CHAPTER V

ANTIMICROBIAL SCREENING

5.1. INTRODUCTION

Medicinal plants are useful to treat curing various disorders in humans from time immemorial and an integral part of the Indian traditional medicinal system, such as the Ayurvedia and Siddha (Basu 2002). It is estimated that about 80,000 species of plants are utilized by the different systems of Indian medicine (Prajapathi et al., 2006). Modern day synthetic and chemical drugs often exhibit some negative effects or side effects but traditional herbals are safer and easy to access (Thomas et al., 2006). Traditional healers and medicine man in India use a few medicinal plants for curing various diseases and they are cheap as compared to pharmaceutical drugs (Singh, 2006). Such plant based drugs are obtained from roots, stem, flower, bark or fruit of different medicinal parts at different stages of their growth and development (Basu, 2002; Singh, 2006).

Plants containing protoberberines and related alkaloids, picralima-type indole alkaloids and garcinia biflavonones are active against a wide variety of microorganisms (Iwu et al., 1999). The overall quantities of plants used medically, in one way or another, are large. Plant based antimicrobials represent a vast untapped source for medicines. Continued and further exploration of plant antimicrobials needs to occur (Farnsworth et al., 1991). The potential for developing antimicrobials into medicines appears rewarding, from both the perspective of drug development and the perspective of phytomedicines (Iwu et al., 1999).

Ethnobotanists and natural plant product based pharmacists and researches have reported that a number of medicinal plants are capable of curing a number of human diseases such as diabetes, hypercholesterolaemia, fever, asthma, malaria, diarrhoea are to name a few of them (Basu, 2002; Dhanukar et al., 2000; Kumar et al., 2006; Thomas et al., 2006). The codified traditions have about 25,000 plant drugs formulations that have emerged from such studies. In addition to this over 50,000 formulations are believed to exist in the folk and tribal traditions (Kumar et al., 2006; Prajapati et al., 2006). Shiva et al. (2006) report that according to World Health
Organisation (WHO) more than billion people all over the world rely on herbals as sources of medicines to a large extent. WHO has listed ~21,000 plants with medicinal uses around the world and considers India as one of the countries rich with medicinal plant resources (Kumar et al., 2006; Shiva et al., 2002).

Researches in the last few decades have documented an alarming increase in the rate of resistance to synthetic antibacterial compounds globally (Baquero 1997; Chopra et al., 1996). Hence it is necessary to screen medicinal plants with antibacterial effects. There are reports on several plants that have antimicrobial effects (Dorman and Deans, 2000; Kumar et al., 2006; Palombo and Semple, 2001). Knowledge of the biological activities and/or chemical constituents of medicinal plants are desirable not only for the discovery of new therapeutic agents, but also for information in discovering new sources of other economic materials (Khaleqzzaman et al., 2002). Phytochemical investigations of medicinal plants including biochemical tests of their crude extracts and isolation pure chemical compounds could provide such knowledge (Lewington, 1990). Natural products have been a major source of new drugs (Vuorelaa et al., 2004). To determine the potential medicinal use of plants it is important to screen them for activity against a wide range of pathogenic bacteria (Fazilatun et al., 2004).

Plants Zingiberaceae are known for a number of medicinal properties (Basu 2002; Kumar et al., 2006; Prajapathi et al., 2006). Rhizome extracts of some members of the medicinal Zingibers are widely used in dietary intake as well as in traditional systems of medicine (Ibrahim et al., 2007). Arambewela (1999) reported that rhizomes and roots of Kaempferia galanga showed antibacterial activity against Escherichia coli and Staphylococcus aureus. Parvez et al. (2005) screened the antimicrobial activities of rhizome in K. galanga.

5.2. Review of literature

Three antimicrobial isoflavanones, named desmodianones A, B and C were isolated from the CH$_2$C$_{12}$-soluble fraction of the ethanol extract of the root of Desmodium canum, following bioactivity directed fractionation. Desmodianones A and B exhibited activity against Bacillus subtilis, Escherichia coli, Candida albicans and Neurospora crassa (Delle et al., 1996). An antimicrobial diterpene was
isolated from *Alpinia galanga* in the screening for potentiators of phytochemical antibiotic action. The structure was elucidated by spectral data and identified as (E) – 8 beta, 17 – epoxylabd – 12 – ene – 15, 16 – dial Diterpene 1 synergistically enhanced the antifungal activity of quarcetin and chalcone against *C. albicans* (Haraguchi *et al.*, 1996).

The petrol chloroform and ethanol extracts of *Daphne pontica* were tested against 8 bacteria and 6 yeasts. The chloroform extracts showed activity inhibiting *Staphylococcus aureus*, *Candida tropicalis*, *C. pseudotropicalis* and *C. guillermondii* (Sur Altiner *et al.*, 1997). Antimicrobial activity of various extracts of *Striga sulphurea* and *Hemidesmus indicus* were reported by Hiiremath *et al.* (1997). Extracts of whole plants of *S. sulphurea* and roots of *H. indicus* were screened for activity against *S. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Aspergillus niger*. The ethanol (95%) extract of *S. sulphurea* showed high activity against all the bacteria tested. The chloroform and ethanol (95%) extracts of *H. indicus* showed antifungal activity.

Ali Shtayeh *et al.* (1997) studied the activity of organic and aqueous extracts of *Micromeria nervosa* of Palestinian area on several micro organisium including bacteria and yeas and all organisms were susceptible to the plant. Tonia Rebe *et al.* (1997) investigated the antibacterial activity of crude extracts of 21 South African plants using agar diffusion and dilution method. Almost all the plants showed activity against *Bacillus subtilis*. One plant inhibited the growth of *Escherichia coli*. The essential oils of *Kaempferia galanga* root and rhizome showed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. No activity was displayed against *Pseudomonas aeruginosa* (Arambewala *et al.*, 1999).

The extracts of stem, bark, leaves and flowers of *Goniothalamus grandiflorus* were screened against six gram-positive bacteria (*E. coli*, *Micrococcus luteus*, *M. roseus*, *Proteus mirabilis*, *Salmonella typhi* and *S. typhimurium*) and six gram-negative bacteria (*Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *S. albus*, *S. epidermidis* and *Streptococcus faecalis*), a parasite (*Trichomonas vaginalis*) and four fungi (*Trichophyton mentagrophytes*, *T. verrucosum*, *Candida albicans* and *C. tropicalis*). All extracts were found to be active against all the bacteria and some
fungi. The flower and the leaf extracts exhibited a superior level of antibacterial activity (Khan et al., 1999).

Atalay et al. (1999) used agar well-diffusion method and have reported that the antimicrobial screening of 16 crude extracts from 8 plants traditionally used in Turkey for treatment of many diseases. The plant species were found to possess an activity against at least one or more test micro-organisms. The water insoluble parts of the methanolic extracts were found to be more effective than the water soluble ones against test microorganisms. The n-hexanic extract from Thymus fallax, methanolic extracts from Nigella sativa, Hypericum scabrum and Allium scorodoprasum showed marked activity against gram-positive bacteria whereas seven were active against Bacillus cereus and eight against Staphylococcus aureus.

Antimicrobial activity in 51 extracts from 29 plant species used in traditional medicine in Trinidad and the Caribbean island was tested against 6 bacteria Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, S. epidermidis and Enterococcus faecalis. This study showed that the most susceptible bacterium was S. aureus (Chariandy et al., 1999).

The in vitro antimicrobial activity of Calendula officinalis flower extracts has been investigated against Staphylococcus aureus, Bacillus subtilis, B. cereus, B. stearothermophilus, E. coli, Vibrio cholerae, Salmonella typhi, Pseudomonas aeruginosa and Klebsiella pneumoniae. The petroleum ether (60 – 80 degrees) and chloroform extracts of C. officinalis showed high activity against nearly all test microorganisms. The inhibitory effects of extracts are very close and identical in magnitude and are comparable with the standard antibiotics used (Sakharkar et al. 2000). Habtemariam and Macpherson (2000) reported cytotoxicity and antimicrobial activity of ethanolic extract from leaves of a herbal drug Eupatorium perfoliatum, against gram-positive organism such as Staphylococcus aureus and Bacillus megaterium.

Perumalsamy and Ignacimuthu (2000) studied the antibacterial properties of 30 Indian folklore medicinal plant used by the tribal healers. The bacteria used in the experimental study were Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Proteus vulgaris, Pseudomonas aeruginosa and
Staphylococcus aureus. Among the 20 plant species the extracts of Cassia occidentalis and C. auriculata exhibited significant broad spectrum of activity against B. subtilis and S. aureus.

Madhumathi et al. (2000) studied antimicrobial properties of crude fruit extracts of J. gossypifolia. Various solvent extracts of dried leaves, stem and fresh fruits of Jatropha gossypifolia using hexane, chloroform and ethyl acetate were tested for their antimicrobial activity against Candida albicans, Aspergillus terreus, Fusarium oxysporum and Pestalotiopsis palmarum, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella flexneri, Proteus mirabilis and Salmonella typhi. Hexane extracts of the fresh fruits showed biological activity against all the test fungi and against two species of bacteria while the hexane extracts of leaf and stem showed a little activity. Similarly chloroform extract of the fruit, leaf and stem also showed little activity only.

The antimicrobial properties of extracts from leaves, roots and stem barks of Horsfieldia helwigii and Melia azederachta were evaluated against bacteria (Bacillus, Micrococcus, Staphylococcus, Streptococcus, Proteus, Salmonella, Lactobacillus casci, Agrobacterium tumifaciens, Citrobacter freundii, Enterobacter aerogens, E. coli, Klebsiella pneumoniae, Neisseria gonorrhoeae, Pseudomonas aeruginosa and Serratia marcescens), protozoan (Trichomonas vaginalis) and fungi (Aspergillus niger, Candida albicans, C. tropicalis and Trichophyton mentagrophytes) by disc diffusion method. All extracts exhibited a range activities against the tested organism except fungi (Khan et al., 2001).

The compound curcumin (1, 7 bis, 4 – hydroxyl – 3 – methoxy – phenyl) heptan 1, 6 – diene, 3, 5 – dione) isolated from rhizomes of Curcuma aromatic (Zingiberaceae) was subjected to antimicrobial studies against bacteria (Staphylococcus aureus) and fungi (Saccharomyces cerevisiae). The compound was found to be significantly active against both bacteria and fungi which activity increasing with increase in concentration. The minimum effective concentration for antifungal activity is lower than that of the antibacterial activity (Deepa Singh et al., 2000).
Man Jingleun et al. (2001) reported the antimicrobial effects of extracts from Chinese chive, Cinnamon and Corni fructus. Extracts were prepared from Chinese chive (Allium tuberosum), Cinnamon (Cinnamomum cassia) and Corni fructus (Cornus officinalis) were used to evaluate the antimicrobial activity on common food borne microorganism (Bacillus subtilis, Escherichia coli, Flavobacterium sp., Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Vibrio paraheamolyticus, Debaryomyces Hansenii, Kloeckera apiculata, Pichia membranaefaciens, Aspergillus flavus, A. niger, Aureobasidium pullulans and Penicillium italicum) alone and in combination. The mixed extract consisting of 3 extracts in equal volumes, showed an entire antimicrobial spectrum and had excellent stability to heat, pH and storage. The mixed extract exhibited better inhibition on growth of E. coli than potassium sorbate at 2 – 5 mg/ml. The mixed extract inhibited the growth of P. membranaefaciens at levels as low as 2 mg/ml.

John Britto (2001) analysed the comparative antibacterial activity of leaf and stem extracts of Solanum incanum against some mutant strains of Escherichia coli. It was found that both the stem and leaf extracts of this plant were active against all strains of E. coli. However the extent on inhibition varied among the strains. In KL 16, the leaf extracts showed relatively more activity than stem extracts.

John Britto and Senthil Kumar (2001) reported the antimicrobial activity of leaf extracts of Solanum incanum. The aqueous and methanolic leaf extracts of this plant was tested against 6 strains of Escherichia coli viz. C2H7, KL 96, KL 16, HFrc, DH₅ α and Y₁₀ 90. The highest antibacterial activity was observed in KL 16, HFrc and Y₁₀ 90 and moderate activity was observed against C₂H₇, KL 96 and DH₅ α. The result indicated that the leaf extracts of this plant had bacteriostatic effects at higher concentrations.

Ramesh et al. (2001) tested aqueous and methanolic extracts and their fractions of Bridelia crenulata Roxb. against 10 human pathogenic bacteria and 4 fungal strains. Inhibitory activity was maximum in the chloroform – methanol (1:1) of the methanolic extract against E. coli, K lebsiella pneumoniae and Pseudomonas aeruginosa which are responsible for the pathogenesis of urinary tract infection.
The antimicrobial activity of the ethyl acetate, ethanol, petroleum ether and aqueous extracts of *Securinega leucopyrus* leaves was tested on 8 micro-organisms (*Bacillus subtilis*, strain MTCC 121, *B. cereus* strain MTCC 1429, *Staphylococcus aureus* strain MTTC 737, *E. coli* strain MTTC 1687, *P. aeruginosa* MTTC 1688, *Proteus vulgaris* strain MTTC 1771, *K. pneumoniae* strain MTTC 109 and *Candida albicans* strain MTTC 183). Ethyl acetate and ethanol extracts showed antimicrobial activities against *B. cereus*, *K. pneumoniae* and *Proteus vulgaris* were resistant to the ethyl acetate and ethanol extracts. Petroleum ether and aqueous extracts did not show antimicrobial activities (Bakshu *et al.*, 2001).

The antimicrobial activity and cytotoxicity of ether, chloroform and methanol extracts from the leaves and bark of *Zanthoxylum budrunga* were evaluated on bacteria (*Klebsiella*, *Bacillus*, *Shigella*, *Staphylococcus aureus* and *Streptococcus beta-hemolyticus* and *E. coli*) and fungi (*Aspergillus fumigatus*, *A. niger*, *Candida albicans*, *Hansenula californica*, *Rhizopus oryzae*, *R. oligosporus*, *Saccharomyces cerevisiae* and *Schizosporum* sp.) by disc diffusion method (Islam *et al.*, 2001). The chloroform extract of the bark showed the most potent antibacterial and antifungal activities.

The antimicrobial properties of the hexane extract from the leaves and twigs of the *Eupatorium glutinosum* was tested against two gram-negative and two gram-positive bacteria (El Seedi *et al.*, 2002). Suri *et al.* (2002) conducted antibacterial assay of *Citrus hystrix* extracts. The volatile oil obtained from leaf and fruit inhibited bacterial growth. All gram-negative bacteria were weakly inhibited by the volatile oil obtained from callus treated with 0.25 mm proline. Leaves were more potent against gram-positive bacteria.

Kuruppusamy *et al.* (2002) reported antimicrobial activity of *Balanites aegyptiaca* (L.) Del. The ethanol extract of *B. aegyptiaca* leaves, stem bark, root bark and fruits were tested for antimicrobial activities against *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Candida albicans*. The extract yield was higher in fruits (40.2 %) and was followed by leaves (27.0 %), stem bark (4.3 %), root bark (3.5 %). The extract of various parts of *B. aegyptiaca* showed
different degree of inhibition (in mm) against tested microorganisms but the largest inhibition zones were recorded upon treatment with the leaf extract.

Chloroform, ethanol and aqueous extracts of Wrightia tinctoria leaves were investigated for antimicrobial activity against Staphylococcus aureus, S. epidermidis, Bacillus subtilis, Klebsiella pneumonia, Salmonella paratyphi, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Aspergillus niger, Penicillium sp., Rhizopus sp., Cryptococcus neoformis and Candida albicans. Among the various extracts maximum antibacterial activity was exhibited by chloroform extract (40, 23 and 22 mm) followed by ethanol extract (40, 20 and 20 mm) and aqueous extract against Staphylococcus aureus, S. epidermidis and Pseudomonas aeruginosa respectively. There is no antifungal activity exhibited by all the three extracts against various fungi used in the study (Anbuganapthi et al., 2002).

Alangium salviifolium is a valuable ethnomedicinal plant used in indigenous systems. 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, Serratia marcescens and Klebsiella pneumoniae by using disc diffusion method. The zone of inhibition were recorded and compared with standard antibiotic drug ie. Chloramphenicol. Ethanolic extract showed the high degree of inhibition when compared with chloroform and aqueous extracts (Natarajan et al., 2003).

Murat et al. (2003) studied the antimicrobial activity of two prapolis samples from Kazan and Marmris regions in Turkey by the disc diffusion method. Antimicrobial activity was tested with 4 different ethanolic extracts (30, 50, 70 and 96% ethanol) of each sample against 7 gram – positive and 4 gram – negative bacteria.

The antibacterial activity of 36 ethanol extracts from 24 plants, all of them currently used in the Peruvian traditional medicine for the treatment of several infections and inflammatory disorders, was tested by means of the agar – well diffusion assay against 4 bacteria and 4 fungi. Twenty five (69%) extracts showed some degree of antimicrobial activity against atleast one microorganism. The plant with the greatest antimicrobial activity was Cestrum auriculatum (Rojas et al., 2003).
Aqueous and methanol extracts of the leaves of *Juniperus oxycedrus* were investigated for their *in vitro* antimicrobial properties against 143 laboratory strains belonging to 56 bacterial species and 31 isolates of 5 fungi species based on the inhibition zone using the disc diffusion assay, Minimal Inhibition Concentration (MIC), Minimal Bactericidal Concentration (MBC) values. The aqueous extracts of *J. oxycedrus* had no antimicrobial effect against the test microorganisms whereas the methanol extract had inhibitory effects on the growth of 57 strains of 24 bacterial species (Karaman *et al*., 2003).

Extracts from 11 plants species belonging to the Zingiberaceae were tested for antifungal activity using disc diffusion bioassay. Extracts from several members, especially *Alpinia galanga*, *Curcuma zedoaria* and *Zingiber purpureum* were found to have pronounced inhibitory activities against a wide variety of human pathogenic fungi including strains resistant to the common antifungals amphotericin B and Ketoconazole (Christine Ficker *et al*., 2003).

The pet - ether (PEB), Chloroform (CEB) and methanol (MEB) leaf extracts of *Blumea balsamifera* DC. were tested for their antibacterial activity (Fazilatun *et al*., 2004). The micro-organisms used in this study included four gram-positive bacteria, *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and eight gram-negative bacteria, *Enterobacter aeruginosa*, *Salmonella typhi*, *S. paratyphi*, *Escherichia coli*, *Shigella sonnei*, *S. flexneri*, *Proteus vulgaris* and *P. mirabilis*. All the extracts showed wide range of activities against gram-positive as well as gram-negative bacteria. But extracts were insensitive to *Salmonella paratyphi* and *Escherichia coli*. The extracts showed comparatively higher activity against a number of bacteria than the standard gentamicin.

The *in vitro* antimicrobial activity of *Nerium oleander* roots, bark and leaf extracts were studied against *Bacillus pumilus*, *B. subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus niger*. The chloroform, ethanol and methanol extracts of *Nerium oleander* showed high activity against all the tested bacteria. None of the crude extracts of the selected plant exhibited activity against *Aspergillus niger*. These results were compared with the zones of inhibition produced by commercially available standard antibiotics. The inhibitory effects of extracts are very close and
identical in magnitude and are comparable with the standard antibiotics used (Hussain and Gorsi, 2004).

Bouhadjera et al. (2005) reported that the ethyl acetate extracts of the leaves of Aristida pungens L. show a significant antibacterial activity on Pseudomonas and on a large spectrum of fungi. Methanol extracts of three Calophaca species, C. citrine, C. lacteal and C. schaereri showed broad spectrum antifungal and antibacterial properties against some human and plant pathogens (Manojlovie et al., 2005).

The methanolic extract of Centaurea diffusa demonstrated antimicrobial activity against Staphylococcus aureus, S. epidermidis, S. intermedius, Pseudomonas aeruginosa, Escherichia coli, Candida albicans, Mycobacterium phlei and Mycobacterium fortuitum (Skliar et al., 2005).

Fernendes et al. (2005) evaluated the in vitro antibacterial activity of three plant species from Cerrado (Hymenaea courbaril, Plathymenia reticulata and Guazuma ulmifolia) on standard gram-positive, gram-negative bacteria and Candida albicans. Minimum inhibitory concentration (MIC) assay was used to quantify antimicrobial activity of the P. reticulata and H. courbaril extracts against mutants Streptococci and Staphylococcus sp. using an agar well diffusion technique. The extracts showed antimicrobial activity for the gram-positive microorganisms, but they did not inhibit the gram-negative microorganisms and Candida albicans. P. reticulata extract inhibited Staphylococcus sp. and mutants of Streptococci. Under the same conditions, H. courbaril extract inhibited Staphylococcus sp. and mutants Streptococci.

Crude extracts and VLC fractions from the stem bark of Barringtonia acutangula were screened for their antimicrobial activities against two gram-positive bacteria, two gram-negative bacteria and two fungi using a microdilution titre assay. Among the crude extracts, petroleum ether extracts showed good activity against all test organisms. The VLC fraction PE 16 was found to be very effective against Bacillus subtilis (MIC = 12.5 micro g/ml) (Rahman et al., 2005).

The antibacterial and antifungal activities of various extracts from the herbs of Cirsium hypoleucum were screened by micro dilution method against both the standard and the isolated strains of Escherichia coli, Pseudomonas aeruginosa,
Enterococcus faecalis, Staphylococcus aureus and the fungi Candida albicans and C. parapsilosis. Methanol extract, ethylacetate ad n- butanol fractions displayed remarkable antibacterial activity against isolated strains of S. aureus at 32 micro g/ml concentration (Ozcelk et al., 2005).

Daud et al. (2005) reported the ethanol extract of flowers of Phrygilanthus acutifolius inhibited the growth of both gram-positive bacteria (Staphylococcus aureus, S. saprophyticus and Enterococcus faecalis) and gram-negative bacteria (Serratia marcescens, Acinetobacter sp. and Pseudomonas aeruginosa). This extract was bactericidal against Staphylococcus aureus and bacteriostatic against Pseudomonas aeruginosa.

Successive chloroform, ethanol and ethyl acetate partitions of extracts from Cynara scolymus L. leaf, head and stem were tested for their antimicrobial activity against 15 microbial species, including 7 food borne bacterial pathogens, Bacillus subtilis, Staphylococcus aureus, Agrobacterium tumefaciens, Micrococcus luteus, Escherichia coli, Salmonella typhimurium and Pseudomonas aeruginosa, 4 yeast, Candida albicans, C. lusitaniae, Saccharomyces cerevisiae and S. uvarum and 4 molds Aspergillus niger, Penicillium oxalicum, Mucor mucdo and Cladosporium cucumerinum using the disc diffusion assay technique. The leaf extract was found to be most effective against all of the tested organisms followed by the head and stem extracts and ethanol fraction showed the most significant antimicrobial activity against all of the tests among 3 soluble fractions of extract, followed by the chloroform and ethyl acetate fractions. The minimum inhibitory concentrations (MICs) of extracts determined by the agar and broth dilution method ranged from 1.25 to 10.0 mg/ml. The MIC of ethanol fraction of leaf extracts was the lowest by comparison with the other 2 extracts. The MIC for fungi was at or below 2.5 mg/ml and for bacteria was at or above 2.5 mg/ml (Zhu et al., 2005).

Takahashi et al. (2004) investigated the antimicrobial activities of Eucalyptus leaf extracts to find effective antibacterial agents. Extracts of Eucalyptus globulus, E. maculate and E. viminalis significantly inhibited the growth of 6 gram-positive bacteria (Staphylococcus aureus, MRSA, Bacillus cereus, Enterococcus faecalis, Alicyclobacillus acidoterrestris, Propionibacterium acnes) and of a fungus
Trichophyton mentagrophytes), but they did not show strong antibacterial activity against gram-negative bacteria (Escherichia coli, Pseudomonas putida).

Mausumi et al. (2006) described the antibacterial activity of ginger and turmericic and mixtures. Extracts of these compounds were studied on three bacterial Escherichia coli, Bacillus subtilis and Staphylococcus aureus. Both aqueous heated and unheated extracts were used for antibacterial assay. Growth inhibition was evaluated by the disc diffusion and agar disc method. The antibacterial activity of heated extracts were found to be statistically greater (PLO.03) than that of the unheated extracts for both the spices used alone or in mixtures there of against all the three bacterial strains.

The antimicrobial activity of Vernonia tenoreana was investigated by Ogundare et al. (2006). Crude extracts and solvent fractions of the leaf and bark of V. tenoreana were obtained using methanol and distilled water (2:3, v:v), ethylacetate, Chloroform and n – hexane as solvent of extraction. The crude and solvent fractions showed broad spectrum activities against Staphylococcus aureus, Streptococcus faecalis, Bacillus subtilis, B. cereus, Shigella dysenteriae, Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus vulgaris. The leaf extracts showed better antimicrobial activities out of all the solvent fractions, the chloroform fractions exhibited the highest antimicrobial activities.

Various organic and aqueous extracts of leaves of Indigofera suffruticosa obtained by infusion and maceration were screened for their antibacterial and antifungal activities. The extracts were tested against 5 different human pathogenic bacterial species and 17 fungal strains by the agar – solid diffusion method. The aqueous extracts of leaves of I. suffruticosa obtained by infusion which showed strong inhibitory activity against the gram-positive bacteria Staphylococcus aureus with a minimal inhibitory concentration (MIC) of 5000 micro g/ml (Leite et al., 2006).

The antibacterial activity of Bryum argenteum ethanol extracts was evaluated by micro-dilution method against four bacterial (Escherichia coli, Bacillus subtilis, Micrococcus luteus and Staphylococcus aureus) and four fungal species (Aspergillus niger, Penicillium ochrochloron, Candida albicans and Trichophyton
mentagrophyces). All the investigated ethanol extracts have been proved to be active against all bacteria and fungi tested (Sabovljevic et al., 2006).

*In vitro* antibacterial activities of *Ageratum conyzoides* extracts on some selected bacterial pathogens were investigated. The susceptibility of *Staphylococcus aureus, Yersinia enterocolitica, Salmonella gallinarum* and *Escherichia coli* to the various extracts as well as the minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) were studied using standard method. Results obtained showed that the hexane extracts of the leaf, stem and root had 100% susceptibility to all the bacterial isolates used. The aqueous leaf extracts gave 75% susceptibility, while methanolic leaf extracts gave 50% (Okwari et al. 2007).

The antibacterial activities of the aqueous and ethanolic extracts of the stem of *Olax subscorpioidea* were evaluated. The ethanolic extract showed considerable activity on both the fungi and bacteria with zones of inhibition ranging from 7.2 mm to 21.5 mm, with minimum inhibitory concentrations ranging from 5 to 45 mg/ml, while the aqueous extract was only active against three of the test organisms used (Ayandele and Adebiyi, 2007).

The methanol and acetone extracts of 14 plants belonging to different families were evaluated for antimicrobial activity against five gram-positive bacteria: *Staphylococcus aureus, S. epidermidis, Bacillus cereus, B. subtilis, Micrococcus flavus*; seven gram-negative bacteria: *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, P. vulgaris, Salmonella typhimurium, Citrobacter freundii* and three fungi: *Candida tropicalis, Cryptococcus luteolus* and *Candida albicans*. The *in vitro* antimicrobial activity was performed by agar disc diffusion method. The extractive yield was more in methanol than acetone extracts. The most susceptible bacterium was *K. pneumoniae* and the most resistant were *P. vulgaris, S. typhimurium, P. aeruginosa* and *E. coli* (Yogeshkumar et al., 2007).

Berahou et al. (2007) reported the antibacterial activity of different extracts of *Quercus ilex* bark (Fagaceae) against *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (BCCM 21055), *Bacillus subtilis* (ATCC 6051), *Klebsiella pneumoniae* (ATCC 13883), *Salmonella typhimurium* (ATCC 43971), *Vibrio cholerae* (ATCC 14033), *Proteus mirabilis* (HITM 20), *Staphylococcus epidermidis* (HITM 60), *Streptococcus pyogenes* (HITM 100) and
Streptococcus agalactiae (HITM 80). The ethyl acetate extract (QE), n-butanol extract (QB) and final aqueous layer (QA) were effective against all bacterial strains tested at MICs ranging from 128 to 512 mg/ml. The n–hexane extract (QH) and dichloromethane extract (QD) showed no activity.

Duraipandian and Ignacimuthu (2007) have reported the antibacterial activities of Cassia fistula. Hexane, chloroform, ethyl acetate, methanol and water extracts from the flower exhibited antibacterial activity against Staphylococcus aureus, S. epidermidis, Bacillus subtilis, Enterococcus faecalis and Pseudomonas aeruginosa.

The effect of petroleum ether, acetone, methanol and aqueous extracts of Terminalia chebula against 11 pathogens, Staphylococcus aureus, Streptococcus mutans, S. salivarius, S. sanguis, Lactobacillus acidophilus, E. coli (MTCC), Klebsiella pneumoniae (MTCC), Micrococcus luteus (MTCC), Bacillus (MTCC), B. megnetherium (MTCC) and Staphylococcus epidermidis (MTCC) have been studied. The plant showed broad spectrum activity against gram-positive and gram-negative bacteria. The methanolic extract showed the strong activity against S. aureus (27 mm) and petroleum ether extract showed the minimum activity against S. sanguis (17 mm) (Prabhat et al., 2008).

Crude extracts of flavonoids, extracted from Adhathoda zeylanica, Ailanthus excels, Barleria prioinitis and Cassia angustifolia and their calli were tested for their antimicrobial activity against Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Candida albicans. Flavonoids extracted from calli of all plant species showed greater antimicrobial activity against micro-organisms because of higher concentrations of flavonoids in them (Asha et al., 2008).

The aqueous and chloroform fractions of stem bark of Distemonanthus benthamianus exhibited significant inhibitory action against twelve bacterial isolates tested at a concentration of 10 mg mL -1. The zones of inhibition due to the aqueous fraction ranged between 10 and 15 mm, while that of chloroform fraction ranged between 8 and 13 mm. The Minimum Inhibitory Concentration (MIC) exhibited by aqueous fraction against the bacterial isolates ranged between 0.625 and 2.5 mg mL -1 while that of chloroform fraction ranged between 0.313 and 5.0 mg mL -1 (Aiyegoro, 2008).
Aqueous pulp extract of *Tamarindus indica* showed antibacterial activity against all the tested bacteria in the order of sensitivity as *Staphylococcus aureus* > *Escherichia coli* > *Pseudomonas aeruginosa* with the exception *Salmonella typhi*. The antibacterial activity of aqueous pulp extract on *S. aureus* was sensitive at 80, 120, 140, 160 and 180 mg mL⁻¹ of extract with 0.2, 0.3, 0.6, 0.8 and 10.0 mm zones of inhibition while *E. coli* revealed 0.2, 0.2, 0.4 and 0.6 mm zones of inhibition at 120, 140, 160 and 180 mg mL⁻¹ of extract, respectively. *P. aeruginosa* was only sensitive at 140, 160 and 180 mg mL⁻¹ of the extract with 0.4, 0.6 and 0.8 mm zones of inhibition (Abukakar, 2008).

De Villers *et al.* (2010) reported that metanolic leaf extracts of *Cussonia* species were active against *Pseudomonas aeruginosa* (MIC of 1-1.5 mg/ml), *Trichomonas vaginalis* (MIC 0.8-1.3 mg/ml) and *Staphylococcus aureus, Cussonia arborea* (1.8 mg/ml).

### 5.3. Materials and Method

#### 5.3.1. Plant materials

*Kaempferia galanga* were collected from Kerala Agricultural University, Vellanikara Trissur district in Kerala, India and identified at Rapinat Herbarium, (RHT), St. Joseph’s College (Autonomous), Tiruchirappalli, Tamil Nadu. A voucher specimen was prepared and deposited in the Rapinat Herbarium. The plants were shade-dried at ambient temperature (31°C) and the dried materials were crushed into fine powder using an electric blender.

#### 5.3.2. Preparation of plant Extract

**5.3.2.1. Continuous hot extraction – using Soxhlet apparatus**

Soxhlet apparatus is used to execute continuous hot extraction. The soxhlet apparatus is for the extraction of coarse powder and is placed in a “thimble” made filter paper and is inserted into the wide tube of the extractor. The solvent, which is taken in the flask, is heated; the vapours, which arise from the solvent, get into the condenser through a side tube and the liquid condensed form the vapors drips into the thimble. The solvent liquid level slowly rises and during this period, the dried materials get extracted of its soluble constituents. When the level of the liquid reaches the top of the siphon, it gets siphoned into the flask. The suction effect of the siphoning assists permeation of the solvent through the drugs again, a portion the solvent from the solution is vaporized leaving constituents in the flask itself and the
process mentioned above is repeated. The same process is repeated again and again; this kind of continuous hot percolation is undertaken when the active constituents are not readily soluble in the cold and thermobile.

5.3.2.2. Solvent extraction

Fifty grams of dried powdered materials (leaves, rhizome and root) were soaked separately in 300 ml each of the solvents viz. (Ethanol, Methanol, Petroleum ether, Chloroform and aqueous) in a soxhlet apparatus for 72 hours at 31°C until complete exhaustion of the material. Each mixture was stirred at every 24 hours using sterile glass rod. At the end of 72 hours, each extract was passed through Whatman No: 1 filter paper and the filtrates were concentrated in vacuum rotary evaporated at 60°C in order to reduce the volume. The paste like extracts were stored in labelled screw caped bottles and kept in refrigerator at 4°C. Each of the extract was individually reconstructed using minimal amounts of the extracting solvent prior to use.

5.3.3. Microorganism

The microorganisms used for the present study were collected from department of Microbiology, institute of Basic Medical Science, University of Madras, Taramani, Chennai, India. It includes both bacterial strains and fungal stains. The gram-positive bacterial strains are: *Staphylococcus aureus* MTCC # 3163, *Streptococcus faecalis* MTCC # 459, *Bacillus cereus* MTCC # 1306, *Bacillus subtillis MTCC #121 and gram-negative: *Salmonella typhi* MTCC # 734, *Escherichia coli* MTCC # 119, *Enterobacter aerogenes* MTCC # 2990, *Pseudomonas aeruginosa MTCC # 2474, Klebsiella pneumoniae* MTCC # 3040 and *Vibrio cholerae*. The fungal strains are *Aspergillus niger* MTCC # 2612, *A. flavus* MTCC # 2813, *A. fumigatus MTCC # 2584 and *Candida albicans* MTCC # 1637.

5.3.3.1. Maintenance of microorganisms

The bacterial strains were maintained in Nutrient Agar (Hi-media Laboratories pvt. Ltd., Mumbai) and the fungal strains were maintained on Potato Dextrose Agar slants (Hi- media Laboratories pvt Ltd., Mumbai). The strains were subcultured bimonthly and the cultured strains were allowed to grow for one week and stored at 5°C for further analysis.
5.3.4. Assay of Antibacterial Activity

5.3.4.1. Disc Diffusion Method (Maruzzella and Henry, 1958)

Principle

The disc–diffusion method provides a simple and reliable test in routine clinical bacteriology in order to find out the effect of a particular substance on a specific bacterium. This method consists of impregnating small circular discs of standard filter paper with given amount of a chosen concentration of substance. The discs are placed on plates of culture medium previously spread with a bacterial inoculum to be tested. After incubation the degree of sensitivity is determined by measuring the inhibition zone produced by the diffusion of the antibiotic substances from the discs into the surrounding medium.

Preparation of Discs

Discs usually consisted of absorbent paper impregnated with the compound (plant extracts). It is most convenient to use whatman No.1 filter paper for preparing the discs. Dry discs of 6 mm diameter were prepared from whatman No.1 filter paper and sterilized in an autoclave. These dry discs were used for the assay.

Procedure

Circular discs of 6 mm diameter were prepared from Whatman No.1 filter paper and sterilized in an autoclave. These paper discs were impregnated with test compounds (Plant extract) in the respective solvents for overnight and placed on nutrient agar plates seeded with the test bacterium. The plates were incubated at 37°C for 24 hours. After 24 hours the zone of inhibition around each disc was measured and the diameter was recorded. Kanamycin 30 µg/disc was used as the reference. A negative control was prepared using only the solvent used for extraction and kept for comparison.

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

Agar well diffusion method is also known as Hole Plate Diffusion Method (Brantner, et al., 1993) or cup diffusion method (Vikas Dhingra, et al., 1999).

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 or cups are made using a sterilized cork borer on the seeded nutrient agar in a petridish to which the test compound is added. The treated petridishes are incubated at 37°C for 24 hours. The inhibition zone formed around each well indicates the antimicrobial activity.

**Procedure**

Nutrient agar was used as the culture medium for this assay. The molten nutrient agar was dispensed in presterilized petridishes (25 ml each) and allowed to cool. These agar plates were homogenously inoculated with the test bacterium previously suspended in tryptose broth \((10^6 \text{ cells/ml})\). The plates were allowed to solidify. After solidification holes/wells of 6 mm diameter were punched into the agar with the help of flamed cork borer. Three cells were prepared for each plate and filled with plant extracts. The petridishes were incubated at 37°C for 24 hours. After this incubation period the diameter of the inhibition zone formed around each hole (well/cup) 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 is positive control (Kanamycin) another one is negative control.

5.3.4.3. Streak Plate Method (Orzechowski, 1972)

**Principle**

The inhibitory effect of plant extract can be easily rated by this steak plate method with unaided eye. The result of this test was usually rated as strong inhibition where no growth of the bacterium occurs, partial inhibition where less growth of the bacterium than the normal occurs and no inhibition where full growth of the bacterium occurs. Once an extract showed a definite inhibitory effect further testing was carried out by means of disc diffusion assay or agar well diffusion assay.

**Procedure**

Nutrient agar medium was prepared and 8 ml of the molten agar medium and 2 ml of the plants extracts were mixed thoroughly and the mixture was poured into a sterile petridish under aseptic condition. It was cooled at room temperature. After cooling the bacterial culture was taken (24 hours old) and using an inoculation needle, the bacterial culture was applied on the surface of the agar medium in the form of
parallel streaks. The plates were incubated at 37°C for 24 hours. After 24 hours, the results were rated as strong inhibition (if no growth of the test bacterium), partial inhibition (if less growth of the bacterium than the normal) and no inhibition (if full growth of the test bacterium). Control plates without the plant extracts were also maintained for reference.

5.3.4.4. The Minimum Inhibitory Concentration (MIC)

Dilution method for antibacterial testing to calculate MIC (Zgoda and Porter, 2001; Kuete et al., 2007; NCCLS, 1990).

1. NB tubes are labelled with name, date of inoculation, experiment number, and name of bacterium.

2. The microdilution was performed in 96-well microtitre plates with U-shaped wells, label with name and date of inoculation, experiment number and name of bacterium.

3. The stock solution was prepared with DSMO (400 mg/ml).

4. 500µl (250 mg) of the extract was added to 4.5 ml of NPBG (Nutrient Broth containing 0.05% Phenol red and supplemented with 10% Glucose).

5. The extract was transferred to 200 µl/well and serially diluted (base 2 logarithmic dilutions) with NPBG (ie. Two fold to obtain concentrations ranges of (4 mg/ml – 0.008 mg/ml) in use the micropipetted to dispense 100 µl of test extract to the first two –fold dilution. The micropipette was used with the same tip to carry out a second two – fold dilution, the series of two–fold dilutions was continued until the 11th well (Column) of the microwell plate. The quantity in the micropipette from this well was discarded.

6. 24 h culture of bacterial strains were stirred with 0.9% NaCl to achieve 0.5 McFarland (108 cells/ml for bacteria and 106 for fungi) diluted 1/100 to achieve 106 and 104 cells /ml for bacteria and fungi respectively and inoculated in the 96 wells plates and 5µl of inoculum was added to each well.

7. The first well consists of the negative control with standard inoculums, the last well (12th well Colum) control of positive control with 15µg [Gentamycin (15 µl of 1mg / 1ml stock) for gram-positive bacteria and Ofloxacine (15 µl of 1mg / 1ml stock) for gram-negative bacteria].

8. Microbial growth was determined by observing the change of growth and yellow when there is growth.

9. The plates were sealed, placed in plastic bags and incubated at 37°C (for bacteria) for 24 hours in ambient atmosphere.
10. Measurement of the MIC is defined as the lowest concentration of extract that exhibited on growth by visual reading. It is expressed in mg or µg/ ml.

11. The plates are discarded by appropriate procedure.

The Minimum Inhibitory Concentration (MIC) was the lowest concentration of extract that completely inhibited the growth of microorganism in the microdilution wells and detected by unaided eye (i.e. red colour when there is no growth negative wells).

5.3.4.5. Minimum Bactericidal Concentrations (MBC)

The main advantage of the ‘Broth dilution’ method for the MIC determination is that it can readily be converted to determine the MBC as well.

Method

Dilutions and inoculations are prepared in the same manner as described for the determination of MIC. The control plate containing no antibiotic is immediately subcultured (before incubation) by spreading a loopful evenly over a quarter of the plate on a medium suitable for the growth of the test organism and incubated at 37°C overnight. The plates are also incubated overnight at 37°C. MIC of the control organism is checked, so that the drug concentrations are correct. The lowest concentration inhibiting growth of the organisms is noted and recorded as the MIC. All plates not showing visible growth in the same manner as the control plate described above are subcultured and incubated at 37°C overnight. The amount of growth from the control plate before incubation is compared, which represents the original inoculum. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. These plates are not subcultured; the purpose of the control is to confirm by its MIC that the drug level is correct.

Reading of result

These subcultures may show

- Similar number of colonies- indicating bacteriostasis only.
- A reduced number of colonies-indicating a partial or slow bactericidal activity.
- No growth- if the whole inoculum has been killed
- The highest dilution showing at least 99% inhibition is taken as MBC.
5.3.5. Assay of Antifungal Activity

The antifungal activity of the plant extract is determined by the following assays.

5.3.5.1. Disc Diffusion Method (Barry and Thorrsberry 1991)

Principle

The antimycotic activity of aqueous and other solvent extracts were evaluated by paper disc agar diffusion method. This method consists of impregnating small circular discs of standard filter paper with given amount of a chosen concentration of substance. The discs are placed on plates of culture medium previously spread with the fungal spores (inoculum). After incubation the degree of sensitivity is determined by measuring the inhibition zone produced by the diffusion of the antibiotic substances from the disc into the surrounding medium.

Preparation of disc

Discs usually consisted of absorbent paper impregnated with the compound (plant extract). It is most convenient to use whatman No.1 filter paper of preparing the discs. Dry discs of 13 mm diameter were prepared from whatman No. 1 filter paper and sterilized in an autoclave. These dry discs were used for the assay.

Procedure

Test plates (petridishes) were prepared with potato dextrose Agar medium and inoculated on the surface with a spore suspension of 10^6 CFU/ml. Sterile paper discs of 6mm diameter impregnated with the extract at the concentration of 10mg/ml were placed over the test plates and were incubated at 25°C for 48 hours. The diameter of growth inhibition zone around each disc was measured against each concentration after 48 hours.

5.3.5.2. Agar Well Diffusion Method (Gbodi and Irobi, 1992)

Principle

The antifungal activity of the plant extract to various fungi was evaluated by agar well diffusion method. The diameter of the inhibition zone is an indicator of the antifungal activity.
**Procedure**

Potato Dextrose Agar was used to culture the fungal organism. The plates were inoculated with 48 hours culture of respective fungi. With the help of a flamed cork borer 8 mm wells were cut out and the plant extracts were aseptically added with the help of sterile syringe. The plates were kept in cold for an hour to facilitate diffusion of the test solution (the extracts). Later the plates were incubated at 25˚C for 48 hours. After incubation the diameter of the inhibition zone was measured. Nystatin was used as a standard for comparison of antifungal activity.

**5.3.5.3. Streak Plate Method (Orzechowski, 1972)**

The inhibitory effect of plant extract on the test fungi can be easily rated by unaided eye using this streak method. The results of this test were rated as strong inhibition (there is no growth of the fungus partial inhibition (where less growth of the fungus than the normal) and no inhibition (full growth of the fungus).

**Procedure**

Eight milliliter of the molten PDA medium and 2 ml of sterile plant extracts were mixed thoroughly and the mixture was poured into a pre–sterilized petridish under aseptic condition. After cooling at room temperature, small streaks of the test fungi were made on the surface of the medium using an inoculation needle. The plates were incubated at 25˚C for 48 hours. After the incubation period the results were recorded as strong inhibition (if no growth of the test fungi), partial inhibition (if less growth of the fungi than the normal) and no inhibition (if full growth of the test fungi) control plates without the plant extract were also maintained for reference.

**5.4. Results and Discussions**

**5.4.1. Antibacterial assays**

The *in vitro* antibacterial activity of the ethanol, methanol, petroleum ether, chloroform and aqueous extracts of various parts of *Kaempferia galanga* (rhizome, leaf and root) was evaluated by various assays against ten pathogenic bacteria. The bacteria include both gram-positive and gram-negative. Based on the screening it showed that all the solvents (ethanol, methanol, petroleum ether, chloroform and aqueous) extracts of *K. galanga* (rhizome, leaf and root) exhibited demonstrable antibacterial activity against all the test pathogenic bacteria.
5.4.1.1. Disc Diffusion method

The antibacterial activity of various solvent extracts of different parts of *Kaempferia galanga* by disc diffusion method has been summarized in the Table 5.1, 5.2, 5.3 (Rhizome, leaf and root respectively). The inhibitory action was observed in terms of diameter of inhibition zone formed around each disc caused by the disc diffusion of antimicrobial substances from the paper discs into the surrounding medium. The rhizome and leaf had the maximum antibacterial activity. Antibacterial activity was the highest in ethanol and methanol whereas moderate in petroleum ether, chloroform and aqueous extracts. The diameter of the inhibition zone for each extract against each microorganism was found to be less than to the standard antibiotic (Kanamycin 30 µg/discs) used in this assay. Among the three parts used, rhizome and leaf exhibited higher degree of antibacterial activity than root. The diameter of inhibition zones were noted in the rhizome, leaf and root extract (Table 5.1, 5.2, 5.3 and Plate 5.1-5.6). The rhizome ethanolic extract showed higher degree of inhibition zone in *Staphylococcus aureus* 21.3±0.08 mm, *Streptococcus faecalis* 19.7± 0.37 mm, *Bacillus subtilis* 19.7±0.20 mm and *B. cereus* 18.4±0.41 mm. The moderate inhibition was noted against *Escherichia coli* 17.8±0.55 mm and *Enterobacter aerogens* 16.2±0.37 mm. The other bacteria showed inhibition zone such as *Salmonella typhi* 15.5±0.17 mm, *Klebsiella pneumoniae* 14.9±0.95 mm, *Vibrio cholerae* 12.3±0.16 mm and *Pseudomonas aeruginosa* 12.1±0.40 mm. The methanol extract showed good activity in gram-positive bacteria such as *S. aureus* 18.1±0.30 mm and *S. faecalis* 16.4±0.34 mm and the gram-negative organism *E. aerogenes* 15.6±0.26 mm and *Escherichia coli* 12.8±0.34 mm. The petroleum ether and chloroform rhizome extract showed higher level of inhibition zone in *B. cereus* 14.3±0.29 mm and 14.5±0.32 mm respectively.

The ethanolic extract of leaf showed the highest degree of inhibition zone in *Staphylococcus aureus* 19.5±0.17 mm, *Streptococcus faecalis* 17.1±0.57 mm, *Bacillus cereus* 16.4±0.13 mm and *B. subtilis* 16.0±0.65 mm and *Escherichia coli* 16.4±0.45 mm. Methanol leaf extract also showed high degree of inhibition zone against gram-positive bacteria. The petroleum ether, chloroform and aqueous leaf extracts showed moderate level of inhibition against all tested bacteria (Table 5.2). The ethanolic root extract showed the highest degree of inhibition zone against *S. aureus* 15.4± 0.37 mm.
followed by *B. subtilis* 14.5±0.34 mm, *Klebsiella pneumoniae* 14.3±0.13 mm and *Escherichia coli* 14.0±0.65 mm. Similar observations were reported by earlier workers such as Hartman *et al.* (1975), Chatterjee *et al.* (1993), Alade and Irobi (1993), Desta (1993), Irobi *et al.* (1994), Darah and Halim (1995), Vikas Dhingra *et al.* (1999), Gadhi *et al.* (2001), Aburjai (2001), Natarajan *et al.* (2003), Christine Ficker *et al.* (2003), and Daud *et al.* (2005).

5.4.1.2. Agar well diffusion method

The data pertaining to the antibacterial potential of the *Kaempferia galanga* extract (rhizome, leaf and root) are presented in Table 5.4, 5.5 and 5.6. The results were observed in terms of inhibition zones around each well or hole caused by diffusion of antibacterial properties from the plant extract impregnated disc into a surrounding medium (Plate 5.7, 5.8 and 5.9). The ethanolic extract of rhizome leaf and root had a stronger and a broader spectrum of antibacterial activity compared with other extracts. Ethanolic extract of rhizome exhibited strong activity against *Staphylococcus aureus* (20.9±0.09 mm), *Streptococcus faecalis* (18.4±0.32 mm), *Enterobacter aerogens* (17.2±0.89 mm) and *Escherichia coli* (16.4±0.28 mm). Methanol rhizome extract showed maximum inhibition zone against *S. aureus* (16.4±0.98 mm). Petroleum ether, chloroform and aqueous extract of rhizome showed moderate activity towards all tested bacteria (Table 5.4). Ethanolic extract of leaf also exhibited good inhibition zone around *S. aureus*, *S. faecalis*, *Bacillus cereus* and *Escherichia coli* when compared to other solvent extracts (Table 5.5). These findings coincide with the previous reports of Arambewala *et al.* (1999), Khan *et al.* (2000), Natarajan *et al.* (2003), Rojas *et al.* (2003), Daud *et al.* (2005) and Mausumi *et al.* (2006). The root extracts demonstrated moderate type of inhibition to all tested bacteria (Table 5.6). Similar results were reported by Vikas Dhingra *et al.* (1999), Gould and Booker (2000) and Madamome and Afolayam (2003). In general, the obtained data by agar well diffusion method revealed that among the ten bacterial species tested *S. aureus* was the most susceptible microbe to most extracts from the studied plant. These reports also coincide with the previous report of Oonmetta-aree *et al.* (2006).
5.4.1.3. Streak method

The in vitro antibacterial sensitivity of various extracts of Kaempferia galanga (Rhizome, leaf and root) against ten pathogenic bacteria by stroke method are depicted in Table 5.7-5.12. The data showed that there is a strong inhibition with the increasing percentage of concentration of the plant extracts. All the gram-positive and gram-negative organism tested were sensitive to the crude plant 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 extract, the growth rate of bacteria was noted to be excessive whereas in experimental plates the degree of growth inhibition increased with the gradual increase with the concentration of plant extracts. Hence the higher concentration viz 75% and 100% strongly or completely inhibit the growth of test bacteria. Almost all bacterial strains especially Staphylococcus aureus, Streptococcus faecalis, Bacillus cereus and Escherichia coli were found to be high by sensitive to the rhizome extract (with ethanol, methanol, petroleum ether, chloroform and aqueous extracts) at higher concentration (75% and 100%). Ethanol was found to be the most active extract demonstrating maximum sensitivity or inhibitory among five solvent used.

The results of leaf extracts on the test bacteria with reference to the sensitivity test has been shown in the Table 5.9 and 5.10. It is evident from the table that almost all the five solvent extracts of leaf exhibited anti microbial activity at higher concentration. Ethanol was found to be the most active extract demonstrating maximum sensitivity. The antimicrobial activity was identified by the magnitude of presence or absence of growth. In control plates of each solvent without plant extract, the growth rate of bacteria was noted to be excessive where as in experimental plates the degree of growth inhibition increased with the gradual increase with the concentration of plant extracts. Therefore in the experimental plates, the higher concentrations (75% and100%) completely inhibit the growth of bacteria.

The results of root extracts on the test bacteria with reference to the sensitivity test has been shown in the Table 5.11 and 5.12. It is evident from the table that almost all the five solvent extracts of root (Ethanol, Methanol, Petroleum ether, Chloroform and Aqueous) exhibited anti microbial activity at higher concentration. Ethanol was found to be the most active extract demonstrating maximum sensitivity.
The antimicrobial activity was identified by the magnitude of presence or absence of growth. In control plates of each solvent without plant extract, the growth rate of bacteria was noted to be excessive where as in experimental plates the degree of growth inhibition increased with the gradual increase with the concentration of plant extracts. Therefore in the experimental plates, the higher concentrations (75% and 100%) completely inhibit the growth of bacteria *Staphylococcus aureus*, *Streptococcus faecalis* and *Escherichia coli*. Similar observations were reported by earlier workers such as Brantner and Grein (1994), Alonso Paz (1995), Vijaya *et al.* (1995) reported that the degree of sensitivity increased with the gradual increase in the concentrations of exracts when gram-positive and gram-negative bacteria were tested under *in vitro* conditions. The present investigation is also in agreement with the previous reports.

5.4.1.4. MIC and MBC determinations

The minimum inhibitory concentrations of ethanolic extracts of *Kaempferia galanga* rhizome and leaf against different pathogenic bacteria are depicted in Table 5.13. MIC test was done selectively against eight organisms that were found susceptible in disc diffusion assay. They are *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus cereus*, *B. subtilis*, *Escherichia coli*, *Enterobacter aerogens*, *Klebsiella pneumoniae* and *Salmonella typhymurium*. MIC values also showed that extracts were able to inhibit gram-positive bacteria at lower concentrations. The lowest MIC for *K. galanga* rhizome ethanol extract was recorded against *S. aureus* (0.25 mg/ml), *B. cereus* (0.5 mg/ml) and *B. subtilis* (1.0 mg/ml) as detailed in Table 5.10 [MIC values < 1.0 mg/ml (1000 µg/ml) were considered to be active] (Plate 5.19). The MIC values of *K. galanga* leaf ethanol extract are also depicted in table 5.13. The rhizome Ethanolic extract gave an MBC of 0.5 mg/ml for *S. aureus*, while for *B. cereus* the MBC values were 1.0 mg/ml, respectively (Table 5.14). For leaf ethanolic extract, the MBC values were Resistant (R) for all the pathogens. The MIC and MBC values tend to support the results obtained in the antibacterial screening by disc diffusion showing clearly that the rhizome ethanolic extracts were more potent than leaf ethanolic extracts. In this study, the MIC values were either the same or slightly lower than the MBC values, similar to the results of Karou *et al.* (2006). The MIC and MBC values are predictive of the efficacy of agents’ *in-vivo*. However, the MBC
values which are obtained after plating various dilutions of the extracts, is more reliable than the MIC values.

5.4.2. Antifungal assays

The effect of ethanol, methanol, petroleum ether, chloroform and aqueous extracts of rhizome, leaf and root in *Kaempferia galanga* were investigated for *in vitro* antifungal activity against four selected fungi by various techniques. The test fungi were *Aspergillus flavus*, *A. fumigatus*, *A. niger* and *Candida albicans*. The antifungal activity which was observed in ethanol extract showed maximum inhibitory action against almost all the fungi used in this study.

5.4.2.1. Disc Diffusion Method

The antifungal activity of various solvent extracts of *Kaempferia galanga* rhizome, leaf and root on different fungi by disc diffusion method has been summarized in Tables 5.15, 5.16 and 5.17. From the table it is evident that antifungal activity was maximum at the ethanol extracts. Rhizome ethanol extract showed maximum inhibition in *Aspergillus niger* (16.4±0.45 mm), *A. flavus* (15.3±0.36), *A. fumigatus* (14.0±0.48) and *Candida albicans* (12.2±0.45 mm) (Plate 5.14). Methanolic rhizome extract showed high inhibition zone against *Aspergillus niger* (14.2±0.26 mm) (Plate 5.15). Petroleum ether chloroform and aqueous extracts exhibited moderate level of inhibition zone against all tested fungi (Table 5.15). Ethanol leaf extract demonstrated the highest inhibition zone in *A. niger* (13.4±0.39 mm) while methanol leaf extract shows maximum inhibition zone against *Candida albicans* (10.3±1.4 mm) (Table 5.16). In the case of root, the extracts exhibited the inhibition zone between 10.4±0.38 mm and 6.0±0.92 mm. This observation is in agreement with the report of earlier workers Sathish Kumar (2008) and Bualeea (2007), who reported that the ethanol extract produced strong inhibitory activities against some of the microorganisms.

5.4.2.2. Agar Well Diffusion Method

The antifungal activity of various solvent extracts of *Kaempferia galanga* rhizome, leaf and root on different fungi by Agar well diffusion method has been summarized in Table 5.18, 5.19 and 5.20. The results indicate that the five solvent extracts (ethanol, methanol, petroleum ether, chloroform and aqueous) exhibited inhibitory action against all fungi used in this study. The effect is noted in the form of
zone of inhibition, formed around each well whose diameter measures the degree of inhibition. The antifungal activity was higher in ethanol extract compared to other extracts. Table 5.18 show ethanolic rhizome had a stronger and broader spectrum of antifungal activities compared with leaf and root. Inhibition zones in ethanolic rhizome extracts were *Aspergillus flavus* (16.2±1.23 mm), *A. niger* (15.0±0.19 mm), *A. fumigatus* (14.7±0.18 mm), and *Candida albicans* (14.21.98 mm) (Plate 5.16). The other solvent extracts showed moderate inhibitory action (12.5±1.19 mm to 7.1±0.39 mm) (Plate 5.17). This work also supported by the previous reports of Purohit et al. (1995), Amphawan et al. (1995) and Portillo et al. (2001) who established that the ethanol extract of many plants inhibited the growth of several fungal strains.

5.4.2.3. Streak method

The effect of various extracts of rhizome, leaf and root in *Kaempferia galanga* on the sensitivity of four selected fungi by stroke method is shown in Table 5.21-5.26.

A close examination of the results pointed out that there is a sharp degree of correlation with percentage of concentration of the extract and the sensitivity of the fungal strains. It is evident from the Tables (5.21-5.26) that as concentration increases the degree of sensitivity also increases. In other words at higher concentration (75 % and 100 %) the degree of sensitivity is also higher. It is evident from plates (Plate 5.18) that the thickness of the streaking line of the initial areas of contact is comparatively denser and thicker while it is progressively becoming thinner and thinner along the streaking lines towards the end. This thickness decreased further as the concentration increased. Almost all species of fungi expressed the same type of result. The sensitivity of the fungi to the various concentrations of the extract has been denoted as complete inhibition (-) partial inhibition (2+) and no inhibition (3+). The control plates of most of the fungi were found to be ++++ and the experimental plate of higher concentrations were found to be (-). The absence of growth indicates the complete or strong inhibitory action of the plant extract. Almost all fungi especially, *Aspergillus niger*, *A. flavus* and *A. fumigatus* exhibited the same type of results in this sensitivity test. In this case also ethanolic rhizome extract showed maximum inhibitory activity than the other solvents. These results coincide with the results of Sabovljевич et al. (2006). They reported that all the ethanol extracts had been proved to be active against all bacteria and fungi tested.