4.1 INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modem drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines.

Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human healthcare since ancient times (Farombi, 2003). Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982) and natural products play an important role in drug development programmes in the pharmaceutical industry (Baker et al., 1995).

There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are
provided by rich natural sources. This burgeoning worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both for the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nair et al., 2005).

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by a number of researchers in different parts of the world (Erdogrul, 2002). It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumour and antimicrobial agents (Chung et al., 1995; Vlietinck et al., 1995). The selection of crude plant extracts for screening programmes has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural product (Kusumoto et al., 1995).

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. Ayurveda has a clinical specialty called rasayana, which prevents diseases and counteracts the ageing process by means of optimization of homeostasis. It has been reported that
the rasayanas are rejuvenators, nutritional supplements and possess strong antioxidant activity (Auddy et al., 2003).

Resistance to antimicrobial agents such as antibiotics is becoming more common in a wide variety of organisms (Babu et al., 2002; Diamond, 1993; Tomin and Tomasz, 1986). Antimicrobials of plant origin have enormous therapeutic potential and are effective in the treatment of infectious diseases simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999). Recently there has been a growing interest to evaluate plants possessing antibacterial activity for wound infections, gastrointestinal disorders, diarrhoea, food poisoning, constipation and typhoid fever (Perumalsamy and Ignacimuthu, 2000; Brantner and Grein, 1994; Clark and Hufford, 1993; Moskalenko, 1986; Bhakuni et al., 1974).

There is nothing more important in the world than the health of a person. Throughout the ages, plants have been used by humans as a source of food, cosmetics, medicines, clothing and even shelter. It has been estimated by the WHO that about 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care. The United States National Cancer Institute has screened well over 100000 plant extracts for anticancer activity and over 30000 for anti-AIDS activity. A number of workers have carried out investigations pertaining to antimicrobial action of plant extracts against an array of pathogens (Khan and Khan 2004; Nickavar et al., 2002; Elgayyar et al., 2000; Gogoi et al., 2000).

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential indeed and that they contained what is currently
characterized as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies. For example, the use of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tea tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents (Heinrich et al., 2004). That being said, it has generally been the essential oils of these plants rather than their extracts that have had the greatest use in the treatment of infectious pathologies in the respiratory system, urinary tract and gastrointestinal as well as on the skin. In the case of *Melaleuca alternifolia*, for example, the use of the essential oil (tea tree oil) is a common therapeutic tool to treat acne and other infectious troubles of the skin (Vanaclocha and Cañigueral, 2003).

Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities. It is believed that these compounds play an important ecological role. They can work as pollinator, attractants and as chemical defenses against insects, herbivores and microorganisms. These antimicrobial compounds produced by plants are active against plants and human pathogenic microorganisms. There are several reports in the literature regarding the antimicrobial activity of plant crude extracts and the bioassays-guided fractionation of them to yield active principles (Rojas et al., 2003).
Natural products contribute in a great extent to the fight against pathogenic microorganisms. Several plants or parts of them are used in food as spices and are thought to display some therapeutic activity or to provide a natural conservation by inhibiting the microbial growth. The anti-microbial activity is another widely studied feature of essential oils (Deans, 1991). Recently, many studies on plants revealed antibacterial and anti-inflammatory activity and therefore, have been made in order to understand their antimicrobial properties (Chen et al., 2001; Resch et al., 2001; Vairappan et al., 2001).

Flavonoid compounds exhibit inhibitory effects against multiple viruses (Critchfield et al., 1996; Watanbe et al., 1996). Since flavones, flavonoids and flavonols are known to be synthesized by plants in response to microbial infections (Dixon et al., 1983), it should not be surprising that they have been found in vitro, to be effective antimicrobial substances against a wide array of microorganisms. Many human physiological activities, such as stimulation of phagocytic cells, host mediated tumour activity and a wide range of anti-infective actions have been assigned to tannins (Haslam, 1996).

Worldwide spending on finding new anti-infective agents is expected to increase 60% from the spending levels in 1993 (Alper, 1998). The human is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. The dangerous bacterial strains from hospital sources demonstrated an increased resistance to the most common antibiotics. Over the last 20 years, a large number of plant species have been evaluated for their antimicrobial activity.
Human infectious diseases caused by bacteria and fungi have emerged during the last few decades (Terrel, 1999). The growth of many bacteria and fungi is especially difficult to control because of their ability to metabolize many substances (Quiroga et al., 2001). The incidence of opportunistic fungal infections in immuno compromised host, especially in AIDS patients has increased dramatically in recent years (Graybill, 1988).

Historically, plants have provided a source of inspiration for novel drug compounds as plant derived medicines have made large contributions to human health and wellbeing. There are numerous illustrations of plant derived drugs which have been used for many years as antimicrobial drugs as well as for the treatment of abscesses (Iwu et al., 1999).

*Streptococcus pneumoniae* is a key causative pathogen of Community Acquired Respiratory Tract Infections (CARTI) (Giglione and Meinnel, 2002). *Bacillus cereus* has been implicated in food borne intoxication (Granum and Lund, 1997). *Bacillus cereus* has been associated with a range of clinical conditions, food spoilage and incidents of food-borne gastroenteritis (Salkinoja et al., 1999). *Klebsiella pneumoniae* cause disease like mastitis, abortions and upper respiratory complications (Fraser, 1986). *Enterococcus faecalis* is becoming an increasingly important nosocomial pathogen (Yu et al., 2002). *Serratia marcescens* is another important nosocomial pathogen which is often resistant to multiple antimicrobial agents (Yum et al., 2002). *Pseudomonas aeruginosa* is a ubiquitous microorganism, inhabitant of fresh waters, soil and plants. Their strains are capable of producing
entrotoxins. These bacteria have been recognized as an enteric pathogen and causative agent of diarrhoea with infants and children (Henderson et al., 1969).

It is estimated that today, plant materials are present in, or have provided the models for 50% Western drugs. Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Robbers et al., 1996).

Worldwide, there has been a renewed interest in natural products. This interest is a result of factors such as: consumer's belief that natural products are superior; consumer's dissatisfaction with conventional medicines; changes in laws allowing structure-function claims which results in more liberal advertising; aging boomers and national concerns for health care cost. Sales of plant based drugs in market have increased dramatically in the last decade. It is vital to be in the position to capitalize on the phytomedicine market, providing environmentally responsible solutions to public health concerns presented by new trends in infectious diseases. Infections induced by pathogenic bacteria and fungi are recognized as emerging threat to global health and socio-economic problem. It is estimated that infectious disease is the underlying cause of death in 8% of the death occurring in the world. Death from infectious diseases, ranked fifth in 1981, has become the third leading cause of death in 1992, an increase of 58% (Iwu et al., 1999).
Due to indiscriminate use, the antibiotics are losing their effectiveness since many of the pathogens especially bacteria and fungi have developed substantial resistance to the antibiotics. Moreover the widespread use of antibiotics has led to the decimation of sensitive organisms from the population with the consequent increase in the number of resistant microorganisms. For instance, after the World War II, rapid development of antibiotics began to increase which ultimately led to the evolution of drug resistant microbes especially bacteria in particular on an alarming rate. At present there is a list of top ten drug resistant microbes which includes *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Shigella dysenteriae*, *Neisseria gonorrhoea*, *Mycobacterium tuberculosis*, *Haemophilus influenzae* and *Enterobacter aerogenes* (John, 2002).

The microbes are resistant to well known antibiotics such as penicillin G, tetracycline, erythromycin, chloramphenicol, vancomycin, polymyxin B, kanamycin, ampicillin and sulfonamides and hence known as multiple drug resistant strains. Many bacteria are naturally resistant to antibiotics due to the permeability barrier afforded by their outer membrane lipopolysaccharides. Also its tendency to colonize surface in a biofilm form makes the cells imperious to therapeutic concentration of antibiotics. In addition, some of the social, economic and political factors contribute to the development of resistance (Giglione and Meinnel, 2002).

Microbial resistance to antibiotics represents a serious problem for human beings since most of the rampant killer diseases are of microbial origin and account for high proportion of mortality in underdeveloped as well as developed countries
(Gundidza and Gaza, 1993). A few decades ago in India, typhoid fever could be cured with three inexpensive drugs namely cephalosporins, penicillin-G and chloramphenicol (John, 2002). But today these drugs are largely inactive against the life threatening typhoid fever. In Eastern Europe and parts of Russia, more than 10% of tuberculosis patients cannot be cured completely because of the drug resistant strains which are insensitive to the most powerful antibiotics viz., streptomycin and rifampicin. In US alone about 14000 people die every year because of drug resistant microbes which infect people (Nosacomial infections) in hospitals. One of the methods to reduce the antibiotic resistance and adverse effect on host is by using antibiotic resistance inhibitors and effective antimicrobials of plant origin.

The search for biologically active compounds has been vigorous in recent years due to the growing cases of microbial resistance to the time honoured antibiotics (Irobi and Banso, 1994; Dimayuga and Garcia, 1991). In addition, antibiotics produce many adverse effects on host which include immuno suppression and allergic reactions (Idose et al., 1968). One approach is to screen local medicinal plants for possible antimicrobial agents for use and they contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties (Cowan, 1999). In recent years, a number of studies have been reported, dealing with antimicrobial screening of extracts of medicinal plants (Perumalsamy and Ignacimuthu, 2000; Gundidza and Gaza, 1993).
4.2 REVIEW OF LITERATURE

Perez and Claudia (1994) have reported the hot water extracts of 132 plant samples from 54 families commonly used in Argentine folk medicine were screened for antibacterial activity against Salmonella typhi. The agar well diffusion method was used with Ampicillin as the standard. It was found that among the 132 plant samples, 24 plant species showed antibacterial activity against Salmonella typhi.

Ashour and Kheiralla (1995) tested the antimicrobial activity of eleven folklore medicinal plants of Egypt against both Gram-negative and Gram-positive organism as well as yeast like fungi. It was found that most extracts showed marked activity against most of the organisms used. Besides fruits of Balanites aegyptiaca showed marked activity against all microorganisms except Kluyveromyces lactis and Candida utilis in ethyl alcohol extract, however benzene, ether, chloroform and ethyl alcohol extract showed complete inhibition toward Salmonella typhi.

Aqueous, methanolic and ethyl acetate extracts of 14 plants used in traditional Zulu medicine for treatment of ailments of an infectious nature were screened for antibacterial activity. Most of the activity detected was against Gram-positive bacteria. Tuber bark extracts of Dioscorea sylvatica had activity against Gram-negative Escherichia coli and extracts of Dioscorea dregeana, Cheilanthes viridis and Vernonia colorata were active against Pseudomonas aeruginosa. The highest antibacterial activity was found in extracts of C. viridis, D. dregeana, D. sylvatica, Melianthus comosus and V. colorata. In general, methanolic extracts exhibited higher activity than aqueous and ethyl acetate extracts (Kelmanson et al., 2000).
Acetone, methanol and water extracts obtained from the shoots of *Arctotis arctotoides* through shaking and homogenization, were investigated for their antimicrobial activities. Growth inhibition using agar dilution assays was determined against ten selected bacterial and six fungal species. Although not fungicidal, extracts from the herb showed significant growth inhibition against all the fungi tested. The homogenized water extract was particularly inhibitory to the growth of the fungi with inhibitory activity ranging from 50.7% to 95.2% on *Aspergillus tamari* and *Penicillium digitatum* respectively. Acetone and methanol extracts were very active against the Gram-positive bacteria. The Gram-negative bacteria were, however, more resistant to the extracts than the Gram-positive ones. None of the extracts inhibited *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Afolayan, 2003).

Camporese *et al.* (2003) screened the twenty one extracts from seven herbal drugs. *Aristolochia trilobata* leaves and bark, *Bursera simaruba* bark, *Guazuma ulmifolia* bark, *Hamelia patens* leaves and *Syngonium podophyllum* leaves and bark from Belize (Central America) were evaluated for their anti-bacterial properties. Activity was tested against standard strains of *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterobacter faecalis*. The results showed that the hexane extracts of *A. trilobata* leaves and bark were the most active extracts against *Staphylococcus aureus*.

Aqueous, hexane and methanol extracts of 12 plant species traditionally used in Kenya for treatment of ailments of infectious and/or inflammatory nature were screened for *in vitro* antibacterial and anti-inflammatory activities. All the
antibacterial activity was against Gram-positive bacteria with nine plant species showing some activity against *Staphylococcus aureus*. The results of the testing showed that the methanol extracts caused higher inhibition than aqueous and hexane extracts (Esther and Staden 2003).

*Alangium salvifolium* is an ethnomedicinal plant used in folklore as a medicine. The leaves were extracted with water, ethanol and chloroform, and each extract was evaluated for antibacterial activity against pathogenic strains of *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, *Enterobacter faecalis*, *Serratia marcescens* and *Klebsiella pneumoniae* by using disc diffusion method. The zones of inhibition were recorded and compared with standard antibiotic drug Chloramphenicol. Ethanolic extract showed the high degree of inhibition when compared with chloroform and aqueous extracts (Natarajan *et al.*, 2003).

Jain *et al.* (2004) had demonstrated that *Mimosa hamata* (Mimosaceae) has demonstrated pronounced bio-efficacy against the selected bacteria (*Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*), fungi (*Aspergillus flavus*, *Fusarium moniliforme* and *Rhizoctonia bataticola*) and viruses (*Herpes simplex*, *Poliomyelitis* and *Vesicular stomatitis*).

Various fraction of *Lantana camara* were tested against different pathogens. Cold water extract was found to be endowed with maximum antimicrobial activity. MIC of cold water and methanol extract were found to be ranged from 40.0 μg/disc to 50.0 μg/disc against all the bacterial species except *E. coli* and 100 μg/disc to 50 μg/disc against fungal species by disc diffusion assay. The MIC by microbroth
dilution assay was found to be ranging from 12.5 mg/ml to 1.56 mg/ml (Dabur et al., 2004).

Ethanolic extract of *Ocimum canum* leaves was screened against *Xanthomonas malvacerum, X. compestris, Agrobacterium tumefaciens, A. radiobacter, Pseudomonas solanacerum* and *P. syringae*. The activity was observed from 2.0 to 5.0 % and the zone of inhibition increased with increasing concentration. High inhibition zone was observed with *P. syringae* (27 mm), *A. tumefaciens* (25 mm), *X. malvacearum* (24 mm) and *X. compestris* (20 mm), *A. radiobacter* and *P. solanacerum* showed zone of inhibition with a range of 17 and 15 mm respectively for 5.0% concentration. MIC was 0.25 mg/ml for *P. syringae, A. tumefaciens, X. malvacearum* and 0.5 mg/ml for *X. compestris, A. radiobacter* and *P. solanacearum* (Shimpi and Bendre, 2004).

Leaf extracts of *Tapinanthus sessilifolius* growing on five different host plants, viz., *Psidium guajava, Citrus limon, Vernonia amygdalina, Persea americana* and *Jatropha curcas* were evaluated. Powdered leaves of *T. sessilifolius* collected from each host plant were divided into two portions. One portion was used for aqueous infusion and the other portion was successively extracted with hexane, ethyl acetate and methanol. Infusion of aqueous extract of powdered leaves did not show antimicrobial effect even at the concentration of 1000 and 2000 μg/ml on test microorganisms. However in broth culture, methanolic and hexane extract had MIC range of 62.5-500 μg/ml and ethyl acetate extract had 250-500 μg/ml. Phytochemical screening of leaf samples collected from different
host plants showed positive test for hydrolysable tannins, saponins, flavonoids, terpenes, cardiac glycoside, reducing sugars and proteins (Tarfa et al., 2004).

The beneficial health effects of extracts from different types of plants that are used for many purposes have been claimed for centuries. The purpose of the study was to examine the effectiveness of three extracts of Ocimum sanctum for control of growth and survival of microorganisms. Inhibition of growth was tested by the paper disc agar diffusion method. Three bacterial strains viz., Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli were used as test microorganisms for the antibacterial activity of O. sanctum. Some of the extracts were found to be highly active against some bacterial strains. The methanolic extract exhibited a higher level of antibacterial activity (Khan, 2004).

Studies on extract of Capsicum annuum were carried out to assess its inhibitory effect on Pseudomonas aeruginosa and Staphylococcus aureus. This was done by preparing crude extract of the pepper with sterile distilled water. The microbes were treated with the extract by agar diffusion and tube dilution methods. The crude extract showed considerable inhibitory effect on the bacteria with 16 mm and 24 mm diameters in the zones of inhibition for P. aeruginosa and S. aureus at 24 hr of incubation respectively. At 48 hr of incubation, the diameter in the area of growth inhibition for S. aureus decreased to 11 mm and that of P. aeruginosa increased to 21 mm. Minimum inhibitory concentrations of the C. annuum were 335 mg/ml and 402 mg/ml for P. aeruginosa and S. aureus respectively (Boboye, 2004).

Extracts of three Sudanese medicinal plants Vernonia adoensis, Khaya senegalensis and Courboria virgata were tested for antibacterial activity against
five bacteria such as two Gram-positive *Bacillus subtilis*, *Staphylococcus aureus*, and three Gram-negative (*E. coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*). The extracts of *V. adoensis* (Asteraceae) were tested against 67 clinical isolates and that of *K. senegalensis* (Meliaceae) tested against 34 clinical isolates, isolated from ear, urine and wound swabs. Extract of *C. virgata* (Capparidaceae) was tested against bacteria. The antifungal activity of the extracts of the three plants was investigated against *Candida albicans*. Reference antibiotics were tested against standard organisms and the results were compared with the activity of the plant extracts. The minimum inhibitory concentration of the extract against the standard organisms was also determined (Elkatib *et al.*, 2004).

Effects of raw and boiled extracts of garlic (*Allium sativum*) on some human pathogenic bacteria viz., *Vibrio cholerae*, *Proteus mirabilis*, *Salmonella paratyphi* B and C, *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were examined by agar diffusion method. MIC of crude garlic extract was determined using *P. aeruginosa* and *S. aureus* by tube dilution method. It was found that raw garlic extract inhibited the growth of all the microbes to varying degrees at 24 hr of incubation. Action of the extract appeared bacteriostatic on *V. cholerae*, *P. mirabilis*, *S. paratyphi* B & C and *S. aureus*. At 48 hr of incubation, it was observed that *P. aeruginosa* and *S. dysenteriae* did not resume growth in the presence of the extract. None of the pathogens was prevented from growing in the presence of boiled garlic extract. The MIC of the crude garlic extract were 134 mg/ml and 161 mg/ml for *P. aeruginosa* and *S. aureus* respectively (Boboye and Dayo-Owoyemi, 2004).
Ethanol extracts of six dye plants viz., *Pterocarpus erinaceous*, *Zingiber officinale*, *Zanthoxylum zanthoxyloides*, *Morinda lucida*, *Bixa orellana* and *Sorghum caudatum* were screened for secondary metabolites and antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The ethanol extracts of *Z. zanthoxyloides*, *Z. officinale* and *M. lucida* were able to inhibit the growth of the three indicator bacteria with the zones of inhibition between 0.50-11.6 mm. *P. erinaceous* inhibited *S. aureus* and *E. coli*, *B. orellana* inhibited *P. aeruginosa* while, *Sorghum caudatum* inhibited *P. aeruginosa* only. All the dye extracts show the presence of at least three secondary metabolites, and these might be responsible for their antibacterial properties and their usefulness as medicinal plants. It may also enhance the stability of the plants colourant as added additives to polymer substrates (Adetuyi *et al.*, 2004).

The antimicrobial comparison of natural plant and callus extracts of *Abrus precatorius* was studied against four bacteria and two fungi by using cup plate method. Various solvents such as petroleum ether, chloroform, ethanol and distilled water. The result reveals that, the stem and leaf callus show significant activity against the tested microorganisms than the natural plant extract. The petroleum ether of stem callus and leaf callus showed maximum inhibitory activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* than that of natural plant extracts and standard. Similarly, the stem callus and leaf callus has also shown significant activity in *Aspergillus niger* and *A. flavus* in chloroform or petroleum ether extract (Kaviraj *et al.*, 2005).
Aqueous, ethanol and chloroform extracts of *Tinospora cordifolia* leaves and stems were investigated individually for *in vitro* antibacterial activity by disc diffusion method against *Escherichia coli*, *Proteus vulgaris*, *Enterobacter faecalis*, *Salmonella typhi*, *Staphylococcus aureus* and *Serratia marcescens*. The highest antibacterial activity was found in ethanolic leaf extracts. It showed the greater activity against *E. coli* and *P. vulgaris* and satisfactory results against other test bacteria. The preliminary results of this study indicate that the leaf extracts have potential antibacterial activity (Jeyachandran and Anand, 2005).

Extract of *Aegle marmelos*, used in traditional medicine for the treatment of various infections were screened for antibacterial activity. In the present investigation Soxhlet extracts of leaves, stem and root of this plant in different solvents at different concentrations were tried against five different strains of bacteria. The extracts tested possessed various degrees of antibacterial activity (Vimal Mohan et al., 2005).

Successive petroleum ether, benzene, chloroform, ethyl acetate, and methanol extracts of *Hemidesmus indicus* roots were tested for their phytochemical constituents and antimicrobial activity against fifteen human pathogenic microorganisms using standard disc diffusion method. The methanol and petroleum ether extracts were active against most of the tested organisms as they showed potential phytochemical constituents. The antimicrobial activities of the extracts were compared with their respective reference antibiotics as MICs. Discs treated with solvents alone served as negative control where as discs treated with standard antibiotics served as positive control in the experiment (Chakradhar et al., 2005).
Different organic and alcoholic extracts of *Calotropis gigantea, Justicia adhatoda, Moringa oleifera* and *Piper betle* were tested for their antimicrobial activity against certain bacterial pathogens *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Klebsiella pneumoniae* and fungal strains of *Aspergillus niger* and *Rhizopus* species. The plant extracts exhibited broader and moderate activity against all the microbial pathogens at 100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml concentrations (Uma and Sasikumar, 2005).

*Galinsoga ciliata* is a small annual herb, which has a wide traditional use. The leaves and inflorescence in dressing the fresh wounds or cuts as well as saddle sores and rawness of the skin to faster was a quick healing. Petroleum ether, benzene, ethyl acetate and acetone extracts of the leaves were found to be completely active against the bacteria tested. The leaf extracts displayed higher activity against the Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) rather than the Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). The toxicity against microorganisms may be due to the high amount of phenolic compounds present (Poonkothai *et al.*, 2005).

Screening of twenty-five plants was undertaken for detection of antibacterial activity. Petroleum ether, acetone and 90% methanol extracts of parts of these plants were tested against standard bacterial cultures of *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa*. Appreciable antibacterial activity against both Gram-positive and Gram-negative bacteria was detected in the acetone and methanol extracts of *Abrus*
precatorius, Boswellia serrata, Careya arborea, Emblica officinalis, Soymida febrifuga, Syzygium cumini, Woodfordia fruticosa and Sphaeranthus indicus. Extracts of some of the other plants were active only against Gram-positive bacteria (Deshpande et al., 2005).

Fractions from a partition of a total extract of Hypericum ascyron and Hypericum japonicum were investigated for their antibacterial activity against a strain of multidrug-resistant Staphylococcus aureus possessing the multidrug-efflux transporter, the major characterized MDR pump in this species. The hexane fraction of Hypericum ascyron and the butanol fraction of H. japonicum both showed appreciable antibacterial MIC values of 128 µg/ml for both fractions. Given the complexity of these extracts and the relevance of the efflux mechanism in clinical isolates of Staphylococcus aureus, these results highlight the potential of the genus Hypericum as a source of new antibacterial drugleads (Mu et al., 2006).

The antimicrobial activity of Phlomis bruguieri Desf., P. herba-venti L., and P. olivieri Benth. (Labiatae), native plants of Iran, were studied using the disk diffusion method and determination of minimum inhibitory concentration values against Staphylococcus aureus, Streptococcus sanguis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Aspergillus niger, and Candida albicans. The methanol extracts of the aerial parts of P. bruguieri, P. herba-venti, and P. olivieri exhibited concentration-dependent antibacterial activity against all tested bacteria. The methanol ext.acts were more active against Gram-positive
microorganisms (*Streptococcus sanguis* and *Staphylococcus aureus*). The extracts did not show antifungal activity (Morteza-Semnani et al., 2006).

The antifungal and antibacterial activity of 10 crude extracts from four different species, all of them used in Mexican folk medicine for the treatment of infectious diseases, were tested *in vitro* for antimicrobial activity against *Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Candida albicans, Trichophyton mentagrophytes* and *Trichophyton rubrum*. All extracts from the above plants showed some degree of antimicrobial activity against at least two microorganisms tested. The strongest antibacterial activity was found in the water extract of *Hibiscus sabdariffa* and the methanol extract of *Lysiloma acapulcensis*, whereas the methanol extract from *Loeselgia mexicana* showed the best antifungal activity against dermatophytes (Navarro Garcia et al., 2006).

Various aqueous and alcoholic extracts of plant parts of *Araiostegia* species were tested against the growth of some human and plant pathogenic bacteria like, *Agrobacterium tumefaciens, Escherichia coli, Salmonella arizonae, Salmonella typhi* and *Staphylococcus aureus*. In this investigation nearly all the extracts were found effective against the bacteria. The positive results so obtained were compared with that of the reference standard antibiotic (Tetracycline) and it was found that many extracts when mixed in equal proportion with the antibiotic and tested against
the bacteria, the mixture of extract + antibiotic was more effective than the antibiotic alone (Parihar and Bohra, 2006).

*Solanum aculeastrum* Dunal (Solanaceae) is used in traditional medicine to treat various human and animal diseases, specifically stomach disorders and various cancers, in the Eastern Cape, South Africa. The fruit and leaf extracts of this plant were investigated for in vitro antimicrobial activity against 10 selected bacterial and 5 fungal strains. The methanolic extracts of both the fruits and the leaves showed appreciable activity against Gram-positive and Gram-negative bacteria ranging from 4.0 to 10.0 mg/ml. whereas the methanol extracts were the most active material, the water extracts showed the least activity against the bacteria (Koduru et al., 2006).

4.3 MATERIAL AND METHODS

Antibacterial Screening of *Zehneria scabra*

Plant materials

The dried plant materials can be used as a good source for secondary plant components. Most scientists have opted to use dry material for several reasons: traditional healers frequently use dried plant material; the time delay between collecting plant material and processing it makes it difficult to work with fresh materials; and there are fewer problems associated with the large scale extraction of dried plant materials. Dried materials are more commonly used since old-dried materials may undergo some quantitative losses and qualitative changes. In the present study, shade dried plant material was used (Martini, 2001).
The plant parts (shoot and root) were carefully examined and old, insect affected, damaged and fungus-infected areas were removed. Healthy plant parts were spread out and dried in the laboratory at room temperature for 5-8 days. Once completely dry, plant parts were ground to a fine powder (Plate 4.1- a & b) using an electronic blender. Plants were stored in a closed container at room temperature until required.

**Tested bacteria**

Eight bacterial species were tested. These bacterial strains were collected from microbial type culture collection, Institute of Microbial Technology, Chandigarh, Punjab. The bacteria such as *Staphylococcus aureus*, Rosenbach (MTCC 1430), *Shigella dysenteriae* Shiga (MTCC 2957), *Bacillus subtilis* (Gram positive Bacteria); *Escherichia coli* Escherich (MTCC 1195), *Salmonella typhi* Eberth (MTCC 733), *Klebsiella pneumoniae* Friedlander (MTCC 2405), *Pseudomonas aeruginosa* Migula (MTCC 2642) and *Enterobacter faecalis* (Gram negative bacteria).

**Maintenance of Microorganisms**

The test bacteria were maintained in Nutrient Agar (Himedia Laboratories Pvt. Ltd., Mumbai). The microbial cultures were subcultured and the cultured strains were allowed to grow for two days and they were stored at 5° C for further studies.

**Solvents used**

Farnsworth (1984) stated that the biggest problem of drug development from plants is choosing the appropriate extracting solvent. Soxhlet extraction of dried
plant material is widely used employing solvents *viz.* water, petroleum ether, chloroform, ethyl acetate and ethanol. This method only works for compounds that can withstand the high temperature of the boiling solvent but cannot be used for thermophilic compounds. The choice of solvents also depends on what is planned with the extract. For antimicrobial screening, the effect of the solvent on subsequent bioassay is important. Solvents used for the extraction of bioactive principles varied from 80% ethanol (Vlietinck *et al.*, 1995), methanol (Taylor *et al.*, 1995), petroleum ether, chloroform, ethanol and water (Sato *et al.*, 2000). In previous reports, it was found that the ethanol seems to be a potential solvent for antibiotic inhibitors than those extracted with petroleum ether, chloroform and water (Eloff, 1998) and was therefore used in the present study to extract the plant secondary substances.

**Aqueous extracts**

Fifty grams of dried powdered plant materials were extracted with 300 ml of sterile distilled water (1:6 w/v). The aqueous extract was maintained in a Soxhlet apparatus over 48 hr, filtered and concentrated.

**Solvent extracts**

Fifty grams of the dried powdered plant materials (shoot and root) were soaked separately with 300 ml of each of the solvents *viz.* ethanol, methanol, ethyl acetate, chloroform and hexane in a Soxhlet apparatus for 48 hr (Plate 4.1-c) until complete extraction of the materials. At the end of 48 hr, each extract was filtered through Whatman No.1 filter paper and filtrates were concentrated at room temperature in order to reduce the volume. The paste like extracts was stored in pre-weighed screw caped bottles and the yield of extract has been weighed. These screw
Plate 4.1

Powdered plant parts of *Zehneria scabra* and Soxhlet apparatus

a. Root powder.

b. Shoot powder.

c. Soxhlet used for extraction of plant materials.
cap bottles were kept in refrigerator at 4° C. Each of the extract was individually reconstituted and the required extract was diluted for use.

**Media preparation**

The readymade Nutrient Agar (NA) medium was used for these studies. Nutrient agar was weighed and dissolved in distilled water made into 1000 ml and autoclaved at 121° C for 15 minutes.

**Nutrient agar (NA)**

The medium having the following composition:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>5 g</td>
</tr>
<tr>
<td>Peptone</td>
<td>5 g</td>
</tr>
<tr>
<td>Beef extract</td>
<td>3 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

**Nutrient broth**

The nutrient broth medium consists of the following composition:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5 g</td>
</tr>
<tr>
<td>Beef extract</td>
<td>3 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5 g</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>
After adding all the ingredients into the distilled water, it was boiled to
dissolve the medium completely and sterilized by autoclaving 15 psi pressure
(121° C) for 15 minutes.

Methods of antibacterial screening

The antibacterial screening of the aqueous and other organic solvent extracts
of shoot and root parts of *Zehneria scabra* were investigated through different
methods such as disc diffusion method, streak plate method and agar well diffusion
method.

**Disc diffusion method** (Maruzella and Henry, 1958)

**Principle**

Disc diffusion method used for the rapid determination of the drug or a
particular substance on a specific bacterium. This method consists of impregnating
small circular disc of standard filter paper with varied concentrations of substance.
The discs are placed on plates of culture medium that has been seeded with a test
bacterial inoculum. After incubation, the diameter of the clear zone of inhibition
surrounding the deposit of substance is taken as a measure of the inhibitory power
of the particular substance against the particular test organism.

**Disc preparation**

The filter paper disc of uniform size is impregnated with the compound (plant
extract) usually it consisted of absorbent paper. It is most convenient to use
Whatman No.1 filter paper for preparing the discs. Dried discs of 6 mm diameter
were prepared from Whatman No.1 filter paper and sterilized in an autoclave. These
dried discs were used for the test.
Procedure

Sterile liquid Nutrient Agar medium (pH 7.4 ± 2) was poured (10-15 ml) into each sterile Petri plates. After solidification, using 100 μl of suspension containing 108 CFU/ml of each test bacteria were spread over Nutrient agar plates. The sterile filter paper discs (6 mm in diameter) were impregnated with 10 μl of the 3 mg/ml extracts (30 μg/disc) placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extract. Streptomycin & Gentamycin (30 μl/disc) were used as positive reference control to determine the sensitivity of plant extract on each bacterial species. The inoculated plates were incubated at 37° C for 24 hr. Antibacterial activity was evaluated by measuring the zones of inhibition against the test organisms. Each assay was conducted in triplicate.

Streak plate method (Orzechowski, 1972)

Principle

The determination of inhibitory effect of plant extract can be easily done by this stroke (streak) method. The different concentrated crude plant extracts on the growth of test bacteria were tested. The result of this test was usually rated as strong inhibition where complete absence of bacterial growth occurs, partial inhibition where less growth of bacterium than the normal growth occurs and no inhibition where complete growth of the bacteria occurs. Once the plant extract showed a strong inhibitory effect, further analyses were carried out.
**Procedure**

For the sensitivity of crude extract different stock solutions (at different concentrations) viz. 0.5 mg/ml (25%), 1.5 mg/ml (50%), 2.5 mg/ml (75%) and 3 mg/ml (100%) were added to 15 ml of melted nutrient agar medium to obtain different concentrations of crude extract on solid agar plates. After solidification, the best bacterial cultures were taken (18-24 hr old) and using an inoculation needle, the bacterial culture was streaked (parallel strokes) on the surface of the agar plates. All the plates were incubated at 37° C and observation for inhibition of growth was made after 24 hr. Each experiment was triplicate. The results were rated as strong inhibition (if complete absence of bacterial growth) partial growth (if less bacterial growth than the normal growth) and no inhibition (if full growth of the test bacterium). Similarly control plates (agar medium without plant extract) were also maintained for reference.

**Agar Well Diffusion Method** (Perez et al., 1990)

Agar well diffusion method is also known as hole plate diffusion method (Brantner et al., 1993).

**Principle**

It is an important method for studying the inhibitory effect of any compound (plant extract or antibiotics) on the growth and multiplication of a particular bacterium. Here wells are made using a sterilized cork borer on the seeded nutrient agar in a Petri dish to which the test compound is added. The treated Petri dishes are incubated at 37° C for 24 hr. The inhibition zone formed around each well indicates the intensity of antimicrobial activity.
**Procedure**

Nutrient agar was used as the culture medium for this assay. The nutrient agar was dispensed in pre-sterilized Petri dishes (25 ml each) and allowed to cool. These agar plates were homogenously inoculated with the test bacterium previously suspended in nutrient broth ($10^6$ cells/ml). The plates were allowed to solidify. After solidification, wells of 6 mm diameter were punched into the agar with the help of flamed cork borer. Six wells were prepared for each plate. Of these six, four holes were filled with 0.2 ml of different concentrations of plant extract and the fifth hole was filled with 0.2 ml of standard antibiotic solution (Gentamycin, 500 μg/ml) and the sixth hole was filled with blank (extracting solvent alone). The Petri dishes were incubated at 37° C for 24 hr. After incubation period, the diameter of the inhibition zone formed around each hole/well was measured and the values were recorded. The antimicrobial activity was expressed as the ratio of the inhibition zone produced by the plant extract and the inhibition zone caused by the standard. Two sets of control were used. One control was the organism control where standard antibiotic solution was used and the other control was the blank where only the extracting solvent was used. This was just to ensure the validity of the test. Testing was carried out for each bacterium in triplicates.
4.4 RESULTS AND DISCUSSION

The antimicrobial activity of *Zehneria scabra* root and shoot solvent (ethanol, methanol, ethyl acetate, chloroform, hexane and aqueous) extracts were determined against *Bacillus subtilis*, *Enterobacter faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae* and *Staphylococcus aureus* using various methods such as disc diffusion method, streak plate method and agar well diffusion method.

**Disc diffusion method**

The antibacterial sensitivity of the plant extract was observed using the disc diffusion method by measuring the diameter of the growth inhibition zone. For disc diffusion method, high range of inhibition zone was exhibited against ethanolic and methanolic root and shoot extracts. The rate of inhibition zones moderate in ethyl acetate and chloroform extracts were followed by hexane and aqueous extracts.

**Root extracts**

The activity of ethanolic root extracts of *Zehneria scabra* roots exhibited significant activity against *Escherichia coli* and moderate inhibition of *Pseudomonas aeruginosa*. The extract showed less activity against *Salmonella typhi*, *Enterobacter faecalis* and poor activity in *Bacillus subtilis* and *Shigella dysenteriae*. Gentamycin antibiotic disc was maintained as a control (Plate 4.2, Table 4.1 and Fig. 4.1). Antibacterial activity was similar both in ethanol and methanol extracts.
Plate 4.2

Zones of inhibition with ethanolic root extracts of *Zehneria scabra* (Disc diffusion method)

a. Control (Gentamycin)  
b. *Escherichia coli*

c. *Pseudomonas aeruginosa*  
d. *Salmonella typhi*

e. *Enterobacter faecalis*  
f. *Bacillus subtilis*
*Escherichia coli* growth rate were controlled by *Zehneria scabra*, ethanolic and methanolic root extracts. Present finding is in concordance with the results of *Ocimum sanctum* (Khan, 2004) and *Ocimum basilicum* (Adiguzel et al., 2005).

*Zehneria scabra* ethyl acetate root extracts affected the activity of *Salmonella typhi* to a greater extent followed by *Escherichia coli* and *Shigella dysenteriae*. The inhibition zone exhibited by *Staphylococcus aureus* and *Bacillus subtilis* were less and poor (Plate 4.3, Table 4.1 and Fig. 4.1). Similar kind of result was reported by Ashour and Kheiralla (1995) in some Egyptian plants.

However, chloroform root extracts exhibited moderate antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* and less activity against *Salmonella typhi*. In contradiction, there was detrimental effect against *Enterobacter faecalis*, *Bacillus subtilis* and *Shigella dysenteriae* in the present investigation (Table 4.1 and Fig. 4.1).

The hexane root extracts affected the activity of *Escherichia coli* followed by *Salmonella typhi* and *Shigella dysenteriae*. There was no inhibition zone against other bacterial strains (Table 4.1 and Fig. 4.1).

The aqueous root extract was less inhibitory against *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. The extract did not show any activity against *Enterobacter faecalis*, *Bacillus subtilis* and *Shigella dysenteriae* (Plate 4.4, Table 4.1 and Fig.4.1).

**Shoot extracts**

In the case of both ethanolic and methanolic shoot extracts of *Zehneria scabra* they revealed similar kind of results, which affected the activity of
Plate 4.3

Zones of inhibition with ethyl acetate root extracts of *Zehneria scabra*
(Disc diffusion method)

a. Gentamycin (control)  b. *Salmonella typhi*

c. *Escherichia coli*  d. *Shigella dysenteriae*

e. *Staphylococcus aureus*  f. *Bacillus subtilis*
Plate 4.4

Zones of inhibition with aqueous root and shoot extracts of *Zehneria scabra*

(Disc diffusion method)

a. *Escherichia coli* (root)  
b. *Pseudomonas aeruginosa* (root)

c. *Salmonella typhi* (root)  
d. *Staphylococcus aureus* (shoot)

e. *Salmonella typhi* (shoot)  
f. *Escherichia coli* (shoot)
Table 4.1
Antibacterial activity of root extract of Zehneria scabra against various bacteria
[Disc diffusion method]

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Ethanol extract</th>
<th>Methanol extract</th>
<th>Ethyl acetate extract</th>
<th>Chloroform extract</th>
<th>Hexane extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>0.17 ± 0.04</td>
<td>0.11 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Enterobacter faecalis</td>
<td>0.20 ± 0.00</td>
<td>0.12 ± 0.04</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.70 ± 0.14*</td>
<td>0.52 ± 0.17*</td>
<td>0.25 ± 0.12</td>
<td>0.30 ± 0.04*</td>
<td>0.33 ± 0.02*</td>
<td>0.10 ± 0.00</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>0.10 ± 0.02</td>
<td>0.10 ± 0.00</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0.40 ± 0.04</td>
<td>0.20 ± 0.02</td>
<td>–</td>
<td>0.30 ± 0.04*</td>
<td>–</td>
<td>0.10 ± 0.09</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>0.27 ± 0.04</td>
<td>0.10 ± 0.01</td>
<td>0.43 ± 0.11*</td>
<td>0.10 ± 0.00</td>
<td>0.23 ± 0.12</td>
<td>–</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>0.17 ± 0.04</td>
<td>0.15 ± 0.04</td>
<td>0.23 ± 0.09</td>
<td>–</td>
<td>0.15 ± 0.04</td>
<td>–</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.14 ± 0.02</td>
<td>0.10 ± 0.02</td>
<td>0.20 ± 0.02</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Values represent diameter of inhibition zone in cm (Mean ± SD)

'–' Represents absence of measurable inhibitory action

* Represents highest range of inhibition zone
Fig. 4.1
Antibacterial activity of root extract of Zehneria scabra against various bacteria
[Disc diffusion method]
*Staphylococcus aureus* to a great extent followed by *Salmonella typhi*. Other bacterial strains induced negligible amount of inhibition zones. Streptomycin (antibiotic disc) was maintained as a control (Plate 4.5, Table 4.2 and Fig. 4.2).

*Zehneria scabra* ethyl acetate shoot extracts showed high degree of inhibition against *Staphylococcus aureus*, *Escherichia coli* and moderate inhibition was observed in *Salmonella typhi* and *Enterobacter faecalis* and there was no inhibition against other microorganisms (Plate 4.6, Table 4.2 and Fig. 4.2).

Both chloroform and hexane shoot extracts exhibited less activity against *Staphylococcus aureus* and no satisfactory results against other tested bacteria (Table 4.2 and Fig. 4.2). Aqueous extracts of the same part were found to possess less inhibition against *Staphylococcus aureus* and there was no inhibition against other pathogenic bacteria (Plate 4.4, Table 4.2 and Fig. 4.2). The concordant results were reported by Koduru *et al.* (2006) in *Solanum aculeastrum*, Yu *et al.* (2004) in *Scutellaria barbata* Caspar *et al.* (2003) in *Plectranthus fruticosus* and Kasali *et al.* (2002) in *Lantana camara*.

**Streak Plate Method**

Based on the disc diffusion result, highly sensitivity bacterial pathogens were tested by streak plate method against respective solvent extracts.

**Root extracts**

The ethanolic root extracts of *Zehneria scabra* result showed that the gradual suppression of growth rate of *Escherichia coli* was exhibited by increasing concentration. The control showed excessive growth. In 25% concentrations, there
Plate 4.5

Zones of inhibition with ethanolic shoot extracts of Zehneria scabra
(Disc diffusion method)

a. Streptomycin (control)       b. *Staphylococcus aureus*

c. *Salmonella typhi*           d. *Shigella dysenteriae*

e. *Klebsiella pneumoniae*      f. *Bacillus subtilis*
Plate 4.6

Zones of inhibition with ethyl acetate shoot extracts of *Zehneria scabra*  
(Disc diffusion method)

a. Streptomycin (control)  
b. *Staphylococcus aureus*

c. *Escherichia coli*  
d. *Salmonella typhi*

e. *Enterobacter faecalis*  
f. *Klebsiella pneumoniae*
Table-4.2

Antibacterial activity of shoot extract of *Zehneria scabra* against various bacteria
[Disc diffusion method]

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Ethanol extract</th>
<th>Methanol extract</th>
<th>Ethyl acetate extract</th>
<th>Chloroform extract</th>
<th>Hexane extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>0.10 ± 0.00</td>
<td>0.10 ± 0.00</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Enterobacter faecalis</em></td>
<td>–</td>
<td>–</td>
<td>0.10 ± 0.04</td>
<td>–</td>
<td>0.10 ± 0.00</td>
<td>–</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0.10 ± 0.00</td>
<td>0.10 ± 0.00</td>
<td>0.20 ± 0.04</td>
<td>0.10 ± 0.02</td>
<td>0.10 ± 0.00</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>0.14 ± 0.04</td>
<td>0.10 ± 0.04</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>0.12 ± 0.02</td>
<td>0.10 ± 0.01</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>0.27 ± 0.06</td>
<td>0.21 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>0.10 ± 0.04</td>
<td>0.10 ± 0.02</td>
<td>0.10 ± 0.00</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>0.17 ± 0.04</td>
<td>0.10 ± 0.09</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.50 ± 0.11*</td>
<td>0.45 ± 0.04*</td>
<td>0.30 ± 0.02*</td>
<td>0.21 ± 0.09*</td>
<td>0.15 ± 0.02*</td>
<td>0.10 ± 0.02</td>
</tr>
</tbody>
</table>

Values represent diameter of inhibition zone in cm (Mean ± SD)

'-' Represents absence of measurable inhibitory action

* Represents highest range of inhibition zone
Fig. 4.2
Antibacterial activity of shoot extract of *Zehneria scabra* against various bacteria
[Disc diffusion method]
was a gradual decline in growth of *E. coli* followed by 50%, 75% and without growth showing 100% concentration (Plate 4.7, Table 4.3 and Fig. 4.3). Similar trend were observed in methanolic root extract of *Zehneria scabra* (Plate 4.8, Table 4.3 and Fig. 4.3). *Alangium salvifolium* (Natarajan *et al.*, 2003) and *Actiniopteris radiata* (Parihar and Bohra, 2004) plant extracts were also controlling the growth rate of *E. coli* in prior studies.

The ethyl acetate root extract of *Zehneria scabra* control led the growth of *Salmonella typhi*. There was less and poor growth in 25%, 50% and 75% and there was no growth in 100% concentration of root extracts (Plate 4.9, Table 4.3 and Fig. 4.3). Similar observation was made in several medicinal plants (Perez and Claudia, 1994).

**Shoot extracts**

The *in vitro* antibacterial sensitivity of shoot extracts of *Zehneria scabra* against eight pathogenic bacteria by streak plate method is depicted in Table 4.4 (Fig. 4.4). The data showed that there is a strong inhibition with the increasing percentage of concentration of the shoot extracts of *Zehneria scabra*. All the Gram-positive and Gram-negative organisms tested were sensitive to the crude extracts at the level of 100% concentration. The antibacterial sensitivity was determined by the magnitude of presence or absence of growth. In control plates of each solvent without plant extracts, the growth rate of bacteria was noted to be excessive whereas in experimental plates, the degree of growth inhibition increased with the gradual increase in the concentration of shoot extracts.
Plate 4.7

Growth rate of *Escherichia coli* against different concentrations of *Zehneria scabra* root ethanol extract

(Streak plate method)

a. Control

b. 25%  

c. 50%

d. 75%  

e. 100%
Plate 4.8

Growth rate of *Escherichia coli* against different concentrations of *Zehneria scabra* root methanol extract

(Streak plate method)

a. Control

b. 25%  
c. 50%

d. 75%  
e. 100%
Plate 4.9
Growth rate of *Salmonella typhi* against different concentrations of *Zehneria scabra* root ethyl acetate extract
*(Streak plate method)*

a. Control

b. 25%       c. 50%
d. 75%       e. 100%
### Table-4.3

Growth rate of different bacterial strains against different concentrations of various root extracts of *Zehneria scabra*

[Streak plate method]

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Ethanol extract (concentrations %)</th>
<th>Methanol extract (concentration %)</th>
<th>Ethyl acetate extract (concentration %)</th>
<th>Chloroform extract (concentration %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C 25 50 75 100</td>
<td>C 25 50 75 100</td>
<td>C 25 50 75 100</td>
<td>C 25 50 75 100</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>3+ 3+ 3+ 2+ 2+</td>
<td>3+ 3+ 3+ 2+ 2+</td>
<td>3+ 3+ 2+ 2+ 2+</td>
<td>3+ 3+ 3+ 2+ 2+</td>
</tr>
<tr>
<td><em>Enterobacter faecalis</em></td>
<td>3+ 3+ 2+ 2+ 2+</td>
<td>3+ 3+ 2+ 2+ 1+</td>
<td>3+ 3+ 3+ 2+ 2+</td>
<td>3+ 3+ 3+ 2+ 1+</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>3+ 2+ 1+ 1+ –</td>
<td>3+ 2+ 1+ 1+ –</td>
<td>3+ 2+ 2+ 1+ 1+</td>
<td>3+ 3+ 3+ 2+ 1+</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>3+ 3+ 2+ 2+ 1+</td>
<td>3+ 3+ 2+ 2+ 1+</td>
<td>3+ 3+ 3+ 2+ 2+</td>
<td>3+ 3+ 3+ 2+ 2+</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>3+ 3+ 3+ 2+ 2+</td>
<td>3+ 3+ 3+ 2+ 2+</td>
<td>3+ 2+ 2+ 1+ 1+</td>
<td>3+ 3+ 3+ 2+ 2+</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>3+ 3+ 2+ 2+ 1+</td>
<td>3+ 3+ 2+ 2+ 1+</td>
<td>3+ 2+ 2+ 1+ –</td>
<td>3+ 3+ 3+ 2+ 1+</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>3+ 3+ 3+ 2+ 2+</td>
<td>3+ 3+ 3+ 2+ 2+</td>
<td>3+ 3+ 2+ 2+ 2+</td>
<td>3+ 3+ 3+ 2+ 1+</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>3+ 3+ 3+ 2+ 2+</td>
<td>3+ 3+ 3+ 2+ 2+</td>
<td>3+ 3+ 2+ 2+ 1+</td>
<td>3+ 3+ 3+ 2+ 2+</td>
</tr>
</tbody>
</table>

**Note:** Hexane and aqueous extracts showing excessive growth in all concentrations.

C : Control
3+ : Excessive growth (No inhibition)
2+ : Moderate growth (Partial inhibition)
1+ : Poor growth (Strong inhibition)
– : No growth (Complete inhibition)
Fig. 4.3
Growth rate of different bacterial strains against different concentrations of various root extracts of *Zehneria scabra* [Streak plate method]

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Chloroform</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Ethyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Growth rate of various organisms:
- Bacillus subtilis
- Enterobacter faecalis
- Escherichia coli
- Klebsiella pneumoniae
- Pseudomonas aeruginosa
- Salmonella typhi
- Shigella dysenteriae
- Staphylococcus aureus
Hence the higher concentrations viz. 75% and 100% strongly or completely inhibit the growth of test bacteria. It is evident from Table 4.4 (Fig. 4.4) that *Staphylococcus aureus* were susceptible to the shoot extracts (ethanol, methanol, ethyl acetate, chloroform and hexane) at higher concentration viz. 75% and 100% where complete inhibition (absence of bacterial growth) was observed. Ethanolic and ethyl acetate shoot extracts were more inhibitory on *Staphylococcus aureus* than the other solvents used (Plates 4.10, 4.11, Table 4.4 and Fig. 4.4).

The growth rate of *Staphylococcus aureus* was controlled by other plants as earlier reported by Mu et al. (2006) in *Hypericum* sps. Morteza-Semnani et al. (2006) *Phlomis* sps. Camporese et al. (2003) in *Aristolochia trilobata* and Esther & Staden (2003) in nine different plants.

The ethanol extract of *Zehneria scabra* effectively inhibited the growth of both the Gram-positive and Gram-negative bacteria. The activity of the aqueous extracts of the plants showed nil activity. There is a general opinion that aqueous extracts do not possess antibacterial activity against various pathogens as reported earlier by Chakrabarty and Brantner (1999) and Aburjai et al. (2001).

**Agar well diffusion method**

The different concentrations of root and shoot extract were poured on the well of the solidified nutrient media with various concentrations (25%, 50%, 75% and 100%) of plant extracts. Both control (distilled water alone) and negative control (particular solvent used for extraction) were also maintained. The inhibition zone around the well was measured after 24 hr.
Plate 4.10
Growth rate of *Staphylococcus aureus* against different concentrations of *Zehneria scabra* shoot ethanol extract
(Streak plate method)

a. Control

b. 25%  
c. 50%

d. 75%  
e. 100%
Plate 4.11

Growth rate of *Staphylococcus aureus* against different concentrations of *Zehneria scabra* shoot ethyl acetate extract

(Streak plate method)

a. Control

b. 25%  
c. 50%

d. 75%  
e. 100%
Table 4.4

Growth rate of different bacterial strains against different concentrations of various shoot extracts of Zehneria scabra
[Streak plate method]

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Ethanol extract (concentration %)</th>
<th>Methanol extract (concentration %)</th>
<th>Ethyl acetate extract (concentration %)</th>
<th>Chloroform extract (concentration %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C 25 50 75 100</td>
<td>C 25 50 75 100</td>
<td>C 25 50 75 100</td>
<td>C 25 50 75 100</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>3+ 3+ 3+ 2+ 2+</td>
<td>3+ 3+ 2+ 2+ 2+</td>
<td>3+ 3+ 2+ 2+ 2+</td>
<td>3+ 3+ 3+ 3+ 3+ 2+ 2+</td>
</tr>
<tr>
<td>Enterobacter faecalis</td>
<td>3+ 3+ 3+ 2+ 2+</td>
<td>3+ 3+ 2+ 2+ 2+</td>
<td>3+ 3+ 3+ 3+ 2+ 2+</td>
<td>3+ 3+ 3+ 3+ 2+ 2+</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>3+ 2+ 2+ 2+ 1+</td>
<td>3+ 2+ 2+ 2+ 1+</td>
<td>3+ 2+ 3+ 2+ 2+</td>
<td>3+ 3+ 3+ 3+ 2+ 1+</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>3+ 3+ 2+ 2+ 2+</td>
<td>3+ 3+ 2+ 2+ 2+</td>
<td>3+ 3+ 3+ 3+ 2+ 1+</td>
<td>3+ 3+ 3+ 3+ 2+ 2+</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>3+ 3+ 2+ 2+ 2+</td>
<td>3+ 3+ 2+ 2+ 2+</td>
<td>3+ 2+ 3+ 2+ 1+</td>
<td>3+ 3+ 3+ 3+ 2+ 2+</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>3+ 3+ 2+ 2+ 1+</td>
<td>3+ 3+ 2+ 2+ 1+</td>
<td>3+ 2+ 2+ 2+ 1+</td>
<td>3+ 3+ 3+ 3+ 2+ 1+</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>3+ 3+ 2+ 2+ 2+</td>
<td>3+ 3+ 3+ 2+ 2+</td>
<td>3+ 3+ 2+ 2+ 2+</td>
<td>3+ 3+ 3+ 3+ 2+ 1+</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>3+ 2+ 1+ 1+ 1+</td>
<td>3+ 2+ 2+ 1+ 1+</td>
<td>3+ 1+ 1+ 1+ 1+</td>
<td>3+ 3+ 2+ 1+ 1+</td>
</tr>
</tbody>
</table>

Note: Hexane and aqueous extracts showing excessive growth in all concentrations.
C : Control
3+ : Excessive growth (No inhibition)
2+ : Moderate growth (Partial inhibition)
1+ : Poor growth (Strong inhibition)
- : No growth (Complete inhibition)
Fig. 4.4
Growth rate of different bacterial strains against different concentrations of various shoot extracts of *Zehneria scabra* [Streak plate method]
A close examination of the result showed that the antibacterial activity of each extract against each of the bacterial strains varies. Among various solvents used for extraction, the ethanol, methanol and ethyl acetate produced comparatively higher activity than the other solvent.

**Root extracts**

Maximum inhibition zone around the well in ethanolic root extract of *Zehneria scabra* was observed in 100% (0.70 ± 0.12 cm) against *E. coli*, the inhibition zone got reduced in subsequent concentrations (75%, 50% and 25%) and 0.10 ± 0.02 cm inhibition zone was observed in negative control (Plate 4.12-a, Table 4.5 and Fig. 4.5). Comparable result was reported by Kelmanson *et al.* (2000) in *Dioscorea sylvatica*.

High rate of inhibition zone around the well in ethyl acetate root extract of *Zehneria scabra* was observed in 100% (0.63 ± 0.02 cm) against *Salmonella typhi* at the same time the gradual suppression of growth rate of *Salmonella typhi* were exhibited by decreasing concentrations (75%, 50% and 25%) of plant extracts and 0.10 ± 0.02 cm inhibition zone around the well was observed in negative control. There is no zone around the control (Plate 4.12-b, Table 4.5 and Fig. 4.5). Concordant results were observed in different plants (Perez and Claudia, 1994; Ashour and Kheiralla, 1995).

**Shoot extracts**

The growth rate of *Staphylococcus aureus* decreases with decreasing concentration of *Zehneria scabra* ethanol shoot extracts. The maximum (0.67 ± 0.02 cm) zone was observed at 100% concentration of plant extracts. Poor growth
Table-4.5
Inhibitory effect of various root extracts of Zehneria scabra against *Escherichia coli* and *Salmonella typhi* (Agar well diffusion method)

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Diameter of inhibition zones in cm (mean ± SD) on different concentrations of root extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Ethanol</td>
<td>–</td>
</tr>
<tr>
<td>Methanol</td>
<td>–</td>
</tr>
<tr>
<td>Ethyl acetate*</td>
<td>–</td>
</tr>
<tr>
<td>Chloroform</td>
<td>–</td>
</tr>
<tr>
<td>Hexane</td>
<td>–</td>
</tr>
<tr>
<td>Aqueous</td>
<td>–</td>
</tr>
</tbody>
</table>

* Against *Salmonella typhi*
Fig. 4.5
Inhibitory effect of various root extracts of *Zehneria scabra* against *Escherichia coli* and *Salmonella typhi* (Agar well diffusion method)
(0.01 ± 0.00 cm) was found in negative control, whereas no growth was observed in the control (Plate 4.12-c, Table 4.6 and Fig. 4.6).

More and effective inhibition zone around the well in ethyl acetate shoot extract of *Zehneria scabra* was observed in 100% (0.56 ± 0.04 cm) against *Staphylococcus aureus*, the inhibition zone was subsequently decreased at concentrations (75%, 50% & 25%) and 0.1 ± 0.02 cm inhibition zone around well was observed in negative control. However, control has nil growth (Plate 4.12-d, Table 4.6 and Fig. 4.6). Similar finding were observed against some other plants *viz.* *Senecio cannabifolius* (Wu *et al.*, 2006) and *Solanum aculeastrum* (Koduru *et al.*, 2006).

The ethanol extract of *Zehneria scabra* showed greater inhibition against both Gram-positive and Gram-negative bacteria. These results were in accordance with the previous findings of Essawi and Srour (2000) who have demonstrated that ethanolic extract of plant parts exhibit more activity than other extracts. In addition, the present results support the evidence observed by Perumalsamy and Ignacimuthu (2000) and Dhingra *et al.* (1999).

On the basis of results obtained, it is suggested that the antibacterial principles present in *Zehneria scabra* may be either polar or non-polar or the plant have both non-polar and polar compounds. These observations suggest that the organic solvent extraction method is better for the isolation of antibacterial compounds. Similar findings and conclusions were drawn by Singh and Singh (2000) in their experiments which represent a very good mechanism of biological control of microorganisms. In addition, the antibacterial nature of plant can be
Table 4.6

Inhibitory effect of shoot extracts of *Zehneria scabra* against *Staphylococcus aureus*
[Agar well diffusion method]

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Diameter of inhibition zones in cm (mean ± SD) on different concentrations of shoot extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Ethanol</td>
<td>–</td>
</tr>
<tr>
<td>Methanol</td>
<td>–</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>–</td>
</tr>
<tr>
<td>Chloroform</td>
<td>–</td>
</tr>
<tr>
<td>Hexane</td>
<td>–</td>
</tr>
<tr>
<td>Aqueous</td>
<td>–</td>
</tr>
</tbody>
</table>
Fig. 4.6
Inhibitory effect of various shoot extracts of *Zehneria scabra* against *Staphylococcus aureus*
(Agar well diffusion method)
attributed not only to a particular compound but also to the cumulative effect of various bioactive substances (Bai, 1990). Some examples include alkaloids, flavonoids, triterpenoids, thymol and other compounds of phenolic nature which are classified as antimicrobial compounds (Rojas et al., 1992). The present study showed the efficacy of antibacterial activity exclusively for bacterial pathogens which really shows the presence of biological principles. It leads to the isolation and characterization of the active principles present in the experimental plant.

The results showed the presence of antimicrobial activities agreeing with comparable results of previous researches using extracts of other plant species like Plumbago zeylanica, Boerhavia diffusa, Tinospora cordifolia, Berberis aristata, Terminalia chebula, Zingiber officinale, Azadirachta indica, Trichosanthes dioica and Picrorhiza kurrooa (Sohni et al., 1995; Prakash et al., 1995; Desta, 1993).