus pneumonia* was inhibited by methanolic extract of ten medicinal plants. The maximum inhibition was observed with *Justicia adhatoda* as 1.2cm zone of inhibition followed by *Epilobium roseum* and *Astilbe rivularis* (1.0cm). Similarly, the lowest inhibition was observed with *Dioscorea bulbifera* and *Mahonia nepalensis* with ZOI of 0.6cm.

The growth of *Shigella flexneri* was inhibited by aqueous extract of eighteen medicinal plants and maximum inhibition was observed with *Rumex nepalensis* and *Stellaria vestita* with 1.1cm zone of inhibition followed by *Dioscorea bulbifera* (1.0cm). In contrast, lowest inhibition was observed with *Justicia adhatoda* with ZOI of 0.5cm. Similarly, *Shigella flexneri* was inhibited by methanolic extract of seventeen medicinal plants. The maximum inhibition was observed with *Epilobium roseum* with 1.9cm zone of inhibition followed by *Bergenia ciliata* (1.5cm). Similarly, the lowest inhibition was observed with *Maconopsis horidula* with ZOI of 0.6cm.

Among seven tested bacteria, *Escherichia coli* were found to be the most resistant bacteria and it was susceptible to extract of only nine medicinal plants. Similarly, *Shigella flexneri*, *Klebsiella pneumonia* and *Salmonella typhi* were found to be the most susceptible bacteria and they were inhibited by extract of nineteen medicinal plants.

# CHAPTER-V

# DISCUSSION

## 5.1 Significance of Antibacterial test

Plants and plant based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments (Abraham 1981). The discovery of medicinal plants has usually depended on the experience of the laymen based on long and dangerous self experiment. Progress over the centuries towards understanding of a plant derived medicine has depended on two factors that have gone hand in hand. One has been the development of increasing strict criteria of proof that a medicine really does what it is claimed to do and the other has been the identification by chemical analysis of the active compound in the plant (Holiman 1989).

Different extracts from traditional medicinal plants have been tested to identify the sources of the therapeutic effects. As a result, some natural products have been approved as new antibacterial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance. Continued further exploration of plant-derived antimicrobials is needed. Now a day, a renewed interest in traditional medicine is observed and there has been an increasing demand for more and more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that “green medicine” is safe and more dependable than the costly synthetic drugs many of which have adverse side effects (Parekh and Chanda 2006).

In the present study, an attempt has been made to evaluate the antibacterial activities of 22 medicinal plants which have been traditionally used in various therapies in Nepal. The Zone of inhibition was determined by disc diffusion method (Bauer *et al.* 1966, Parekh and Chanda 2007). The seven different strains of bacteria were used to test the antibacterial activity of crude aqueous and methanol extract of selected plants. Among seven strains of tested bacteria, two strains were Gram positive viz. *Staphylococcus aureus* and *Streptococcus pneumonia* and five Gram negative bacteria viz. *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumonia*, *Vibrio cholera* and *Shigella flexneri*.

### 5.2 Extraction of Medicinal Plants

Successive isolation of phytochemicals from plant material is largely dependent on the type of solvent used in extraction procedures. Different extracts are used to test the antimicrobial properties of medicinal plants. The traditional healers use primarily water as the solvents. But a number of researchers found that plant extracts prepared with methanol and ethanol as solvent provided more consistent antimicrobial activity (Allero and Afolagan 2006, Parekh and Chanda 2007). Alcohol extracts provide a more complete extraction, including less polar compounds, and many of these extracts have been found to possesses antimicrobial properties (Ali-Shtayeh and Abu Ghdeib 1999). The methanol solvent is known with its ability to isolate more antimicrobials from plants including tannins, polyphenols, terpenoids, saponins, xanthoxyllines, totarol, quassinoids, lactones, flavones and phenones; while the water solvent extracts contain only anthocyanins, starches, tannins, saponins, polypeptides and lectins (Cowan 1999). In the present study, the solvent used both water and methanol were reliable. Most of the identified components from plants which are active against microorganisms are aromatic or saturated organic compounds, they are often obtained through initial ethanol or methanol extraction (Viges *et al.* 1997).

The amount of extracts was obtained by soaking the ground medicinal plants on water and methanol at room temperature. The amount of extract varies on different plants. It is because the yield depends on various factors like parts and type of plant materials, duration of extraction and extent of dryness. Older plants yield less as compared to younger ones. Not only this, the incomplete extraction results lower yield and if the solvent is not totally removed, it falsely results higher yield.

### 5.3 Screening of Antibacterial Activities

Different kinds of diseases like cough, cold, tonsillitis, fever, boils, cuts, urinary diseases, respiratory diseases, diarrhea, jaundice, dysentery and wounds were caused by different pathogenic bacteria. The inhibition zone was produced by plant extracts which contains antibacterial substances and were able to kill or inhibit the growth of tasted bacteria in the given concentration.

The study revealed that *Rubus nepalensis, Rumex nepalensis, Smilex aspara, Justicia adhatoda* and *Aloe vera* were the most effective medicinal plants for the aqueous

solutions. Similar types of results obtained by Bonjar (2004) where *Cuscuta epithymum* and *Smilax sp.* were active against bacterial strain like *Escherichia coli* and *Staphylococcus aureus*. Similarly, *Epolobium roseum, Murraya koenigii* and *Justicia adhatoda* were most effective medicinal plants for the methanol extracts. These plants showed the activity against all the tested strains of bacteria. Panthi and Chaudhary (2006) also found that *Justicia adhatoda* and *Bergenia ciliata* were the most effective medicinal plants against gram positive bacteria where as gram negative bacteria (*Escherichia coli)* was controlled by *Paris polyphyla*. Similarly, according to Kaladhar, (2010), methanolic and ethyl acetate leaf extract of *Dioscorrea hamiltonii* has shown good antimicrobial activity against gram positive bacteria. Similarly, essential oil of *Vetiver zizanoides* shows significant antibacterial activity against different bacteria (Hammar *et al.* 1999) and methanolic extract of *Astilbe rivularis* was most effective against *Escherichia coli* (Adhikary *et al.* 2012).

### 5.4 Evaluation of Antibacterial Activity

Screening process only indicates whether or not any compound inhibits or kills particular bacteria. It may not suggest about potency of antibacterial substance. Plant extract having antibacterial activity against large numbers of bacterial strains may have little or no importance if the potency of extract is very low. That is why evaluation of the potency of medicinal plants extract is also most essential step during new drug research process from natural products.

In regard to evaluation of antibacterial activity, natural compound having broad spectrum may be considered little potency and such compounds have little value in developments of new drugs (Parekh and Chanda 2007). For this, disc diffusion method was employed. In disc diffusion method, the antibacterial substance diffusing in the media kill or inhibit the bacteria and thus zone of inhibition appears around the disc in agar surface. There is the gradual decrease in the concentration of antibacterial substance as the distance from disc is increased. A critical point arises after certain distance. After this point there will be growth of bacteria, the concentration of antibacterial substance at that critical point is actually minimum inhibitory concentration. By measuring the diameter of zone of inhibition we can simply evaluate the potency of the antibacterial drugs. The evaluation of such antibacterial

drug becomes the essential step during new drug research from natural product (Parekh and Chanda 2007).

Among all the tested plants, largest ZOI (1.6cm) was shown by *Cuscuta reflexa* on bacteria *Staphylococcus aureus* and *Escherichia coli*. This plant showed ZOI of 0.9cm and 0.7cm against bacteria *Klebsiella pneumonia* and *Salmonella typhi* respectively in aqueous solutions. Although it showed comparative narrow spectrum of activity but had broader ZOI. This may due to the antibacterial substances present in it may have higher diffusibility (Panthi and Chaudhary 2006). Similarly, two plants *Epilobium roseum* and *Murraya koenigii* showed broad spectrum of activity but had comparative lesser value of ZOI in aqueous and methanolic extract. It may be due to the low diffusibility of antibacterial substances present on those plants part (Panthi and Chaudhary 2006, Kaladhar 2010). The diffusing capacity of the chemical substances may be affected by the presence of aromatic gases, oils, resin or wax in the extract (Parekh and Chanda 2006). Presence of these substances may cause the low diffusibility in the agar media. The diffused extracts were more active than the extract having undiffused character (Parekh and Chanda 2006).

Amongst the gram-positive and gram-negative bacteria, gram-positive bacterial strains were more susceptible to the extracts as compared to gram-negative bacteria. Previous study reveals that plant extracts are more active against gram-positive bacteria than gram-negative bacteria (Vlietinck *et al*. 1995; Rabe and Van Staden 1997; Lin *et al.* 1999; Parekh and Chanda 2007). While comparing the resistivity, strains of gram-positive bacteria were more resistant as compared to gram-negative bacteria. In this study, *Taxus baccata* is only one of plant among all experimented plants, has shown antibacterial properties with all tested gram negative bacteria where as all tested gram positive bacteria are resistant. But all other tested plants has shown similar results to the corresponding findings. These differences may be attributed to the fact that the cell wall in gram-positive bacteria are of a single layered where as that of gram-negative are multilayered (Yao *et al.* 1995). So the passage of the active compound through the gram-negative cell wall may be inhibited. In addition, the microorganisms show the variable sensitivity to chemical substances related to different resistant levels between strains (Cetin and Gurler, 1989).

## CHAPTER-VI

## CONCLUSIONS

The present study concludes that most of the medicinal plants used in traditional way to treat against bacterial diseases have antibacterial properties. The antibacterial effects are due to synergistic effects of one or more than one compounds present in them. The aqueous extract and methanolic extract of different parts of plants used in the medicinal purposes gave varying yield.

Twenty two different medicinal plants were selected for screening purpose against seven different bacteria among them two are gram-positive (*Staphylococcus aureus* and *Streptococcus pneumonia*) and five are gram-negative (*Salmonella typhi, Escherichia coli, Klebsiella pneumonia, Vibrio cholera* and *Shigella flexneri*). Among these bacterial strains gram-negative bacteria were more resistant as compared to gram-positive bacteria.

Among twenty two medicinal plants tested, in the present study, all plants show activity against at least one bacteria for aqueous extract and two bacteria for methanolic extract. *Rubus nepalensis* and *Rumex nepalensis* inhibited all the tested bacteria in aqueous solution and *Epilobium roseum* inhibited all the tested bacteria in methanolic solution.. Similarly, *Murraya koenigii* and *Smilex aspara* were effective against six bacteria in aqueous solutions and *Astilbe rivularis*, *Gaultheria fragrantissima*, *Rubia manjith*, *Rumex nepalensis*, and *Murraya koenigii* were effective against six bacteria in methanolic extract. Five plants inhibit the growth of five bacteria, two plants inhibit the growth of four bacteria, seven plants inhibit the growth of three bacteria, three plants inhibit the growth of two bacteria and one plant inhibit the growth of one bacteria in aqueous extract. Seven plants inhibit the growth of five bacteria, two plants inhibit the growth of four bacteria, four plants inhibit the growth of three bacteria, and three plants inhibit the growth of two bacteria in methanolic extract.

The ZOI value ranges from 0.3 to 1.6cm for aqueous extract and 0.5 to 2.0cm for methanolic extract. The maximum value of ZOI (1.6cm) was shown by plant *Cuscuta reflexa* on bacteria *Staphylococcus aureus* and *Escherichia coli* in aqueous extract. Similarly the maximum value of ZOI (2.0cm) was shown by plant *Cuscuta reflexa* on bacteria *Staphylococcus aureus* in methanolic extract.

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**Appendix A**

**Graphs showing antibacterial activity of different medicinal plants on tested bacteria**

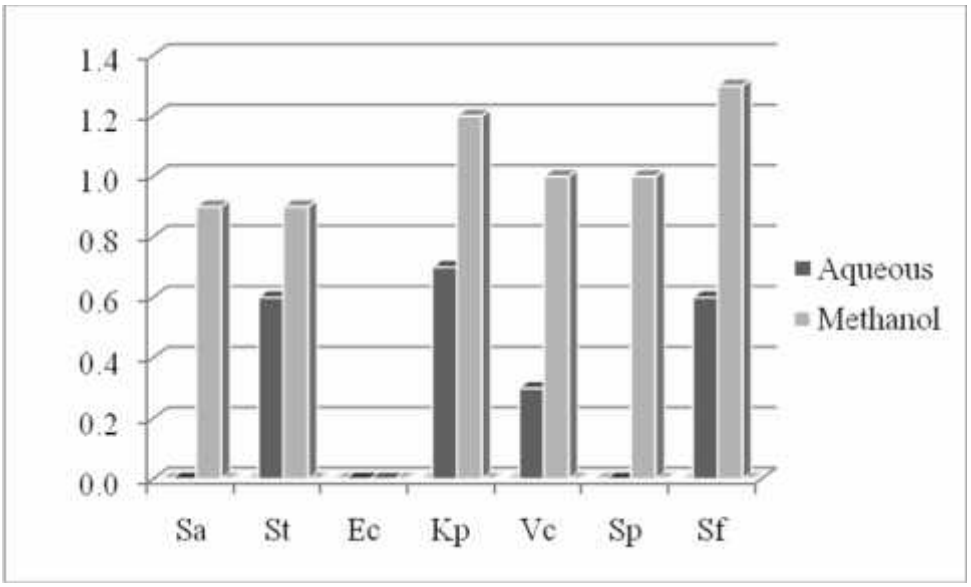


Figure 1: Zone of inhibition of *Astilbe rivularis* (Abbreviations: Sa – *Staphylococcus aureus,* St – *Salmonella typhi,* Ec – *Escherichia coli*, Kp - *Klebsiella pneumonia,* Vc - *Vibrio cholera,* Sp - *Streptococcus pneumonia*, Sf - *Shigella flexneri*).

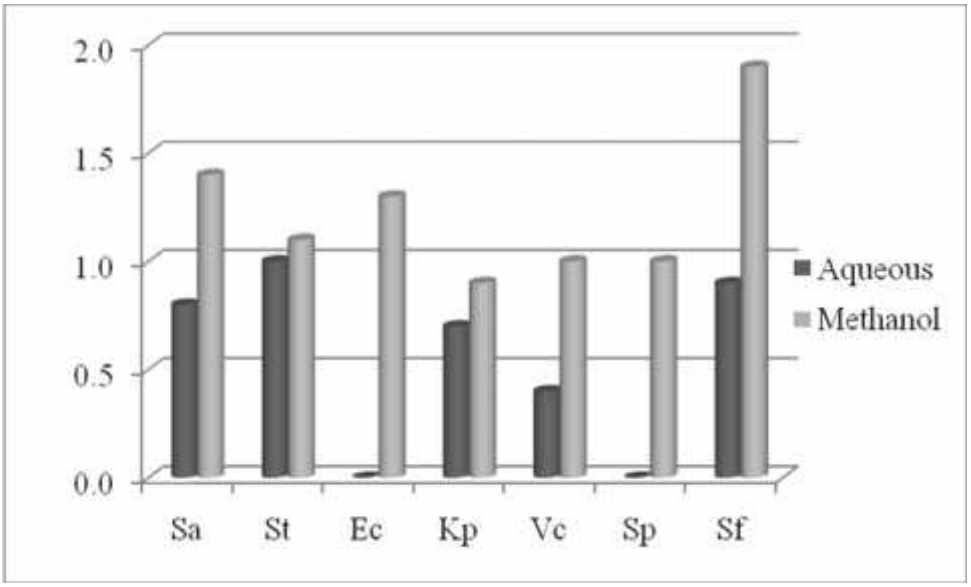


Figure 2: Zone of inhibition of *Epilobium roseum*

(Abbreviations: Sa – *Staphylococcus aureus,* St – *Salmonella typhi,* Ec – *Escherichia coli,* Kp - *Klebsiella pneumonia,* Vc - *Vibrio cholera,* Sp - *Streptococcus pneumonia*, Sf - *Shigella flexneri*).

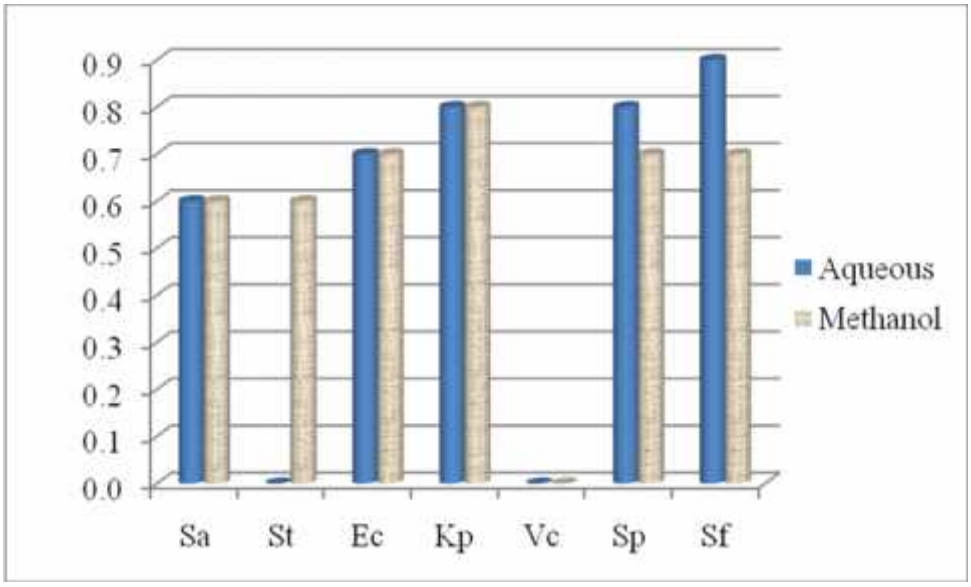


Figure 3: Zone of inhibition of *Rubia manjith*

(Abbreviations: Sa – *Staphylococcus aureus*, St – *Salmonella typhi*, Ec – *Escherichia coli*, Kp - *Klebsiella pneumonia*, Vc - *Vibrio cholera*, Sp - *Streptococcus pneumonia*, Sf - *Shigella flexneri*).

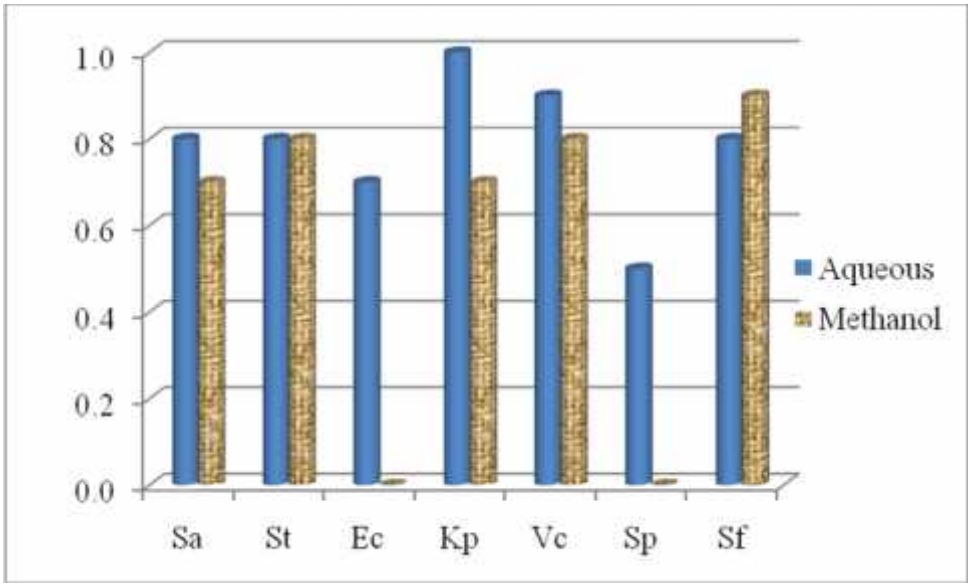


Figure 4: Zone of inhibition of *Rubus nepalensis*

(Abbreviations: Sa – *Staphylococcus aureus*, St – *Salmonella typhi*, Ec – *Escherichia coli*, Kp - *Klebsiella pneumonia*, Vc - *Vibrio cholera*, Sp - *Streptococcus pneumonia*, Sf - *Shigella flexneri*).

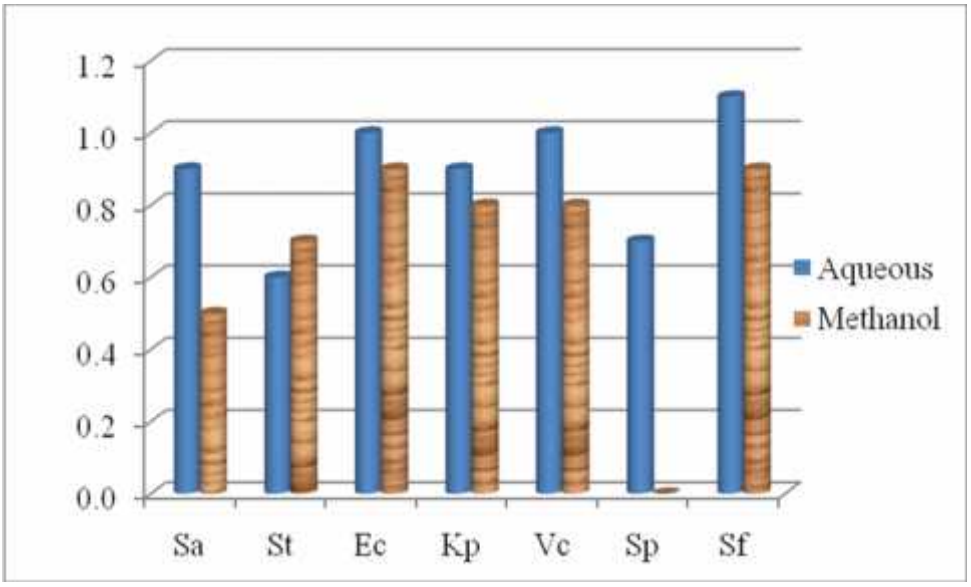


Figure 5: Zone of inhibition of *Rumex nepalensis*

(Abbreviations: Sa – *Staphylococcus aureus*, St – *Salmonella typhi*, Ec – *Escherichia coli*, Kp - *Klebsiella pneumonia*, Vc - *Vibrio cholera*, Sp - *Streptococcus pneumonia*, Sf - *Shigella flexneri*).

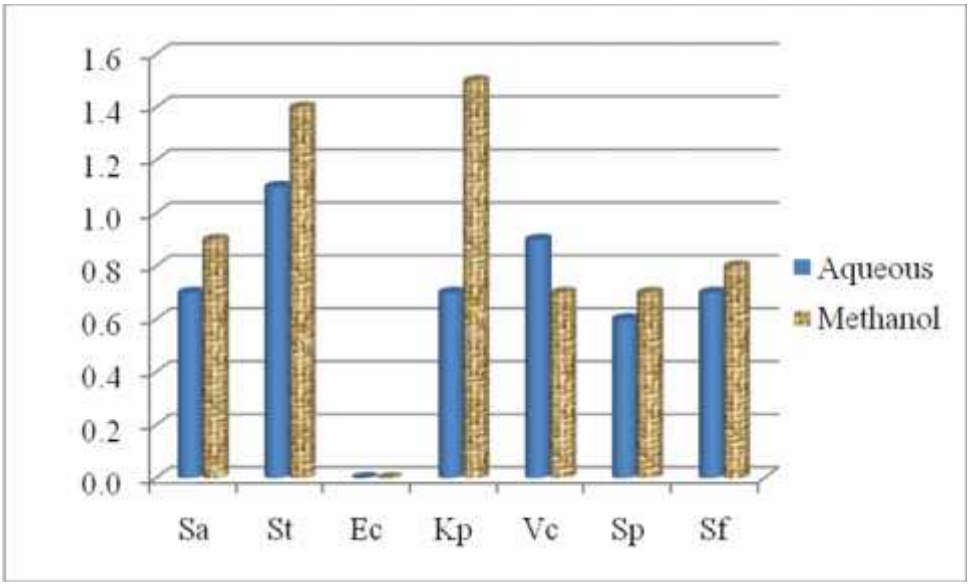


Figure 6: Zone of inhibition of *Murraya koenigii*

(Abbreviations: Sa – *Staphylococcus aureus*, St – *Salmonella typhi*, Ec – *Escherichia coli*, Kp - *Klebsiella pneumonia*, Vc - *Vibrio cholera*, Sp - *Streptococcus pneumonia*, Sf - *Shigella flexneri*).

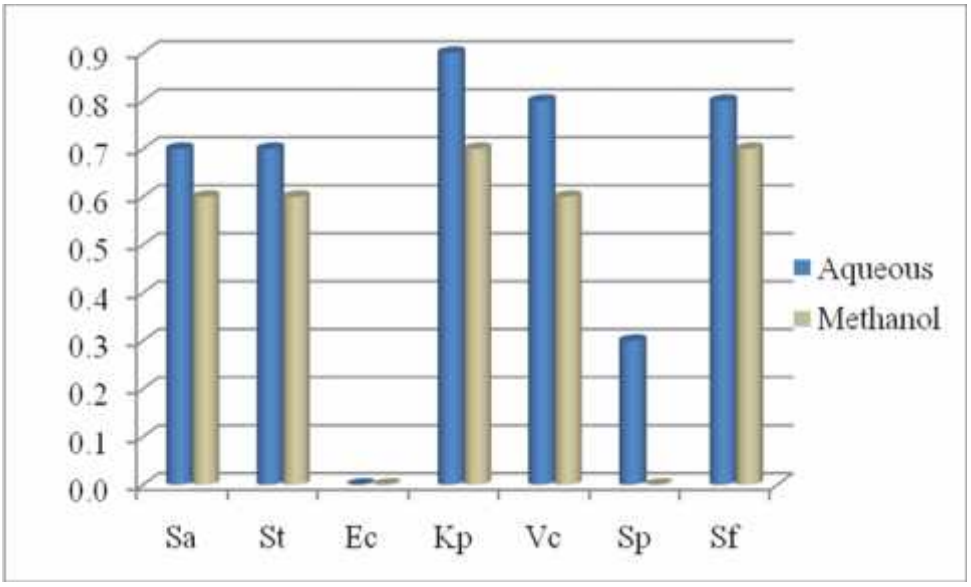


Figure 7: Zone of inhibition of *Smilex aspara*

(Abbreviations: Sa – *Staphylococcus aureus*, St – *Salmonella typhi*, Ec – *Escherichia coli*, Kp - *Klebsiella pneumonia*, Vc - *Vibrio cholera*, Sp - *Streptococcus pneumonia*, Sf - *Shigella flexneri*).

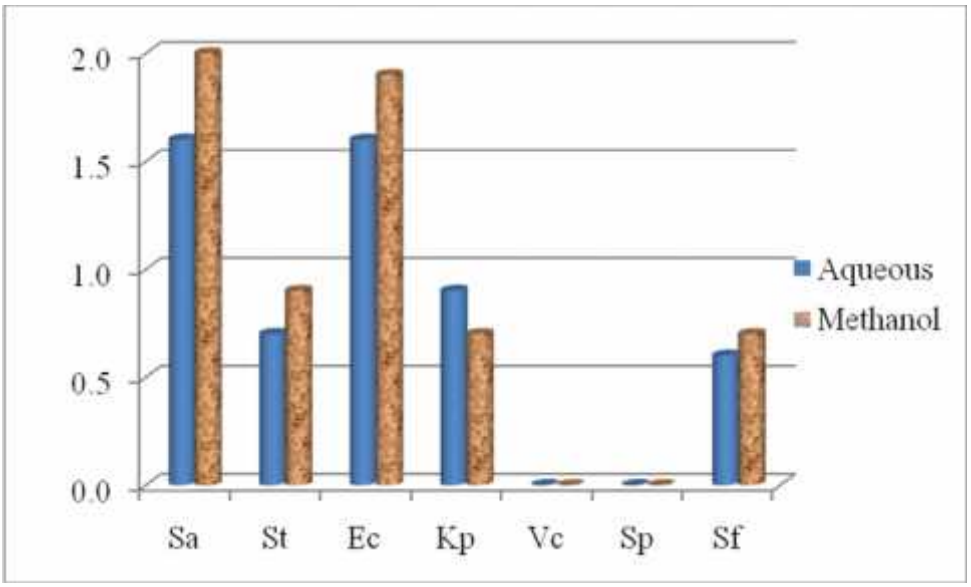


Figure 8: Zone of inhibition of *Cuscuta reflexa*

(Abbreviations: Sa – *Staphylococcus aureus*, St – *Salmonella typhi*, Ec – *Escherichia coli*, Kp - *Klebsiella pneumonia*, Vc - *Vibrio cholera*, Sp - *Streptococcus pneumonia*, Sf - *Shigella flexneri*).

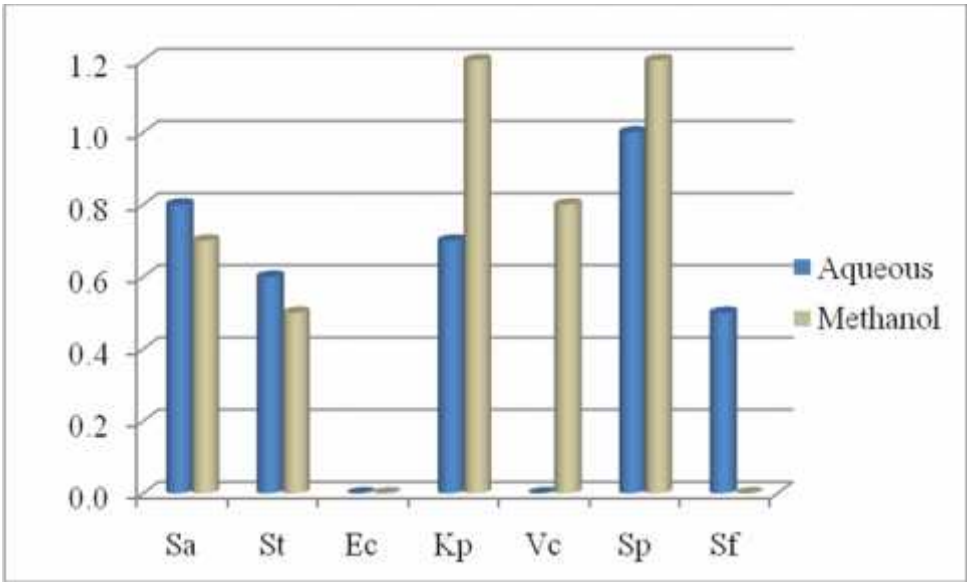


Figure 9: Zone of inhibition of *Justicia adhatoda*

(Abbreviations: Sa – *Staphylococcus aureus*, St – *Salmonella typhi*, Ec – *Escherichia coli*, Kp - *Klebsiella pneumonia*, Vc - *Vibrio cholera*, Sp - *Streptococcus pneumonia*, Sf - *Shigella flexneri*).

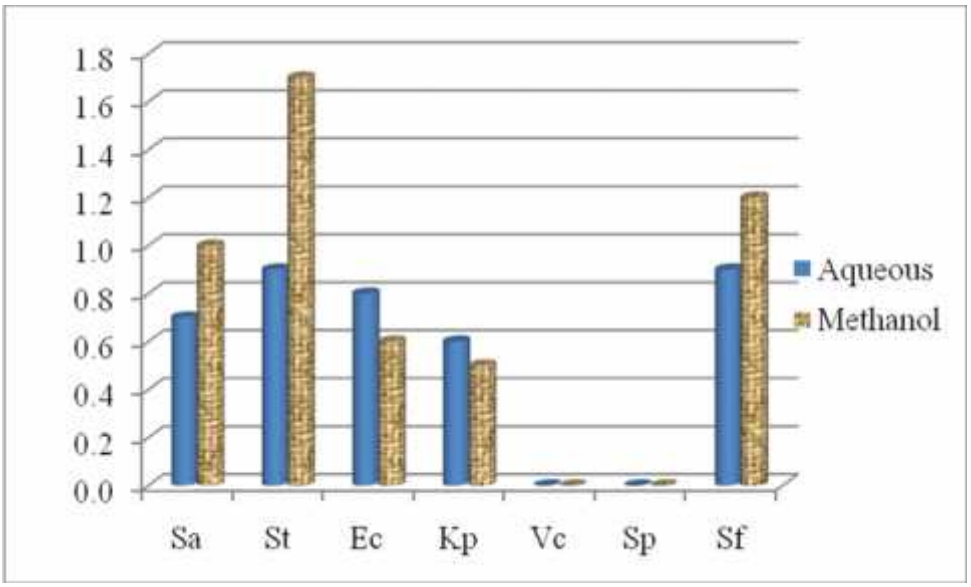


Figure 10: Zone of inhibition of *Aloe vera*

(Abbreviations: Sa – *Staphylococcus aureus*, St – *Salmonella typhi*, Ec – *Escherichia coli*, Kp - *Klebsiella pneumonia*, Vc - *Vibrio cholera*, Sp - *Streptococcus pneumonia*, Sf - *Shigella flexneri*).

## Appendix B

**Description of Medicinal Plants Used in the Evaluation of Antibacterial Activities**

Plants are described on the basis of Annotated Checklist of the Flowering Plants of Nepal (Press *et al*., 2000).

**1.** ***Aloe vera*** (L.) Burm. f.

**Family:** Liliaceae

**Common name:** Gheukumari

**Description:** It is a perennial herb which is about 2-3 feet high. It has short stem and bears fleshy leaves tapering to a blunt point. Leaves are pale green, smooth and possess horny prickles on their margins.

**Distribution:** West, Cent., East. Nepal

**Part(s) used:** Leaves

**Uses:** Leaves are used for curing skin disease, fever, vomiting, tumors, enlargement of spleen and other glands, gonorrhea, constipation, menstrual suppression, piles, jaundice and rheumatic affection. It is also used to cure cough, cold and intestinal worms in children. The sap of leaves is widely used in inflamed parts and scalds.

**2.** ***Astilbe rivularis*** Buch.-Ham.ex D. Don.

**Family:** Saxifragacea**e**

**Common name:** Goat's beard

**Description:** Wild or cultivated, perennial, herbaceous, fern-like foliage, and dense, feathery plumes of flowers. They are widely adapted to shade and water-logged conditions, hence they are particularly associated with pond-side planting. They also tolerate clay soils well. Flowers give pleasant aroma.

**Distribution:** Central, East. Nepal

**Part(s) used:** rhizome

**Uses:** This plant is mainly used to treat diarrhea and dysentery.

**3.** ***Bergenia ciliata*** (Haw.) Sternb.

**Family:** Saxifragaceae

**Common name:** Paashanabed/Pakhanbed

**Description:** Perennial herb with creeping rootstocks and thick leaves. Flowering stems up to 25 cm. Leaves rosette, short petiole, broadly ovate, rounded at the base and apex, glabrous but margin ciliate with stiff hairs. Flowers white, pink or purple arranged in a cyme supported by a long leafless peduncle. Capsule rounded.

**Distribution:** West, Cent., East. Nepal

**Part(s) used:** Rhizome, whole plant.

**Uses:** It is useful for Cough diarrhea, indigestion, fevers, tumors, urinary stones diseases. Ayurvedic herbs are often taken in combination with others to neutralize the toxicity one herb with the opposing effect of other.

4. ***Cuscuta reflexa*** Roxb.

**Family:** Convolvulaceae

**Common name:** Aakasbeli

**Description:** Herbaceous parasitic climber, stems yellow, branched, leaves reduced to scale, flowers creamy white to pink, bell shaped, fragrant, fruit fleshy, four seeded.

**Distribution:** West, Cent., East. Nepal

**Part(s) used:** Whole plant.

**Uses: Used** for cough and stomach disorders, juice is taken to treat jaundice (Chaudhary *et al.* 2002; Oli and Nepal 2003), antihelmintic, and to treat wound and bone fractures (Chaudhary *et al.* 2002).

5. ***Daphne bholua*** Buch.-Ham. ex D. Don

**Family:** Thymelaeaceae

**Common name:** Lokata

**Description:** Errect, evergreen shrub, 1-3 m high, leaves entire, elliptic, glabrous, leathery with very short petiole, flowers sweet scented, white flushed, fruit drupe, black when ripe (Ghimire *et al.* 2008)

**Distribution:** West, Cent., East. Nepal

**Part(s) used:** Stem, bark, root.

**Uses:** The juice of the roots combined with molasses is used in the treatment of fevers and intestinal problems. A decoction of the bark is used to treat fevers. The

powdered seeds are antihelmintic. A very good quality paper is made from the inner bark. It is one of the principal sources of handmade paper in Nepal. The fiber in the inner bark can be used to make rope (DPR, 2007).

6. ***Digitalis purpurea*** L**.**

**Family:** Plantaginaceae

**Common name:** Foxglove

**Description:** It is herbaceous biennial plant. The leaves are spirally arranged, simple, 10-35 cm long and 5-12 cm board, grey-green, downy, and with a finely toothed margin: they form a tight rosette at ground level in the first year. The flowering stem develops in the second year. The flowers are arranged in a showy, terminal, elongated cluster, each tubular, pendent, purple. They are also spotted inside bottom of the tube. The fruit is a capsule which splits open at maturity to release the numerous tiny (0.1-0.2 mm) seeds.

**Distribution:** West, Cent., East. Nepal

**Part(s) used:** Flowers and leaves

**Uses:** It is used in rheumatic fever. It is also used as a molecular probe to detect DNA or RNA. Its leaves are mainly used to control bleeding from smaller arteries. Due to the presence of the cardiac glycoside digitoxin, the leaves, flowers and seeds of this plant are all poisonous to humans and some animals and can be fatal if eaten.

7. ***Dioscorea bulbifera*** L.

**Family:** Dioscoreaceae

**Common name:** Gitthe tarul

**Description:** Perennial vine, two types of storage organs, bulbils in the leaf axils of the twining stems, and tubers beneath the ground, tubers are small, oblong , twining, long-stemmed herbaceous vine which may arise from an underground tuber, stems round to slightly angled in cross section and they twine coutner-clockwise the leaves are attractive, alternate, broadly heart-shaped, up to 20 cm long and attached by long petioles.

**Distribution:** West, Central, East. Nepal

**Part(s) used:** leaves, tuber

**Uses:** used to treat diarrhea, dysentery, jaundice, stomach pain, bone fracture, anorexia, diuretic, anti-fungal, anti-tumor. It can also be used for lower cholesterol level, relieve pain and lower blood pressure.

8. ***Epilobium roseum*** var. ***cylindricum*** (D. Don) C. B. Clarke

   **Family:** Onagraceae

   **Description:** Errect, perennial herb, leaf ovate, dentate, opposite, hairy stem, flower erect.

   **Distribution:** West, Cent., East. Nepal

   **Part(s) used:** Whole plant (Bark, root, flower, leaves)

   **Uses:** Used to treat prostrate problem, bladder problem, gastrointestinal disorder, kidney disorder, rectal bleeding, menstrual disorders, urinary infections, diarrhea.

9. ***Gaultheria fragrantissima*** Wall.

   **Family:** Ericaceae

   **Common name:** Dhasingare

   **Description:** perennial shrub, height up to 3.2 meter, branched stem, leaves are mostly 12 cm long, leathery, dotted, with glands, flowers small, fruits mostly circular, blew when ripe.

   **Distribution:** West, Cent., East. Nepal

   **Part(s) used:** Leaves

   **Uses:** The oil obtained from its leaves is used in the treatment of atrophic arthrities, destroy hookworm, stimulant, antitumeric, antiseptic, aromatic, carminative, stimulant, stomach trouble, toothache, killing mosquitoes and other insects.

10. ***Justicia adhatoda*** L.

    **Family:** Acanthaceae

    **Common name:** Asuro (Nep.), Vashak (Tam.), Asur (Tha.)

    **Description:** Small, evergreen, gregarious shrub found about 1-2m in height. The leaves of this plant are elliptic lanceolate. Flowers are white in colour with red spots and streaks within. Flowers are two lipped and borne in dense short terminal and axillary spikes with conspicuous ovate overlapping bracts.

**Distribution:** West, Cent., East. Nepal

**Part(s) used:** Leaves

**Uses:** The decoction of leaves is used as antipyretic and to treat asthma. Leaves juice is used to treat scabies, skin disease, fever, bronchitis, cough, stomach pain, alkaloid obtained from the leaf is powerful expectorant and antispasmodic and commonly used in chest disease. A poultice of leaf is used applied over fresh wounds, rheumatic joints and inflammatory swelling. The root is expectorant, antispasmodic, antiseptic, antiperiodic and antihelmintic. It is used in the treatment of malarial fever, respiratory diseases,diphtheria and gonorrhea.

**11.** ***Lobelia pyramidalis*** Wall.

**Family:** Campanulaceae

**Common name:** Ekle bir

**Description:** Annual herb, verities in flower colour, mostly blue, simple, alternate leaves, flowers tubular and tow lipped, each with five lobed.

**Distribution:** West, Cent,Nepal

**Part(s) used:** Root, leaves, flowers.

**Uses:** It can be used to treat muscular and respiratory disorder, as a purgative, for syphilis. It is ineffective in helping people to quit smoking.

**12.** ***Mahonia napaulensis*** DC.

**Family:** Berberidaceae

**Common name:** Jamane mandro

**Description:** Evergreen shrub about 3 m height, branched stem, leaves pinnate, oblong-lanceolate, leaves sessile, ovate to lanceolate, with spiny marginal teeth and pointed apex, flowers yellow, terminal spike.

**Distribution:** West, Cent., East, Nepal

**Part(s) used:** Stem, leaves fruit.

**Uses:** Used for treatment of urinary diseases, used to prepare local alcohol, ripe fruit are eaten for sour and sweet taste.

**13.** ***Meconopsis horridula*** var. ***rudis*** Hook. f. and Thomson.

**Family:** Papaveraceae

**Common name:** Dangin-da

**Description:** Herb with long, cylinder tap root, leaves mostly basal in rosette, elliptic, to linear-oblong, subacute, margin entire, covered with bristly spines, stem leaves absent, flowers light blue, in spike like clusters.

**Distribution:** West, Cent., East. Nepal

**Part(s) used:** Flowers, leaves, roots, seeds.

**Uses:** Used in headache, upper back pain, fever, heart problem, skin disease, sinusitis, lone and bile disorder and wounds. It is also used for blood purifications and to heal fractured bones (Ghimire *et al.* 2001; Lama *et al*. 2001). Root, leaves, flowers are also used in kidney disorder and to remove accumulated body fluid (Pandey 2006).

**14.** ***Murraya koenigii*** (L.) Spreng.

**Family:** Rutaceae

**Common name:** Mitho neem, Mechiya saag

**Description:** Small tree growing 4-6 m tall. The leaves are exstipulate, bipinnate, with 11-21 leaflets, each leaflets 2-4 cm long and lanceolate, highly aromatic. Flowers are small, white and fragrant. Fruit berry and edible but seeds are poisonous.

**Distribution:** West, East Nepal

**Part(s) used:** Stem, leaves

**Uses:** Leaves are used as vegetables, tonic and stomachic, antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, hypercholesterolemic.

**15.** ***Paris polyphylla*** Sm.

**Family:** Trilliaceae

**Common name:** Satuwa

**Description:** Errect, perennial herb, 40-70 m high, rhizome stout, creeping, leaves 4-9 in whorled, elliptic, oblong or lanceolate, terminal, short stalked, flower yellow or purple, fruit globular.

**Distribution:** West, Cent., East. Nepal

**Part(s) used:** Roots, rhizomes.

**Uses:** Fever, root paste is applied to cure wounds and root powder is used as a remedy for diarrhea (Pohle, 1990), intestinal worms (Bhattarai *et al.* 2006). Root powder is taken as antihelmintic drug (Dangol, 2002).

**16. *Rubia manjith*** Roxb. ex Fleming

**Family:** Rubiaceae

**Common name:** Majitho

**Description:** It is a climbing prickly herb grows up to 10 m long. Stem quadrate shape and hairy. Leaves ovate or heart shaped, 2-8 in whorl. Flowers are small white or radish brown. Fruits are globes.

**Distribution:** Cent., East.

**Part(s) used:** Root, stem, root

**Uses:** Root is credited with tonic, astringent, anti-dysenteric, antiseptic. Paste used for ulcers, inflammation and skin troubles. A valuable red dye is obtained from the root and stems.

**17. *Rubus nepalensis*** (Hook. f.) Kuntze

**Family:** Rosaceae

**Common name:** bhuin Ainselu

**Description:** Evergreen shrub, creeper grows up to 1 m in diameter and 20 cm in height. Leaves are trifoliate and hairy.The flowers are five-petaled and white. Fruit is aggregate drupes, small, edible.

**Distribution:** West, Cent., East. Nepal

**Part(s) used:** Leaves, fruit, roots.

**Uses:** Decoction of leaves is used to cure cough, cold and fever. Ripe fruit is useful for dysentery. Root paste is applied in wounds.

**18. *Rumex nepalensis*** Spreng.

**Family:** Polygonaceae

**Common name:** Halhale saag

**Description:** Robust perennial herb, about 1 m tall with stout rootstock. Leaves entire, lower leaves oblong-ovate, long petiole, upper leaves smaller, lanceolate, sessile. Flowers bisexual, greenish, borne in whorls in leafless racemes.

**Distribution:** West, Cent., East. Nepal

**Part(s) used:** Roots, leaves, stem, seeds.

**Uses:** Seeds are used in mouth disorder and stem in lung and liver disease, constipation, sores and skin disease (Ghimire *et al.*, 2001; Lama *et al.*, 2001), fever, fractured, reduced body pain.

**19. *Smilax aspera*** L.

**Family:** Smilacaceae

**Common name:** Kukurdaino

**Description:** Large climbing shrub, leaves variable, triangular to hastate with rounded or cordate or ovate, alternate, margin entire, apex acute, tendril paired, flowers small, white, fragrant. Fruit is a red berry, turning blue-black when ripe.

**Distribution:** West, Cent., East. Nepal

**Part(s) used:** Leaves, roots.

**Uses:** Leaf juice is applied to treat scabies, wounds caused by muddy water. Root juice is taken to treat stomachache, bowel complaints and vomiting.

**20. *Stellaria vestita*** Kurz.

**Family:** Caryophyllaceae

**Common name:** Corn ful jhar

**Description:** Stem is 15-150 cm high, leaves are lanceolate, cylinder, sharp up to 4 cm in length, petal long, seeds are numerous, wrinkled, poisonous plant.

**Distribution:** West, Cent., East, Nepal

**Part(s) used:** Leaves, flower

**Uses:** It is employed as a treatment for dry, cracked and inflamed tissue, used to treat rheumatic pain, arthritis, bronchitis, psoriasis, asthma, conjunctivitis, constipation, obesity, blood disorders, diuretic, laxative, liver tonic.

**21.** ***Taxus baccata*** subsp. ***wallichiana*** L.

**Family:** Taxaceae

**Common name:** Launth Sallo

**Description:** Medium sized to tall evergreen tree with dark grey bark. Leaves linear flattened, curved, spiny-tipped. Flowers usually dioecious, ovoid, solitary axillary. Fruit red fleshy, single seeded.

**Distribution:** West, Cent., East. Nepal

**Part(s) used:** Bark, wood, leaves.

**Uses:** Bark is used in muscular pain and fever (Ghimire *et al.,* 2001), herbal tea, ripe fruit are edible. Leaf extract is consumed to cure asthma, bronchitis and other respiratory disease. The extract "taxol" is highly medicinal value.

**22.** ***Vetiveria zizanoides*** (L.) Hash

**Family:** Poaceae

**Common name:** Khas-khas grass, vetiver

**Description:** Tall, stout perennial with an oblong panicle over 30 cm long which has whorled branches bearing spikelets 5-6 mm long, with a few tubercle-based short bristles.

**Distribution:** West, Central, East. Nepal

**Part(s) used:** Root

**Uses:** Prevention form soil erosion. Essential oils obtained from its root is used in high end perfumes, anti-inflammatory, antiseptic, tonic bath, rheumatism, arthritis, muscular pain, sedatives (Acharya, 2005).

## Appendix C

## Short Description of Bacteria Involved in the Present Study and Their Pathogenicity

### 1. *Staphylococcus aureus*

### a. Morphology and Biochemical Characters

It is gram positive, spherical bacteria that occur in microscopic clusters resembling grapes. On nutrient agar at 37$^{o}$C it forms colonies 1-3 mm in diameter with smooth, low convex, opaque, and of butyrins 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 range of 15 to 45$^{o}$C and at NaCl concentrations as high as 15 per cent. 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 phylogenic 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*. 1999).

### 2. *Salmonella typhi*

### a. Morphology and Biochemical Character

It is a gram-negative, facultative rod-shaped, non–capsulated, and non-sporing bacterium. It can grow wide range of media at the temperature range 15-45°C. Selentine F. broth is probably the best media for its growth. It is non-lactose fermenter; produce gas during fermentation of sugar. Unlike other *Salmonella* it cannot ferment glucose. It is indole, Voges-Proskauer, urease negative. It produces $H_2S$ in TSI with production of acid and no gas. It is methyl red positive (Collee *et al.* 1999).

**b. Pathogenicity**

Strains of *Salmonella* are mostly responsible for enteric fever which includes typhoid fever. Other *Salmonella* causes gastrointestinal tract infections, osteomyelitis in children with sickle cell diseases (Cheesbrough 1993).

**3. *Escherichia coli***

**a. Morphological 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 36-37ºC.

**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 disease 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 defenses (Todar, 2008). On the basis of their pathogenicity, they are divided into four groups viz. enterotoxigenic *E. coli* (ETEC) strains causes an acute watery diarrhea, enteroinvasive strains of *E. coli* (EIEC) can cause shigella like 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.* 1999)

**4. *Klebsiella pneumonia***

**a. Morphology and Biochemical Character**

It is a Gram negative, non motile encapsulated, lactose fermenting, facultative, anaerobic rod shaped bacteria. It can synthesize ATP by aerobic respiration, but can also switch an anaerobic fermentation for driving energy. It is found naturally in the soil, water and vegetable. Some of the strains of *Klebsiella pneumonia* have an ability to fix atmospheric nitrogen in a more useable form for plants. In human, it can be found in the skin, pharynx and gastrointestinal tract. However in certain condition, it causes serious infection.

**b. Pathogenicity:**

*Klebsiella pneumonia* causes pneumonia in human. It can also causes urinary tract infection and abdominal infections. It is second pathogen after *Escherichia coli*. It normally affects persons with low immune system such as hospital patients, diabetes patients and people with chronic lung diseases. Alcoholics also suffer from *Klebsiella pneumonia* infections (Collee *et al*., 1999)

**5. *Vibrio cholerae***

**a. Morphology and Biochemical Character**

*Vibrio cholerae* is a facultatively anaerobic, Gram-negative, non-spore forming, curved rod, about 1.4–2.6 µm long bacteria with a single, polar flagellum for movement and capable of respiratory and fermentative metabolism. There are numerous strains of *Vibrio cholerae,* some of which are pathogenic and some of which are not. The entire genome of the virulent strain has been sequenced, and contains two circular chromosomes. The first chromosome is larger that contains the crucial genes for toxicity, regulation of toxicity and important cellular functions, such as transcription and translation. The second chromosome is determined to be different from a plasmid or megaplasmid due to the inclusion of housekeeping and other essential genes in the genome, including essential genes for metabolism When *Vibrio cholerae* are growing during the logarithmic phase there is little or no change to the cellular envelope. However, towards the end of logarithmic phase and into the begining of stationary phase cholera toxin is produced, which is accompanied by internal swelling of the cell and permeability changes to the cellular envelope.

**b. Pathogenicity:**

This bacteria infects the intestine and increases mucous production causing diarrhea and vomiting which result in extreme dehydration and, if not treated, death. It is usually transmitted through the feces of an infected person, often by way of unclean drinking water or contaminated food. It is for this great risk to human health that makes it so worthy of studying and sequencing. And because of the variety of strains, it could be possible to determine the pathogenicity of new strains by comparing their genomes to strains of known pathogenic status.

**6. *Streptococcus pneumoniae***

**a. Morphology and Biochemical Characters**

*Streptococcus pneumoniae* is a Gram-positive, alpha-hemolytic, aero-tolerant anaerobic member of the genus *Streptococcus*. A significant human pathogenic bacterium, *S. pneumoniae* was recognized as a major cause of pneumonia in the late $19^{th}$ century, and is the subject of many humoral immunity studies. *S. pneumoniae* played a central role in demonstrating genetic material consists of DNA. In 1928, Frederick Griffith demonstrated transformation of life, turning harmless pneumococcus into a lethal form by co-inoculating the live *Pneumococci* into a mouse along with heat-killed, virulent *Pneumococci*. In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty demonstrated the transforming factor in Griffith's experiment was DNA, not protein, as was widely believed at the time. Avery's work marked the birth of the molecular era of genetics. The genome of *S. pneumoniae* is a closed, circular DNA structure that contains between 2.0 and 2.1 million basepairs, depending on the strain. It has a core set of 1553 genes, plus 154 genes in its virulome, which contribute to virulence, and 176 genes that maintain a noninvasive phenotype. Genetic information can vary up to 10% between strains.

**b. Pathogenicity**

The organism causes many types of pneumococcal infections other than pneumonia. These invasive pneumooccal disease include acute sinusitis, meningitis, bacteremia, sepsis, osteomyelitis, septicarthritis, endocarditis, peritonitis,pericarditis, cellulitis, and brain abscess. *S. pneumoniae* is one of the most common causes of bacterial meningitis in adults and young adults. The organism was termed *Diplococcus pneumoniae* from 1920 because of its characteristic appearance in Gram-

stained sputum. It was renamed *Streptococcus pneumoniae* in 1974 because of its growth in chains in liquid media.

### 7. *Shigella flexneri*

#### a. Morphology and Biochemical Characters

*Shigella flexneri* is a bacteria belonging to the family Enterobacteriaceae. This species is a Gram-negative, rod shaped and non-lactose fermenting. This species is non-motile due to the lack of flagella and makes ATP by aerobic respiration but it is also capable of switching to fermentation acts as facultative anaerobe. *Shigella* can ferment carbohydrate with the production of acid and grow in translucent white colonies. *Shigella* is closely related to Escherichia coli and *Salmonella typhi* and share common antigens with one another and other entire bacteria. *Shigella* species have a large virulent plasmid that carries the genes necessary for invasion and colonization of the epithelial cell layer of the human gut resulting in dysentery (Dorman, 2009). It moves around form cell to cell by using protein (IcsA) that action polymerization in the host cell in a "rocket" propulsion fashion (Taylor, 2008). It usually becomes resistant to antibiotics quickly and so antibiotics are prescribed when absolutely necessary, especially in case of epidermis. Once infected, the person is not likely to get infected by that same strain for a while, but is still risk of getting infected by other strains of *Shigella*. Due to the large number of cases of Shigellosis and subsequent death around the world vaccines to prevent the disease, especially in developing countries, are well under way (Bhardwaj and Panhotra, 1985).

#### b. Pathogenicity

*Schigella flexneri* causes shigellosis an infectious disease that causes watery loose stool, diarrhea, vomiting, stomach cramps and high fever. The infection lasts five to seven days but children, the elderly and individuals who are immune-suppressed are more prone to severe cases of shigellosis. In fact if left untreated diarrhea can cause death and seizures can result due to high fever.

## Appendix D

## Photo plate I

**Medicinal Plants**

Photo#: 1 *Aloe vera*

Photo#: 2 *Astilbe rivularis*

Photo#: 3 *Bergenia ciliata*

Photo#: 4 *Cuscuta reflexa*

Photo#:5 *Daphne bholua*

Photo#: 6 *Digitalis purpurea*

Photo#: 7 *Dioscorea bulbifera*

Photo#: 8 *Epilobium roseum*

Photo#: 9 *Gaultheria fragrantissima*

*Photo#:10 Justicia adhatoda*

Photo#: 11 *Mahonia nepalensis*

## Photo plate II

### Medicinal Plants

Photo#: 1 *Meconopsis horidula*

Photo#: 2 *Murraya koenigii*

Photo#: 3 *Paris polyphyla*

Photo#: 4 *Rubia manjith*

Photo#: 5 *Rubus nepalensis*

Photo#: 6 *Rumex nepalensis*

Photo#: 7 *Smilex aspara*

Photo#: 8 *Taxus baccata*

Photo#: 9 *Vetiveria zizanoides*

## Photo plate III

**Antibacterial Test**

Photo#: 1 Bacerial strains of *Escherichia coli* cultures on nutrient media.

Photo#: 2 Bacerial strains of *Streptococcus pneumoni* cultures on nutrient media.

Photo#: 3 Aqueous and methanol extract of plants.

Photo#: 4 ZOI shown by plant extracts of *Rubia manjith* on methanolic extract against *Escherichia coli*.

Photo#: 5 ZOI shown by plant extracts of *Lobelia pyramidalis* on methanolic extract against *Klebsiella pneumonia*.

Photo#: 6 ZOI shown by plant extracts of *Epilobium roseum* on aqueous extract against *Staphylococcus aureus*.

## Photo plate IV

### Antibacterial Test

Photo#: 1 ZOI shown by plant extracts of *Epilobium roseum* on methanolic extract against *Klebsiella pneumonia*.

Photo#: 2 ZOI shown by plant extracts of *Stellaria vestita* on aqueous extract against *Shigella flexneri*.

Photo#: 3 ZOI shown by plant extracts of *Cuscuta reflexa* on methanolic extract against *Staphylococcus aureus*.

Photo#: 4 ZOI shown by plant extracts of *Rubus nepalensis* on aqueous extract against *Shigella flexneri*.

Photo#: 5 Methanolic extracts of some selected plants.

Photo#: 6 Aqueous extracts of s ome selected plants.