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 & Kurmi 2006).

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 & 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 19^{th} 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 1985). 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 Activitiy 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 antimicrobials from higher plants appears rewarding as it will lead to the development of phytomedicine to act against microbes (Iwu *et al.* 1999). Plants with possible antimicrobial effect should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by large number of researcher in different parts of world especially in medicinal plants of Africa and Nigeria. But very limited work has been done in this field in Nepal (Panthi & 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 1980s most major infectious diseases in the 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 & 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 & 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 & 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, auxotrophic or metabolic by-pass (Dax 1997).

1.1.3 Natural Products from Plants

Bioactive natural products are mainly secondary metabolites which are used by the host plants as defensive and protective mechanisms against their enemies and predators (Patrick 2001). Generally, screening of these secondary metabolites and development of drugs is a very hard task requiring much effort starting from botanical identification, collection, extraction, isolation, purification, and compound identification to pharmacological and clinical testing. Biologically active compounds present in the medicinal plants have always been of great interest to phytochemist it is

because the medicinal value of the plant lies on these compounds. In recent year, secondary chemical metabolites previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents (Krishnaraju *et al.* 2005).

The phytochemical study of the medicinal plants provide valuable material base for the development and discovery of new drugs of natural origin. The medicinal value of plant lies on some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds. The phytochemical research based on ethnopharmacological information is generally considered as an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan *et al.* 2006). Knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab *et al.* 2003). Chemical constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are inert chemical constituents (Iyengar 1995).

1.2 Objectives

1.2.1 General objective

The overall aim of the study was to access the antimicrobial activities of few medicinal plants of Nepal.

1.2.2 Specific objectives

- To screen and evaluate the antibacterial activity of crude methanol extract of some medicinal plants.
- To perform the preliminary phytochemical screening of those plants.

1.3 Rationale of the Study

The different medicinal plants are being used by tribal people on different bacterial disease. 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 plants 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.

1.4 Limitations of the Study

Due to the time constraints this study was limited to only six strains of bacteria. Minimum inhibitory concentration (MIC) is also an important basis to evaluate the antibacterial activity, only zone of inhibition (ZOI) was determined but MIC was avoided.

Qualitative phytochemical analysis was conducted but due to the unavailability of chemicals and equipments, their quantification was not performed in this study.

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 estimates that 80% of the population living in rural areas use or depend on herbal medicine for their health care (WHO 2002). 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 and etc. (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 & Morris 1976).

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.

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 & 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.* 2004, and Mahato & Chaudhary 2005). Not only this, phytochemical screening of the plants were coupled with antibacterial effects to link the main groups of phytochemicals and their effects on bacteria (Raghavendra *et al.* 2005, Parekh & Chanda 2006, Nandagopal *et al.* 2007, Sanni *et al*. 2008, Chhetri *et al*. 2008, Gyawali *et al.* 2008).

2.1.1 Work Outside the Country

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.*

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*.

Barbour *et al.* (2004) 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.

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.

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.

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.

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 bacterial 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.

2.1.2 Work Inside the Country

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.

Shakya (1988) studied 45 medicinal plants and 18 types of essential oils for antimicrobial activities by filter paper diffusion method. The test organisms employed were *Staphylococcus aureus, Bacillus subtilis, Shigella dysenteriae, Escherichia coli, Candida albicans,* and *Saccharomyces cerevesiae.* Some of the plant extract showed weak activities but extracts of *Berberis aristata* and *Anagalis arvensis* showed moderate activities on the bacteria and fungi respectively. Most of the essential oils showed moderate, encouraging, and strong activities.

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.

Shrestha and Sharma (1998) 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.

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.

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.

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.

Gyawali *et al.* (2008) carried out the phytochemical screening of 47 Nepalese medicinal plants. Screening of medicinal plants was performed to test the presence of alkaloids, flavanoids, glycosides, saponins, tannins and terpenoids. Most of the species contain secondary metabolites but their concentration varied, and there was also found definite correlation between the traditional application of plants and possession of secondary metabolites.

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.

CHAPTER-III

MATERIALS AND METHODS

3.1 Sample Material Preparation

3.1.1 Selection of Medicinal Plants

Eighteen 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. All the materials used to accomplish this study are given in the Appendix-A, and their description and uses are provided in Appendix-B.

3.1.2 Collection of Plant Materials

Fresh plant or plant parts were collected from different places- Kathmandu and Syangja 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. Exposure to sunlight was avoided to prevent the loss of active components.

3.1.3 Plant Identification

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

3.1.4 Packaging and Storage

The completely dried samples were packed in water proof bags and incompletely dried samples were kept in cotton bags which enhance the air circulation and prevent rotting during storage. The packed samples were stored in room temperature avoiding direct sunlight.

3.1.5 Grinding

The completely dried samples of each plant parts were ground separately into fine powder with the help of electric grinder. The powdered material was stored on polythene bags and used as needed.

3.2 Antibacterial Test

Inhibition of bacterial growth was tested by using the paper disc diffusion method (Bauer *et al*.1966, Parekh & 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 (Whatman 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 (25mLx3 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 Central Department of Microbiology, T. U. The studied strains include six different types of bacteria, two gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and four gram-negative (*Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* 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 Whatman 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, while tetracycline paper discs were used as positive control. For tetracycline paper discs 10mL solution was prepared mixing 0.8mL tetracycline solution (prepared by dissolving 500mg tablets of tetracycline in 20mL methanol) with 9.2mL of methanol. The final concentration of tetracycline was 0.25mg/mL.

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. 28g 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. 13g 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.

3.3 Phytochemical Test

The powdered plant material was used for phytochemical screening test. Chemical tests were carried out on the aqueous and alcoholic extracts using standard procedures to identify the constituent as described in literatures (Harborne 1973, Somolenski *et al.* 1974, Rizk 1982, Salehi *et al.* 1992).

3.3.1 Test for Alkaloids

About 2.5g of sample was extracted with 10mL methanol and evaporated to dryness and the residue was heated on a boiling water bath with 2N HCl (5mL). The resulting mixture was centrifuged for 10 minute at 3000 rpm to remove filtrate. First 1mL of the filtrate was treated with a few drops of Mayer's reagent and the second 1mL portion was treated with equal amounts of Wagner's reagent. The samples were then observed for presence of precipitation.

3.3.2 Test for Glycosides

About 0.2g of powdered medicinal plant was taken in a test tube with 5mL of water and warmed it on a water bath for two minutes. Resulting solution of plant extract was filtered and pipette out the supernatant liquid. 0.1mL of Fehling's A solution was added and then Fehling's B solution until the resulting solution becomes alkaline. Warmed the resulting solution on a water bath for two minutes and observed for precipitation.

3.3.3 Test for Saponins

About 2.5gm of the plant material was extracted with 10mL of boiling water. After cooling, the extract was shaken vigorously to froth and was then allowed to stand for 15-20min and classified for saponins content as follows: no froth-negative; froth less than 1cm-weakly positive; froth 1.2cm high-positive; and froth greater than 2cm-strongly positive.

3.3.4 Test for Tannins

About 0.5g of sample was boiled in 20mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride ($FeCl_3$) solution was added and observed for brownish green or blue black coloration. A blue–black precipitate was taken as evidence for the presence of tannins.

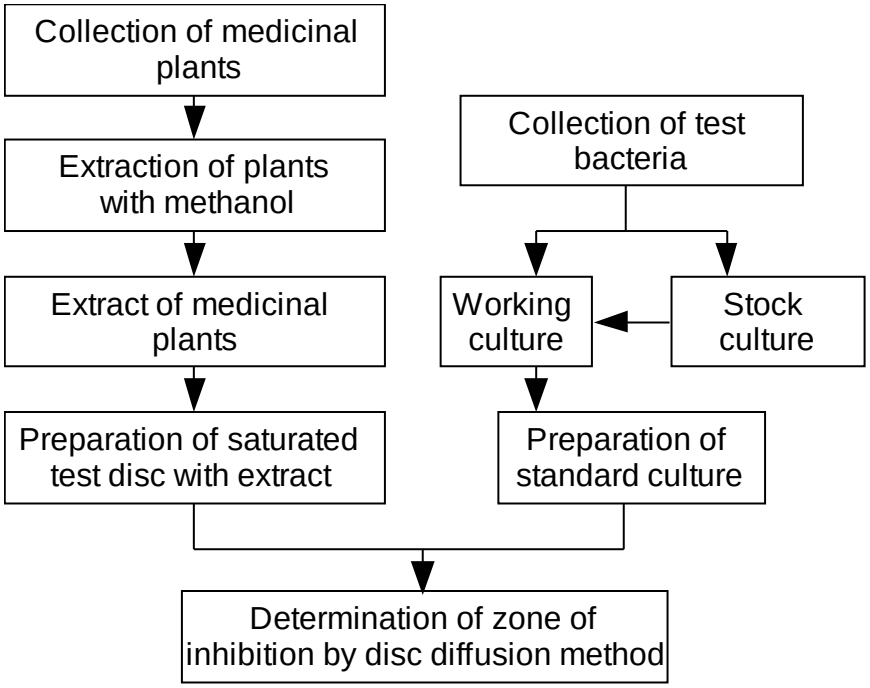
3.3.5 Test for Terpenoids (Salkowski Test)

5mL methanol extract, corresponding to 2.5g of plant material, was mixed in 2mL chloroform, and concentrated H_2SO_4 (3mL) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

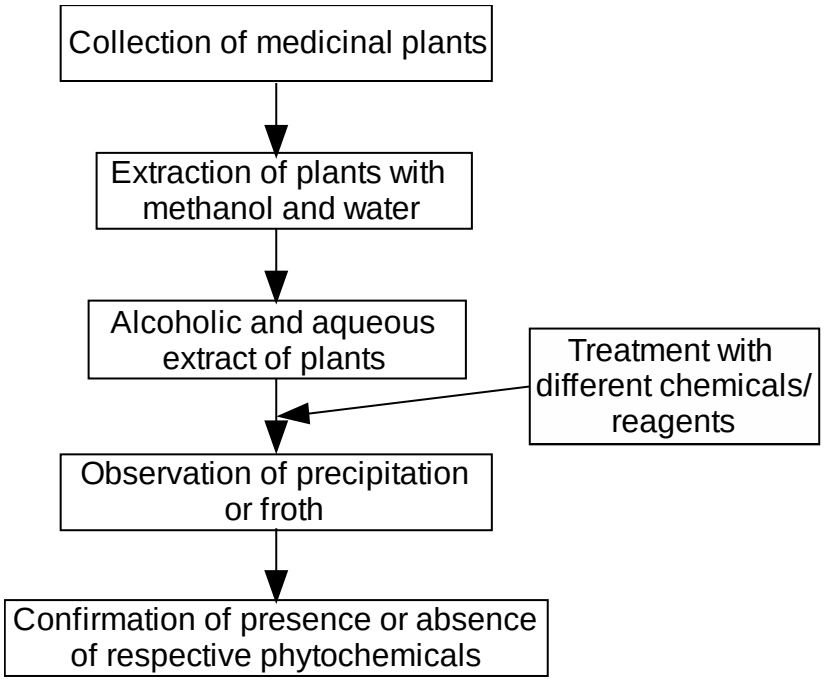
Note: A (+) score was recorded if the reagent produced only slight opaqueness; a (++) score was recorded if a definite turbidity, but no flocculation was observed and a (+++) score was recorded if a definite heavy precipitation of flocculation was produced.

Flow Chart of the Methods

I. Antibacterial Test



II. Phytochemical Test



CHAPTER-IV

RESULTS

4.1 Yield of Methanol Crude Extract of Medicinal Plants

The percentage yield of the crude extract of medicinal plants by soaking process is shown in table 1. The amount of extract varied among the medicinal plants. *Drymaria cordata* gave the highest yield (20%), followed by *Vitex negundo* (18%), *Taraxacum officinale* (17%), *Ageratum conyzoides* (13%), *Bauhinia purpurea* (13%) etc. The lowest yield was obtained from *Zizyphus mauritiana* (5%) and *Urtica dioica* (4%).

4.2 Screening of Medicinal Plants for Antibacterial Activity

Table 2 summarizes the result obtained from screening of methanolic extract of different medicinal plants against tested bacteria. It is revealed from the table that all the tested medicinal plants were effective against the employed bacteria. Among 18

Table 1: Percentage yields of crude methanol extract of medicinal plants

S.N.	Plant species	Part used	% yield
1.	*Euphorbia hirta*	Whole plant	11
2.	*Drymaria cordata*	Whole plant	20
3.	*Taraxacum officinale*	Roots	17
4.	*Bauhinia purpurea*	Flower	13
5.	*Ageratum conyzoides*	Leaves	13
6.	*Ficus religiosa*	Leaves	7
7.	*Urtica dioica*	Leaves	4
8.	*Lantana camara*	Leaves	8
9.	*Phyllanthus amarus*	Whole plant	9
10.	*Cinnamomum tamala*	Leaves	7
11.	*Melia azedarach*	Fruit	8
12.	*Vitex negundo*	Leaves	18
13.	*Oxalis corniculata*	Whole plant	10
14.	*Achyranthes bidentata*	Roots	5
15.	*Zizyphus mauritiana*	Roots	5
16.	*Mimosa pudica*	Roots	8
17.	*Cissampelos pareira*	Leaves	9
18.	*Rhus javanica*	Fruit	12

medicinal plants tested, in the present study, all plants show activity against at least two bacteria. *Phyllanthus amarus* and *Rhus javanica* inhibited all the tested bacteria. Similarly, *Drymaria cordata* was effective against 83% of tested bacteria. Nine plants inhibit the growth of 67% and five plants were effective against only 50% of screened bacteria and remaining one plant *Ficus religiosa* inhibited growth of 33% of the screened bacteria (Table 3).

Among the tested bacteria, *Escherichia coli* were most resistant bacteria. Only five plants among 18 plants employed in this screening could inhibit its growth where as *Staphylococcus aureus* was the most susceptible bacteria which were inhibited by 17 plants out of 18 plants tested.

4.3 Evaluation of Antibacterial Activity of Medicinal Plants

The mean zone of inhibition (ZOI) of methanol extract of plants, which were able to show significant zone of inhibition (≥8mm) during qualitative screening process is shown in table 4.

Phyllanthus amarus and *Rhus javanica* had broad spectrum activity. Both of the plants inhibited the growth of all the bacteria tested. *Phyllanthus amarus* showed the strongest ZOI (13mm) towards *Staphylococcus aureus* and weakest ZOI (8.5mm) towards *Escherichia coli*. Similarly, *Rhus javanica* showed strongest ZOI (15.5mm) towards *Staphylococcus aureus* and weakest ZOI (9mm) towards *Escherichia coli.*

Drymaria cordata also showed the comparative broad spectrum activity. It inhibited the growth of five bacteria out of six bacteria screened. It could not inhibit the growth of *Escherichia coli*, showed strongest activity against *Staphylococcus aureus* with 23mm ZOI and low activity against *Salmonella typhi* with 8mm ZOI.

Five plants *Bauhinia purpurea*, *Urtica dioica*, *Vitex negundo*, *Oxalis corniculata* and *Zizyphus mauritiana* showed somewhat similar results. All plants inhibited the growth of four bacteria viz. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Proteus vulgaris*. Two bacteria *Escherichia coli* and *Salmonella typhi* are resistant towards these plants.

Bauhinia purpurea showed highest ZOI (13.5mm) with *Staphylococcus aureus* and lowest ZOI (8.5 mm) with *Pseudomonas aeruginosa* whereas *Urtica dioica* had strongest ZOI (10.5mm) with *Staphylococcus aureus* and weakest ZOI (8mm) with *Bacillus subtilis* and *Proteus vulgaris*. Similar trend was showed by *Vitex negundo* which had strongest ZOI (17mm) towards *Staphylococcus aureus* and weakest ZOI (10 mm) with *Proteus vulgaris*. *Oxalis corniculata* showed strongest ZOI (15.5mm) against *Staphylococcus aureus* and weakest ZOI (8mm) against *Bacillus subtilis*

Table 2: Antibacterial property of methanolic extracts of different medicinal plants against tested bacteria

Plant Names	Pa	Sa	Ec	St	Bs	Pv
Euphorbia hirta	-	+	-	-	+	+
Drymaria cordata	+	+	-	+	+	+
Taraxacum officinale	-	+	-	-	+	+
Bauhinia purpurea	+	+	-	-	+	+
Ageratum conyzoides	-	+	-	+	+	+
Ficus religiosa	-	-	-	+	+	-
Urtica dioica	+	+	-	-	+	+
Lantana camara	+	+	+	-	-	+
Phyllanthus amarus	+	+	+	+	+	+
Cinnamomum tamala	-	+	+	+	-	+
Melia azedarach	+	+	+	-	+	-
Vitex negundo	+	+	-	-	+	+
Oxalis corniculata	+	+	-	-	+	+
Achyranthes bidentata	-	+	-	-	+	+
Zizyphus mauritiana	+	+	-	-	+	+
Mimosa pudica	+	+	-	-	+	-
Cissampelos pareira	-	+	-	-	+	+
Rhus javanica	+	+	+	+	+	+

(Note: all the plants show positive result for positive and negative result for negative control)

Abbreviations: Pa – *Pseudomonas aeruginosa*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, St – *Salmonella typhi*, Bs – *Bacillus subtilis*, Pv- *Proteus vulgaris*.

Zizyphus mauritiana also showed the similar trend as *Bauhinia purpurea*. It showed strongest ZOI (14.5mm) against *Staphylococcus aureus* and weakest ZOI (10.5mm) against *Pseudomonas aeruginosa*.

Four plants *Ageratum conyzoides*, *Lantana camara*, *Cinnamomum tamala*, and *Melia azedarach* all inhibited the growth of four bacteria out of six bacteria tested.

Ageratum conyzoides inhibited the growth of four bacteria viz. *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, and *Proteus vulgaris* while other two bacteria *Pseudomonas aeruginosa* and *Escherichia coli* were resistant to it. It showed highest ZOI (13mm) with bacteria *Staphylococcus aureus* whereas bacteria *Salmonella typhi* showed smallest ZOI (8mm).

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

S.N.	Plant Names	No. of bacteria inhibited	% of bacteria inhibited
1.	*Euphorbia hirta*	3	50
2.	*Drymaria cordata*	5	83
3.	*Taraxacum officinale*	3	50
4.	*Bauhinia purpurea*	4	67
5.	*Ageratum conyzoides*	4	67
6.	*Ficus religiosa*	2	33
7.	*Urtica dioica*	4	67
8.	*Lantana camara*	4	67
9.	*Phyllanthus amarus*	6	100
10.	*Cinnamomum tamala*	4	67
11.	*Melia azedarach*	4	67
12.	*Vitex negundo*	4	67
13.	*Oxalis corniculata*	4	67
14.	*Achyranthes bidentata*	3	50
15.	*Zizyphus mauritiana*	4	67
16.	*Mimosa pudica*	3	50
17.	*Cissampelos pareira*	3	50
18.	*Rhus javanica*	6	100

Lantana camara was active against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris*. Remaining two bacteria *Bacillus subtilis* and *Salmonella typhi* were resistant towards it. *Proteus vulgaris* showed highest ZOI (17mm) whereas *Escherichia coli* showed weakest ZOI of 8mm.

The methanolic extract of *Cinnamomum tamala* inhibited the growth of *Staphylococcus aureus*, *Escherichia coli, Salmonella typhi,* and *Proteus vulgaris* but it showed comparative narrow range of ZOI (8-9mm) against all these bacteria. Two bacteria *Pseudomonas aeruginosa* and *Bacillus subtilis* were resistant to it.

Melia azedarach inhibited the growth of *Pseudomonas aeruginosa, Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. Two bacteria *Salmonella typhi* and

Table 4: Mean zone of inhibition (ZOI) shown by different medicinal plants against tested bacteria

Plant Names	Pa	Sa	Ec	St	Bs	Pv
Euphorbia hirta	-	25	-	-	17.5	16.5
Drymaria cordata	14.5	23	-	8	15	11
Taraxacum officinale	-	17.5	-	-	10.5	12
Bauhinia purpurea	8.5	13.5	-	-	10	10
Ageratum conyzoides	-	13	-	8	9.5	10
Ficus religiosa	-	-	-	8	8	-
Urtica dioica	9	10.5	-	-	8	8
Lantana camara	8.5	15.5	8	-	-	17
Phyllanthus amarus	9.5	13	8.5	9.5	12.5	9.5
Cinnamomum tamala	-	9	8	8	-	9
Melia azedarach	11.5	14.5	8	-	9	-
Vitex negundo	10.5	17	-	-	15	10
Oxalis corniculata	8.5	15.5	-	-	8	15
Achyranthes bidentata	-	11.5	-	-	8.5	10
Zizyphus mauritiana	10.5	14.5	-	-	11.5	13
Mimosa pudica	9.5	8	-	-	8	-
Cissampelos pareira	-	15	-	-	12	11
Rhus javanica	11	15.5	9	9.5	10	13

Abbreviations: Pa – *Pseudomonas aeruginosa*, Sa – *Staphylococcus aureus*, Ec – *Escherichia coli*, St – *Salmonella typhi*, Bs – *Bacillus subtilis*, Pv- *Proteus vulgaris*.
The mean ZOI was in mm including the diameter of disc (6mm).

Proteus vulgaris were resistant to it. The value of ZOI ranges from 14.5 mm against *Staphylococcus aureus* to 8 mm against *Escherichia coli*.

Four plants *Euphorbia hirta*, *Taraxacum officinale*, *Achyranthes bidentata* and *Cissampelos pareira* showed comparative narrow spectrum of activity. These plants

inhibited the growth of only three bacteria viz. *Staphylococcus aureus, Bacillus subtilis*, and *Proteus vulgaris* remaining three bacteria were resistant to these plants.

Euphorbia hirta showed strongest activity against *Staphylococcus aureus* with biggest ZOI of 25mm and weakest ZOI of 16.5mm against *Proteus vulgaris*. It could not inhibit the growth of *Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi*.

Taraxacum officinale showed strongest activity against *Staphylococcus aureus* with ZOI of 17.5mm and weakest activity against *Bacillus subtilis* with ZOI of 10.5mm.

Achyranthes bidentata showed strongest activity against *Staphylococcus aureus* with ZOI of 11.5mm whereas weakest activity was exhibited by *Bacillus subtilis* with ZOI of 8.5mm.

Cissampelos pareira showed largest ZOI of 15mm against *Staphylococcus aureus* and weakest ZOI of 11mm against *Proteus vulgaris.*

Another plant *Mimosa pudica* also inhibited the growth of three bacteria viz. *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. The ZOI shown by the plants were 9.5mm, 8mm and 8mm respectively.

Ficus religiosa showed weakest activity among the tested plants. It can inhibit the growth of only two bacteria *Salmonella typhi* and *Bacillus subtilis* and the ZOI (8mm each) was also comparatively very low.

4.4 Evaluation of Susceptibility of the Tested Bacteria

Susceptibility of the bacteria is evaluated in term of number of medicinal plants which affected the bacteria and the extent of it i.e. size of ZOI produced by them. *Staphylococcus aureus* was the most susceptible bacteria being inhibited by 17 out of 18 medicinal plants. *Escherichia coli* were found to be the most resistant bacteria being susceptible to only five plants.

The growth of *Pseudomonas aeruginosa* was inhibited by 11 plant extracts and maximum inhibition was observed with *Drymaria cordata* as 14.5mm zone of inhibition followed by *Melia azedarach* (11.5mm), *Rhus javanica* (11mm), *Vitex*

negundo (10.5mm), and *Zizyphus mauritiana* (10.5mm). In contrast, lowest inhibition was observed with *Bauhinia purpurea*, *Lantana camara*, and *Oxalis corniculata* each with lowest ZOI of 8.5mm each.

Staphylococcus aureus was found to be most susceptible bacteria and it was inhibited by 17 medicinal plants. All plants except *Ficus religiosa* can inhibit the growth of this bacterium. Among them highest ZOI was produced by *Euphorbia hirta* (25mm) followed by *Drymaria cordata* (23mm), *Taraxacum officinale* (17.5mm) and lowest ZOI was given by *Mimosa pudica* (8mm).

Escherichia coli were found to be the most resistant bacteria and it was susceptible to extract of five medicinal plants. *Rhus javanica* showed highest activity with 9mm ZOI where as three plants *Lantana camara*, *Cinnamomum tamala* and *Melia azedarach* showed the low activity with ZOI of 8mm each.

The growth of *Salmonella typhi* was inhibited by the six plants out of 18 plants tested. The maximum ZOI (9.5mm) was showed by *Rhus javanica* and *Phyllanthus amarus* whereas *Drymaria cordata*, *Ageratum conyzoides*, *Ficus religiosa* and *Cinnamomum tamala* showed the low value of ZOI of 8mm.

Bacillus subtilis was inhibited by 16 medicinal plants. Largest value of ZOI (17.5mm) was exhibited by *Euphorbia hirta* followed by *Drymaria cordata* (15mm), *Vitex negundo* (15mm), and *Phyllanthus amarus* (12.5mm) whereas *Ficus religiosa*, *Urtica dioica*, *Oxalis corniculata* and *Mimosa pudica* showed the low value of ZOI of 8mm each.

Proteus vulgaris were found to be the most susceptible bacteria among gram-negative bacteria employed in this evaluation. These bacteria were inhibited by 15 plant extracts. Among them *Lantana camara* showed the highest ZOI (17mm) followed by *Euphorbia hirta* (16.5mm), and *Oxalis corniculata* (15mm) whereas *Urtica dioica* showed the lowest ZOI of 8mm.

4.5 Phytochemical Screening of Medicinal Plants

The Phytochemical screening was carried out on 18 medicinal plants. These plants are used in traditional medicinal systems of Nepal to cure different bacterial diseases. The

investigation revealed the presence of secondary metabolites in all plants but their concentration varies (Table 5). Among the investigated plants 67% of plants showed the presence of alkaloids, glycosides, saponins and terpenoids were found to be present in 78% of plants evaluated and 72% plants contain tannin.

4.5.1 Alkaloids

Among evaluated plants, 12 plants were found to contain alkaloids. From this study it is found that *Urtica dioica* and *Mimosa pudica* were rich in alkaloids. *Euphorbia hirta, Taraxacum officinale, Ageratum conyzoides, Ficus religiosa, Lantana camara* and *Cissampelos pareira* contain considerable amount of alkaloids. The trace amount of alkaloids was detected in plants *Bauhinia purpurea, Cinnamomum tamala, Melia azedarach* and *Vitex negundo.*

4.5.2 Glycosides

Altogether 14 medicinal plants were found to contain glycosides. Among them *Drymaria cordata* and *Ageratum conyzoides* are rich in glycosides. Considerable amount of glycosides was detected from *Bauhinia purpurea, Ficus religiosa, Phyllanthus amarus, Oxalis corniculata, Mimosa pudica* and *Rhus javanica,* Glycosides was also detected in trace amount form *Urtica dioica, Cinnamomum tamala, Melia azedarach, Vitex negundo, Achyranthes bidentata* and *Cissampelos pareira*.

4.5.3 Saponins

Fourteen medicinal plants are found to contain saponins. It was detected in rich amount in plants *Drymaria cordata, Vitex negundo, Achyranthes bidentata, Zizyphus mauritiana* and *Mimosa pudica*. Considerable amount of it was also detected from *Lantana camara,* C*innamomum tamala, Cissampelos pareira* and *Rhus javanica*. Saponins was also detected in trace amount from *Taraxacum officinale, Bauhinia purpurea, Ageratum conyzoides, Urtica dioica* and *Oxalis corniculata*.

4.5.4 Tannins

Tannin was found to present in altogether 13 plants among the 18 plants investigated. *Euphorbia hirta, Urtica dioica, Phyllanthus amarus* and *Zizyphus mauritiana* contain

rich amount of tannins. Considerable amount of it was also detected in *Drymaria cordata*, *Taraxacum officinale*, *Bauhinia purpurea*, *Lantana camara*, *Oxalis corniculata* and *Mimosa pudica*. Tannin is also found in trace amount in *Achyranthes bidentata*, *Cissampelos pareira* and *Rhus javanica*.

Table 5: Chemical constituents of medicinal plants

Plant species	Alkaloids	Glycosides	Saponins	Tannins	Terpenoids
Euphorbia hirta	++	-	-	+++	++
Drymaria cordata	-	+++	+++	++	+
Taraxacum officinale	++	-	+	++	+
Bauhinia purpurea	+	++	+	++	+
Ageratum conyzoides	++	+++	+	-	+
Ficus religiosa	++	++	-	-	-
Urtica dioica	+++	+	+	+++	-
Lantana camara	++	-	++	++	++
Phyllanthus amarus	-	++	-	+++	++
Cinnamomum tamala	+	+	++	-	+
Melia azedarach	+	+	-	-	+++
Vitex negundo	+	+	+++	-	++
Oxalis corniculata	-	++	+	++	+
Achyranthes bidentata	-	+	+++	+	+
Zizyphus mauritiana	-	-	+++	+++	-
Mimosa pudica	+++	++	+++	++	++
Cissampelos pareira	++	+	++	+	-
Rhus javanica	-	++	++	+	++

Note: A (+) score was recorded in the reagent produced only slight opaqueness; a (++) score was recorded if a definite turbidity, but no flocculation was observed and a (+++) score was recorded if a definite heavy precipitation of flocculation was produced.

Table 6: No. of plants constituting respective phytochemicals

Phytochemicals	No. of plants	% of plants
Alkaloids	12	67
Glycosides	14	78
Saponins	14	78
Tannins	13	72
Terpenoids	14	78

4.5.5 Terpenoids

Terpenoids was detected from 14 plants among the tested plants. *Melia azedarach* was rich in terpenoids. Plant species *Euphorbia hirta*, *Lantana camara*, *Phyllanthus amarus*, *Vitex negundo*, *Mimosa pudica* and *Rhus javanica* contain good amount of terpenoids. It is also detected in trace amount from *Drymaria cordata*, *Taraxacum officinale*, *Bauhinia purpurea*, *Ageratum conyzoides*, *Cinnamomum tamala*, *Oxalis corniculata* and *Achyranthes bidentata.*

CHAPTER-V

DISSCUSSION

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 populace based on long and dangerous self experiment. Progress over the centuries towards a better 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 & Chanda 2006).

In the present study, an attempt has been made to evaluate the antibacterial activities of 18 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 *et al*. 2007) with slight modifications. The six different strains of bacteria were employed to test the antibacterial activity of crude methanol extract of selected plants. The preliminary phytochemical screening was also performed to observe the presence of secondary metabolites on those plants.

5.1 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 & Afolagan 2006, Parekh & Chanda 2007). Alcohol extracts provide a more complete extraction, including less polar compounds, and many of these extracts have been found to possess antimicrobial properties (Ali-Shtayeh & 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 could contain only anthocyanins, starches, tannins, saponins, polypeptides and lectins (Cowan 1999). 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 methanol. The yield varied in different medicinal plants largely. It ranges from 4% to 20%. 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. Plant materials with large amount of leave gives large amount of yield. 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.

5.2 Screening of Antibacterial Activities

The study revealed that the *Rhus javanica* and *Phyllanthus amarus* were the most effective medicinal plants. These two plants showed the activity against all the tested strains of bacteria. Timsina (2003) also found that *Rhus javanica* was effective against more than 80% of tested microorganisms whereas, Mahato and Chaudhary (2005) reported 100% activity of methanolic extract of fruit of *Rhus javanica* against four

bacterial strains viz. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*.

Shakya *et al.* (2008) found that 50% ethanolic extract of whole plant of *Drymaria cordata* was effective against two strains of bacteria among eight strains of microorganisms tested. But in this study methanolic extract of *Drymaria cordata* was effective against 83% of bacteria. The difference in result may be due to the difference in solvent used and different strains of bacteria employed.

Bauhinia purpurea, *Ageratum conyzoides*, *Urtica dioica*, *Lantana camara*, *Cinnamomum tamala*, *Melia azedarach*, *Vitex negundo*, *Oxalis corniculata* and *Zizyphus mauritiana* were effective against 67% of the tested bacteria. Mahato and Chaudhary (2005) reported the 100% activity of *Ageratum conyzoides* leaves against four strains of bacteria two gram-positive and two gram-negative. This result was somewhat similar to Parajuli *et al.* (2001). According to Shakya *et al*. (2008) whole plant extracts of *Oxalis corniculata* did not show any activity. But in the present study, it inhibited the growth of four strains of bacteria. However, the present result was supported by Raghavendra *et al*. (2006). Similarly, Parekh *et al*. (2007) tested leaf extracts of *Cinnamomum tamala* that showed no activities with the studied bacteria but Shakya *et al.* (2008) reported moderate activities of 50% methanolic extract of *Cinnamomum tamala* leaves on two bacterial strains *Escherichia coli* and *Salmonella typhi*. The investigation of Taylor *et al.* (1995) on root extract of *Zizyphus mauritiana* showed antibacterial activity on *Bacillus subtilis* and *Staphylococcus aureus* but it did not show activity on *Escherichia coli* which supports present result. Mahato and Chaudhary (2005) found that the steam bark of *Melia azedarach* show no zone of inhibition against tested bacteria while in the present study, fruit of *Melia azedarach* were assayed which showed that it was active against 67% of bacteria.

Euphorbia hirta, *Taraxacum officinale*, *Achyranthes bidentata*, *Mimosa pudica* and *Cissampelos pareira* showed activity with 50% of tested bacteria. Shakya *et al.* (2008) reported 50% ethanolic extract of whole plant of *Euphorbia hirta* was active against *Bacillus subtilis* and *Escherichia coli*. The same researcher also reported the activity of 50% ethanolic extract of tuber of *Cissampelos pareira* with *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi*.

Methanolic extract of leaves of *Ficus religiosa* showed comparatively narrow spectrum of activity. It inhibited growth of *Salmonella typhi* and *Bacillus subtilis*.

5.3 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.

Evaluation of antibacterial activity was aspect of this study as compound having broad spectrum may have little potency and such compounds have little value in developments of new drugs. The evaluation of antibacterial substance becomes the essential step during new drug research from natural product. 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.

Among the tested plants, largest ZOI (25mm) was shown by *Euphorbia hirta* on bacteria *Staphylococcus aureus*. This plant showed ZOI of 17.5 and 16.5mm against bacteria *Bacillus subtilis* and *Proteus vulgaris* respectively. 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. *Drymaria cordata* also showed similar trend of results. It showed highest ZOI of 23mm with *Staphylococcus aureus*.

Two plants *Rhus javanica* and *Phyllanthus amarus* showed broad spectrum of activity but had comparative lesser value of ZOI. It may be due to the low diffusibility of antibacterial substances present on those plants. The diffusing capacity of the chemical substances may be affected by the presence of fat, oils, resin or wax in the extract. Presence of these substances may cause the low diffusibility in the agar media. The defatted extracts were more active than the extract without defatting (Parekh & Chanda 2006).

Nine plants inhibited the growth of four bacteria and show high spectrum activity. The ZOI value shown by these plants ranges widely. Among these plants *Vitex negundo* showed highest value of ZOI. It ranges from 10-17mm. But *Urtica dioica* (8-10.5mm) and *Cinnamomum tamala* (8-9mm) have low and narrow range of ZOI value.

Five plants show narrow spectrum activity and inhibited the growth of three bacteria. Among them the plant *Euphorbia hirta* has high value of ZOI. It ranges from 16.5-25mm, where as *Mimosa pudica* has very low value of ZOI and it ranges from 8-9.5mm.

One plant *Ficus religiosa* showed narrow spectrum activity by inhibiting only two bacteria. It also has the lowest value of ZOI (8mm). So this plant has very less potency in antibacterial activity.

Among the tested bacteria, *Staphylococcus aureus* showed high value of ZOI (8-25mm) where as bacteria *Escherichia coli* showed low value of ZOI (8-9mm). Amongst the gram-positive and gram-negative bacteria, gram-positive bacterial strains were more susceptible to the extracts as compared to gram-negative bacteria. This is in agreement with previous reports that plant extracts are more active against gram-positive bacteria than gram-negative bacteria (Vlietinck *et al*. 1995, Rabe & Van Staden 1997, Lin *et al.* 1999, Parekh & Chanda 2007). 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 & Gurler 1989).

5.4 Phytochemical Analysis

All the plants which were used to evaluate antibacterial activities were also subjected to preliminary phytochemical screening for detection of phytochemical constituents present in them. The main purpose of phytochemical screening was to identify the main group of chemical constituents present in different plant extracts by their color reaction with different specific reagents and chemicals. Phytochemical screening of 18 medicinal plants showed that these plants are rich in different Phytochemicals.

It is known that the plants which are rich in wide variety of secondary metabolites belonging to chemical classes such as tannins, terpenoids, alkaloids, and polyphenols are generally superior in medicinal properties and exhibit physiological activity (Gurib-Facim 2006, Muetzel & Becker 2006). Most important bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Cowan 1999). Most of the plants in the present study contain glycosides, saponins and terpenoids whereas comparative fewer plants contain tannin and alkaloids. The antibacterial properties exhibited by extracts may be associated with presence of tannins, saponins, cardiac glycosides and alkaloids found in the plant extract (Bastista *et al.* 1994, Boris 1996, Akujobi *et al.* 2006).

Many alkaloids contain at least one nitrogen atom in an amine type structure that makes them pharmacologically active. In the present study, twelve plants were found to contain alkaloids in different concentration. Root of *Mimosa pudica* and leaves of *Cinnamomum tamala* found to contain alkaloids but this result was in contrast with the result of Karanjit *et al.* (2007). This difference may be due to the difference in solvent used in extraction process. In the later study, 50% ethanol was used to extract the plants. Karanjit *et al.* (2007) also found to contain alkaloids in ethanolic extract of leaves of *Urtica dioica* and *Cissampelos pareira.* This result is similar to present study. Hadi (2002) isolated the antimicrobial alkaloids: lombine, cononaridine and mataranine A and B from the plant *Vocanga foetida*. These alkaloids were reported to

have strong activity against *Staphylococcus aureus*. A mixture of mataranine A and B was found active *in vitro* against *Plasmodium falciparum.*

Thirteen plants were found to contain tannins. The plants which contain tannin show more antibacterial activities in the present study. *Lantana camara, Cissampelos pareira, Mimosa pudica* were found to contain tannins. These results are similar to Karanjit *et al*. (2007). Uma Devi *et al*. (2007) reported that the ethanolic root extracts of *Achyranthes bidentata* contain tannins. This result favors present outcome. Tannins are also known antimicrobial agents. Tannins (commonly referred to as tannic acid) are water-soluble polyphenols. It has been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sodipo *et al.* 1991). Phytotherapeutically tannin-containing plants are used to treat nonspecific diarrhea, inflammations of mouth and throat and slightly injured skins (Westendarp 2006). Tannin was reported to possess physiological astringent properties, which hasten wound healing and ameliorate in flamed mucus membrane (Tyler *et al.* 1988). It also has haemostatic properties (Awosika 1991).

Fourteen plants were found to contain saponins in different concentrations. *Urtica dioica* was found to contain saponins, that result was also supported by the result of Gyawali *et al.* (2008). *Cinnamomum tamala, Lantana camara, Mimosa pudica* contain saponins. This result was similar to findings of Karanjit *et al.* (2007). Saponins are a special class of glycosides which have soapy characteristics (Fluck, 1973). It has also been shown that saponins are active antifungal agents (Sodipo *et al.* 1991). Saponin has expectorant action, which is very useful in management of upper respiratory tract inflammation; saponins present in plants are cardiotonic in nature (Finar 1989, Trease & Evans 1989).

Fourteen plants were found to contain glycosides in varying concentrations. *Urtica dioica* was found to contain glycosides. This result was similar to the result of Gyawali *et al.* (2008). *Mimosa pudica* and *Cissampelos pareira* found to contain no glycosides in the study of Karanjit *et al.* (2007), present result contradicts this finding, and this may be due to the difference in solvent used during extraction process. But *Cinnamomum tamala, Lantana camara* contain glycosides this result is similar to that

of Karanjit *et al.* (2007). Raghavendra *et al*. (2006) reported the presence of glycosides in ethanolic and methanolic extract of *Oxalis corniculata* that supports present result. Fourteen plants were found to contain terpenoids in different concentrations. Terpenoids was absent in *Urtica dioica* this result was supported by the work of Gyawali *et al.* (2008).

CHAPTER-VI

CONCLUSIONS AND FUTURE DIRECTIONS

6.1 Conclusions

The present study concludes that the most 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 methanolic extract of different parts of plants used in the medicinal purposes gave varying yield. It ranges from 4 to 20%.

Eighteen different medicinal plants were selected for screening purpose against six different bacteria among them two are gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and four are gram-negative (*Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi* and *Proteus vulgaris*). Among these bacterial strains gram-negative bacteria were more resistant as compared to gram-positive bacteria.

Among 18 medicinal plants tested, in the present study, all plants show activity against at least two bacteria. *Phyllanthus amarus* and *Rhus javanica* inhibited all the tested bacteria. Similarly, *Drymaria cordata* was effective against five bacteria. Nine plants inhibit the growth of four and five plants were effective against only three of the screened bacteria and remaining one plant *Ficus religiosa* inhibited growth of two of the screened bacteria. The ZOI value ranges up to 25mm. The maximum value of ZOI (25mm) was shown by plant *Euphorbia hirta* on bacteria *Staphylococcus aureus* followed by *Drymaria cordata* (23mm) on same bacteria.

The phytochemical screening also suggests that these plants are rich in secondary metabolites such as alkaloids, glycosides, saponins, tannins and terpenoids. Twelve plants were found to contain alkaloids, 13 plants were found to contain tannins and 14 plants indicate the presence of each glycosides, saponin and terpenoids.

6.2 Future Directions

The present work is a preliminary study of antibacterial and phytochemical screening of some medicinal plants. Due to time constrains limited work has been done in this research. From this study, following points can be noted as future directions.

- Only methanolic extract of medicinal plants have been used in this experiment. Further trials should be done by using solvent of various polarities which will extract the other active compounds present in plants.
- The plants *Phyllanthus amarus* and *Rhus javanica* showed the broad spectrum antibacterial activity. These plants can be further subjected to isolation of the therapeutic antibacterial compounds and carry out further pharmacological evaluation.
- The plants which showed remarkable activity towards *in vitro* test should be further carried out towards *in vivo* test.
- Only qualitative phytochemical screening has been conducted. The plants contained source of various secondary metabolites. The plants which show encouraging result should be studied for the isolation of active phytochemicals which may lead to the discovery of more potent drugs.

Photo plate I

Photo#:1

Photo#:2

Photo#:3

Photo#:4

Photo#:5

Photo#:6

Photo#: 7

Photo#: 8

Medicinal plants

Photo plate II

Photo#:1

Photo#:2

Photo#:3

Photo#:4

Photo#: 5

Photo#: 6

Photo#: 7

Photo#: 8

Medicinal plants

Photo plate III

Photo#:1

Photo#:2

Photo#:3

Photo#:4

Photo#:5

Photo#:6

Photo#: 7

Photo#: 8

Antibacterial and Phytochemical Test

Photoplate I

Medicinal Plants

Photo#: 1 *Urtica dioica*

Photo#: 2 *Lantana camara*

Photo#: 3 *Drymaria cordata*

Photo#: 4 *Oxalis corniculata*

Photo#: 5 *Phyllanthus amarus*

Photo#: 6 *Cinnamomum tamala*

Photo#: 7 *Zizyphus mauritiana*

Photo#: 8 *Mimosa pudica*

Photoplate II

Medicinal Plants

Photo#: 1 *Vitex negundo*

Photo#: 2 *Taraxacum officinale*

Photo#: 3 *Melia azedarach*

Photo#: 4 *Ageratum conyzoides*

Photo#: 5 *Rhus javanica*

Photo#: 6 *Cissampelos pareira*

Photo#: 7 *Ficus religiosa*

Photo#: 8 *Euphorbia hirta*

Photoplate III

Antibacterial and Phytochemical Test

Photo#: 1 Methanol extracts of plants

Photo#: 2 ZOI shown by plant extracts on *Proteus vulgaris*
From lower left side *Oxalis corniculata, Achyranthes bidentata, Zizyphus mauritiana* (anticlockwise)

Photo#: 3 ZOI shown by plant extracts on *Staphylococcus aureus*
From lower left side *Mimosa pudica, Cissampelos pareira, Rhus javanica* (anticlockwise)

Photo#: 4 ZOI shown by plant extracts on *Bacillus subtilis*
From lower left side *Euphorbia hirta, Drymaria cordata, Taraxacum officinale, Bauhinia purpurea* (anticlockwise)

Photo#: 5 Test for terpenoids

Photo#: 6 Test for saponins

Photo#: 7 Test for alkaloids

Photo#: 8 Test for glycosides

REFERENCES

Abraham, Z., 1981. *Glimpses of Indian Ethnobotany*. Oxford and Publishing Co., New Delhi.

Adomi, P.O. 2008. Screening of the leaves of three Nigerian medicinal plants for antibacterial activity. *Afr. J. of Biotechnol.,* **7**(15):2540-2542.

Adwan, G., B. Abu-Shanab, K. Adwan and F. Abu-Shanab. 2006. Antibacterial effects of nutraceutical plants growing in Palestine on *Pseudomonas aeruginosa*. *Turk. J. Biol.*, **30**:239-242.

Agarwal, A. 2005. Critical issues in quality control of herbal products, *Pharm. Times*, **37**(6):09-11.

Akerele, O. 1984. *WHO's traditional medicine programme: progress and perspective*, WHO chronicals, social science and medicine. **38**:78-81.

Akujobi, C.O., J.N. Ogbulie and U.N. Uchegbu. 2006. Antibacterial activities and preliminary phytochemical screening of *Vernonia amygdalina* and *Citrus aurantifolia*. *Niger. J. of Microbiol.,* **20**(1):649-654.

Ali-Shtayeh, M.S., F Ahmed, M.K. Pervez, I. Azhar, S.A Ibrahim and Parkintin. 2005. A new flavanone with epoxy-isopentyl moiety from *Parkinsonia aculeate* Linn, *Nat Prod Res*., **19**(1):53-60.

Ali-Shtayeh, M.S.and S.I. Abu Ghdeib. 1999. Antifungal activity of plant extracts against dermatophytes. *Mycoses*, **42**:665–672.

Allero, A.A. and A.J. Afolagan. 2006. Antibacterial activitiy of *Solanum tomentosum*. *Afr. J. Biotechnol*, **5**:369-372.

Awosika, F. 1991. Local medicinal plants and health of consumers. *Clin. Pharm. Herbal Med.*, **9**:28-29.

Baral, S.R. and P.P. Kurmi. 2006. *A Compendium of Medicinal Plants in Nepal.* Mass Printing Press, Chauni, Kathmandu, Nepal.

Bastista, O., O. Duarte, S. Nascimento and M.F. Simones. 1994. Structure and antimicrobial activity of differences from the root of *Plectranthus hereoensis*. *J. Nat. Prod*., **57**:279-237.

Bauer, A.W., W.M.M. Kirby, S.C. Sherries and M. Turk. 1966. Antibacterial susceptibility testing by a standard single disc method. *Am. J. Clini. Pathol*., **36**:492-496.

Boris, R.P. 1996. Natural products research perspectives from a major pharmaceutical company. *J. Ethnopharamacol.,* **51**:29-38.

Buckingham, J. (Excecutive editor).1994. *Dictionary of Natural Products.* Vol. VII.Chapman and Hall, London.

Cetin, T.E., N Gurler. 1989. Bakterilerin antibiyotiklere duyaarlilik deneyinin yapilmasi. *Kukem Dergisi*, **12**:2-5.

Chaudhary, R.P. 1998. *Biodiversity in Nepal: Status and Conservation*. S. Devi, Saharanpur, India and Craftsman Press, Bangkok, Thailand.

Cheesbrough, M. 1993. *Medical Laboratory Manual for Tropical Countries*. Microbiology. Linacre house, Jordan Hill Oxford.

Chehregani, A., S.J. Sabounchi and V. Jodaian. 2007. Antibacterial effect of N-Naphtylen Diamine Platinum (II) Choloride as novel compound. *Pakistan J. Biol. Sci.,* **10**:641–644.

Chhetri, H.P., N.S. Yogol, J. Sherchan, K.C. Anup, S. Mansoor, and P. Thapa. 2008. Phytochemical and Antimicrobial Evaluations of Some Medicinal Plants of Nepal. *Kath. Univ. J. of Sci., Engi. and Tech.,* **1**(5):49-54.

Coates A, Y. Hu, R. Bax and C. Page. 2002. The future challenges facing the development of new antimicrobial drugs. *Nat. Rev. Drug Discov*., **1**:895-910.

Collee, J.G., A.G. Fraser, B.P. Marmion and A. Simmon. 1999. *Mackie and McCartney Practical Medical Microbiology*. Churchill Living Stone.

Colombo, M.L. and E. Bosisio. 1996. Pharmacological activities of *Chelidonium majus* L. (Papaveraceae). *Pharmacol. Res.*, **33**:127-134.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clinc. Microbio. Rev.*, **12:**546-582.

Dax, S.L. 1997. *Antibacterial chemotherapeutic agents*. Blackie Academic and Professinal, Chapman and Hall, London, UK.

Devi, P., U.S. Murugan, S. Suju, S. Selvi, P. Chinnaswamy and E. Vijayanand. 2007. Antibacterial, *in vitro* Lipid per oxidation and phytochemial observation on *Achyranthes bidentata* Blume. *Pak. J. of Nutr.,* **6**(5):447-451.

Devkota, A., G.P.S. Ghimire, and C.B. Baniya. 1999. Antibiotic activities of some Lichen species. *Tribu. Univ. J.,* **22**(1):45-51.

DPR. 2007. *Medicinal Plants of Nepal*. Ministry of Forest and Soil Conservation, DPR, Kathmandu, Nepal.

Duraipandiyan, V., M. Ayyanar and S. Ignacimuthu. 2006. Antimicrobial activity of some ethnomedical plants used by Paliyar tribe from Tamil Nadu, India. *BMC Comp. and Alt. Med.,* **6**:35-41.

Farmsworth, N.R. and R.W. Morris. 1976. Higher plants-the sleeping giant of drug development. *Am. J. Pharm.,* **147**:46-50.

Finar, I.L. 1989. *Organic Chemistry: Stereochemistry and Chemistry of Natural Products*. Longman group, UK.

Fluck, H. 1973. *Medicinal plants and their uses*. W. Feulshom and comp. Ltd, New York.

Gupta, S.S. 1994. Prospects and perspectives of natural plant products in medicine. *Indian J. Pharmacol.,* **26**:1-12.

Gurib-Facim, A. 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Mole. Aspects of Med.,* **27**:1-93.

Gyawali, R., D. Gyawali and K. Kyong-Su. 2008. Phytochemical Screening of Some Species of Nepalese Medicinal Plants. In: P.K. Jha, S. B. Karmacharya, M. K. Chhetri, C. B. Thapa and B. B. Shreshta (eds.) *Medicinal Plants in Nepal: An Anthology of Contemporary Research*. Ecological Society (ECOS), Kathmandu, Nepal. pp.43-49.

Hadi, S. 2002. *Bioactive alkaloids from medicinal plants of Lombok*. PhD Thesis, University of Wollongong: Wollongong.

Harbone, J.B. 1973. *Phytochemical methods: A Guide to Modern Technique of Plant Analysis*, Chapman and Hall, London.

Holiman, A. 1989. *Plants in Medicine*. The Chelsea Phyc Garden Co. Ltd.

Husain, A., O.P. Virmani, S.P. Popali, L.N. Mishra, M.M. Gupta, G.N. Shrivastav, Z. Abraham and A.K. Singh. 1992. *Dictionary of Indian Medicinal Plants*. Central Institute of Medicinal and Aromatic Plants, Lukhnow, India.

IUCN. 2004. *National Register of Medicinal and Aromatic Plants*. IUCN-The World Conservation Union, Nepal.

Iwu, M.W., A.R. Duncan and C.O. Okunji, 1999. *Leatherhead International Limited, Royal Society of New antimicrobials of plant origin.* In: Janick, J. (ed.), Chemistry. Great Britain. pp 127-129.

Iyengar, M.A. 1995. *Study of Crude Drugs*. Manipal Power Press, Manipal, India

Janovska, D., K. Kubikova and L. Kokoska, 2003. Screening for antimicrobial activity of some medicinal plants species of traditional Chinese medicine. *Czech J. Food Sci.,* **21**:107-110.

Joshi, K.K. and S.D. Joshi. 2001. *Genetic Heritage of Medicinal and Aromatic Plants of Nepal Himalaya.* Buddha Academic Publishers and Distibuters Pvt. Ltd. Kathmandu, Nepal.

Karanjit, N. K. Shakya, N.B. Pradhan, L.N. Gautam, and D. Shakya. 2007. A compilation of preliminary phytochemical and biological screening of some medicinal plants of Nepal. *Plant Resources*, **29**:79-96.

Kelnanson, J.E., A.K. Jagar and J. Staden. 2000. Zulu medicinal plants with antibacterial activity. *J. of Ethnopharmacol.,* **69**(3):241-246.

Krishnaraju, A.V., T.V.N. Rao and D. Sundararaju. 2005 Assessment of bioactivity of Indian medicinal plants using Brine shrimp (Artemiasalina) lethality assay. *Int. J. Appl. Sci. Eng.,* **2**:125-134.

Latha, S. and K. Kannabiran 2006. Antimicrobial activity and phytochemicals of *Solanum trinobatum* Linn. *Afr. J. Biotechnol.,* **5**(23):2402-2404.

Leggadrio, R. 1995. Emerging drug resistant bacteria: the wake-up call has come. *Stn. Med. J.,* **88**:884-885.

Levy, S.B. 1998. The challenge of antibiotic resistance. *Scient. Americ.*, **278**:46-53.

Lin, J., A.R., Opoku, M. Geheeb-Keller, A.D. Hutchings, S.E. Terblanche, A.K. Jager and J. Van Staden. 1999. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antimicrobial activities. *J. Ethnopharmacol.,* **68**:267-274.

Lipsitch, M .2001. The rise and fall of antimicrobial resistance. *Trends in Microb. Rev.* **9**:438-444.

Mahato, R.B. and R.P. Chaudhary. 2005. Ethnomedicinal study and antibacterial activities of selected plants of Palpa district, Nepal. *Sci. World*, **3**(3):26-31.

Malla, S.B. 1999. An overview of plant resources in Nepal. *In:* Shrestha, R. and Shrestha, B. (eds.), *Wild relatives of cultivated plants in Nepal*. The Green Energy Mission, Nepal. pp.1-11.

Manandhar, N.P. 1985. Ethnobotanical notes on certain medicinal plants use by Tharus of Dang-Deokhuri District, Nepal. *Int. J. of Crude Drug Res.*, **23**(4):153-159.

Manandhar, N.P. 2002. *Plants and People of Nepal*. Timber Press, Oregon, USA.

Marchese, A. and G. Shito. 2001. Resistance patterns of lower respiratory tract pathogens in Europe. *Int. J. Antimicrobial Agents,* **16**:25-29.

Mitscher, L.A., S.P. Pillai, E.J. Gentry and D.M. Shankel. 1999. Multiple drug resistance. *Med. Res. Rev*., **19**:477-496.

Mojab, F., M. Kamalinejad, N. Ghaderi and H. Vahidipour. 2003. Phytochemical screening of some Iranian plants. *Iran. J. of Pharmaceutic. Res.*, 77-82.

Muetzel, S. and K. Becker. 2006. Extractability and biological activity of tannins from various tree leaves determined by chemical and biological assays as affected by drying procedure. *Ani. Feed Sci. and Tech.,* **125**:139-149.

Nair, R., T. Kalaria and S. Chanda. 2005. Antibacterial activity of some selected Indian medicinal flora. *Turk. J. Biol.,* **29**:41-47.

Nandagopal, S. and B.D. Ranjita Kumari. 2007. phytochemical and antibacterial studies of Chicory (*Cichorium intybus* L.) a multipurpose medicinal plant. *Adv. in Biol. Res.*. **1**(1-2):17-21.

Panthi, M.P. and R.P. Chaudhary. 2006. Antibacterial activities of some selected folklore medicinal plants from west Nepal. *Sci. World,* **4**(4):16-21

Parajuli, S., R.P. Chaudhary, R.S.L. Taylor. 2001. Antibacterial Activity of Medicinal Plants Used to Treat Skin Ailments in Kaski Distirct, Nepal. In*:* P.K. Jha, S.R. Baral, S.B. Karmacharya, H.D. Lekhak, P. Lacoul and C.B. Baniya (eds.) *Environment and Agriculture: Biodiversity, Agriculture and Pollution in South Asia*. Ecological Society (ECOS), Kathmandu, Nepal. pp.230-273.

Parekh, J. and S. Chanda. 2007. *In vitro* screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. *Afri. J. of Microbiol. Res.,* **1**(6):92-99.

Parekh, J. and S. Chanda.2006. *In-vitro* antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L.(Cyperaceae). *Afri. J. of Biomed. Res.*, **9**:89-93.

Patrick, G. 2001. *Medicinal Chemistry-Instant Notes*. Bios Scientific Publishers Limited, UK.

Perumal, G., K. Srinivastava, D. Natarajan and C. Mohanasundari. 2004. *Indian J. Environ*. *Ecoplan*., **8**:161-162.

Press, J.R., K.K. Shrestha and D.A. Sutton. 2000. *Annotated Checklist of the Flowering Plants of Nepal*. The Natural History Museum, London, UK and Central Department of Botany, Tribhuvan University, Kathmandu,Nepal.

Rabe, T. and J. Van Staden. 1997 Antibacterial activity of South African plants used for medicinal purposes. *J.of Ethnopharmacol*., **56**:81-87.

Raghavendra, M.P., S. Satish and K.A. Raveesha. 2006. Phytochemical analysis and antibacterial activity of *Oxalis corniculata*; a known medicinal plant. *myScience* **1**(1):72-78.

Rai, S.K. 2004.Medicinal plants used by Meche people of Jhapa district, Eastern Nepal. *Our Nature*, **2**:27-32.

Rajbhandari, K.R. 2001. *Ethnobotany of Nepal*. Ethnobotanical Society of Nepal (ESON), Kathmandu, Nepal.

Rizk, A.M. 1982. Constituents of plants growing in Quatar. I. A chemical survey of six plants. *Fitoterapia*, **52**:35-44.

Sairam, K.S., A. Hemalatha, T. Kumar, J. Srinivasan, M. Ganesh, S. Shankar and S. Venkataraman. 2002. Evaluation of anti-diarrheal activity of seed extracts of *Mangifera indica*. *J.of Ethnopharmacol.*, **84**:11-15.

Salehi Surmagi, M.H., Y. Aynehchi, G.H. Amin and Z. Mahmoodi. 1992. Survey of Iranian plants for saponins, alkaloids, flavonoids and tannins.*IV Daru.* **2**:281-291.

Samy, R.P. and S. Ignacimuthu. 2000. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. *J. of Ethnopharmacol.*, **69**:63-71.

Sanni, S. P.A. Onyeyili and F.S. Sanni. 2008. Phytochemical analysis, elemental determination and some *in vitro* antibacterial activity of *Ocimum basilicum* L. leaf extracts. *Research J. of Phytochem*., **2**(2):77-83.

Scazzocchio, F., M.F. Comets, L. Tomassini and M. Palmery. 2001. Antibacterial activity of *Hydrastis canadensis* extract and its major isolated alkaloids. *Planta Med*., **67**:561-563.

Shakya, D.M., R. Pradhan and R. Ranjitkar, 2008. A preliminary screening of some Nepalese medicinal plants for antimicrobial activity. *Plant Res.*, **30**:87-94.

Shakya, P.M. 1982. Preliminary studies on some medicinal plants for their antimicrobial activities. In: *Proceedings of National Science and Technology Conference (NSTC)*, pp. 136-143. RONAST, Kathmandu, Nepal.

Shreshta, R. and A.P. Sharma. 1988. Antimicrobial activity of some essential oils. *In: First Regional Conference of Association of Plant Physiologists of SAARC*

Countries (APPSC) on the role of Plant Physiology and Biotechnology in Plant Productivity.

Sieradzki, K., R.B. Roberts, S.W. Haber and A. Tomasz. 1999. The development of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection. *N. Engl. J. Med..*, **340**:517–523.

Sodipo, O.A., M.A. Akanji, F.B. Kolawole and A.A. Odutuga. 1991. Saponin is the active antifungal principle in Garcinia kola, heckle seed. *Biosci. Res. Commun*., **3**:171.

Somolenski, S.J., H. Silinis and N.R. Farnsworth. 1974. Alkaloid screening. *I. Lloydia.* **37**:506-536.

Taylor, R.S., N.P. Manandhar, and G.H.N. Towers. 1995. Screening of selected medicinal plants of Nepal for antimicrobial activities. *J. of Ethnopharmacol*., **46**:153-159.

Thomas, E., J. Shanmugham and M.M. Raf. 1999. *In vitro* antimicrobial activity of certain medicinal plants. *Biomedicine*, **19**(3):185-190.

Timsina, G. 2003. *Evaluation of antimicrobial activities of some medicinal plants used in traditional medicine in Nepal*. M. Sc. Thesis, Central Department of Botany, Tribhuvan University, Kathmandu, Nepal.

Tiwari, N.N. 1999. Wild relatives of cultivated medicinal and aromatic plants in Nepal. In: R. Shrestha, and B. Shrestha (eds.) *Proceedings of National Conference on Wild Relatives of Cultivated Plants in Nepal*. Green Energy Mission, Nepal. pp.141-148.

Todar, K. 2008. *Todar's Online Textbook of Bacteriology*.
(URL: http://textbookofbacteriology.net)

Tomoko N., A. Takashi, T. Hiromu, I. Yuka, M. Hiroko, I. Munekazu, T. Totshiyuki, I. Tetsuro, A. Fujio, I. Iriya, N. Tsutomu and W. Kazuhito. 2002. Antibacterial activity of extract prepared from tropical and subtropical plants on methicillin-resistant *Staphylococcus aureus*. *J. Health Sci.*, **48**:273-276.

Trease, G.E. and M.D. Evans. 1989. *A Text Book of Pharmacognosy*. Baillier, Tindal and Caussel, London.

Tyler, V.E, L.R. Braddy and J.E. Roberts. 1988. *Pharmacology*. Lea and Ferbiger, Philadelphia.

Uma Devi, P., S. Murugan, S. Suja, S. Selvi, P. Chinnaswamy and E. Vijayanand. 2007. *Antibacterial, In vitro* Lipid per Oxidation and Phytochemical Observation on *Achyranthes Bidentata* Blume. *Pak. J. of Nutri.,* **6**(5):447-451.

Viges, J.H., E. DcMarchi and F.M. Lancas. 1997. Extraction of low polarity compounds (with emphasis on coumarin and kaurenoic acids) from *Mikania glomerata* (guaco) leaves. *Phytochem Anal.,* **8**:266-270.

Vlietinck, A.J., L. Van Hoof, J. Totte, A. Lasure, D.Vanden Berghe, P.C. Rwangobo and J. Mvukiyuniwami. 1995. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *J. of Ethnopharmacol*., **46**:31-47.

Warrier, P.K., V.P.K. Nambiar and C. Ramankutty 1996. *Indian Medicinal Plants*. Vol. III. Orient Longman Pvt. Ltd., Chennai, India.

Warrier, P.K., V.P.K. Nambiar and C. Ramankutty. 2006a *Indian Medicinal Plants*. Vol. II. Orient Longman Pvt. Ltd., Chennai, India.

Warrier, P.K., V.P.K. Nambiar and C. Ramankutty. 2006b. *Indian Medicinal Plants*. Vol. IV. Orient Longman Pvt. Ltd., Chennai, India.

Warrier, P.K., V.P.K. Nambiar and C. Ramankutty. 2006c. *Indian Medicinal Plants*. Vol. V. Orient Longman Pvt. Ltd., Chennai, India.

Watanabe, T., K.R. Rajbhandari, K.J. Malla, and S. Yahara. 2005. *A Handbook of Medicinal and Aromatic Plants of Nepal*; Bangkok, Kobfai Publishing Project.

Westendarp, H. 2000. Effects of tannins in animal nutrition. *Dtsch Tierarztl Wochenschr.* **113**(7):264-268.

WHO. 2002. *Traditional Medicine Strategy*. WHO Publication.

Williams, R.A.D., P.A. Lambert and P. Singleton. 1996. *Antimicrobial drug action*. Bios Scientific Publishers Ltd., UK.

Yao, J. and R. Moellering. 1995. Antibacterial agents. In: P. Murray, E. Baron, M. Pfaller, F. Tenover, R. Yolken (eds.) *Manual of Clini. Microbiol.* ASM, Washington DC. pp.1281-1290.

Appendix A

Code of Plants	Botanical Name	Family	Parts Used	Place and Date of Collection	Medicinal Uses	References
MP 1	*Euphorbia hirta*	Euphorbiaceae	Whole plant	Kirtipur, Ktm. Sep., 2008	Dysentery, Colic troubles, Cut wounds	Joshi & Joshi 2001, Rajbhandari 2001, Watanabe *et al.* 2005,
MP2	*Drymaria cordata*	Caryophyllaceae	Whole plant	Kirtipur, Ktm. Sep., 2008	Diarrhea, dysentery	Rajbhandari 2001, Manandhar 2002, Rai 2004
MP3	*Taraxacum officinale*	Asteraceae	Tuber	Kirtipur, Ktm. Sep., 2008	Antibacterial	Joshi & Joshi 2001, Warrier *et al.* 2006b, DPR 2007
MP4	*Bauhinia purpurea*	Leguminosae	Flowers	Kirtipur, Ktm. Sep., 2008	Diarrhea, dysentery	IUCN 2004, Mahato 2005, DPR 2007
MP5	*Ageratum conyzoides*	Asteraceae	Leaves	Kirtipur, Ktm. Sep., 2008	Dysentery, diarrhea, Cut wounds	Rajbhandari 2001, Warrier *et al.* 2005, DPR 2007.
MP6	*Ficus religiosa*	Moraceae	Leaves	Kirtipur, Ktm. Sep., 2008	Antibacterial	Warrier *et al.* 1996, Manandhar 2002, Baral & Kurmi 2006
MP7	*Urtica dioica*	Urticaceae	Leaves	Kirtipur, Ktm. Sep., 2008	diarrhea and dysentery, cut and wounds	Rajbhanari 2001, Joshi & Joshi 2001, Manandhar 2002.
MP8	*Lantana camara*	Verbanaceae	Leaves	Kirtipur, Ktm. Sep., 2008	Cut and wound, malaria	Warrier et al. 1996, Baral & Kurmi 2005
MP9	*Phyllanthus amarus*	Euphorbiaceae	Whole plant	Kirtipur, Ktm. Sep., 2008	Cut and wounds , diarrhea dysentery	Joshi & Joshi 2001, IUCN 2004, Warrier *et al.* 2006b
MP10	*Cinnamomum tamala*	Lauraceae	Leaves	Kirtipur, Ktm. Sep., 2008	Diarrhea, Colic pain	IUCN 2004, Watanabe *et al.* 2005, Warrier *et al.* 2006a
MP11	*Melia azedarach*	Meliaceae	Fruits	Kirtipur, Ktm. Sep., 2008	Typhoid fever	Joshi & Joshi 2001, Warrier *et al.* 2006b, Baral & Kurmi 2006
MP12	*Vitex negundo*	Verbenaceae	Leaves	Kirtipur, Ktm. Sep., 2008	Antiseptic, germicidal	Warrier *et al.* 2006c, IUCN 2004, Baral & Kurmi 2006
MP13	*Oxalis corniculata*	Oxalidaceae	Whole plant	Birgha, Syan. Oct.,2008	Diarrhea, dysentery, cut and wounds	Joshi & Joshi 2001, Watanabe *et al.* 2005, Warrier *et al.* 2006c.
MP14	*Achyranthes bidentata*	Amaranthaceae	Roots	Birgha, Syan. Oct.,2008	Dysentery, pneumonia	Joshi & Joshi 2001, IUCN 2004, Rai 2004.
MP15	*Zizyphus mauritiana*	Rhamnaceae	Root bark	Birgha, Syan. Oct.,2008	Dysentery diarrhea , wound &ulcers	Joshi & Joshi 2001, Manandhar 2002, Warrier *et al.* 2006c.
MP16	*Mimosa pudica*	Leguminosae	Root	Birgha, Syan. Oct.,2008	Dysentery, diarrhea	Joshi & Joshi 2001, Watanabe et al. 2005, Warrier *et al.* 2006b.
MP17	*Cissampelos pareira*	Menispermaceae	Leaves	Birgha, Syan. Oct.,2008	Diarrhoea	Rajbhandari 2001, Mahato 2005, Baral & Kurmi 2006
MP18	*Rhus javanica*	Anacardiaceae	Fruits	Birgha, Syan. Oct.,2008	Dysentery, diarrhea	Rajbhandari 2001, Baral & Kurmi 2006, Mahato 2005

List of Medicinal Plants Used in Antibacterial and Phytochemical Study

Appendix B

Description of Medicinal Plants Used in the Evaluation of Antibacterial Activities

1. Ageratum conyzoides L., *Sp. Pl.*: 839 (1753).

Family: Asteraceae

Common name: Gandhejhar (Nep.)

Description: Erect annual herb, 30-90cm high. Leaves petioled, ovate. Heads pale-blue or white, in dense terminal corymbs. Achenes black.

Distribution: WCE. alt.: 200-2000m. Pantropical.

Part(s) used: Root, leaf and flowers.

Uses: Root is digestive, appetizer and juice of root is antilithic; Leaves are applied to cut and sores (DPR 2007).

Chemical Constituents: β-sitosterol, dotriacontene, conyzorigun, 7-methoxy-2,2-dimethylchromene (Husain *et al*. 1992).

2. Achyranthes bidentata Blume, *Bijdr. Fl. Ned. Ind*. **11**: 545 (1826).

Family: Amaranthaceae

Common name: Datiwan (Nep.)

Description: Herb; 0.3-1m high. Leaves 2.5-8x1-3.5cm, opposite, petiolate, elliptic, lanceolate, acuminate, pubescent, and membranous. Flowers in slender, pink.

Distribution: CE. alt.: 1200-2100m. Tropical Africa, Himalaya, India, east to China, Malaysia.

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

Uses: White variety used in dysentery, itching and pain in abdomen, red variety emetic, constipating, and dried plant is given in colic (Joshi & Joshi 2001).

Chemical Constituents: 4-methylheptatriacont-en-ol, 2-tetracontanol, 7-cyclo-exyl-heptacosan-7-ol (Watanabe *et al*. 2005).

3. Bauhinia purpurea L., *Sp. Pl.*: 375 (1753).

Family: Leguminosae

Common name: Taki (Nep.)

Description: Tree up to 6m tall. Leaves alternate, petiolate, shallowly cordate, lobes obtuse, entire, glabrous, Flowers in terminal and axillary corymbs, pink. Fruit pod, flat, glabrous.

Distribution: WCE. alt.: 300-1600m. Tropical Himalaya (Kashmir to Bhutan), India, SE Asia, W. & S. China.

Part(s) used: Flowers, bark, roots.

Uses: Bark is astringent and used in diarrhea and dysentery (DPR 2007, IUCN 2004).

4. Cinnamomum tamala (Buch.-Ham.) Nees & Eberm., *Handb. Med. Pharm. Bot*. **2**:426 (1831).

Family: Lauraceae

Common name: Tejpat (Nep.)

Description: Medium sized evergreen tree about 8m tall. Bark thin dark brown. Leaves simple, short stalked, ovate-lanceolate, long pointed 10-15 cm long with 3 conspicuous nearly parallel veins arising from near the base. Leaves bright pink when young in spring, aromatic when crushed. Flowers pale yellow, in terminus and axillary-branched clusters. Fruit ovoid drupe, black, succulent.

Distribution: WCE. alt.: 450-2000m. Himalaya (Kashmir to Bhutan), NE India (Assam, Meghalaya).

Part(s) used: Leaves and barks.

Uses: Leaves are carminative and used to control diarrhea and colic pain (Warrier *et al*. 2006a, Joshi & Joshi 2001). Bark is useful in diarrhea, flatulence and nausea (Watanabe *et al*. 2005). Leaves and bark astringent stimulant and carminative; used in rheumatism, colic, diarrhea, useful in checking nausea and vomiting (IUCN 2004).

Chemical Constituents: Trans-Caryophyllene, p-Eugenol, Myricetin (Watanabe *et al*. 2005)

5. Cissampelos pareira L., *Sp. Pl.:* 1031 (1753) var. **hirsuta** (Buch.-Ham. ex DC.) Forman, *Kew Bull*. **22:**356 (1968).

Family: Menispermaceae

Common name: Batulpate (Nep.)

Description: Climbing shrub. Leaves petiolate, orbicular or broadly ovate, peltate, obtuse, entire, glabrous. Flowers axillary; male flowers cymose; female flowers raceme, yellowish.

Distribution: WCE. alt.: 150-2200m. Pantropical.

Part(s) used: Whole plant.

Uses: Plant is used in diarrhea, ulcers, to cure skin erruption, burning (IUCN 2004).

Chemical Constituents: Cissampeloflavone, Pareitropone (Watanabe *et al*. 2005), Cissamine, insularine, alkaloids-cissamparein and cissampine (Husain *et al*. 1992).

6. Drymaria cordata (L.) Willd. ex Roem. & Schult., *Syst. Veg*. **5**:406 (1819).

Family: Caryophyllaceae

Common name: Abijalo (Nep.).

Description: Annual, prostrate or decumbent herb. Leaves ovate-orbicular, apiculate, stipules lanceolate. Flowers greenish white or white, in lax, glandular-puberulous, repeatedly forked cymes. Pedicels shorter than the calyx, spathulate, deeply bifid; lobes oblong, obtuse; stamens3; style 2to3 fid. Capsule3-gonous.

Distribution: WCE. alt.: 2200-4300m. Africa, America, naturalized in Nepal and India, Pacific islands.

Part(s) used: Whole plant.

Uses: Extraction of plant is given to treat diarrhea and dysentery and fever, Plant paste is applied on the forehead to treat headache (Rajbhandari 2001). Plant extract is taken in cold throat trouble, diarrhea and dysentery (Manandhar 2002).

Chemical Constituents: 4-methoxycanthin-6-one (Buckingham 1994).

7. Euphorbia hirta L., *Sp. Pl.*: 454 (1753).

Family: Euphorbiaceae

Common name: Dudhe jhar (Nep.).

Description: A prostrate or ascending herb, 10-30cm tall. Leaves opposite, shortly stalked, elliptic-oblong, ovate-lanceolate or oblong-lanceolate. Flowers unisexual, in axillary and terminal dense-flowered sessile or peduncled cyathea.

Distribution: WCE. alt.:150-1500m. Pantropical.

Part(s) used: Whole plant.

Uses: The plants are useful in treatment of colic troubles, dysentery, cough, asthma vomiting and worms (Joshi & Joshi 2001). Plant extraction is applied to heal wound (Watanabe *et al*. 2005).

Chemical Constituents: 3, 4-Di-O-galloylquinic acid, 12-Deoxy-4α-hydro-xyphorbol, 13-dodecanoate 20-acetate (Watanabe *et al*. 2005).

8. Ficus religiosa L., *Sp. Pl.*: 1059 (1753).

Family: Moraceae

Common name: Peepal (Nep.)

Description: Large glabrous tree. Leaves leathery, broadly ovate, entire, petiolate. Flowers unisexual, male flower very few, only near the mouth of some receptacles; female flower sessile.

Distribution: WCE. alt.: 150-4500m. Widely cultivated in Nepal, India and SE Asia.

Part(s) used: Bark, latex, leaf bud, leaves, fruits, seeds.

Uses: Bark decoction is used to treat gonorrhea, skin diseases, also has antibacterial activity, juice of bark is given in diarrhea, dysentery, and latex of plant is prescribed for toothache (Manandhar 2002).

Chemical Constituents: The bark contains tannins and phytosterolin (DPR 2007)

9. Lantana camara L., *Sp. Pl.*: 627 (1753).

Family: Verbenaceae

Common name: Ban Phanda (Nep.)

Description: Rambling rough-hairy evergreen shrub with 4-sided branches; twigs often more or less prickly; with ovate toothed leaves. Leaves opposite, shortly stalked. Flowers in a long-stalked rounded heads, numerous white, pale purple, or commonly orange or yellow, flowers with a curved corolla-tube to 1cm and with four spreading rounded lobes. Fruits stalked cylindrical cluster; drupes black shining.

Distribution: CE. alt.: 400-1300m. Himalaya (Nepal), India, Myanmar, China, Indo-China, Malaya. Native of America widely naturalized in Nepal, India and other parts of Asia.

Part(s) used: Stem, leaves, fruits.

Uses: Decoction of fresh root is used by hill tribes in all kind of dysentery, powdered leaves are used in cut wounds, ulcers and swellings (Warrier *et al*. 1996).

10. Mimosa pudica L., *Sp. Pl.:* 518 (1853).

Family: Leguminosae

Common name: Lajjawati (Nep.)

Description: A diffuse prickly under-shrub with leaves very sensitive to touch. Leaves bipinnately compound, pinnae 2-4. Flowers pink in globose heads, usually in pairs, axillary. Fruits bristly pod, in globular clusters, flat, straw colored, consisting of 3-5 one seeded segments.

Distribution: CE. alt.: 200-1200m. Pantropical.

Part(s) used: Leaves and roots.

Uses: The root is used in the treatment of asthma, fever, cough, dysentery and vaginal and uterine ailments (Watanabe *et al*. 2005, Joshi & Joshi 2001). Roots are also used in jaundice, smallpox and fever, and leaves in cut and wounds, conjunctivitis (Warrier *et al*. 2006b).

Chemical Constituents: Mimopudine, Strophanthidin-3-O-β-D-glucopyranosyl-(1-4)-O-β-D-xylopyranoside (Watanabe *et al*. 2005), mimosine (Joshi & Joshi 2001).

11. Melia azedarach L., *Sp. Pl.:* 384 (1753)

Family: Meliaceae.

Common name: Bakaino (Nep.)

Description: A moderate sized deciduous tree about 12m tall. Bark dark gray with shallow longitudinal furrows. Leaves bi- or tri-pinnate, pinnae opposite or alternate, ovate or lanceolate, glabrous on both surfaces. Flowers in long peduncled axillary panicles, sweet scented lilac. Fruit ellipsoid-globose, 4-seeded, drupe, yellow when ripe.

Distribution: WE. alt.: 700-1100m. Iran, Himalaya, east to China. Cultivated.

Part(s) used: Bark, root, flowers, fruits and seeds.

Uses: The roots are astringent, anodyne, antiseptic, anthelmintic, and tonic (Warrier *et al*. 2006b). Fruits are poisonous, induce vomiting and develop the symptoms of paralysis when eaten. (Joshi & Joshi 2001).

Chemical Constituents: Azadirachtin M, 22, 23-Dihydronimocinol (Watanabe *et al*. 2005), Azaridine and paraisine (Joshi & Joshi 2001).

12. Oxalis corniculata L., *Sp. Pl*.: 435 (1753).

Family: Oxalidaceae

Common name: Chari amilo (Nep.)

Description: A perennial spreading herb with prostrate stems. Leave 3-foliate, long-petioled leaflets broadly obcordate, hairy, 6-15mm broad. Flowers yellow, in umbels. Fruit capsule, cylindrical and hairy.

Distribution: WCE. alt.: 300-2900m. Almost cosmopolitan.

Part(s) used: Leaves and flower.

Uses: It is used as antibacterial and antiseptic, in diarrhea, dysentery, fever, scurvy, warts leaves juice is used in treatment of cataract (Warrier *et al*. 2006c, Joshi & Joshi 2001). Leaves and flowers are used internally in fevers dysentery and scurvy and externally to remove warts (Watanabe *et al*. 2005).

Chemical Constituents: β-Sitosterol, Isovitexin (Watanabe *et al.* 2005), oxalate (Joshi & Joshi 2001).

13. Phyllanthus amarus Schum. & Thonn., *Kong. Danske Vid. Selsk. Skr*. **4**:195 (1829).

Family: Euphorbiaceae

Common name: Bhui amala (Nep.)

Description: A small glabrous pale green herb. Leaves alternate, obovate, tip rounded entire. Flowers in axillary clusters, creamy, fruit an ovoid or globose capsule.

Distribution: CE. alt.: 470-900m. Pantropical.

Part(s) used: Whole plant.

Uses: Plant is used in bronchitis, leprosy, anemia, urinary discharges asthma and hiccough (Joshi & Joshi 2001). It is also used as diuretic and menorrhagia, jaundice, pimples, cuts and wounds (IUCN 2004).

Chemical Constituents: Phyllanthine and hypophyllanthine (Joshi & Joshi 2001),

14. Rhus javanica L**.,** *Sp. Pl*.: 265 (1753).

Family: Anacardiaceae

Common name: Bhakiamilo (Nep.)

Description: Shrub or small tree with young parts, leaf-stalks and inflorescence hairy. Leaves pinnate with 5-13 leaflets and with the upper part of the rachis narrowly winged; the leaves turn red during autumn. Flowers c. 3mm, pale yellowish-green, very numerous, in large branched pyramidal clusters c. 30cm and nearly as long as the leaves. Fruit c. 4mm, wooly, reddish-brown.

Distribution: WCE. alt.: 1300-2400m. Himalaya (Kashmir to Bhutan), India. Shrilanka, Myanmar, East to China, Korea, Japan.

Part(s) used: Fruits.

Uses: Fruit is used in diarrhea; ripe fruit is appetizer. Fruit decoction, seed powder is taken internally during dysentery (Rajbhandari 2001).

Chemical Constituents: Flavonoid-pongapin, tetramethoxyfistein, demetho-xykanugin and abibenzoylmethaneovalitenone (Husain *et al.* 1992).

15. Taraxacum officinale F. H. Wigg., *Fl. Brit. India* **3(8)**:401 (1881).

Family: Asteraceae

Common name: Tukiphool (Nep.)

Description: Perennial herb, root cylindrical, externally yellowish brown, longitudinally wrinkled, internally whitish. Leaves entire, radical, sessile, oblanceolate, and acute. Heads 8-50mm in diameter, bracteate, inner white, outer short; flowers yellow.

Distribution: WCE, Subtropical to Supalpine.

Part(s) used: Root, leaves and flower.

Uses: Plants are diuretic, tonic and used in remedy for chronic disorder of kidney and liver (DPR 2007). Plant juice valued for chronic hepatitis, and intermittent fever (Joshi & Joshi 2001).

16. Urtica dioica L., *Sp. Pl.*: 984 (1753).

Family: Urticaceae

Common name: Sisnu (Nep.)

Description: Erect herb. Leaves ovate or lanceolate, cordate, pointed, teeth large, coarse, regular, acute. Flowers small, green, male and female on separate plants.

Distribution: WC. alt.: 3000-4500m. Europe, N. Africa, W. Siberia, C. Asia, Himalaya, W. China, naturalized widely in other temperate regions.

Part(s) used: Whole plant.

Uses: Leaf paste is useful in diarrhea, dysentery and to stop uterine bleeding. The root paste is used to treat cuts, wounds and dog bites (Manandhar 2002). The plant is used as haemostatic in vomiting of blood, uterine hemorrhage, nose bleed and also given for ease delivery (Joshi & Joshi 2001). Fruit paste is used to treat bone dislocation (Rajbhandari 2001).

Chemical Constituents: Betaline, Choline, Plastoquinones (Husain *et al*. 1992).

17. Vitex negundo L. *Sp. Pl.*: 638 (1753) var. **negundo**.

Family: Verbenaceae

Common name: Simali (Nep.)

Description: An aromatic large shrub or small tree, up to 3m high with quadrangular branches. Leaves opposite, exstipulate, long petioled, 3-foliate, leaflets lanceolate, acuminate, entire or rarely crenate, white-tomentose beneath. Flowers blue in cymes forming large terminal panicles.

Distribution: WEC. alt. 100-1200m. Himalaya (Nepal to Bhutan), Afghanistan, India, Shrilanka, China, Myanmar, Indo-China, Malaysia.

Part(s) used: Whole plant

Uses: The plant is antiseptic, antipyretic, the roots are useful in dysentery, wounds, ulcers, malarial fever, and the flowers are used in diarrhea, cholera, fever (Warrier *et al*. 2006c). Leaves used in common cold, fever, enteritis, diarrhea, vaginal discharge, and dermatitis (IUCN 2004).

Chemical Constituents: An alkaloid-histindine; flavonoids-5-hydroxy-3,6,7,3',4'-pentamethoxyflavonee and casticin (Husain *et al*. 1992).

18. Zizyphus mauritiana Lam., *Encycl*. **3(1)**:319 (1789).

Family: Rhamnaceae

Common name: Bayar (Nep.)

Description: More or less evergreen shrub or small tree with spreading drooping softly hairy branches armed with curved prickles. Leaves elliptic-ovate or orbicular, entire or regularly toothed, unequal at base, dark green and with 3 prominent veins above, pale and wooly-hairy beneath. Flowers greenish-yellow, in axillary clusters; petals spathulate, concave, reflexed. Fruit globose, fleshy, yellow becoming orange red.

Distribution: WCE. alt.: 200-1200m. Tropical Asia, Australia, widely cultivated.

Part(s) used: Root, barks, fruits.

Uses: Bark is used in diarrhea, dysentery, and used in externally in boils. Fruit cooling, aphrodisiac, tonic and laxative (Joshi & Joshi 2001). Pulp of ripe fruits is useful in fever, ulcer, wounds and digestion and also purifies blood (Manandhar 2002, Warrier *et al.* 2006c).

Chemical Constituents: Leucopelargonidin, betulinic acid, and ceanothic acid (Husain *et al*. 1992).

(Note: The nomenclature of plant is based on *Annotated Checklist of Flowering Plants of Nepal* and the description of plants is based on *An Compendium of Medicinal Plants of Nepal* and *Genetic Heritage and of Medicinal and Aromatic Plants of Nepal Himalaya.*)

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 butyrous consistency within 18-24 hours. It forms a fairly large yellow colony on rich medium. It is often hemolytic on blood agar. The bacteria are catalase-positive and oxidase-negative. It can grow at a temperature range of 15 to 45ºC 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 pyogenic infections including folliculitis, impetigo, furuncles, carbuncles, breast abscess, post-operative wound infections, cellulites, pyomyositis, osteomyelitis, septic arthritis, bronchopneumonia, lungs abscess etc. It also causes boils, secondary infections, septicemia, pneumonia, meningitis, acute endocarditis, conjunctivitis, toxic shock syndrome and more commonly food poisoning (Collee *et al*. 1996).

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.* 1996).

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. Pseudomonas aeruginosa

a. Morphology and Biochemical Characters

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

b. Pathogenicity

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

4. Bacillus subtilis

a. Morphology and Biochemical Characters

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

b. Pathogenicity

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

5. 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.* 1996)

6. Proteus vulgaris

a. Morphology and Biochemical Characters

It is gram negative, actively motile aerobic bacillus with characteristic swarming growth on many, even well dried, and solid laboratory media. The swarming growth with its fishy odor may cover most or all of the agar surface as well as colonies of

other organisms. Swarming is inhibited by MacConkey's agar and on DCA by bile salt and on CLED agar by the absence of electrolytes. They are lactose non-fermenter, methyl red positive. They can hydrolyze urea rapidly and are positive in gelatinase and lipase tests (Collee *et al.* 1996).

b. Pathogenicity

They are opportunistic pathogens and after *E. coli* they are commonest cause of urinary tract infections. They have also been recovered from infected wounds and abscesses and from causes of otitis media, meningitis, septicemia and osteomyelitis.

Appendix D

Lists of Materials used for the study

Glassware

Screw capped bottle	Funnels	Petri plates
Round bottomed flasks	Test tubes	Glass rods
Measuring cylinder	Pipettes	Beakers

Apparatus and Equipments

Electric grinder	Micropipette	Refrigerator
Incubator	Dropper	Hot air oven
Electric balance	Filter papers	Vortex shaker
Centrifuge	Aluminum foils	Cotton swabs
Inoculating loops	Polythene bags	Forceps
pH meter	Cotton bags	Cotton rolls
Water distillation plant	Sticker	Wash bottle
Plant cutter	Autoclave	Camara
Note book/Markers/pencils		

Media for culture, chemicals and reagents

Nutrient Broth (NB)	Tetracycline tablets	Methanol
Nutrient Agar (NA)	Copper Sulphate	Conc. H_2SO4
Fehling A solution	Buffer tablets	Iodine
Fehling B solution	Ferric chlorides	Spirit
Mayer’s reagent	Wagner’s reagent	Conc. HCl
Mercuric chloride	Potassium iodide	Chloroform

Appendix E

Composition of Some Media and Reagents Used in the Study

Nutrient Media

1. Nutrient Agar (NA)

Composition	Grams/Liter
Peptone	5.00
Sodium Chloride	5.00
Beef extract	1.50
Yeast extract	1.50
Agar	15.00
Final pH (at 25ºC)	7.4±0.2

Procedure

28g of media was dissolved in 1000mL of distilled water and heated to dissolve the media. The media was sterilized by autoclaving at 15lbs pressure at 121ºC for 15 minutes.

2. Nutrient Broth (NB)

Composition	Grams/Liter
Peptone	5.00
Sodium Chloride	5.00
Beef Extract	1.50
Yeast Extract	1.50
Final pH (at 25ºC)	7.4±0.2

Procedure

13g of media was dissolved in 1000mL of distilled water and heated to dissolve the media. The media was sterilized by autoclaving at 15lbs pressure at 121ºC for 15 minutes.

Reagents

Fehling's Solution A

Dissolve 35g $CuSO_4.5H_2O$ in water to make up the volume to 500mL.

Fehling's Solution B

Dissolve 120g of KOH and 173g Na-K tartarate (Rochelle salt) in water and make the final volume to 500mL.

Mayer's reagent

Dissolve 1.358g of $HgCl_2$ in 60ml of water and pour into a solution of KI in 10mL of H_2O. Add sufficient water to make 100mL.

(It shows white precipitation with most alkaloids in slightly acid solutions.)

Wagner's reagent (Iodo-potasium iodide)

Dissolve 2g of iodine and 6g of KI in 100mL of water.

Appendix F

Graphs showing antibacterial activity of different medicinal plants on individual bacteria

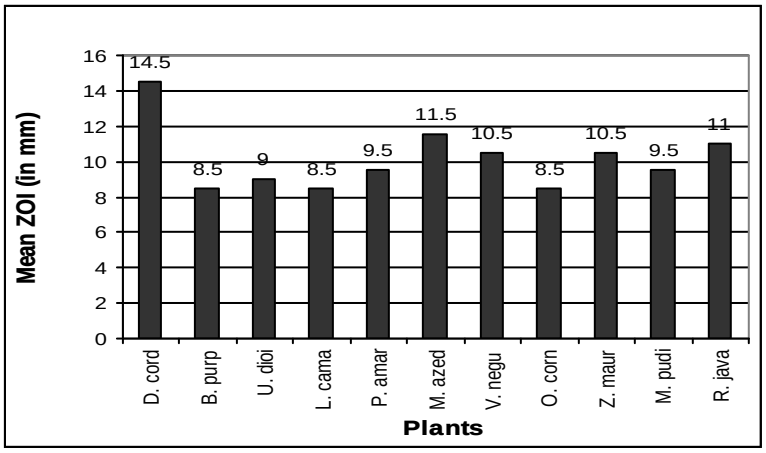


Figure 1: Zone of inhibition for bacterium *Pseudomonas aeruginosa*. (Abbreviations: D.cord – *Drymaria cordata*, B. purp – *Bauhinia purpurea* , U. dioi – *Urtica dioica*, L. cam- *Lantana camara*, P. amar – *Phyllanthus amarus*, M. azed – *Melia azedarach*, V. negu – *Vitex negundo*, O. corn – *Oxalis corniculata*, Z. maur - *Zizyphus mauritiana*, M. pudi – *Mimosa pudica*, R. java – *Rhus javanica*).

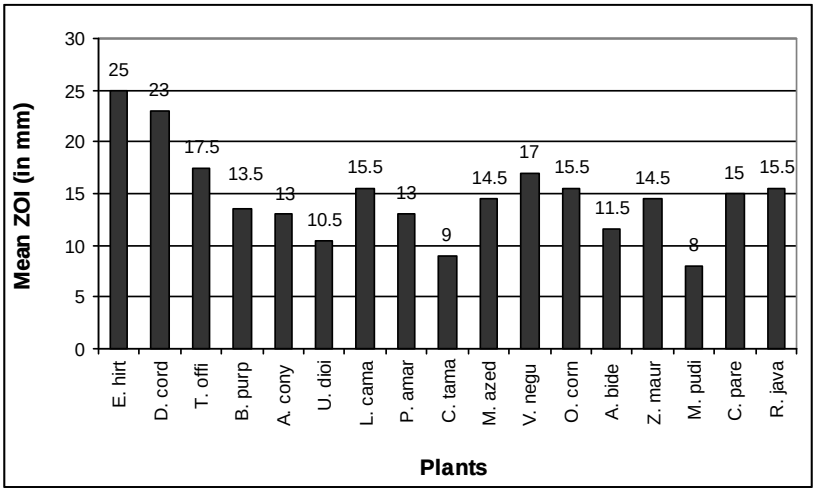


Figure 2: Zone of inhibition for bacterium *Staphylococcus aureus.* (Abbreviations: E. hirt – *Euphorbia hirta*, D. cord – *Drymaria cordata*, T. offi- *Taraxacum officinale*, B. purp – *Bauhinia purpurea*, A. cony – *Ageratum conyzoides*, U. dioi – *Urtica dioica*, L. cam – *Lantana camara*, P. amar – *Phyllanthus amarus*, C. tama – *Cinnamomum tamala*, M. azed – *Melia azedarach*, V. negu – *Vitex negundo*, O. corn – *Oxalis corniculata*, A. bide – *Achyranthes bidentata*, Z. maur – *Zizyphus mauritiana*, M. pudi – *Mimosa pudica*, C. pare – *Cissampelos pareira*, R. java – *Rhus javanica*).

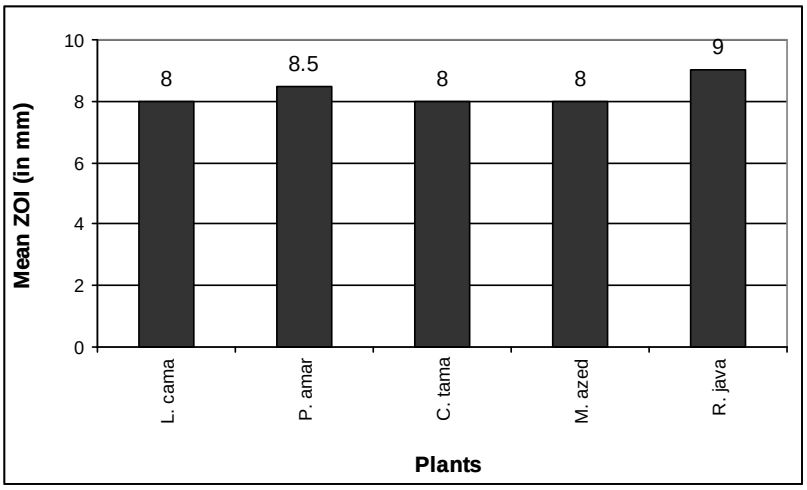


Figure 3: Zone of inhibition for bacterium *Escherichia coli*. (Abbreviation: L. cama – *Lantana camara*, P. amar – *Phyllanthus amarus*, C. tama – *Cinnamomum tamala*, M. azed – *Melia azedarach*, R. java – *Rhus javanica*).

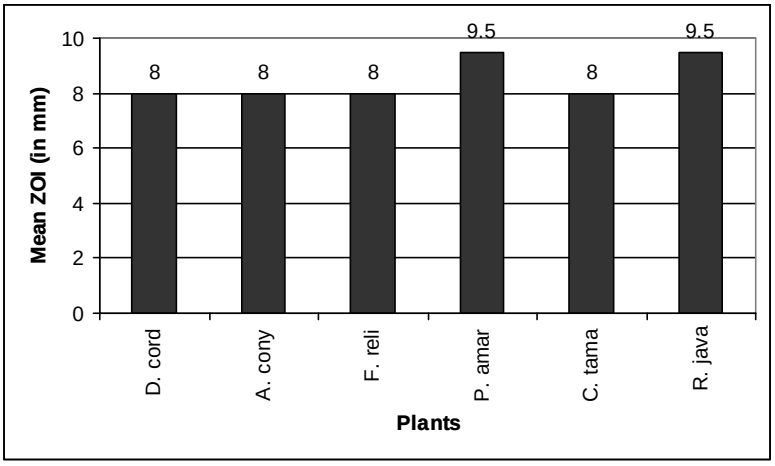


Figure 4: Zone of inhibition for bacterium *Salmonella typhi*. (Abbreviations: D. cord – *Drymaria cordata*, A. cony – *Ageratum conyzoides*, F. reli – *Ficus religiosa*, P. amar – *Phyllanthus amarus*, C. tama – *Cinnamomum tamala*, R. java – *Rhus javanica*).

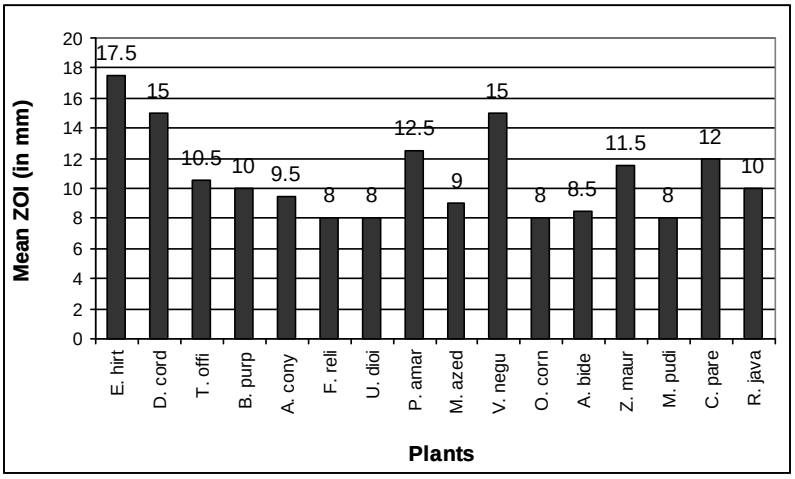


Figure 5: Zone of inhibition for bacterium *Bacillus subtilis*. (Abbreviations: E. hirt – *Euphorbia hirta*, D. cord – *Drymaria cordata*, T. offi – *Taraxacum officinale*, B. purp – *Bauhinia purpurea*, A. cony – *Ageratum conyzoides*, F. reli – *Ficus religiosa*, U. dioi – *Urtica dioica*, P. amar – *Phyllanthus amarus*, M. azed – *Melia azedarach*, V. negu – *Vitex negundo*, O. corn – *Oxalis corniculata*, A. bide – *Achyranthes bidentata*, Z. maur – *Zizyphus mauritiana*, M. pudi –*Mimosa pudica*, C. pare – *Cissampelos pareira*, R. java – *Rhus javanica*).

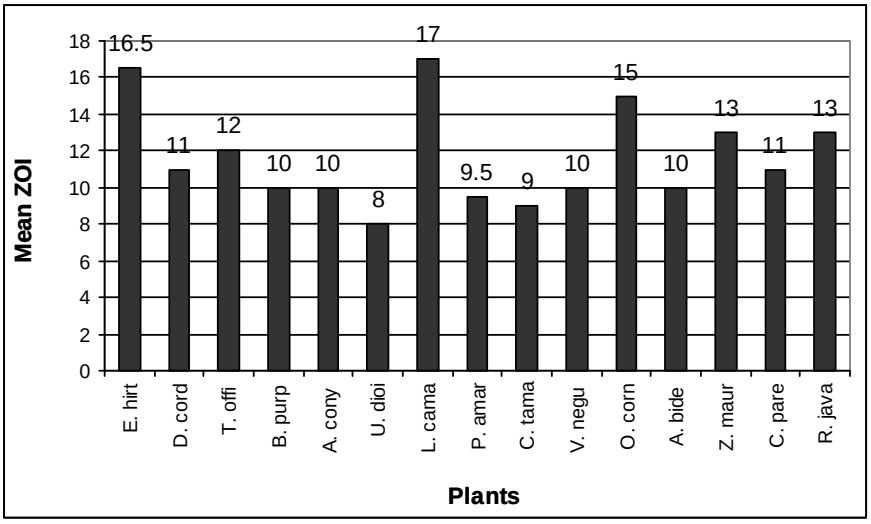


Figure 6: Zone of inhibition for bacterium *Proteus vulgaris*. (Abbreviations: E. hirt – *Euphorbia hirta*, D. cord – *Drymaria cordata*, T. offi – *Taraxum officinale*, B. purp – *Bauhinia purpurea*, A. cony – *Ageratum conyzoides*, U. dioi – *Urtica dioica*, L. cama – *Lantana camara*, P. amar – *Phyllanthus amarus*, C. tama – *Cinnamomum tamala*, V. negu – *Vitex negundo*, O. corn – *Oxalis corniculata*, A. bide – *Achyranthes bidentata*, Z. maur –*Zizyphus mauritiana*, C. pare – *Cissampelos pareira*, R. java – *Rhus javanica*).