CHAPTER- I

Introduction

1.1 General Background

From time immemorial people around the globe have been relying on plant based natural resources for food, clothes, shelter, dyes, treatment of diseases and other health related problems. Fossil record date, human used plants as medicine at least to the middle Paleolithic age some 60,000 years ago. Most of the plants from high altitude are used by local people as medicine based on their experience by hit and trial method and establish knowledge then passed generation to generation. The accumulation of knowledge provides an area of interest to the scientist which inspire them to do something meaningful (Farmsworth *et al*., 1976).

Nature has been the source of medicinal agents for thousand 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). 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 effect compared to modern conventional pharmaceuticals and their easily availability (Adomi, 2008).

The history of medicine and medicinal plants in Nepal can be traced back to the Vedic period, where Nepal Himalaya was mentioned as ascred heaven of potent medicinal and aromatic plants (Baral and kurmi, 2006). The earlisest medicinal used in Hindu culture is found in "Rig-Veda" (4500BC and 1600BC) is supposed to be the oldest repository of human knowledge. Enough information of the ethnobotanical and medicinal use of Nepalese plants is described in "Chandra Nighantu" (Malla, 1999). Even today plant materials continue to play major role in

primary health care as therapeutic remedies in many parts of countries. (Manandhar, 1985). It is estimated that various communities in Nepal use approximately 1000 species of wild plants in traditional medicinal practices (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 of Nepal (Tiwari, 1999).

1.1.1 Antibacterial Activity of Medicinal Plants

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

Another big concern is the development of resistance to antibiotic in current clinical use. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. The drug resistance bacteria and fungal pathogens have further complicated the treatment of infectious diseases in AIDS and cancer patients. Many efforts have been made to discover the new antimicrobial compounds from several of sources such as microorganisms, animals and plants. One of such sources is folk medicine. Systematic screening of such folk medicine may result in the discovery of novel effective compounds (Tomoko *et al.*, 2002). In contrary to the synthetic drugs, antimicrobial effects of plants origin are not associated with side effect 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 appropirate microbial model to confirm the activity and to ascertain the parameters associated with it. The effect of plant extract on bacteria has been studied by large number of researcher in different parts of the world especially in medicinal plants of Africa and Nigeria. But very limited work has been done in this field in Nepal. (Panthi and Chaudhary, 2006). Among them most work is related to ethnomedicinal palnts.

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

1.1.2 Development of Antibacterial Resistance

In most developing countries, the flora remains virtually unxplored from the point of view of practical utilization. Yet past experiences showed many valuable drugs which have been derived from plants. Information that a plant is used in traditional medicine is often an indication that it is worth for scientific study (Farmsworth and Morris, 1976). A wide range of medicinal plants parts are used for extract as raw drugs and they possess varied medicinal properties.

Many antibacterial drugs were developed in the late 1940s, following the first report of resistence in *Staphylococcus aureus* (1941) and in *Mycobacterium tuberculosis* (1940) (Dax, 1997). However, by the 1980S most major infectious disease 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 found world wide (Levy, 1998). A *Streptococcus* that causes noscomial infection showed innate resistance to drugs including cephalosporin, clindamycin and Aminoglycoside (Dox, 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 resistant exhibited by the pathogenic microbial infectious agents has lead to the screening of several medicinal plants for their potential antimicrobial activities (Colombo and Bosisio, 1996, Scazzocchio *et al.,* 2001). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics call attention for search of new species to fight against disease (Sieradzki *et al.,* 1999). Thus there is need to develop new antibiotic which is a global challenge preoccupying research institutions, pharmaceutical companies and academic institutions (Latha and Kannabiran, 2006). However, the past record of rapid wider spread emergence of resistance to newly introduce 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 infectious is now one of the serious medical problems (Marchese and Shito, 2001).

1.2 Objectives

1.2.1 General Objective

The aim of the study is includes to find out the antimicrobial activity of some medicinal plants of folklore bearing importance, which are used by people for medicinal purpose.

Specific Objectives

- To screen the antibacterial activity of crude methanol extract of some medicinal plants of Nepal.
- To evaluate the antibacterial property of those medicinal plants.
- To study medicinal value of those plants used by ethnic group.

1.3 Rationale of the Study

The medicinal plants are being used by people of rural area for different bacterial diseases and other diseases. These plants are also being used by people of rural area in Nepal. The people of rural areas suffer from various bacterial disease like desyntery, diarrhea, jaundice, pneumoniae etc and they mainly depend on primary health care such as treatment provided by traditional healers by using medicinal plants. Due to the lack of modern facilities of hospital, doctors, allopathic medicine and economic problems, rural people depend on traditional healers. These plants may contain some antibacterial activity, so, to know 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. The effects of plants on bacteria have been studied by large number of researchers in the different parts of the world. In Nepal, few plants are screened for antimicrobial activity. The study of antimicrobial compounds on plants is the basis to prepare the antimicrobial compounds to be used in allopathic system of medication. The need of this study is to evaluate the different plants to investigate

different compounds which can fight against the bacteria possessing multiple resistances. This study also provides guidelines to extracts active constituents present in plants having antibacterial properties, which can be further utilized in the allopathic system of treatments.

1.4 Limitation of the Study

Due to the limitation of time and resources, this study was limited to only seven strains of pathogenic bacteria; few medicinal plants were screened for antibacterial activity. The minimum inhibitory concentration (MIC) is also an important basis to evaluate the antibacterial activity but only zone of inhibition (ZOI) was determined but minimum inhibitory concentration (MIC) was avoided.

CHAPTER - II

Literature Reviews

2.1 Plants and Plant Products Used in Medicine

Countries like Nepal and India have been using crude plants as medicines since Vedic period. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources (Panthi and Chaudhary, 2006). Biologically active compounds present in the medicinal plants have always been of great interest to scientists working in this field. In recent years this interest to evaluate plants processing antibacterial activity for various diseases is growing (Clark and Hufford, 1993). Herbal medicinal practice plays an important role in the primary health care system in most developing countries. WHO estimates that 80% of populations 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 medicines, the study of plants for external uses 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 popularised in the developing and developed countries owing to its natural origin and lesser side effects. In olden times *Vaidyas* used to treat patient on individual basis and prepared drugs according to the requirements of the patients. But the scene has been changed now, herbal medicines are being manufactured on a large scale in medical units, where manufactures are facing many problems such as availability of good quality of raw materials, authenticaiton of raw materials, availability of standard, proper standardization methodology of drugs and formulation, quality control parameters and etc (Ali *et al*., 2005, Agarwal, 2005).

The discovery of bacteria in 1663 by Anton Van leuwenhook helped man kind to understand the infectious pathogens and approximately developed antiseptic and antibiotic protocol in the following years. By the beginning of 20th century, Paul Ehrlich purposed the principle of chemotherapy and this work including structure activity. Relationship significantly contributed for shaping synthetic protocol 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 plants substances which were toxic to those microorganisms.

Large number of works had been done to test antibacterial activities of plants throughout the world (Taylor *et al.,* 1995, Dagmer *et al.*, 2003, Parekh and Chand, 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, and Mahato and Chaudhary, 2005) Not only this, phytochemical screening of the plants were coupled with antibacterial effects to link the main group of phytochemicals and their effects on bacteria (Raghavendra *et al.,* 2005, Parekh and Chanda 2006, Nandagopal *et al.*, 2007, Saani *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 medicinal plants 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, Cordiospermum holicacabum Euphorbia hitra, Murraya koenigii, Oldenlandia corymbosa* and *Phyllanthus niruri.*

Samy and Ignacimutha (2000) studied the antimicrobial activity of 30 Indian folklore medicinal plants used by tribal healers to treat infectious by using disc diffusion method against *Bacillus subtilis, Escherichia coli, Klebsiella*

pneumoniae and *Staphylococcus aureus,* 20 plant species showed activities against one or more strains of tested bacteria. The leaf extract of *Cassia occidentalis* and *Cassia auriculata* exhibited significant broad spectrum against *Bacillus subtilis and Stophylococcus aureus.*

Kelmanson *et al.* (2000) studied the antibacterial activity of Zulu medicinal plants. Extracts of 14 medicinal 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 extract of *Dioscorea sylvatica* had activity against Gram negative bacteria *Escherichia coli* and extracts of *Dioscorea dregenoid, Cheilanthes viridis* and *Veronia colorata* were active against *Pseudomonas aeruginosa*.

Uma Devi *et al.* (2000) evaluate the antibacterial efficacy of *Achynanthes bidentata* against seven different bacterial strains. *Escherichia coli, Bacillus subtilis, Proteus vulgaris, Salmonella typhi, Staphylococcus aureus*, *Pseudomonas* sps. and *Klebsiella pneumoniae*. All the extracts of root, stem, leaves and flowers showed high sensitive to *Proteus vulgaris, Escherichia coli, Bacillus subtilis, Salmonella typhi* and *Klebsiella pneumoniae* but moderate and less sensitive to *Staphylococcus aureus* and *Pseudomonas* species.

Sairan *et al.* (2002) evaluated the antibacterial 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 Pneumoniae*. The result illustrate that the extracts of *Mangifera indica* have significant antidirrheal activity.

Nisanta *et al.* (2002) were screened 32 medicinal plant species for antibacterial activities against 2 Gram positive and 3 Gram negative bacteria. The methanol extracts of 29 species were active against at least one microorganisms of

Gram positive. However, only two texa, *Lantana camara* and *Phyllanthus* sp. were active against Gram negative bacteria *Pseudomonas aeruginosa*. None of the species were active against the other Gram negative bacteria.

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 albican.* Among the 10 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 forfara.*

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

Sanaa *et al.* (2005) used Petroleum ether, Methanol and aqueous extracts of *Helianthus annus*, Leaves of *Azadirachta indica*, bulbs of *Allium cepa* and seeds of *Portulaca oleracea* were tested against Gram positive *(Bacillus sublits* and *Staphylococcus aureus)* and Gram negative bacteria *(Escherichia coli, Proteus vulgaris, Pseudomons aeruginosa* and *Salmonella typhi*). The methanol extract of *Azadirachta indica* showed active antibacterial activity against all tested bacteria except *E. coli.*

Nair *et al. (*2005) screened nine plants for potential antibacterial activity by agar disc diffusion method. In evaluating antibacterial activity both aqueous and organic solvents were used. The plants screened were *Sapindus emarginatus, Hibiscus rosa-sinensis, Mirabilis jalpa, Rheo discolor, Nyctanthes arbortritis*

Colocasia esculenta, *Gracilaria corticata* and *Dictyota* species. The test organisms employed were *Pseudomonas testosterone, Staphylococcus epidermidis, Klebsiella pneumoniae, Bacillus subtilis, Proteus 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.

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

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

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

mlotica and *Sida cardifolia* leaf extracts showed highest antibacterial activity against bacillus subtilis. Root and leaf extracts of *sida cardifolia* recorded significant activity against all the tested bacteria.

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

Kumar *et al.* (2009) were screened the plant *Syzygium cumini* for investigation of phytocompounds by the methanol extracts of the seed. The methonol extracts of seed of *Syzygium cumini* showed the presence of alkaloid, flavonoids, glycosides, saponins, tannins, phytosterol, amino acids, steroids and terpenoids in variable concentration.

Anju Kumar *et al.* (2010) screened the plant *Curculigo orchioides* for the investigation of phytocompounts by methanol extracts of root tuber or rhizome. The methanol extract of rhizome of the *Curculigo orchioides* showed the presence of alkaloids, phytosteroides, carbohydrates, glycosides, saponins, protein, amino acid and phytosteroides. The compounds carbohydrate, glycosides and saponins were found in high concentration and other were found in low concentration.

2.1.2 Work Inside the Country

Shakya (1982) performed the preliminary antibacterial activities of 45 indigenous medicinal plants by disc diffusion method on the dried extract of petroleum ether (40-69), 95% alcohol and sterile water. The test organisms were *Staphylococcus aureus, Agrobacterium tumefaciens, Bacillus subtilis, Salmonella typhi, Eschericha coli, Shigella dysenteriae, Condida albicans, Saccharomyces*

cervisiae and *Candida neoformans*. Some plant species showed weak and moderate activities on both bacteria and fungi while some extracts 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 cervesiae.* Some of the plant extracts showed weak activities but extracts of *Barberis aristata* and *Anagalis arvensis* showed moderate activity against bacteria and fungi respectively. Most of the essential oil 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 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 the exposure to light

. Sharma and Sharma (1998) observed the antimicrobial activity of some essential oil viz *Mentha arvensis, Acrous calamus, Xanthoxylum oxyphyllum* and *Terpentine* oil against some fungi and bacteria. The extract of efficacy of the essential oil was studied on two different growth stages of filamentous fungi and non-filamentous fungi Gram positive bacteria *(Staphylococcus* sp. and *Streptococcus* sp.) and Gram negative bacteria *(Escherichia coli* and *Pseudomenas* sp.) by minimum inhibitory concentration (MIC) technique and spore germination test *Terpentine oil* exhibited strong activity against tested bactera.

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.

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

Mahato and Chaudhary (2005) documented 25 plant species of palpa district, Nepal for their ethmonedicinal uses and screened for their antibacterial activity. The disc 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 extract of 13 species (52%) showed positive response against at least one of the tested bacteria while the extract of 11 species (44%) showed positive response at least two strains of tested bacteria, similarly the extract of 10 species (40%) showed positive response against 3 bacteria and nine species (36%) showed positive response against all of the tested four bacteria. However, the extract 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.

Chaudhary and Panthi (2006) tested eighteen medicinal plant species used in folklore medicine in west Nepal for their antibacterial activity by the disc 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.

Pokhrel *et al*. (2008) performed the antimicrobial activity of *Acrous calamus, Curcuma longes, Emblica officinalis, Glycryrrhia globra* (non-indegenous to Nepal), *Justicia adhatoda* and *Xanthoxylum armatum* by disc diffusion method against *Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus.* Among them *Emblica officinalis* was found to be best antibacterial plant and other were found moderate antibacterial plants.

Shakya *et al.* (2008) studied the alcoholic extract of 215 medicinal plants for their antimicrobial properties amnong 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 moderately active against five microorganisms. The plants *Andrographis paniculata, Baberis aristata, Campylandra aurntica, Rheum australe* and *Cinnamomum tamala* showed antimicrobial activity against four microbes. These plants may have effective board spectrum antimicrobial phytocompounds. Only *Compylandra aurntica* showed encouraging activity against fungi.

Gyawali *et al.* (2008) carried out the phytochemical screening of 17 Nepalese medicinal plants was performed to test the presence of alkaloids, favonoids, 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.

CHAPTER- III
Materials and Methodes

3.1 Sample Material Preparation

3.1.1 Selection of Medicinal Plants

Different medicinal plants were selected on the basis of plant materials used for treatment of differrent bacterial diseases like diarrhea dysentery, pneumoniae, cholera, jaundice, typhoids, cuts and wounds etc. The plant materials were collected on the basis of their importance against different diseases. Collection was done with the help local healers. The information about the importance of medicinal plants against different diseases. The description and uses of these plants are provided in Appendix-A.

3.1.2 Collection of Plant Materials

Fresh plants or plant parts were collected from different places of Pyuthan and Kapilvastu during October, November and December 2009. Fresh plant materials were washed with the help of fresh 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 compounds.

3.1.3 Plant Indentification

The collected 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 enhanced 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 ground powder was stored in polythene bags separately 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 and Chand, 2007) with some modifications considering the access and availability of equipments and chemicals.

3.2.1 Preparation of Extract

Two grams of ground powdered materials was soaked in 25ml of methanol for 24 hours and filtered by using standard filter paper (Whiteman no-1 paper). The residue was soaked again with 25ml of fresh methanol and filtered after 24 hours. Same process was repeated ones again. The extract after treating with 75 ml (25ml x 3 times) methanol was filtered. The filtrate was transferred in to beakers and allowed to evaporate until completely dry. Once dried, the extract was resuspended in 2ml of methanol. The concentration of final extract was 1gm material /1ml methanol.

3.2.2 Collection of Test Organisms

The microbial strains used were identified from Central Department of Microbiology TU. The studied strains include seven different types of bacteria, two Gram positive *(Bacillus subtilis, Staphylococcus aureus)* and five Gram negative *(Escherichia coli, Preteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumoniae).* 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-B.

3.2.3 Preparation of the Test Discs

Sterile test discs were prepared by dipping and saturating sterilized filter paper disces (6 mm diameter) are made by cutting the Whiteman number- 1 filter paper with the help of punching machine and absorved the same volume of extracts.

3.2.4 Preparation of Culture Media

3.2.4.1 Nutrient Agar

Nnutrient agar was prepared with the help of manufactures (Hi-media) Recommendations 28gram of nutrient agar was weighed and dissolved into distilled water to make final volume of 1000ml. It was sterilize by autoclaving the media inside the round bottomed flask at 15 lbs pressure at 121^{oC} for 15 minutes. It was then cooled to 50^{oC} about 20ml of media was poured to sterilized petriplates aseptically and labelled properly. For the slant preparation, the required amount of media was poured in appropirate sized screw capped bottle, autoclaved and cooled in position to make slant

3.2.4.2 Nutrient Broth

Nutrient broth was also prepared with the help of manufactures (Hi-media) recommendation 13g of powder was weighed and dissolved in distilled water to make final volume of 1000ml. It was sterilized by autoclaving at 15 lbs pressure and 121^{oC} 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 organisms 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 innoculated overnight inside the incubator at 37^{oC}.

3.2.6 Transfer of Bacteria on Petriplates

The agar plates for the assay were prepared by labelling them with the date, the name of bacteria and the name of code of 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 innoculums by pressing the standard 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 petriplates at 90^{o} and containing 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 dises were transferred on bacterial lawn under aseptic condition using flame sterilized foreceps each time. Each disc was placed gently on the agar surface on equidistance and potted with the forceps to ensure the disc adhere to the surface of agar. The petriplates were incubated in an inverted position for 24 hours at $37^{oC.}$

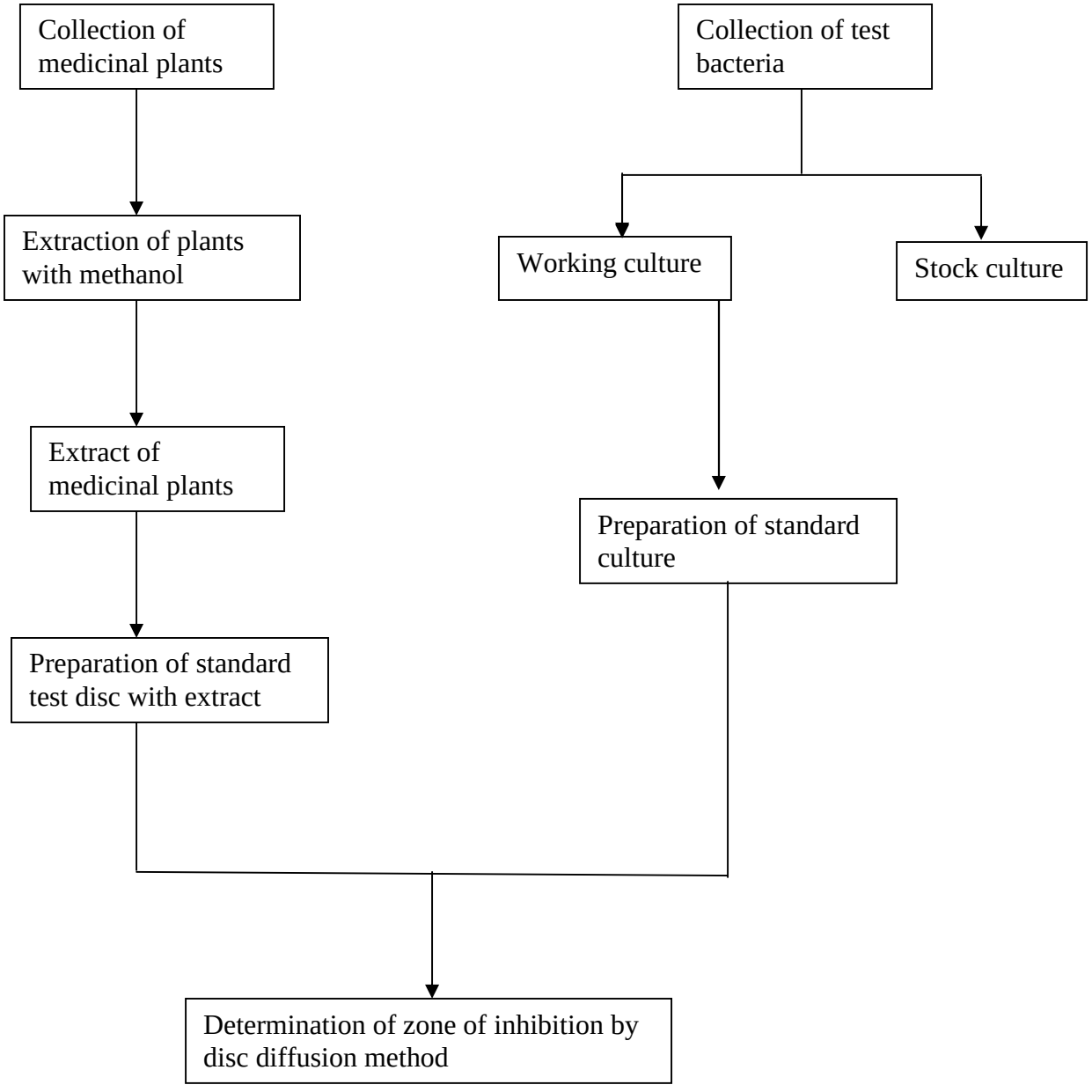
3.2.8 Observation of Result

After 24 hours of incubation at 37^{oC}, the results were recorded as the presence or absence of inhibition zones. Resulting zones of inhibition were observed and recorded as "+" or "-".The diameter of zone of inhibition produced by plant extract on particular bacteria was also measured with the help of millimeter ruler. The inhibitory zone around test paper discs indicate absence of (ZOI) bacterial growth and that was recorded as positive and the inhibitory zone not found around the test paper discs indicate presence of bacterial growth and that

was recoded as negative. The tests were repeated three times to insure the reliability of the result.

Flow Chart of the Method

I. Antibacterial Test



CHAPTER - IV

Result

4.1 Yield of Methanol Crude Extracts of Medicinal Plants

During the methanol crude extraction, variation of extracts of medicinal plants had resulted. The weight and percentage yield of the crude extracts of medicinal plants by soaking process is shown in table-1. The amount of extract varied among the medicinal plnats. According to the result of this study, *Azadirachta indica* gave the highest yield (23.5%), followed by *Bauhania variegata* (22.5%), *Swertia chirayita* (18%), *Justicia adhatoda* (17.32%), *Oroxylum indicum* (14.11%), *Mentha spicata* (13.31%), *Withania somnifera* (13%), *Acmella clava* (12.50%), *Thalictrum foliolosum* (11.33%), *Curculigo orchioides* (11.25%), *Phyllanthus emblica* (9.21%) etc. The lowest yields were obtained from *piper longum* (6.05%) and *Achyranthes aspera* (5.60%).

Table -1 Percnetage yield of crude methanol extract of medicinal plants.

S.N	Plant Name	Parts used	wt.2g the extract	% yield
1	*Piper longum*	Fruit	0.121 gm	6.05
2	*Achyranthes aspera*	root	0.112 gm	5.60
3	*Oroxylum indicum*	Stem bark	0.282 gm	14.1
4	*Acmella clava*	Flower	0.250 gm	12.5
5	*Thalictrum foliolosum*	Root	0.226 gm	11.33
6	*Bauhinia variegata*	Stem Bark	0.45 gm	22.5
7	*Phyllathus emblica*	Fruit	0.184 gm	9.21
8	*Swertia chirayita*	Whole plant	0.36 gm	18
9	*Mentha spicata*	Whole Plant	0.260 gm	13.31
10	*Azadirachta indica*	Leaves	0.47 gm	23.50
11	*Curculigo orchioides*	Rhizome	0.225 gm	11.25
12	*Withania somnifera*	Root	0.26 gm	13
13	*Justicia adhatoda*	Leaf	0.346 gm	17.32

4.2 Screening of Medicinal Plants for Antibacterial Activity

The methanol extract of used parts of different plants were prepared and antibacterial activity was tested by using disc diffusion method. Table-2 summarizes the result obtained from screening of methanolic extract of different medicinal plants against tested bacteria. The +ve sign indicate the production of inhibition zone and -ve sign indicate the absence of inhibition zone or allow the growth of microorganisms. It is revealed from the table that all the tested medicinal plants were effective against the employed bacteria.

Table 2 - Antibacterial properties of methanolic extracts of differnt medicinal plants against tested bacteria.

S.N.	Plant Name	Pa	Sa	Bs	Pv	St	Ec	KP
1	*Piper longum*	-ve	+ve	+ve	+ve	-ve	-ve	+ve
2	*Achyranthes aspera*	-ve	+ve	+ve	-ve	+ve	+ve	+ve
3	*Oroxylum indicum*	+ve	-ve	+ve	+ve	+ve	-ve	+ve
4	*Acmella clava*	+ve	+ve	+ve	+ve	+ve	+ve	-ve
5	*Thalictrum foliolosum*	+ve	+ve	+ve	+ve	+ve	+ve	+ve
6	*Bauhinia variegata*	+ve	+ve	+ve	+ve	+ve	+ve	-ve
7	*Phyllathus emblica*	+ve	+ve	+ve	+ve	+ve	+ve	+ve
8	*Swertia chirayita*	+ve	+ve	+ve	+ve	+ve	+ve	+ve
9	*Mentha spicata*	+ve	-ve	+ve	+ve	+ve	+ve	+ve
10	*Azadirachta indica*	+ve	+ve	+ve	-ve	+ve	-ve	+ve
11	*Curculigo orchioides*	-ve	+ve	+ve	-ve	+ve	-ve	-ve
12	*Withania somnifera*	+ve	+ve	+ve	+ve	+ve	+ve	+ve
13	*Justicia adhatoda*	-ve	+ve	+ve	-ve	-ve	-ve	-ve

(Note: All the plants show positive result for positive and negative result for negative control) Abbreviations, Pa = *Pseudomonas aeruginosa*, Sa = *Staphylococcus aureus*, Bs = *Bacillus subtilis*, Pv = *Proteus vulgaris*, St = *Salmonella typhi*, Ec = *Escherichia* coli and Kp = *Klebsiella pneumoniae).*

Among 13 medicinal plants tested, in the present study, all the medicinal plants showed activity against at least two bacteria. *Swertia chirayita, Phyllanthus emblica, Withania somnifera* and *Thalictrum foliolosum* inhibited all the tested bacteria. Similarly, *Acmella clava, Mentha spicata* and *Bauhinia variegata* were effective against 86% of tested bacteria. Three plants inhibit the growth of 71%. Like as *Piper longum* was effective against 57% of tested bacteria, *Curculigo orchioides* was effective against 43% of tested bacteria and *Justicia adhatoda* was effective against 29% of tested bacteria (Table 3). Among the seven tested bacteria *Escherichia coli* were most resistant bacteria. Only eight plants among 13 plants employed in this study could inhibit its growth whereas *Bacillus subtilis* was the most susceptible bacteria which inhibit its growth by all medicinal plants tested.

4.3 Evaluation of Antibacterial Activity of Medicinal Plants

The result obtained from this study was mentioned as the mean zone of inhibition (ZOI) of methanol extracts of medicinal plants, Which were able to show significant zone of inhibition (| 7.66mm) during the qualitative screening process which is shown in table-4.

Four medicinal plants viz *Swertia chirayita, Phyllauthus emblica, Withania somnifera* and *Thalictrum foliolosum* had broad spectrum activity. They inhibited the growth of all the tested bacteria. None of the seven tested bacteria were resistant towards these four plants. *Thalictrum foliolosum* showed the strongest ZOI (19.33mm) towards the *Staphylococcus aureus* and weakest ZOI (7.66mm) towards the *Escherichia coli* Similarly, *Phyllathus emblica* showed strongest ZOI (12.66mm) towards the *Bacillus subtilis* and weakest ZOI (9.66mm) towards *Proteus vulgaris. Swertia chirayita* showed the strongest ZOI (12.33mm) against *Pseudomonas aeruginosa* and weakest ZOI (9.33mm) against *Escherichia coli* and *Withnia somnifera* showed the strongest ZOI (12.33) against *Klebsiella pneumoniae* and weakest ZOI (8.33) against *Escherichia coli, Pseudomonas aeruginosa, Staphyllococcus aureus* and *Salmonella typhi.*

Three plants viz *Acmella clava, Bauhinia variegata* and *Mentha spicata* showed broad spectrum activity. They inhibit the growth of 6 tested bacteria out of seven tested bacteria screened. *Acmella clava* could not inhibit the growth of *Klebsiella pneumoniae* i.e this bacteria was resistant towards the *Acmella clava* similarly *Bauhinia variegata* could not inhibit the growth of *Klebsiella pneumoniae, Mentha spicata* could not inhibit the growth of *Staphylococcus aureus.*

At least one bacteria screened were resistant towards these three medicinal plants viz *Aanella clava, Bauhinia variegata*, and *Mentha spicata.*

Acmella clava showed highest ZOI (14.33mm) with *Bacillus subtilis* and lowest ZOI (10mm) with *Proteus vulgaris*. *Bauhinia variegata* showed highest ZOI (12.33mm) with *Staphylococcus aureus, Proteus vulgaris, Salmonilla typhi* and *Escherichia coli* and lowest ZOI (11.66mm) with *Pseudomonas aeruginosa* and *Bacillus subtilis* and *Mentha spicata* showed the highest ZOI (12.33mm) with *Salmonella typhi* and lowest ZOI (8.33mm) with *Escherichia coli*.

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

S.N.	Plant Name	No.of bacteria inhibited	% of bacteria inhibited
1	*Piper longum*	4	57
2	*Achyranthes aspera*	5	71
3	*Oroxylum indicum*	5	71
4	*Acmella clava*	6	86
5	*Thalictrum foliolosum*	7	100
6	*Bauhinia variegata*	6	86
7	*Phyllathus emblica*	7	100
8	*Swertia chirayita*	7	100
9	*Mentha spicata*	6	86
10	*Azadirachta indica*	5	71
11	*Curculigo orchioides*	3	43
12	*Withania somnifera*	7	100
13	*Justicia adhatoda*	2	29

Three medicinal plants viz *Achyranthes aspera, Oroxylum indicum* and *Azadirachta indica* showed somewhat similar result. These four plants inhibit the growth of five tested bacteria out of seven tested bacteria screened. *Achyranthes aspera* could not inhibit the growth of *Pseudomonas aureginosa* and *Proteus vulgaris, Oroxylum indicum* could not inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* and *Azadirachta indica* could not inhibit the growth of *Proteus vulgaris* and *Escherichia coli.* According to the obtained

Table- 4 Mean zone of inhibition (ZOI) shown by different medicinal plants against tested bacteria including standard deviation

S.N	Plants Name	Parts	Bacterial strains (MI SD) mm						
			Pa	Sa	Bs	Pv	St	Ec	kp
1	*Piper longum*	Fruit	0	9.33±2.08	16.66±1.52	9±1	0	0	9.33±1.15
2	*Achyranthes aspera*	root	0	11.66±3.21	13.66±0.57	0	10.33±0.57	10.33±1.52	8.33±0.57
3	*Oroxylum indicum*	Stem bark	15.66±1.52	0	9.33±1.52	8.33±0.57	10.66±0.57	0	11±1
4	*Acmella clava*	Flower	10.33±0.57	10.33±2.08	14.33±1.52	10±1	10.33±0.57	11.66±1.15	0
5	*Thalictrum foliolosum*	Root	17.33±0.57	19.33±1.15	16.33±2.08	10.33±2.51	15.33±2.51	7.66±0.57	11±3
6	*Bauhinia variagata*	Stem Bark	11.66±2.08	12.33±0.57	11.66±1.52	12.33±2.08	12.33±2.08	12.33±0.57	0
7	*Phyllathus emblica*	Fruit	11.33±1.15	11.66±1.52	12.66±1.15	9.66±0.57	1.66±0.57	12.33±0.57	10±1
8	*Swertia chirayita*	Whole plant	12.33±2.08	10.33±0.57	19.33±2.51	10±0	9.33±1.15	9.33±0.57	12.33±0.57
9	*Mentha spicata*	Whole Plant	10.33±0.57	0	10.33±2.08	10±1.73	12.33±0.57	8.33±0.57	11.33±0.57
10	*Azadirachta indica*	Leaves	7.66±0.57	8.33±0.57	10±0	0	10.33±0.57	0	9.66±1.52
11	*Curculigo orchioides*	Rhizome	0	13.66±0.57	9.66±1.52	0	12.66±0.57	0	0
12	*Withania somnifera*	Root	8.33±0.57	8.33±0.57	10.33±1.52	11±0.57	8.33±0.57	8.33±0.57	12.33±0.57
13	*Justicia adhatoda*	Leaf	0	9.33±0.57	9.66±1.52	0	0	0	0

(Abbreviations, Pa = *Pseudomonas aeruginosa*, Sa = *Staphylococcus aureus*, Bs = *Bacillus subtilis*, Pv = *Proteus vulgaris*, St = *Salmonella typhi*, Ec = *Escherichia coli* and Kp = *Klebsiella pneumoniae).*

result, at least two tested bacteria were resistant towards these three medicinal plants.

Achyranthes aspera showed the strongest ZOI (13.66mm) against *Bacillus subtilis* and weakest ZOI (8.33mm) against *Klebsiella pneumoniaee*, *Oroxylum indicum* showed the strongest ZOI (15.66mm) against *Pseudomonas aeruginosa* and weakest ZOI (8.33mm) against *Proteus vulgaris* and *Azadirachta indica* showed the highest ZOI (10.33mm) against *Pseudomonas aeruginosa. Piper longum* inhibited the growth of 4 bacteria. It showed highest ZOI (16.66mm) against *Bacillus subtilis* and lowest ZOI (9mm) against *Salmonella typhi.*

Curculigo orchioides plant inhibited the growth of three bacteria viz *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella typhi* while other four tested bacteria viz *Pseudomonas aeruginosa, Proteus vulgaris, Escharichia coli* and *Klebsiella pneumoniae* were resistant to it. It showed highest ZOI (13.66mm) against *Staphylococcus aureus* and lowest ZOI (9.66mm) against *Bacillus subtilis. Justicia adhatoda* medicinal plant inhibited the growth of two bacteria viz *Bacillus subtilis* and *Staphylococcus aureus*. But other five tested bacteria viz *Pseudomonas aeruginosa Salmonella typhi, Proteus vulgaris, Escherichia coli* and *Klebsiella pneumoniae* were resistant to it. It showed strongest ZOI (9.66mm) against *Bacillus subtilis* and weakest ZOI (9.33mm) against *Salmonlla typhi, Justicia adhatoda* showed weakest activity among the tested plants and ZOI also comparatively very low.

4.4 Evaluation of Susceptibility of the Tested Bacteria

Susceptibility of the bacteria is evaluated in terms of the number of medicinal plants which affect the growth of bacteria and extent of bacteria i.e size of zone of inhibition (ZOI) produced by them. In this study, *Bacillus subtilis* was the most susceptible bacteria being inhibited by all 13 medicinal plants tested. *Escherichia coli* were the most resistant bacteria being susceptible to only eight medicinal plants among 13 medicinal plants tested.

The growth of *Pseudomonas aeruginosa* was inhibited by nine plants extracts out of thirteen plants tested. The maximum ZOI was observed with

Thalictrum foliolosum as (17.33mm) and the ZOI followed by *Oroxylum indicum* (15.66mm), *Swertia chirayita* (12.33mm), *Bauhinia variegata* (11.66mm), *Phyllanthus emblica* (11.33mm), *Acmella clava* and *Mentha spicata* (10.33mm), *Withania somnifera,* (8.33mm) and the lowest ZOI was observed with *Azadirachta indica* (7.66mm).

Staphylococcus aureus was found more resistant than *Bacillus subtilis* among these two Gram positive bacteria. The growth of *Staphylococcus aureus* was inhibited by eleven medicinal plants extracts out of 13 plants. The maximum ZOI was found with *Thalictrum folilosum* (19.33mm) and the ZOI was followed by *Curculigo orchioides* (13.66), *Bauhania variegata* (12.33mm), *Phyllanthus emblica* and *Achyranthes aspera* (11.66mm), *Swertia chirayita* and *Acmella clava* (10.33), *Justicia adhatda and Piper longum* (9.33mm). The lowest ZOI was found with *Azadirachta indica* and *Withania somnifera* (8.33mm).

Bacillus subtilis was found to be most susceptible bacteria. It is Gram positive bacteria and it was inhibited by all the plants axtracts tested. Among them, *Swertia chirayita* showed the highest ZOI (19.33mm) with it. The ZOI followed by *Piper longum* (16.66mm), *Thalictrum foliolosum* (16.33mm), *Acmella clava*(14.33mm), *Achyranthes aspera* (13.66mm) *Phyllanthus emblica* (12.66mm), *Bauhinia variegata* (11.66mm), *Mentha spicata* (10.33mm), *Withania somnifera* (10.33mm), *Azadirachta indica* (10mm), *Justicia adhatoda* and *Curculigo orchioides* (9.66mm), and the lowest ZOI was resulted by *Oroxylum indicum* (9.33mm) with it.

Proteus vulgaris was found to be most resistant bacteria after *Escherichia coli*. It is Gram negative bacteria and it was susceptible to extracts of only nine medicinal plants among 13 medicinal plants tested. The strongest ZOI (12.33mm) was shown by *Bauhinia variegata* with it and lowest ZOI (8.33mm) was shown by *Oroxylum indicum* (8.33mm). The ZOI followed by *Withania somnifera* (11mm),

Thalictrum foliolosum (10.33mm), *Swertia chirayita*, *Acmella clava* and *Mentha spicata* (10mm), *Phyllanthus emblica* (9.66mm) and *Piper longum* (9mm).

Salmonella typhi was found to be most susceptible bacteria among Gram negative bacteria. Eleven plant extracts inhibited its growth among thirteen plants. The highest ZOI was shown by *Thalictrum foliolosum* (15.33mm) with it. The ZOI followed by *Curculigo orchioides* (12.66mm), *Bauhinia variegata* and *Mentha spicata* (12.33mm), *Phyllanthus emblica* (11.66mm), *Oroxylum indicum* (10.66mm), *Achyranthes aspera*, *Acmella clava* and *Azadirachta indica* (10.33mm), *Swertia chirayita* (9.33mm). The lowest ZOI was shown by *Withania Somnifera* (8.33mm) with it. *Escherichia coli* were found to be most resistant bacteria than other 6 bacteria. The growth of *Escherichia coli* was inhibited by eight plants extract among thirteen medicinal plants. Among them the highest ZOI was shown by *Bauhinia variegata* and *Phyllanthus emblica* (12.33mm). The ZOI followed by other plants were, *Acmella clava* (11.66mm), *Achyrathes aspera* (10.33mm), *Swertia chirayita* (9.33mm), *Mentha spicata* and *Withania somnifera* (8.33mm). The lowest zone of inhibition was shown by *Thalicturm foliolosum* (7.66mm).

Klebsiella pneumoniae is susceptible towards 9 medicinal plants out of thirteen plants tested. The ZOI (12.33mm) shown by *Withania somnifera* and *Swertia chirayita* is highest. TheZOI followed by other plant extracts were *Mentha spicata* (11.33mm), *Oroxylum indicum* and *Thalictrum foliolosum* (11mm), *Phyllanthus emblia* (10mm), *Azadirachta indica* (9.66mm) and *Piper longum* (9.3mm). The lowest ZOI shown by *Achyranthes aspera* was (8.33mm) with it.

CHAPTER – V

Discussion

In most developing countries, the flora remains virtually unexplored from the point of view of practical utilization. Previous reports showed that many valuable drugs have been derived from plants. Plant is used in traditional medicine is worth to study scientifically. (Farmsworth and Morris, 1976). Medicinal properties of the plants are due to the active chemical constituents present in differnt parts of the plants (Mitcher *et al*., 1999).

Medicinal plants continue to be an important therapeutic aid for alleviating the aliments of human kinds. The search for eternal health and longevity and for remedies to relieve pain and discomfort drove early man to explore his immediate natural surroundings and lead to the use of many plants and the developments of a variety of therapeutic agents. Today, there is a renewed intrest in traditional medicine and an increasing demand for more drugs from plants sources. This revival of intrest in plants derived drugs is mainly due to the widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effect (Rathish and Chanda, 2007). In the contex, about 70-80 percent of people living in the mountain region depend on traditional medicines for health care (Manandhar, 1980). Different researchers have been carried out the antibacterial activities on differnt medicinal plants found in differnt parts of Nepal.

In the present study, altogether thirteen medicinal plants species have been screened to evaluate the antibacterial activity, 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. In the present study, seven different strains of bacteria were used to test the antibacterial activity of crude methanol extracts of selected plants. Among seven strains of bacteria two strains were Gram positive viz. *Staphylococcus*

aureus and *Bacillus subtilis* and five Gram negative viz *Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli* and *Klebsiella pneumoniae.*

5.1 Extraction from Medicinal Plants:

The isolation of phytochemicals from medicinal plants is mostly dependent on the type of solvent used in the extraction process. Differnt extracts are used to test the antimicrobial properties of medicinal plants. The traditional healers were used water as a solvent. According to numbers of researchers, plant extracts prepared with methanol and ethanol as solvents provide a more complete extraction, including less polar compounds and many of these extracts have been found to possess antimicrobial properties (Ali-Shtayeh and Abu Ghdeib, 1999). The methanol solvent also helped in the complete extraction of antimicrobials from plants includings tannins, polyphenols, terpenoids, saponins, xanthoxyllines, totarol, quassionoides, 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 ethanol or methanol extraction (Viges *et al.,* 1997).

It this study, the amount of extracts was obtained by soaking the ground medicinal plants on methanol. The yield of extracts was varied in differnt medicinal plants. Among thirteen differnt medicinal plants, large amount of extracts (23.50%) were obtained from *Azadirachta indica* whereas, least amount of extracts (5.60%) were obtained from *Achyranthes aspera,* which showed that methanol is not suitable solvent for the extraction of *Achyranthes aspera*. Beside this, the variation of amount of extracts on differnt plants depend on various factors like parts and type of plants, with large amount of leaves gives large amount of extracts. Younger plant yield large amount of extracts than older plant

materials. Not only these factors, but also the incomplete extraction result less yield and if the solvent is not completely dried, it resulted higher yield.

5.2 Screening of Antibacterial Activities

Differnt kinds of diseases like cough, cold, tonsillitis, fever, urinary disesases, respiratory diseases, diarrhea, desentey jaundice, boils, cuts and wounds were caused by differnt pathogenic bacteria. The inhibition zone was produced by plant extracts which contained antibacterial substances and were able to kill or inhibit the growth of tested bacteria in the given conentration 1gm/1ml methanol.

In this study, *Phyllauthus emblica, Swertia chirayita, Withania somnifera,* and *Thalictrum foliolosum* were most effective medicinal plants. The four plants showed the activity against all the used strains of bacteria. Hussain *et al.* (2010) also found that the ethanolic extracts of *Phyllanthus emblica* was effective against all the tested bacteria where as Panthi and Chaudhary (2006) reported that the methanolic extracts of *Phyllanthus emblica* was effective against Gram positive bacteria viz *Staphylococcus aureus* but did not show the effect against Gram negative bacteria viz *Escherichia coli* and *Pseudomonas aeruginosa*. The result was simillar with Gram +ve bacteria and dissimilar with Gram -ve bacteria. This difference in result may be due to the difference in time of collection of plant materials and habitat of the plant materials. Similarly *Withania somnifera* was the most effective medicinal plant. The extracts of these plants showed the activity against all the tested bacteria. Rajendran (2009) also found the simillar result. According to his result, the methanolic extract of *Withania somnifera* was effective against *Staphylococcus aureus, Klebsiella pheumoniae, Pseudomonas aeruginosa* and *Escherichia coli. Swertia chiraiyita* also effective against all the tested bacteria. Devkota *et al.* (2000) also found that ethanol extracts of *Swertia chirayita* showed the similar result on these strains of bacteria viz *Pseudomonas auriginosa, Staphylococcus aureus, Escherichia coli* and *Salmonella typhi.*

Four medicinal plants viz *Acmella clava, Mentha spicata* and *Bauhinia variegata* were effective against 86% of tested bacteria. Chaudhary and Panthi (2006) reported that, the methanolic extracts of *Acmella clava* inflorescence were effective against 50% of tested bacteria. The result is some what dissimillar with this present study. This few dissimillar results may be due to the difference in plant parts used, employed bacteria and habitat of plant. In this study, the whole plant of *Acmella clava* was used for screening process.

Mahato and Chaudhary (2005) reported that, the leaf extracts of *Mentha spicata* did not show any activity with all bacteria tested. In this present study whole plant of *Mentha spicata* extracts inhibited growth of 86% of tested bacteria. This difference may be due to the difference in parts used in screening and time of collection of plant.

Three medicinal plants viz, *Achyranthes aspera, Oroxylum indicum* and *Azadirachta indica* were effective against 71% of tested bacterial strains Sanaa *et al.* (2005) reported that, the methanolic extracts of *Azadirachta indica* leaves showed somewhat simillar result. Hussain *et al.* (2010) also found the simillar result. According to Hussain *et al.* (2010), the ethnolic extracts of *Azadirachta indica* inhibit the growth of *Bacillus subtilis, Staphyllococcus aureus, Salmonella typhi* and *Escherichia coli*. Devkota *et al.* (2000) also support the present result.

Justicia adhatoda was effective against 29% of tested bactera. The methanol leaf extracts of *Justicia adhatoda* did not show the zone of inhibition against. *Psendomenas aeruginosa, Salmonella typhi, Proteus vulgaris, Escherichia coli* and *Klebsiella Pneumoniae* but showed the ZOI against *Bacillus subtilis* and *Staphylococcus aureus.* This result was some what simillar to that of Mahato and Chaudhary (2005) reported that, the methanol extracts of *Justicia adhatoda* leaves inhibit the growth of *Bacillus subtilis Staphylococcus aureus* and *Salmonella typhi* but did not show the activity against *Klebsiella pneumoniae, Proteus vulgaris, Pseudsmonas aeruginosa* and *Escherichia coli.*

5.3 Evaluation of Antibacterial Activity

The evaluation process of antibacterial activity was the main aspect of this study. Those compounds having broad spectrum may have little potency and such little potency compounmds have little value in the development of new drugs. The evaluation of antibacterial substances becomes the important steps during the new drugs research from medicinal plant. For this purpose disc diffusion method was employed. In disc diffusion process, the antibacterial substances present in plant extracts diffusing in the agar media kill or inhibit the bacteria and thus zone of inhibition appears around the disc in agar surface. The zone of inhibition appears around the disc in agar becomes broad, if the potency of the plant extracts is more. There is the gradual decrease in the concentration of antibacterial substances as the diameter of inhibition zone is increased from the disc. Thus a critical point arises after certain distance. After this critical point, there will be the growth of bacteria. The concentration of antibacterial substances at the 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.

Screening process can not suggest about the potency of antibacterial substance of medicinal plant. Screening process only provide an idea whether or not compound inhibit or kills the particular bacteria. If the potency of the plant extracts is very low, then they have little or no importance although plant extracts have antibacterial activity against large number of bacterial strains. Thus evaluation of potency of medicinal plant extracts is also most important step during new drug research process from medicinal plants.

Among the tested plants, highest zone of inhibition 19.33mm was shown by *Thalictrum foliolosum* on bacteria, *Staphylococcus aureus* This plant showed ZOI (17.33mm) and (16.33mm) against bacteria *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively. This plant extracts showed broad spectrum of activity and had broader zone of inhibition. This broader zone of inhibition may be

due to the antibacterial substances present in it may have higher diffusibility. Similar trend of result was shown by *Swertia chirayita*. It also showed highest zone of inhibition (19.33 mm) with *Bacillus subtilis.* Two plants *Phyllanthus emblica* and *Withania somnifera* showed borad spectrum of activity but had comparatively lesser value of zone of inhibition. It may be due to the low diffusibility of antibacterial substances present on these plants. The diffusing capacity of the chemical substances present in the medicinal plants extracts may be affected by the presence of fat, oils, resin or wax present in the plant extracts.

The extracts of *Acmella clava, Bauhinia variegata* and *Mentha spicata* inhibited the growth of six bacterial strains and showed high spectrum activity. The zone inhibition of these plants ranges widely between different bacteria. Among them *Acmella clava* showed highest value of ZOI (14.33 mm). It ranges from 10-14.33 mm. Similarly *Mentha spicata* ranges from 8.33-12.33 mm.

The extract of plants viz *Piper longum, Achyranthes aspera, Oroxylum indicum* and *Azadirachta indica* showed moderate spectrum activity. These plants also showed wide range of ZOI. Among these plants *Piper longum* showed highest ZOI. It ranges from 9-16.66 mm. But *Oroxylum indicum* ranges from 8.33-15.66 mm, *Achyranthes aspera* ranges from 8.33-13.66 mm and *Azadirachta indica* ranges from 7.66-10.33 mm. Two plants *Curculigo orchioides* and *Justicia adhatoda* showed narrow spectrum activity. Among these two plants, *Curculigo orchioides* inhibited growth of only three tested bactera among seven bactera tested but *Justicia adhatoda* inhibited only 2 bacteria. They also have low value of ZOI. *Curculigo orchioides* showed the ZOI ranges from 9.33-13.66 mm and *Justicia adhatoda* showed the ZOI from 9.33-9.66 mm. This plant *Justicia adhatoda* showed less spectrum activity and low ZOI so it has less potency in antibacteral activity.

In present study, Gram positive and Gram negative bacterial strains were tested with thirteen plant extracts. Among them Gram positive bacterial strains

were more susceptile to the extracts as compared to Gram negative bacteria. Gram positive bacteria showed comparatively high spectrum activity and high zone of inhibition than Gram negative bacteria with these plant extracts. This is supported by previous reports that, the antibaterial activity as Gram positive or Gram negative, it would be generally be expected that a much greater number would be active against Gram positive than Gram negative bacteria (Mccutcheon *et al.*, 1992). Plant extracts are more active against Gram positive than Gram negative bacteria (Rabe and Van Staden 1997, Parekh and Chanda, 2007). These difference may attributed to the fact that, the cell wall in Gram positive bacteria are of single layered whereas that of Gram negative bacteria are multilayered (Yao *et al.*, 1995). So the passage of the active compound through the Gram negative cell wall may be inhibited thus the microorganisms show variable sensitivity to chemical substances related to different resistant level between strains (Cetin and Gurler, 1989).

CHAPTER - VI

Conclusion and Recommendation

6.1 Conclusions

Present study is focused on screening and evaluation of antibacterial activities of tested medicinal plants used by local people against different bacterial diseases. This study concluded that the most of the medicinal plants used in traditional way by "hit and trial" method by local people to treat bacterial diseases, which have antibacterial properties. The antibacterial effect of these plants is due to active action of one or more compounds present in them. The methanolic extracts of different parts of plants used in the medicinal purpose gave different yield. The methanolic extract ranges from 5.60 to 23.50%.

In the present study, thirteen medicinal plants were selected for screening purpose against seven different pathogenic bacteria. Among them, two are Gram +ve (*Staphylococcus aureus* and *Bacillus subtilis*) and five are Gram -ve (*Pseudomonas aeruginosa, Proteus vulgaries, Salmonella typhi, Escherichia coli* and *Klebsiella penumoniae*). Among these bacterial strains, Gram-ve bacterial strains were more resistant as compaired to Gram (+ve) bacterial strains.

Altogether thirteen medicinal plants were tested in the present study. All plants showed activity against at least two bacteria. *Phyllanthus emblica, Swertia chirayita, Withania somnifera* and *Thalictrum foliolosum* inhibited the growth of all tested bacteria. Similarly, three plants were effective against six bacteria tested; three plants were effective against five tested bacteria. *Piper longum, Curculigo orchioides* and *Justicia adhatoda* inhibited the growth of four, three and two tested bacteria respectively. The zone of inhibition value range up to 19.33mm was shown by *Thalictrum foliolosum* and *Swertia chirayita* on bacteria *Staphylococcus aureus* and *Bacillus subtilis* respectively. High ZOI indicate the potency of the antibacterial substances of the medicinal plant is high.

2. Recommendation

The present research work is the study of screening and enaluation of antibacterial activity of some folklore medicinal plants. The limited work has been done in this research due to the shortage of time and other factors. The following recommendations are drawn from the present research work.

- During this research work, the methanol was only used as extracting solvent. Other extracting solvents of various polarities should be tried for experiment, which will extract the other active compounds present in plants.
- Those plants which showed encouraging activity towards *in vitro* test, they should be further carried out toward *in vivo* test.
- The plants *Thalictrum foliolosum, Swertia chirayita, Phyllanthus emblica* and *Withania somnifera* showed broad spectrum antibacterial activity. These plants can be further subjected for the isolation of the therapeutic antibacterial compounds and carry out further pharmacological evaluation.
- Potency of antibacterial substance may vary due to the change in ecological parameter, so it is also necessary to evaluate plants from different habitat.

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Photoplates I

Antibacterial Test

Photo #: 1 Methanol extracts of plants

Photo #: 2 ZOI shown by plants extract of 1) *Piper longum, 2) Achyranthes aspera, 3) Oroxylem indicum, 4) Acmella clava, 5) Thalictrum foliolosum and 6) Bauhinia variegata* on *Staphylococcus aureus.*

Photo #: 3 ZOI shown by plants extract of 1) *Piper longum, 2) Achyranthe aspera, 3) Oroxylem indicum, 4) Acmella clava, 5) Thalictrum foliolosum and 6) Bauhinia variegata* on *Salmonella typhi.*

Photo #: 4 ZOI shown by plants extract of 1) *Piper longum, 2) Achyranthe aspera, 3) Oroxylem indicum, 4) Acmella clava, and 5) Thalictrum foliolosum* on *Bacillus subtilis*.

Methanol extracts of plants

ZOI of Staphylococcus *aureus*

ZOI of Salmonella *typhi*

ZOI of *Bacillus subtilis*

Photographs II

Photoplate 1- medicinal plants

Photo #: 1 *Phyllanthus emblica*

Photo #: 2 *Piper longum*

Photo #: 3 *Acmella clava*

Photo #: 4 *Bauhinia variegata*

Photo # : 5 *Justicia adhatoda*

Photo # : 6 *Mentha spicata*

Phylanthus emblica

Piper longum

Acmella clava

Bauhinia variegata

Justicia adhatoda

Mentha spicata

Photoplate III Medicinal Plants

Photo # : 7 *Achyranthes aspera*

Photo # : 8 *Azadirachta indica*

Photo # : 9 *Oroxylum indicum*

Photo #: 10 *Curculigo orchioides*

Photo #: 11 *Withania somnifera.*

Photo #: 12 *Thalicturm foliolosum*

Achyranthes aspera

Azadirachta indica

Oroxylum indicum

Curculigo orchioides

Withania somnifera

Thalictrum foliolosum

APPENDIX- A

Description of medicinal plants used in the evaluation of antibacterial activities.

1. *Piper longum* L., sp. Pl.: 29 (1753).

Family: Piperaceae

Common name: Pipalaa (Nep.)

Description: A slender ascending or prostrate or trailing aromatic plant. Leaves simple, alternate, ovate-cordate with broad rounded lobes at the base, lower leaves 6-10x3-5 cm. Flowers in solitary spikes, male spikes narrow and female circular. Fruit ovoid, yellowish orange, fleshy spike.

Distribution: 200-800 m alt. east to west of Nepal.

Part used: Fruits

Uses: Dried fruits are used as medicine and spices. These are used to relieve from cough, common cold, respiratory tract, bronchitis, asthma etc.

2) *Achyranthes aspera* L., sp. Pl.: 204 (1753) var.

Family: Amaranthaceae

Common name: Datiwan (Nep.)

Description: A 30 cm to 90 cm tall herbs with quadrangular branches thickended just above the node. Leaves simple, short, opposite, velvety, tomentose, 10-12 cm long and 7.5 cm wide, rounded at the apex, elliptic, ovate or orbicular. Flowers small, greenish and white. Fruit easily disarticulating. Plant is pungent.

Distribution: Alt 600-1800 m east to west of Nepal.

Parts used: Whole plant.

Uses: Plant is purgative, diuretic and used in dropsy, piles, boils, skin eruptions, coleic and snake bite. Infusion of roots is astringent.

3. *Oroxylum indicum* (L) kurz, forest, Fl. Burma **2:** 237.

Family: Bignoniaceae

Common name: Tatelo (Nep.)

Description: A Small or medium sized deciduous tree. It is 7-12 m tall. Leaves are very large, 0.9-1.5 m long, pinnate or bi-pinnate or tri-pinnate, leaflet ovate or elliptic. Flowers numerous in large erect, 30-60 cm long, racemes, and lurid purple, reaching 10 cm long and fleshy. Fruits large capsule, flat and sword shaped up to 90 cm long and 9cm wide.

Distribution: Alt 400-1400 m east to west of Nepal.

Parts used: Stem bark, fruit and seed.

Uses: Bark is astringent to the bowel. It is cooling, tonic and increase appetite and is useful in diarrhea and dysentery. Infusion of bark is used in acute rheumatism. Stem is useful in scorpion stings.

4. *Acmella clava* (DC.) Jansen, **8:**41(1985).

Family: Compositae

Common name: Marethee

Description: Herb, 15-40 cm tall. Leaves simple, opposite, stalked, ovate. Flowers yellowish white to yellow in long peduncle heads.

Distribution: 300-2300 m east to west of Nepal.

Part used: Whole plant / Inflorescence.

Uses: Plant paste is applied as an antidote to snake bite. Root Juice is used in throat trouble. Floral heads are chewed to cure headache, stomach pain, tooth decay and toothache.

5. *Swertia chirayita* (Roxb., ex. Flem.) Karsten

Family: Gentianaceae.

Common name: Chiraito/Tite (Nep.)

Description: An erect biannual herb, 16-125 cm tall. Leaves simple, opposite, sub-sessile about 10x3 cm, lanceolate. Flower pale green tinged with purple in large panicles, each petal lobe having a pair of green glands. Fruits capsules 6 mm ovoid. The plant is bitter.

Distribution: Alt-1500 m-2500 m east to central Nepal.

Parts used: Whole plant.

Uses: The plant is excellent drug for fever, skin diseases intestinal worms and bronchial asthma. It is also used in diarrhea and lever problems.

6. *Bauhinia variegata* L., sp. pl: 375 (1753).

Family: Leguminosae

Common name: Koiraalo (Nep.)

Description: A medium sized deciduous tree. Leaves simple, alternate stalked, cleft at the apex into two rounded lobes resembling a camel's foot print, 10-15 cm long palmately veined. Flowers in short racemes at the end of branches or in the axial of leaf, fragrant and fourth petal white and fifth darker with purple vein. Flowers appear in leafless branches. Fruit flat and hard pods, 20-30 cm long, 25 cm broad, slightly curved seeds 10-15 in each pod.

Distribution: Alt 150 m-1900 m east to west of Nepal.

Parts used: Stem bark and root.

Uses: The bark is alternative, tonic and blood purifier. It is also useful in diarrhea, dysentery, piles and lever complaints.

7. *Phyllanthus emblica* L., sp. pl.: 982(1753)

Family: Euphorbiaceae

Common name: Amalaa (Nep.).

Description: A deciduous small tree up to 15 m tall. Leaves small, simple, sub-sessile, narrowly oblong, 1-1.5 x 0.2-0.3cm long, pointed, close together in two opposite rows in the branches giving the appearance of pinnately compound leaves. Flower greenish yellow, raceme on branches, male flowers many, female flower few. Fruit globose, obscurely 6-lobed. Fruits are sour, sweet and acrid.

Distribution: Alt-150 m -1400 m east to west of Nepal.

Parts used: Fruit, stem bark, root and flower.

Uses: Fruits are cooling, refrigerant, diuretic and laxative, useful in hemorrhage, diarrhea and dysentery and also in anemia, jaundice and dyspepsia. Fruits are good source of vitamin-C. Dried and powdered fruit is one of the ingredients of "Triphala".

8. *Justicia adhatoda* L., sp. pl. 15 (1753).

Family*:* Acanthaceae.

Common name: Asuro (Nep.)

Description: An evergreen, strong, smelling shrubs growing on waste land, shrubberies often planted as hedge plant, 1.25-2.75 m tall. Leaves simple, opposite, short stalked, elliptic-lanceolate, entire 13-25x5-7cm. Flowers in short compact terminal and auxiliary spikes and with conspicuous ovate overlapping bract, white with purple veins. Fruits 4 seeded club shaped capsule.

Distribution: Alt. 150-1600 m east to west of Nepal.

Parts used Leaves, flowers and root.

Uses: The dry vasaka comprises the fresh or dried leaves of the plant. It is mainly used in cough and bronchitis to facilitate the sputum come out. Plant is used as an expectorant. It is administered as juice, liquid extract, syrup or tincture leaves and roots are used in cough, chronic bronchitis, asthma and phthisis. Leaves are used in rheumatism and also as insecticidal. Flowers, leaves and roots are antispasmodic.

9. ***Mentha spicata*** L., sp. Pl: 576(1753)

Family: Labiatae

Common name: Pudinaa/Baabaree. (Nep.)

Description: An herb about 60 cm tall. Leaves simple, opposite, usually sessile or short stalked, ovate, 3.8 cm long, acute, dentate, wrinkled surface, flower small white.

Distribution: Alt 1800-2700 m east to west of Nepal

Parts used: Leaves and flowering tops.

Uses: Plant is aromatic, stimulant, stomachic, carminative, used for allaying nausea, diarrhea, vomiting and gastric coleic. Plant oil is antiseptic, stimulant and carminative.

10. ***Azadirachta indica*** A. juss., Mem. Mus. Hist. Nat. **19**:220, t.2, n.5 (1830).

Family: Meliaceae

Common name: Neem (Nep.)

Description: A 15-20 m tall evergreen tree. Leaves compound, imparipinnate, 13-17 in number, 7x5 cm, lanceolate, serrated and very oblique at the base. Flower small, white, sweet scented, loose clusters. Fruits one seeded drupe, greenish yellow when ripe. Plant is bitter.

Distribution: Alt 100-900 m east to west of Nepal.

Parts used: Bark of stem, leaves, flowers and seed.

Uses: Leaves are useful in skin diseases, intestinal worms, ulcers, malarial and intermittent fever, liver complaint and diabetes. Stem bark is also used as in the same way as leaves. Leaves are used as insecticide. Fruits are highly recommended for urinary disease, piles, intestinal worms and leprosy. Seeds are useful in tumors, leprosy, intestinal worms, pulmonary tuberculosis, wounds, ulcers and diabetes. Oil is used in skin diseases, ulcers, ringworms, scabies and malarial fevers.

11. *Curculigo orchioides.* Gaertn., fruct. Sem. Pl. 163, t. 13(1788).

Family: Hypoxidaceae

Common name: Kaalo Musalee (Nep.)

Description: A tuberous perennial herbs. Leaves simple, crowded on the short stem, sessile or short stalked with sheathing leaf base, linear or lanceolate, 15-45 cm long. Flowers bright yellow on the very short escape, lowermost flowers are bisexual and upper ones are male. Fruits are capsule.

Distribution: Alt. 500 m-1800 m east to central Nepal.

Parts used: Rhizome.

Uses: Rhizomes are demulcent, diuretic, tonic, aphrodisiac and are used in piles, jaundice, asthma, diarrhea and gonorrhea. The poultice of rhizome is also used in itch and skin diseases.

12. ***Withania somnifera*** L., Dunal.

Family: Solanaceae.

Common name: Ashwogandha

Description: An erect under shrubs up to 1.m tall. Leaves simple, short stalked, alternate, ovate and 5-10 cm long. Flower greenish or lurid yellow in umbelli form cymes. Fruits globose berries, orange when matured, enclosed in a persistent calyx.

Distribution: Cultivated in Nepal, 150- 1500m alt.

Parts used: Roots, leaves, fruits and seeds.

Uses: Roots are alternative, aphrodisiac, tonic, diuretic, narcotic and also used in debility from old age. Leaves are used for fever. Bruised leaves and ground roots are locally applied to painful swelling and ulcers. Seeds are used for coagulating milk.

13. *Thalictrum foliolosum* DC., Syst. Nat. **1:** 175(1817)

Family: Ranunculaceae.

Common name: Daampaate, Bansulee (Nep.).

Description: A perennial, glabrous herb, 1.2-2.6 m tall. Roots are yellow. Leaves pinnately compounds, alternate, stalked, 15-45 cm long, many times divided into leaflets, leaflets 3-lobed, oblong-ovate, rounded-toothed, 1-1.5cm long. Flower white to dull greenish purple, many in branched often dense clusters.

Distribution: 1000 m to 2400 m alt east to west of Nepal.

Parts used: Root

Uses: Roots are tonic, purgative, diuretic, febrifuge, good remedy for chronic dyspepsia, useful in convalescence after acute diseases and useful for jaundice.

Note: The nomenclature of plant is based on **"Annotated Checklist of Flowering Plants of Nepal"** and the description of plants is based on **"Medicinal Plants of Nepal"** (DPR 2007) and **"Plants and People of Nepal".**

APPENDIX - B

Short Description of Bacteria involved in the Present Study and Their Pathogenicity.

1. *Proteus vulgaris*

a. Morphology and Biochemical Characters:

It is Gram negative, motile aerobic bacillus with characteristics 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 surfaces well as colonies of other organisms. Swarming is inhibited by Mac-Conkey'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 one +ve in gelatinase and lipase tests. (Colee *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 cause of otitis media, meningitis, septicemia and osteomyelitis.

2) *Escherichia coli*

a. Morphology and Biochemical Character:

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 constituents. Wild type of *Escherichia coli* has no growth factor requirements and metabolically it can transform glucose in to 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 characteristics "mixed acid and gas" as the 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 fermentor and produce green metallic sheen on EMB agar. Their optimal growth temperature is $36\text{-}37^{0C}$

b. Pathogenicity

Pathogenic strains of *Escherichia coli* are responsible for three types of infections in humans: urinary tract infection (UTI), neonatal meningitis and intestinal diseases (gastroenteritis). The disease caused (or not caused) by particular strains of *Escherichia coli* depend on distribution and expression of an orray 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 *Escherichia coli* (ETEC) strains causes an acute watery diarrhea, enter invasive strains of *Escherichia coli* can cause shigella like blood and mucus in stool, verocytotoxin producing also termed enterohaemorrhagic *Escherichia coli* (VTEC/EHEC) cause hemorrhagic colitis and enteropathogenic *Escherichia coli* (EPEC) which is of minor importance (Colee *et al*., 1996).

3. *Bacillus subtilis.*

a. Morphology and Biochemical characters.

It is a Gram positive rod shaped bacteria that grow aerobically on nutrient agar and it forms resistant endospores. Spores are ellipsoidal, not bulging sporangium, centrally located and heat resistant. It is common saprophyte found as contaminants in food, clinical specimens and laboratory culture. It is facultative thermophile, capable of growth over the range $12\text{-}25^{oC}$. 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).

4. *Pseudomonas aeruginosa*

a. Morphology and Biochemical Character

It is Gram negative, motile, non-spore forming, non-fermentative, aerobic rod. It is widely distributed in the nature. It grows readily on minimal media. Cetrimide agar and *Pseudomonas* isolation agar are best selective media for *Pseudomonas aeruginosa.* Its optimum temp for growth is 37^{oC} and it is also to grow temp. at as high as $42^{oC.}$ *P. aeruginosa* may produce three colony types (Todar, 2008). *Pseudomonas aeruginosa* strains produce 2 types of soluble pigments, pyoverdin and blue pigments pyocyanin. The latter is produced abundantly in media of low-iron content and functions in iron metabolism in the bacterium.

b. Pathogenesity:

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

5. *Staphylococcus aureus*

a. Morphology and Biochemical Character:

It is a Gram positive, spherical bacterium that occurs in microscopic cluster resembling grape on nutrient agar at 37^{oC}. It forms colonies 1-3mm 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 temp. range of15 to 45^{oc} and at Nacl concentrations as high as 15 per cent. Nearly all strains of *Staphylococcus aureus* produce the enzyme coagulase (Todar, 2008).

b. <u>Pathogenicity</u>:

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

6. *Salmonella typhi*

a. <u>Morphology and Biochemical Character</u>

It is Gram negative, facultative, rod shaped non-capsulated and non-sporing bacterium. It can grow wide range of media at the temperature range 15-45^{oc}, 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 can not ferment glucose. It is indole, Voges- Proskaauer, urease negative. It produces H_2O in TSI with production of acid and no gas. It is methyl red positive (Colee *et al.*, 1996).

b. <u>Pathogenicity</u>

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 disease (Cheesbrough, 1993).

7. *Klebsiella pneumonia*

a. Morphology and Biochemical Character:

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

b. Pathogenicity:

Klebsiella pneumonia causes pneumonia in human. It can also cause urinary tract infection and abdominal infections. It is second pathogen after *Escherichia coli.* It normally affects persons with low immune system such as hospital patients, diabetes patents and people with chronic long diseases. Alcoholics also suffer from *Klebsiella pneumonia* infections (Colee *et al.,* 1996).

APPENDIX-C

Glassware

i)	Screw Capped Bottle	iv) Funnels	vii) Petriplates
ii)	Round Bottomed Flask	v) Test Tubes	viii) Glass Rods
iii)	Measuring Cylinder	vi) Pipettes	ix) Beakers

Apparatus and Equipments

i) Electric Grinder	x) Micropipette	xix) Hot Air Oven
ii) Incubator	xi) Dropper	xx) Vortex Shaker
iii) Electric Balance	xii) Filter Paper	xxi) Cotton Swabs
iv) Centrifuge	xiii) Aluminum Foils	xxii) Forceps
V) Inoculating Loops	xiv) Polythene Bags	xxiii) Cotton Rolls
vi) P^H meter	xv) Cotton Bags	xxiv) Wash Bottle
vii) Water Distillation plant	xvi) Sticker	xxv) Camera
Viii) Clean Cut Cutter	xvii) Autoclave	
xi) Not Book/ Marker/Pencils	xviii) Refrigerator.	

Media for Culture, Chemicals and Reagents

Nutrient Broth (NB)	Tetracycline Tablets	Methanol
Nutrient Agar (NA)	Copper Sulphate	Conc. H_2so_4
Fehlings 'A' Solution	Buffer Tablets	Iodine
Fehlings 'B' Solution	Ferric Chloride	Spirit
Mayer's Reagent	Wagner's Reagent	Conc. Hcl.
Mercuric Chloride	Potassium Iodide	Chloroform

APPENDIX-D

Composition of Some Media and Regents used in the Study

Nutrient Media

1. Nutrient Agar (N.A)

Composition	Grams/liter
Peptone	5.00
Sodium chloride	5.00
Beaf extract	1.50
Yeast extract	1.50
Agar	15.00
Final P^H (at 25^{oc})	7.4 ≀ 0.2

Procedure

28gm of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was sterilized by autoclaving at 15 lbs pressure at 121^{oC} for15 minutes.

1 Nutrient Broth (NB)

Composition	Gram/ liter
Peptone	5.00
Sodium chloride	5.00
Beaf extract	1.50
Yeast extract	1.50
Final P^H (at 25^{oC})	7.4 ≀ 0.2

Procedure:

13 gm of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was sterilized by autoclaving at 15 lbs pressure at 121^{oC} for 15 minutes.

APPENDIX -E

Graphs showing antibacterial activity of different medicinal plants on individual bacteria.

Figure No.1

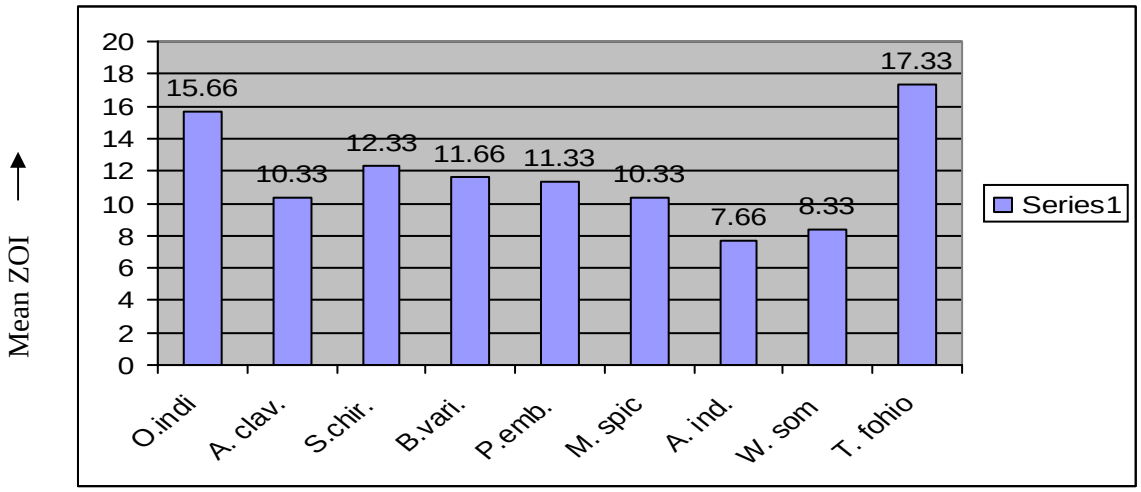

Fig-1: Zone of inhibition for bacterium *Pseudomonas aeruginosa* (Abbreviations: O. indi. = *Oraxylum indicum*, A. clav = *Acmella clava*, S. chir. = *Swertia chirayita*, B. vari. = *Bawhinia variegata*, P. emb. = *Phyllanthus emblica*, M. spic= *Mentha spicata*, A. ind. = *Azadirachta indica*, W. som.= *Withania somnifera*. T. folio = *Thalictrum foliolosu*m).

Figure No.2

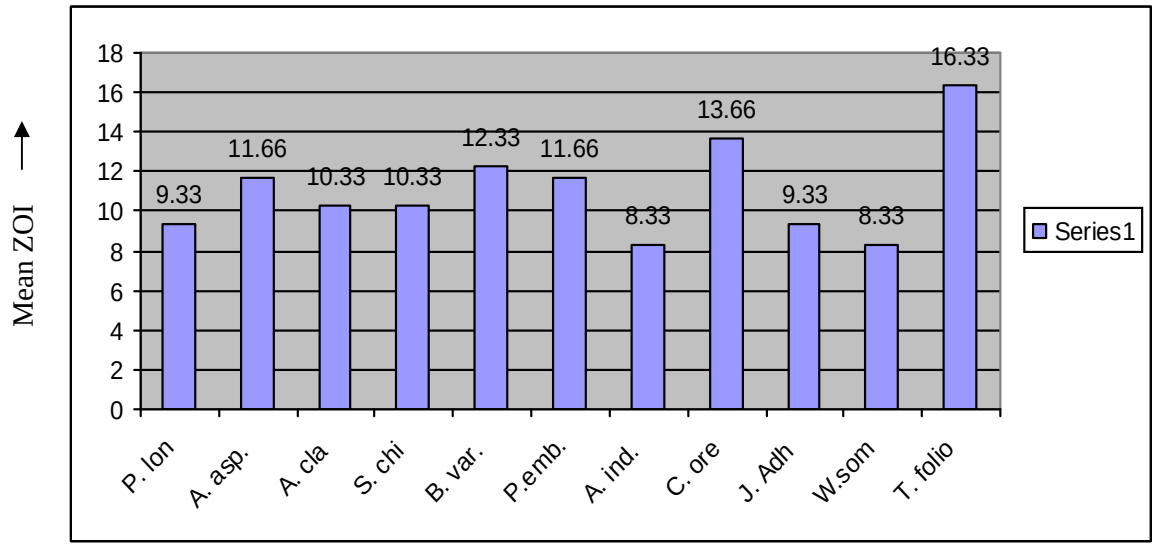

Fig: Zone of inhibition for bacterium *Staphylococcus aureus* (Abbreviations: P. lon. = *Piper longum*, A. asp= *Achyranthes aspera*, A. cla=*Acmella clava*, S. chi. = *Swertia chirayita*, B. vari. = *Bauhinia variegata*, P. emb= *Phyllanthus emblica*, A. ind. = *Azadirachta indica*, C. orch. = *Curculigo orchioides*, J. adh. = *Justicia adhatoda*, W. som. = *Withania somnifera*. T. fol. = *Thalictrum foliolosum).*

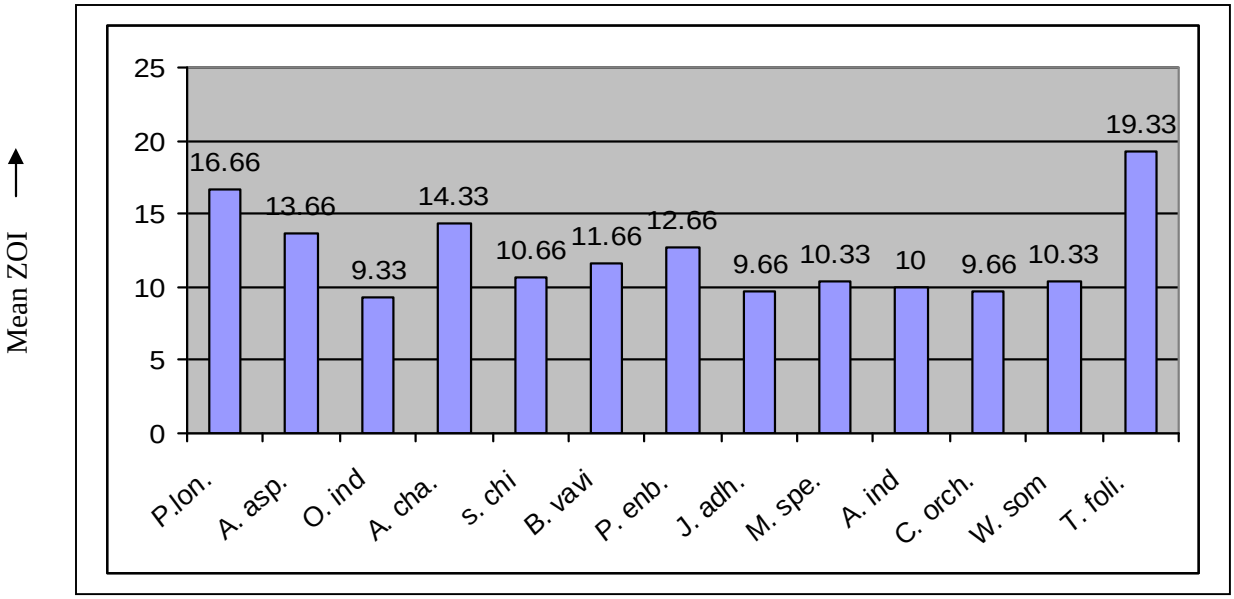

Fig. 3: Zone of inhibition for bacterium *Bacillus subtilis* (Abbreviation: P. lon. = *Piper longum*, A. asp. =*Achranthes aspera*, O. in. = *Oroxylum indicum*, A cla. = *Acmella clava*, S. chi = *Swertia chirayita*, B. vari = *Bauhinia variegata*, P. emb = *Phyllanthus emblic*a, J. adh. = *Justicia adhatoda*, M. spi = *Mentha spicata*, A. ind. = *Azadirachta indica*, C. orch. = *Curculigo orchioides*, W. som. = *Withania somnifera*. T. fol. = *Thalictrum foliolosum*).

Figure No.4

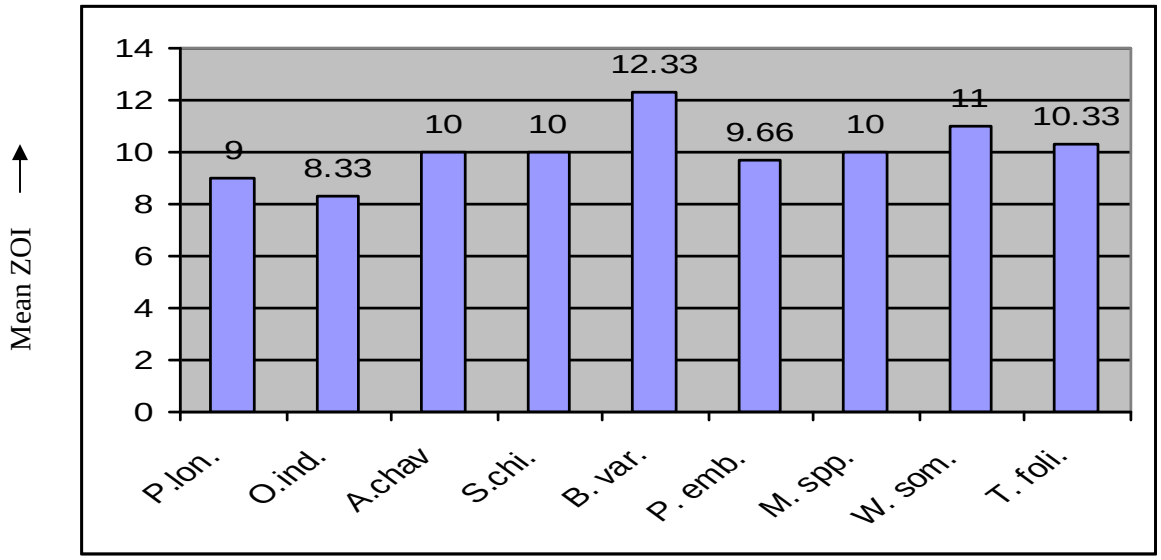

Fig. 4: Zone of inhibition for bacterium *Proteus vulgaris* (Abbreviations: P. lon = *Piper longum*, O. ind. = *Oroxylum indicum*, A. clav = *Acmella clava*, S. chi = *Swertia chirayita*, B. var. = *Bauhinia variegata*, P. emb = *Phyllanthus emblica*, M. spi. = *Mentha spicata*, W. som. = *withania somnifera*. T. foli. = *Thalictrum foliolosum).*

Figure No.5

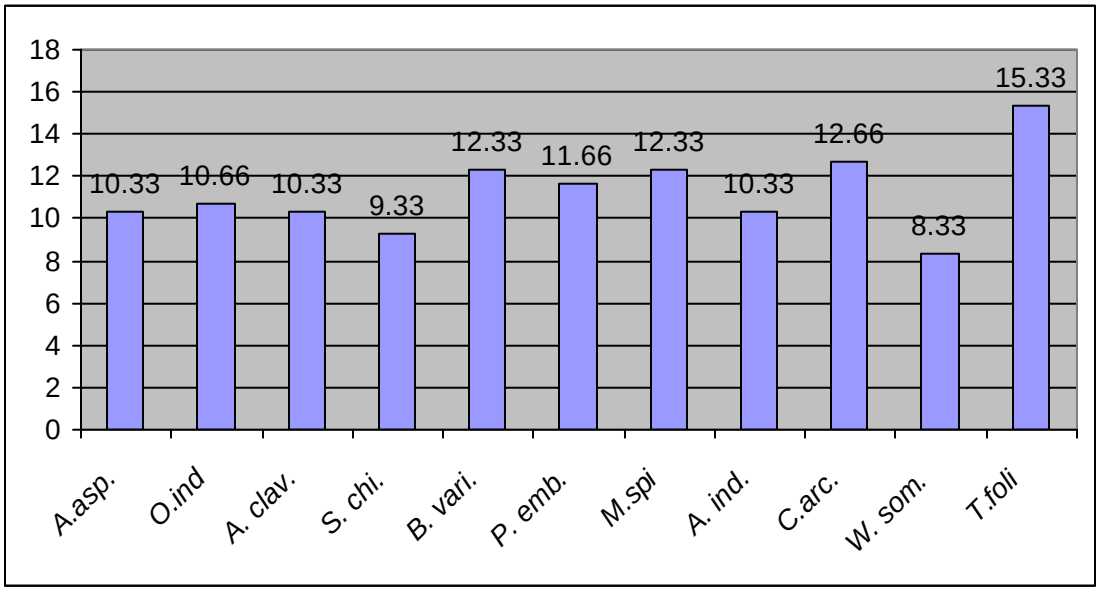

Fig. 6: Zone of inhibition for bacterium *Salmonella typhi* (Abbreviation A. asp. = *Achyranthes aspera*, A. cla. = *Acmella clava*, S.chi= *Swertia chirayita*, P. emb. = *Phyllanthus emblica*, M. spi= *Mentha spicata*, B. vari= *Banhinia variegata*, W.spm. = *Withania somnifera*. T. foli. = *Thalictrum foliolosum.).*

Figure No.6

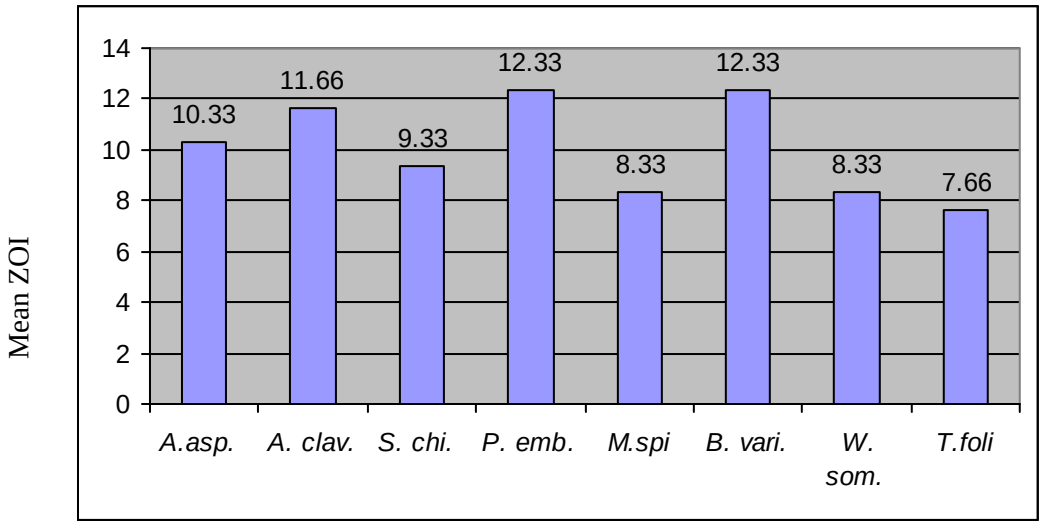

Fig. 6: Zone of inhibition for bacterium *Escherichia coli* (Abbreviation: A. asp. = *Achyranthes aspera*, A. cla. = *Acmella clava*, S.chi= *Swertia chirayita*, P. emb.= *Phyllanthus emblica*, M. Spi.= *Mentha spicata*, B. vari= *Banhinia variegata, W. Som. = Withania somnifera.* T. foli. = *Thalictrum foliolosum).*

Figure No.7

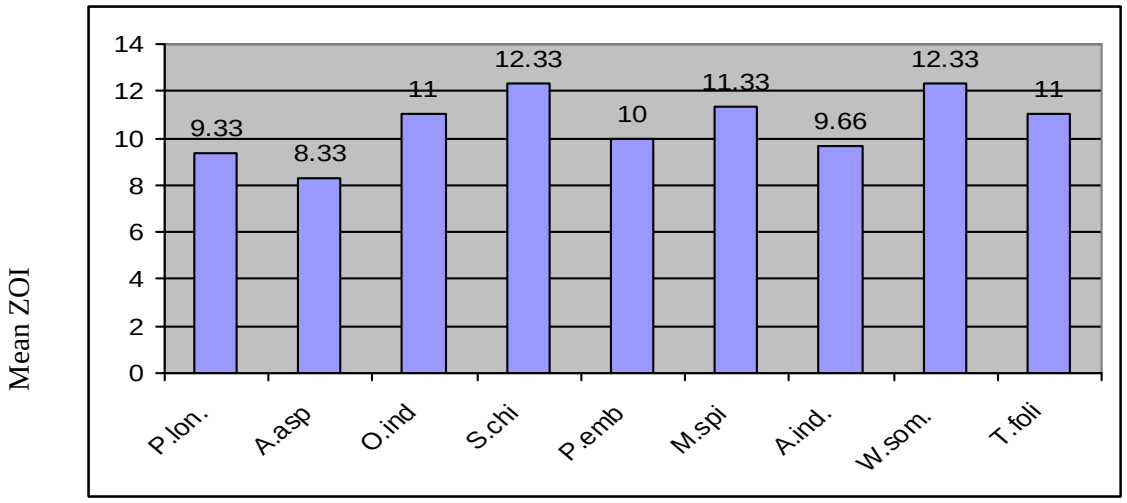

Fig. 7: Zone of inhibition for bacterium *Klebsiella pneumonia* (Abbreviation P. lon. = *Piper longu*m, A.asp. = *Achyranthes aspera*, O.ind = *Orxylum indicum*, S.chi = *Swertia chirayita*, P. emb. = *Phyllanthus emblica*, M. spi= *Mentha spicata*, A.ind. = *Azdirachata indica*, W. som. = *Withania somnifera*. T. foli= *Thalictrum foliolosum).*