INTRODUCTION

Infectious diseases are the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Death from infectious diseases, ranked 5th in 1981, has become the 3rd leading cause of death in 1992, with an increase of 58% (Maridass and De Britto, 2008). Medicinal plants are most of the important sources for drugs. The uses of different parts of several medicinal plants to cure human ailments have been in vogue from ancient times. Ayurvedic remains one of the ancient and yet the tradition practiced widely in developing and developed countries having a sound philosophical and experimental basis (Chopra and Diophode, 2002). Natural product based drug discovery will be more holistic, personalized and involves wise use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be rendered to patients and community (Patwardan and Hopper, 1992). The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. The increase has been attributed to indiscriminate use of broad spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidemic of HIV infection (Dean, 1996; Gonzalez et al., 1996). This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants (Cordell, 2000).

Synthetic drugs are not only expensive and inadequate for the treatment of disease but are also often with adulterations and side effects. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections (Sieradzki, 1999). Plant extracts have been used for centuries as a popular method for treating several health disorders. Numerous studies have been carried out on various natural products screening their antimicrobial activity (Nita et al., 2002; Ates, 2003; Bhatterjee et al., 2006; Parekh, 2006; Parekh, 2007; Vaghasiya et al., 2007). In the past two decades, antibacterial properties of various plants and parts like roots, stem, leaves, seeds and flowers (Lever, 1979; Parekh, 2005) have been well documented for some of the medicinal plants (Vaghasiya et al., 2007). Higher and aromatic plants have been
used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeast (Hulin, 1998). Biologically active compounds from natural sources (Perumal Samy, 2000) have been always a great interest for scientist working on infectious diseases (Nandagopal et al., 2007).

Most of the phytochemical classified as secondary metabolites are mainly products of the shoot part of the plant with unknown function, but provide defense against plant pathogens. Hence the preliminary phytochemical studies are pronounced important because the crude extracts posses varied composition of secondary metabolites (Balandrin, 1985; Wink, 1999). Based on earlier reports, among the variety of secondary metabolites found in plants, phenolics and terpenoids represent the main antimicrobial agent. Aromatics compounds such as phenols, flavonoids, phenolic acids, alkaloids and lectins have been identified as antimicrobials (Siddiqui, 2009). Mainstream medicine is increasingly receptive of the use of antimicrobial and other drugs derived from plants, as traditional antibiotics become ineffective and because of the rapid rate of plant species extinction (Ncube et al., 2008).

Plants have limitless ability to synthesize aromatic secondary metabolites, most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963). Important subclasses in this group of compounds include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. These groups of compounds show antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms. Simple phenols and phenolic acid are bioactive phytochemicals consisting of a single substituted phenolic ring. Phenolic toxicity to microorganisms is due to the site(s) and number of hydroxyl groups present in the phenolic compound (Urs et al., 1975; Scalbert, 1991). Quinones are characteristically highly reactive, colored compounds with two ketone substitutions in aromatic ring. Flavones, flavonoids and flavonols are phenolic structure with one carbonyl group. They are synthesized by plants in response to microbial infection (Dixon et al., 1983) and are often found effective in vitro as antimicrobial substance against a wide array of microorganisms. Tannins are polymeric phenolic substances possessing astringent
property. These compounds are soluble in water, alcohol and acetone and precipitates with proteins (Basri and Fan, 2005).

Coumarins are phenolic substances made up of fused benzene and pyrone rings (O’Kennedy and Thornes, 1997). They have a characteristic odor and several of them have antimicrobial properties. Fragrance of plant is carried by essential oil fractions which are secondary metabolites and highly enriched in isoprene structure based compounds. They are called terpenes but when the compound contains an additional element as oxygen they are termed as terpenoids. Essential oils also possess strong antimicrobial properties. It was reported early that 60% of the essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria (Chaurasia and Vyas, 1977). Naturally found alkaloids are heterocyclic nitrogenous compounds and are commonly found to have antimicrobial property (Omulokoli et al., 1997). Many of the earliest isolated pure compounds with biological activity were alkaloids and were found in pharmacogenically active basic principles of flowering plants. Apart from the above mentioned major phytochemical groups, there are reports of antimicrobial properties associated with polyamines (Flayeh and Sulayman, 1987), isothyocyanates (Dornberger et al., 1975; Iwu et al., 1991), thiosulfinates (Tada et al., 1988) and glycosides (Rucker et al., 1992; Murakami et al., 1993). Since many of these compounds are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, clinicians need to consider the consequences of patients self-medicating with these preparations (Cowan, 1999).

Traditionally used medicinal plants have recently attracted the attention of the biological scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations (Taylor et al., 2001). Many of the plant secondary metabolites are constitutive, existing in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated in response to tissue damage or pathogen attack. The most commonly used solvents for investigations of antimicrobial activity in plants are methanol, ethanol, and water. Traditional healers use primarily water but plant
extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extracts. Most antimicrobial active components that have been identified are water insoluble and thus organic solvent extracts have been found to be more potent (Ncube et al., 2008). Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become ineffective. Another driving factor for the renewed interest in plant antimicrobials in the past 20 years has been the rapid rate of plant species extinction (Lewis et al., 1995) as there is a feeling among natural-products chemists and microbiologists alike that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at risk of being lost irretrievably (Borris, 1996). Several investigators have worked on antibacterial activity using medicinal plants like *Quercus infectoria* (Basri and Fan, 2005), *Senna alata* L. (Idu et al., 2007), *Croton zambesicus* Muell. Arg. (Rueben et al., 2008), *Bridelia ferruginea* (Jose and Kayode, 2009) and *Solanum nigrum* (Sridhar and Naidu, 2011). *Bridelia retusa* belonging to *Bridelia* has been reported to have medicinal properties (Jayasingh et al., 2003).

Helmintic infections are among the most common infections in man, affecting a large proportion of population all over the world. In developing countries they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travelers who have visited those areas, and some of them can be developed in temperate climates (Bundy, 1994). Parasitoses have been of concern to the medical field for centuries and the helminthes still cause considerable problems for human beings and animals. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminths and the indiscriminate use of some drugs has generated several cases of resistance. Furthermore, it has been recognized recently that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health (Patel et al., 2011). Parasitic diseases
cause severe morbidity affecting mainly population in endemic areas with major economic and social consequences. The gastro-intestinal helminthes becomes resistant to currently available antihelmintic drugs therefore, there is a foremost problem in treatment of helminthes diseases; hence there is an increasing demand towards natural antihelmintics (Kosalge and Fursule, 2009). Internal parasitic infection is a great threat to the productivity of the sheep and goat industry. Tremendous progress has been made in the development of antihelmintic drugs in the past 50 years. During this period, the current classes of synthetic drugs were developed, including the benzimidazoles and imidazothiazoles (such as levamisole). Another major step was achieved with the introduction of the avermectin class of macrolactones in the early 1980s. The discovery of this compound class led to antihelmintic drugs, such as ivermectin and doramectin, which have excellent broad-spectrum activity and superior potency. However, resistance to all of these classes of drugs has been observed, leading to the continuing need for further research to discover new classes of antihelmintics, especially those with novel modes of action (Ayers et al., 2010).

In recent years, several reports of apparent failures in the treatment of human nematodes have been published (De Clercq et al., 1997; Reynoldson et al., 1997). Although the interpretation and the implications of these studies are still being debated, they have led to an increased awareness of the potential problem of antihelmintic resistance (AR) in the treatment and control of human helminths. The concerns about AR are not superfluous in the context of serious issues of development of drug resistance in majority of the nematodes infested animals (Waller et al., 1995, 1996; Van Wyk et al., 1997). Intestinal helminth infections of livestock and humans are predominantly controlled by treatment with three classes of synthetic drugs, but some livestock nematodes have now developed resistance to all three classes and there are signs that human hookworms are becoming less responsive to the two classes (benzimidazoles and the nicotinic acetylcholine agonists) that are licensed for treatment of humans. New antihelmintics are urgently needed, and whilst development of new synthetic drugs is ongoing, it is slow and there are no signs yet that novel compounds operating through different modes of action, will be available in the market in the
current decade. The development of naturally-occurring compounds as medicines for human use and for treatment of animals is fraught with problems (Behnke et al., 2008). It would, therefore, be imperative to explore possibilities of developing new antihelmintic compounds. This has drawn attention of researchers to the validation of traditionally used botanical antihelmintics (Hammond et al., 1997; Akhtar et al., 2000; Waller et al., 2001; Iqbal et al., 2003). Because of easy availability, earthworms have been used widely for the evaluation of antihelmintic compounds in vitro (Jain and Jain, 1972; Sallaman, 1981; Dash et al., 2002; Shivkar and Kumar, 2003). Few species of plants recognized as antihelmentics include *Embelia ribes*, *Chenopodium ambrosioides*, *Dryopteris* sps and *Artemisia* sps (Prakash and Mehrotra, 1987). The antihelmintic property of plants is dependent on numerous substances that are found in them. These could be alkaloids, sugars, saponins, aromatic oils, resins and other medicinally useful chemicals (Lejoly et al., 1996).

Among other reports, Fernan-Nunez (1927) reported a centuries-old custom of native people in Columbia who used the sap of the *Ficus laurifolia* (known locally as Leche de Higueron) as a treatment for worm infections, especially *Trichuris trichiura*. Caldwell and Caldwell (1929) reported successful treatment of patients at the Searcy Hospital for the insane at Mt. Vernon, Alabama where Trichiuriasis was extensive, with Higuero latex. The reported documents on faecal egg count reductions of up to 91%, and averaging 89.5%, exceeding quite markedly the average efficacy of modern antihelmintics (Robbins, 1930) for *T. trichiura* infections. Higuero latex was also found to be effective against *Ascaris lumbricoides*, reducing faecal egg counts by 89.7%. The commercially available formulation Vermizym, manufactured in Germany, (based on papain) was shown to be effective against human pinworm (*Enterobius vermicularis*) and round worm (*Ascaris lumbricoides*) infections in a trial involving 60 subjects, who were apparently completely cleared of worms (Weise, 1950). Jonxis and Bekins (1953), in Holland, used a preparation called Velardon, also based on papain, to treat children less than 8 years old for *Ascaris* infections. Stransky and Reyes (1955), working in the Philippines, assessed Vermizyn, treating subjects with three doses at about one and a half hour intervals, each with a teaspoon of Vermizyn. They reported very high efficacy
reflected in reduced faecal egg counts after treatment and expulsion of adult *Ascaris* from treated subjects. They also confirmed that *Trichuris* infections were markedly reduced but failed to detect any effect on hookworm infections.

The stem bark of *Bridelia retusa* was dried, powdered and subjected to sequential extraction using organic and aqueous solvents in increasing order of their polarity and the crude extracts were subjected to phytochemical analysis as mentioned in Chapter I. Petroleum ether showed the presence of proteins, aromatic amino acids, triterpenoids, quinones and sterols. Chloroform extract was detected positive for proteins, quinones and sterols only. Reducing sugars, proteins, tannins, saponins, triterpenoids, sterols and glycosides were detected in ethanol extract. Aqueous extract gave positive results for reducing sugars, proteins, tannins, saponins, sterols and glycosides. Monosaccharides, amino acids, phenols, flavonoids, alkaloids, lignin, gums, mucilage, oils and fats were not detected in any of the extracts. Alkaloids showed no fluorescence while flavonoids, anthracene derivatives, bitter principles, coumarins, saponins and glycosides showed their respective color zones when phytochemical analysis of the *Bridelia retusa* S. stem bark extract was carried out using HPTLC. Studies on the dynamics of secondary metabolism indicated that there is a definite turnover of these compounds evidenced by diurnal variation, seasonal variation and different stages of development (Daniel, 2006). Plants will have different constituents depending on the climatic conditions in which it is growing (Ncube et al., 2008). Hence, the present study on the bark material was undertaken to study the antimicrobial and antihelmentic activity of stem bark extracts of *B. retusa* S.
MATERIALS AND METHODS

Microrganisms

*In vitro* antimicrobial assay was performed using two Gram positive bacteria: *Bacillus subtilis* NCIM2063, *Staphylococcus aureus* NCIM29135; two Gram negative bacteria: *Psuedomonas aeruginosa* NCIM2036, *Escherichia coli* K strain; and two yeast: *Candida albicans* NCIM3102 and *Cryptococcus luteolus* NCIM3238 obtained from National Collection of Industrial Microrganisms (NCIM), National Chemical Laboratory, Pune, India. All the microorganisms and two fungal cultures of *Aspergillus niger* and *Fusarium equisetii* were subcultured and stored at 4°C. Cultures of bacteria, yeast and fungi were inoculated in 50 ml nutrient broth, malt glucose yeast peptone and sabouraud’s broth respectively. The bacterial, yeast and fungal cultures were incubated at 37°C for 24 hrs, 22±2°C for 2-3 days and 22±2°C for 3-4 days respectively (Ncube, *et al.*, 2008).

Antimicrobial assay

The antimicrobial activity was assayed by agar disc diffusion method (Mackie and McCartney, 1996; Vaghasiya and Chanda, 2007). Molten Mueller Hilton agar (Himedia) for antibacterial study, Yeast Malt agar and Antifungal Assay agar (Himedia) for antifungal study were poured into sterile plates and allowed to solidify. The media plates were inoculated by spreading 100 μl of broth cultures. The 7 mm discs (Himedia) were saturated with 25, 50, 75 and 100 mg of extracts dissolved in 1% dimethyl sulfoxide (DMSO), allowed to dry and introduced into the inoculated agar plates. For each microbial strain, controls were maintained using only 1% DMSO. The results were obtained by measuring the zone diameter using HiAntibiotic zone scale (Himedia). The results were compared with standard antibiotics: ampicillin (25 mcg/disc), ceftriaxone (30 mcg/disc) and gentamicin (50 mcg/disc); antifungal agents: amphotericin B (20 mcg/disc) and flucanazole (10 mcg/disc).
**Antihelmintic assay**

The antihelmintic activity of the petroleum ether, chloroform, ethanol and aqueous extracts of bark of *B. retusa* was determined by using the method of Patra *et al.* (2008). The activity was evaluated on adult African night crawlers (*Eudrilus euginae*) earthworms due to its anatomical and physiological similarity with the intestinal roundworm parasites of human being. The earthworms were obtained from University of Agricultural Sciences (UAS, Dharwad) along with compost. They were washed in saline, earthworms of uniform length were chosen for the study. The extract solution containing each of petroleum ether, chloroform, alcoholic and aqueous extract (50 mg/ml in normal saline) were prepared and ten worms were placed in it. Piperazine citrate (50 mg/ml) was used as reference standard while normal saline as control. The time taken for paralyses and death of individual worms was observed. Paralysis was noted when the worms became immobile even in the normal saline solution. Death was concluded when the worms lost their motility followed by fading away of their body color.

**Statistical analysis**

The statistical analysis has been performed according to routine formulae found in standard work on biological statistics (Fisch, 1936; Snedecor, 1946; Wilks, 1949).

Following abbreviations and formulae are used.

\[
\begin{align*}
X &= \text{independent variable} \\
n &= \text{number of observations} \\
df &= \text{degree of freedom}
\end{align*}
\]

Whenever the numerical data provided, it is expressed as \((X \pm SE)\). The standard deviation and standard error of the mean were calculated by using the following formulae.
1. Standard deviation \[ S = \sqrt{(X_1-X_2)^2/n-1} \]

2. Standard error \[ SE = S/\sqrt{n} \] where S is Standard deviation

3. Degree of freedom for a difference of two means \( n_1 \) and \( n_2 \) variable

\[ df = n_1 + n_2 - 2 \]

4. Statistical significant (\( P \leq 0.05 \)) among the various parameters assessed was established by using ANOVA and Dunnet’s test (1955).
OBSERVATIONS

a. Antibacterial activity of extracts of stem bark of Bridelia retusa with different solvents and standard antibiotics (Table 1; Graphs 1, 2, 3 and 4).

Antibacterial activity was investigated using different concentrations of all four extracts on Gram positive and Gram negative bacteria and compared with standard antibiotics. No zones of inhibition were observed by Control (1% DMSO) against all the bacteria. Petroleum ether extract showed inhibition zones only against Pseudomonas aeruginosa inhibition zones of 10, 10, 14 and 19 mm for 25, 50, 75 and 100 mg respectively. Pseudomonas aeruginosa was inhibited by chloroform showing 10 mm zones for all concentrations. Petroleum ether extract showed no inhibition zones against Bacillus subtilis, Staphylococcus aureus and Escherichia coli similar to chloroform and aqueous extract. Zones of inhibition against all bacteria of Bacillus subtilis was 18, 18, 19 and 20 mm, Staphylococcus aureus was 12, 13, 14 and 15 mm, Escherichia coli was 14, 17, 21 and 17 mm and Pseudomonas aeruginosa was 14, 17, 23 and 28 mm for 25, 50, 75 and 100 mg respectively was shown by ethanol extract. The aqueous extract gave inhibition zones of 10, 11, 12 and 14 mm for 25, 50, 75 and 100 mg respectively (Figs. 9-12). Standard antibiotics of ampicillin showed zones of 26 and 40 mm, respectively against Bacillus subtilis and Staphylococcus aureus while gentamicin and ceftriaxone inhibited Bacillus subtilis by 38 and 34 mm, respectively, Staphylococcus aureus by 37 and 23 mm, respectively, Escherichia coli by 26 and 36 mm, respectively and Pseudomonas aeruginosa by 19 and 30 mm, respectively (Figs. 1-4).

In conclusion, significant antibacterial activity was observed of different extracts on the bacteria used in the study which increased with increasing concentration when compared with that of standard antibiotics. Gram positive bacteria were inhibited significantly by ethanol extract showing maximum zones of 20 and 15 mm while minimum zones of 18 and 12 mm against Bacillus subtilis and Staphylococcus aureus respectively. Amongst the Gram negative bacteria, only against Pseudomonas aeruginosa significant antibacterial activity with all the extracts tested showing maximum zones (28 mm for 100 mg) against ethanol extract while minimum zones of 14 mm for Escherichia coli. Ampicillin showed antibacterial activity only against Gram
positive bacteria while gentamicin and ceftriaxone inhibited both the bacteria. The maximum zones of inhibition shown by standard antibiotics of ampicillin, ceftriaxone and gentamicin were 40 mm (Staphylococcus aureus), 38 mm and 34 mm (Bacillus subtilis) respectively. Gram negative bacteria and Gram positive bacteria were inhibited by gentamicin and ceftriaxone with maximum inhibition against Escherichia coli.

b. Antifungal activity of extracts of stem bark of Bridelia retusa (SBEB) with different solvents and standard antifungal agents (Table 1; Graphs 1, 2, 3 and 4).

Antifungal activity was assayed against different fungal and yeast organisms using different concentrations of all four extracts and compared with standard agents. No zones of inhibition were observed by Control (1% DMSO), petroleum ether, chloroform and aqueous extract. Ethanol extract showed inhibition zones against Candida albicans was 9, 10, 12 and 13 mm for 25, 50, 75 and 100 mg respectively and Cryptococcus luteolus of 14, 16, 18 and 20 mm for 25, 50, 75 and 100 mg respectively (Figs.13-17). No antifungal effects were observed against Aspergillus niger and Fusarium equesiti. Standard drugs of Amphotericin B and flucanazole showed inhibition zones against Candida albicans of 15 and 32 mm, respectively and Cryptococcus luteolus of 21 and 29 mm, respectively while Aspergillus niger and Fusarium equesiti were inhibited to 10 mm only by Amphotericin B (Figs. 5-8). In conclusion, significant effect was observed of different extracts on the fungal species used in the study when compared with that of standard antifungal agents. Candida albicans and Cryptococcus luteolus gave significant maximum inhibitory zones of 13 and 20 mm for 100 mg while minimum zones of 9 and 14 mm for 25 mg respectively against ethanol extracts. Flucanazole showed higher antifungal activity than amphotericin B. Amphotericin B showed inhibition against all organisms and similar zones against Aspergillus niger and Fusarium equesiti.

The antimicrobial activity was studied using different concentrations of all four extracts on different microorganisms and compared with standard antimicrobial agents. All the extracts showed dose dependent significant activity which was lesser when compared with that of the respective standard drugs used in the study. Gram positive
bacteria were inhibited by ethanol extract showing maximum zones while minimum zones against *Bacillus subtilis* and *Staphylococcus aureus* respectively. Amongst the Gram negative bacteria, only *Pseudomonas aeruginosa* gave antibacterial activity for all the extracts tested while *Escherishia coli* was inhibited by ethanol and petroleum ether to same extent by 50 and 100 mg of extracts. The maximum and minimum zones for ethanol extract were observed against Gram negative of *Pseudomonas aeruginosa* while for chloroform same zones were obtained at all concentrations. *Candida albicans* and *Cryptococcus luteolus* gave maximum inhibitory zones only against ethanol extracts. No antifungal effects were observed against *Aspergillus niger* and *Fusarium equesiti*. Maximum inhibition was observed at a concentration of 100 mg of the extracts. The maximum zones of inhibition shown by standard antibiotics of ampicillin against *Staphylococcus aureus* while ceftriaxone and gentamicin against *Bacillus subtilis*. Amphotericin B showed similar zones against *Aspergillus niger* and *Fusarium equesiti*. Flucanazole showed highest antifungal activity compared with that of Amphotericin B against both *Candida albicans* and *Cryptococcus luteolus*.

c. Antihelmintic activity of extracts of stem bark of *Bridelia retusa* (SBEB) with different solvents and standard agent (Table 2; Graph 5).

Antihelmintic activity was investigated at 50 mg/ml of all four extracts on earthworms and compared with piperazine citrate. The time of paralysis of the earthworms for piperazine citrate, petroleum ether, chloroform, ethanol and aqueous extract was 69.60, 122.20, 0.43, 61.25 and 185.42 min while time of death was 71.14, 142.05, 0.67, 74.19 and 204.65 min. However, in control group, worms were observed for 24 hours and no paralysis or death was found during that period. In the present study, the extracts obtained using polar and non polar solvents were used for antihelmintic activity against earthworms. Comparing all extracts and standard of piperazine citrate at the same concentration (50 mg/ml), it was clear that chloroform extract showed significantly better effect and hence higher antihelmintic activity in comparison to petroleum ether, chloroform and aqueous extract as well as that of standard.
DISCUSSION

Medicinal plants are major sources of obtaining antimicrobial agents (Sofowora, 1986). Plants are used medicinally worldwide as sources of many potent drugs (Iwu et al., 1999). Traditional medical practitioners use a variety of herbal preparations (Sofowora, 1993) to treat different kinds of diseases including microbial infections (Mann et al., 2008). The use of plant compounds to treat infections is an age old practice in a large parts of the world, especially in developing countries, where there is dependence on traditional medicines for a variety of diseases (Gangoue-Pieboji et al., 2006; Shiba, 2005). Interest in plants with antimicrobial properties has revived as a result of current problems (Shiota et al., 2004; Abu-Shanab, 2006) associated with the use of antibiotics. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. The antimicrobial properties of plant drugs may also be addressed to the phytochemicals present (Cowan, 1999).

Plant-derived substances have recently become of great owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. It has been estimated that 14 - 28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethnomedicinal use of the plants. A number of interesting outcomes have been found with the use of a mixture of natural products to treat diseases, most notably the synergistic effects and polypharmacological application of plant extracts. The development of pharmaceuticals begins with identification of active principles, detailed biological assays and dosage formulations, followed by clinical studies to establish safety, efficacy and pharmacokinetic profile of the new drug which follows for plant therapeutic agents. Thorough biological evaluation of plant extracts is vital to ensure their efficacy and safety. These factors are of importance if plant extracts are to be accepted as valid medicinal agents. Many plants have been used because of their
antimicrobial traits and the antimicrobial properties of these plants have been investigated (Ncube et al., 2008).

Helminthes are recognized as a major problem to livestock production throughout tropics (Adejimi and Harrison 1997). Most diseases caused by helminthes are of a chronic and debilitating in nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. The parasitic gastroenteritis is caused by mixed infection with several species of stomach and intestinal worms, which results in weakness, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity (Gibbs, 1986). Chemotherapy is the only treatment and effective tool to cure and control helminth infection, as effective vaccines against have not been developed so far. Indiscriminate use of synthetic anthelmintics can lead to resistance of parasites (Singh et al., 2002). Herbal drugs have been in use since ancient times for the treatment of parasitic disease in human and could be of value in preventing the development of resistance (Chopra et al., 1956; Hammond et al., 1997). A large number of medicinal plants are claimed to possess antihelmintic activity in traditional systems of medicine and also utilized by ethnic groups worldwide. Following the folk claims, several medicinal plants, their products thereof and isolated phytoprinciples have been scrutinized for their antihelmintic activity to achieve lead molecules in the search of novel antihelmintic drugs (Satyavati, 1990).

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963). Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 1978). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavor (e.g., the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds (Cowan, 1999).
a. Antibacterial activity of extracts of stem bark of *Bridelia retusa* with different solvents and standard antibiotics

In the present study, the extracts obtained using polar and non polar solvents were used for antibacterial activity. The ethanol extract showed significantly better effect and hence higher antibacterial activity in comparison to petroleum ether, chloroform and aqueous extract. The findings of earlier investigations also suggests that methanol stem bark extracts of *Alstonia scholaris* R. Br. possessed maximum antibacterial activity (Deepti *et al.*, 2008). Observations of earlier studies indicated that the antibacterial activity of acetone extract was higher than other plant extracts of *Stevia rebaudiana*, whereas aqueous extract was practically ineffective against test organisms (Jayaram *et al.*, 2008). In the present study ethanol extract was found to be the most effective against all the bacterial cultures. Earlier investigation on chloroform and petroleum ether flower extracts of *Senna alata* was inactive on all the organisms except *Bacillus subtilis* and *Escherichia coli* indicating impotency of the extracts on the organisms similar to present study (Idu *et al.*, 2007). In the present study, the zones of inhibition of same diameter were obtained for 25, 50, 75 and 100 mg against *Pseudomonas aeruginosa* using chloroform extract. In the present study, from all the extracts highest antibacterial activity was shown by ethanol extract against *Pseudomonas aeruginosa*. From earlier studies conducted on *Origanum vulgare* and *Althea officinalis*, methanol extract showed more antibacterial activity in comparison to other organic extracts (Babu *et al.*, 2007). Similarly, hexane extracts of chicory root showed maximum inhibition at 50 μl and 100 μl in comparison to other extracts (Nandagopal and Ranjitha, 2007) which was achieved at 100 mg in the present study for the extracts studied. In the present study, aqueous extract showed inhibitory effect only against *Pseudomonas aeruginosa* but not against other organisms. In a study performed on stem aqueous extracts of *Gnandropsis pentaphylla* no inhibition was observed against the growth of *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger* and *Aspergillus flavus*. Traditional healers use primarily water as solvent, but plant extracts extracted in organic solvents have profoundly distinct antibacterial activity (Vaghasiya and Chanda, 2007) as seen in present study as well. This is interesting as water is one of
the medium through which traditional healers prescribe to their clients (Borgio et al., 2008).

Gram negative bacteria differ from gram positive in having a thick lipopolysaccharide coated cell wall (Prescott, 2002). Petroleum ether, chloroform and aqueous extract showed antibacterial activity only against *Pseudomonas aeruginosa* but had no effect on Gram positive bacteria in the present study. In the present study, the ethanol extract showed significant inhibition towards gram positive and gram negative bacteria hence proved to have a broad spectrum of antibacterial properties. Gram negative bacteria were inhibited significantly to a greater extent in comparison to Gram positive bacteria. From the present studies conducted, the most susceptible bacteria amongst the Gram negative bacteria was *Pseudomonas aeruginosa* and the most resistant Gram positive bacteria was *Staphylococcus aureus*. The activity against Gram positive and Gram negative bacteria may be indicative of the presence of broad spectrum antimicrobial compounds in *Bridelia retusa*. Acidic extract of leaves of *Basilicum polystachyon* showed higher activity against Gram positive (Chakroborty et al., 2007) contrary to present study which showed higher activity against gram negative. From the earlier studies conducted, the activity of *Cnidoscolus aconitifolius* against two different strains of bacteria (Gram positive and Gram negative bacteria) justified its application as a broad spectrum antimicrobial agent with the largest efficacy associated with ethanol extract (Awoyinka et al., 2007). Similarly, methanolic extract of *Croton zambesicus* Muell. Arg. (Euphorbiaceae) showed a wide spectrum of inhibition against most of the bacterial tested and *Proteus vulgaris* was found to be the most resistant bacteria (Rueben et al., 2008). The antimicrobial activity of the *Senna alata* L. flower extracts against both Gram positive and Gram negative bacteria were reported in earlier studies (Idu et al., 2007). Both *Musa paradisiaca, Cocos nucifera* plant extracts produced outstanding antibacterial activity against Gram positive with the greater zone of inhibition than the gram negative bacteria. Contrary to the present study, the Gram positive bacteria are more susceptible than Gram negatives (Karadi et al., 2011). In the previous work (Akter et al., 2010), the twig extracts of *Lawsonia inermis* and *Mimosa pudica* have been determined for their antibacterial properties for controlling some Gram-positive and Gram-negative bacteria. Among the three extracts, ethanol extract was more effective.
than petroleum ether and chloroform extracts against Gram-negative bacteria than Gram-positive bacteria. Khan et al. (2009) also reported that the ethanol extract of Achyranthus aspera was much affective against Gram-negative bacteria than Gram-positive one as seen in the present study. These observations may be attributed to the nature of biological active components whose activity can be increased in the presence of ethanol. Several types of alkaloids, glycosides, steroids and proteins have been reported to have the antibacterial activity (Barnabas and Nagarajan, 1988).

The antibacterial activity in the present study was observed to have increased with increasing concentrations of the extracts but the inhibitory effect was lesser than that of standard antimicrobial agents. Similar results of antimicrobial activity in ethanol extracts were reported from two varieties of Acalypha wilkesiana L. (Euphorbiaceae family) when compared with standard agents (Oladunmoye, 2006). Siddiqui et al. (1995) has also reported dose dependent antibacterial activity in Umbelliferae members. Contrary to present study, antibacterial activity reported showed high sensitivity in Solanum nigrum ethyl acetate seed extract than standard agents (Sridhar and Naidu, 2011), hence, in the present study all the extracts could not achieve results of standard drugs. Thus the indicating the extracts were not as potent as standard antibiotics at lower concentration though a further study on higher concentrations needs to be carried out. Earlier reports on Bridelia ferruginea bark extracts revealed that the methanol extract was most effective than the other extracts on Bacillus substilis and Escherichial coli while ethanol extract was most effective on Staphylococcus aureus (Jose and Kayode, 2009). Thus the present study, confirmed the medicinal properties of Bridelia retusa as reported by Shahid et al., (2009) and Tatiya et al., 2(011), even though the geographical region and the season of the Bridelia retusa used in the study differed.

In conclusion, the ethanol extract showed significantly better activity in comparison to petroleum ether, chloroform and aqueous extract. In the present study, the ethanol extract showed significant inhibition towards gram positive and gram negative bacteria hence, proved to have a broad spectrum of antibacterial properties. Gram negative bacteria were inhibited significantly to a greater extent in comparison to Gram
positive bacteria. From the present studies conducted, the most susceptible bacteria amongst the Gram negative bacteria was *Pseudomonas aeruginosa* and the most resistant Gram positive bacteria was *Staphylococcus aureus*. The antibacterial activity in the present study was observed to dose dependent effect but the inhibitory effect was lesser when compared to standard antimicrobial agents.

b. Antifungal activity of extracts of stem bark of *Bridelia retusa* with different solvents and standard antibiotics

In the present study significant antifungal activity was obtained with ethanol extract which gave the smallest inhibitory zone against *Candida albicans* and maximum inhibitory effect against *Cryptococcus luteolus*. The ethanol extract showed significant antifungal activity while petroleum ether, chloroform and aqueous extract had no effect. Similarly, amongst all the solvents ethanol extract of *Solanum nigrum* seeds showed significantly maximum activity against all fungal strains tested (Sridhar and Naidu 2011). Earlier studies indicated that the chloroform, ethyl acetate, acetone and aqueous leaf extracts of *Stevia rebaudiana* were active against *Epidermophyton species* and *Candida albicans* but ethyl acetate extract showed the highest activity against *Trichophyton mentagrophytes* and *Epidermophyton species* (Jayaram et al., 2008) but in the present study other extracts had no antifungal activity except for ethanol extract. Antifungal and anti-yeast activities of the solvent extracts of *Stevia rebaudiana* have shown that all the extracts had inhibitory effect on the growth of *Epidermophyton species*, *Cryptococcus neoformans* and *Trichophyton mentagrophytes*. The ethyl acetate extract showed highest activity against *Trichophyton mentagrophytes* and *Epidermophyton species*, and this may be due to the greater stability of the active principles in the solvent over a longer period of time (Jayaraman et al., 2008) which may also be the case in the present study. No antifungal activity was observed in the present study by all extracts against *Aspergillus niger* and *Fusarium equisetii*. Contrary in earlier studies, ethanolic and ethyl acetate seed extract showed strong activity against *Fusarium oxysporum* and *Aspergillus niger*, respectively (Sridhar and Naidu, 2011).
The antifungal activity of the extracts in the present study showed inhibitory effect lesser when compared to standard antimicrobial agents. Contrary to the present study, the antifungal activity reported showed high sensitivity in ethyl acetate extract of seeds of *Solanum nigrum* against all fungal strains tested than standard agents (Sridhar and Naidu 2011). Thus confirming medicinal properties of the stem bark of *Bridelia retusa* of the family Euphorbiaceae reported by Jayasingh *et al.*, (2003). In the fungi isolates the ethanol extracts of bark extracts of *Bridelia ferruginea* had the highest growth inhibition obtained but considerably lower than those of the control experiments in the two fungal species of *Aspergillus niger* and *Fusarium solani* (Jose and Kayode, 2009) contrary to present study showed no activity against *A. niger* and *F. equisetii*. Like in present study except for *Aspergillus niger*, fluconazole (the standard antifungal drug used), showed greater zone of inhibition against *Candida albicans*, *Candida tropicalis* and *Aspergillus niger* (Karadi *et al.*, 2011).

In conclusion, the present study significant antifungal activity was obtained with ethanol extract gave the smallest inhibitory zone against *Candida albicans* and maximum inhibitory effect against *Cryptococcus luteolus*. The ethanol extract showed significant antifungal activity while petroleum ether, chloroform and aqueous extract had no effect. No antifungal activity was observed in the present study against *Aspergillus niger* and *Fusarium equisetii*. The antifungal activity of the extracts in the present study showed inhibitory effect lesser when compared to standard antimicrobial agents.

Comparison between antibacterial and antifungal activity of *Bridelia retusa* S. stem bark extract with different solvents in the present study indicated that the antifungal activity was less compared to antibacterial activity. The antimicrobial activity observed in earlier studies in fourteen plant extracts showed antifungal activity relatively less than antibacterial activity (Vaghasiya and Chanda, 2007). Similar to the present result, *Musa paradisiaca*, *Cocos nucifera* had potential antibacterial activity as it shown larger zone of inhibition against bacterial strains than the fungal strains used in earlier studies (Karadi *et al.*, 2011). Similar results of higher antibacterial activity than
antifungal activity was observed in methanolic extract of *Mahonia leschenaultia*, Takeda root and root bark. The methanolic extracts also showed more antibacterial activity than acetone extract and broad spectrum against Gram positive and Gram negative bacteria (Duraiswamy *et al.*, 2006) as seen in the ethanolic extract of the present study. Similarly, methanolic extract of *Croton zambesicus* Muell. Arg. (Euphorbiaceae) showed a wide spectrum of inhibition against most of the bacterial and fungal strains tested (Rueben *et al.*, 2008). *Solanum nigrum* crude extract have been reported to possess a broad spectrum of activity against a panel of bacterial and fungal pathogens responsible for common microbial infections. Ethanolic extract possessed both these activities which showed more effectiveness than other extracts used in the present study. Alcoholic extracts was found to most effective against bacterial and fungal species (Jose and Kayode, 2009). Parekh and Chanda (2006) also reported that alcoholic extract of 50 Indian plants is better than aqueous extract. Overall bacterial and fungal cultures were inhibited indicating versatility as antimicrobials as also observed by Jarfa *et al.* (2004) in leaf extract of *Tapinanthus sessifolius*. Thus, the present study indicated that the antifungal activity was less compared to antibacterial activity. Ethanolic extract possessed both these activities which showed more effectiveness than other extracts used in the present study. The promissory extracts open the possibility of finding new clinically effective antimicrobial compounds (Sridhar and Naidu 2011). Different plants posses different constituents and in different concentrations, which accounts for differential antimicrobial effects (Parekh and Chanda, 2007; Ncube *et al.*, 2008). The activity of antimicrobial agent is concentration dependent (Prescott, 2002). The position of the zone edge (diameter of inhibition) is determined by initial population, density of the organisms, their growth and rate of diffusion of the antimicrobial agent (Hugo *et al.*, 1998). The discovery of potent remedy from plant origin will be a great advancement in fungal and bacterial infection therapies. However, further investigations become important to understand the exact mechanism of antimicrobial activity of *Bridelia retusa* stem bark extracts.

Phytoconstituents may be responsible for the antimicrobial activity of *Bridelia retusa* S. stem bark extract (BSBE) with different solvents. The phytochemical analysis
of stem bark of *Bridelia retusa* crude extracts in the previous chapter revealed that petroleum ether showed the presence of proteins, aromatic amino acids, triterpenoids, quinones and sterols. Monosaccharides, amino acids, phenols, flavonoids, alkaloids, lignin, gums, mucilage, oils and fats were not detected in any of the extracts. Alkaloids showed no fluorescence while anthracene derivatives, flavonoids, bitter principles, coumarins, saponins and glycosides showed their respective color zones when phytochemical analysis of the *Bridelia retusa* S. stem bark extract was carried out using HPTLC. The various phytoconstituents detected in the present study may be responsible for various properties, hence further investigation is necessary. Hence recognizing these compounds responsible for antimicrobial activity becomes necessary. These compounds posse antibacterial, bacteriostatic, antifungal activity may be due to influence on growth rate, lag time and maximum growth of microbes (Berchandt *et al.*, 2008).

Many of the earliest isolated pure compounds with biological activity were alkaloids. Naturally occurring alkaloids are nitrogenous compounds that constitute the pharmacogenically active basic principles of flowering plants. The true alkaloids are derived from amino acids, are basic and contain nitrogen in a heterocyclic ring for example, nicotine (Ncube *et al.*, 2008) which was not detected in the present study as well as not reported earlier (Banerjee and Kulkarni, 2009). The mechanism of action of highly aromatic planar quaternary alkaloids may be attributed to their ability to intercalate DNA (Phillipson and Neil, 1987). A benzylisoquinoline alkaloid, papaverine was shown to have inhibitory effect on several viruses and indoquinoline alkaloids from *Cryptolepis sanguinolenta* displayed activity against a number of gram negative bacteria and yeast (Silva *et al.*, 1996). Quinine, an alkaloid, is popular for its anti-amoebal activity against the malaria parasite (Iwu *et al.*, 1999).

Anthracene glycosides are oxygenated derivatives of pharmacological importance that are used as laxatives or cathartics, anti-inflammatory, antibacterial, antifungal and also as natural dyes. The terpenes are one of the largest and most diverse groups of plant secondary metabolites. They include sterols and triterpenes, complex compounds that are formed by the cyclization of 2,3-oxidosqualene. Sterols and
triterpenes can accumulate as glycoside conjugates in substantial quantities in plants which were detected in the present study and may be the active principle(s). These glycosides, which include steroidal glycoalkaloids, are commonly referred to as saponins. A number of studies have shown saponins to have inhibitory effects on protozoa. Saponins from *Quillaja saponaria* and *Acacia auriculoformis* were found to be antiprotozoal *in-vitro* with butanol as the main active component (Wallace, 2004). Another important sub class of compounds under the terpenes are the essential oils of which monoterpenes, diterpenes and sesquiterpenes form the majority of this sub-class. Essential oils possess biological activity including antibacterial, antiviral, antifungal and anti-inflammatory effects. Oils from *Cinnamomum osmophloeum* have been shown to possess antibacterial activity against *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* (including methicillin resistant *S. aureus*) and *Vibrio parahaemolyticus*, with cinnamaldehyde being the main antibacterial component isolated. This compound has also been widely used in antiseptic mouthwashes because of its activity against oral bacteria. Some essential oils are effective against some higher organisms such as nematodes, helminthes and insects (Wallace, 2004; Acamovic and Brooker, 2005).

The phenolics and polyphenols have been accounted as another group that has been reported to exhibit antimicrobial activity (Bennet and Wallsgrove, 1994). The mechanism thought to be responsible for phenolic toxicity to microrganisms include enzyme inhibition by the oxidized compounds possibly through reaction with sulphydryl groups or through more non specific interactions with the proteins (Mason and Wasserman, 1987) which was detected in the present study. Important subclasses in this group of compounds which have been found to have antimicrobial activity include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. Flavones, flavonoids and flavonols have been known to be synthesized by plants in response to microbial infection so it is not surprising that they have been found, *in vitro*, to be effective antimicrobial substances against a wide array of microorganisms (Bennet and Wallsgrove, 1994). The activity of flavonoids may be due to its ability to complex with extracellular and soluble proteins and complex with bacterial cell walls
which may also be the reason in the present study. The lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya et al., 1996). Tannins have been traditionally used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, hemorrhoids and diarrhea (Ogunleye and Ibitoye, 2003). As a group, coumarins have been found to stimulate macrophages, which could have an indirect negative effect on infections (Cowan, 1999). Both coumarins and tannins were also detected in the present study. One of their molecular actions may be by complexing with proteins through non specific forces such as hydrogen bonds, hydrophobic bonds and covalent bonds (Haslam, 1996 and Stern et al., 1996). Thus their mode of antimicrobial action is related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins, etc. Hence recognizing the exact mode in the present study of these phyoconstituents will need further investigation (Ya et al., 1988).

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils (Jansen et al., 1987) as well as in tannin (Saxena et al., 1994). External plant surfaces are often protected by biopolymers for example, waxes fatty acid esters such as cutin and suberin. In addition, external tissues can be rich in phenolic compounds, alkaloids, diterpenoids, steroid alkaloids and other compounds which inhibit the development of fungi and bacteria. Cell walls of at least some monocotyledons also contain antimicrobial proteins, referred to as thionins (Angeh, 2006). Thus in the present study, the overall antimicrobial activity may be attributed to the presence of one or more phytochemicals detected which can have individual or cumulative effect.
The medicinal plants occupy significant place in the modern medicine as raw material of some important drugs. However, WHO also recognized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for botanical medicine. Among bacterial strains, *Bacillus subtilis* a gram negative bacterium that contaminates wounds, *Staphylococcus aureus* is one of causative bacterium community acquired pneumonia (Prescott *et al.*, 1999: Strainer *et al.*, 1986). *Staphylococcus aureus* is one of the most common Gram-positive bacteria causing food poisoning. An enterohemorrhagic strain of *Escherichia coli* has caused serious cases of food poisoning and preservatives to eliminate its growth are needed. *E.coli* accounts for about 90% of the urinary tract infections occurring in young women (Brooks *et al.*, 2002; Usman *et al.*, 2007). *Candida albicans* is the microbe responsible for most clinical yeast infections, e.g. in mouth infections (Gulcin *et al.*, 2004). *C albicans* can cause pulmonary (Sharma *et al.*, 2002) and oral (Alexopolous and mimis, 1993) candiadisis. *Aspergillus* are pathogenic to humans and cause Aspergillosis, a disease of lungs which is quite common in patients with malignancies specially leukemia, lymphoma and also in immunosuppressed patients (Alexopolous and mimis, 1993). Fungi are achlorophyllylous and heterotrophic in nature and comprise out of 1.5 million species only 74,000 are described while more than 300 species are potentially allergy in man (Gupta *et al.*, 2002). Many species of fungi cause serious diseases of useful plants like wheat, rice, maize, etc. Hence, fungi are regarded as causative agents of plant pathology. Similarly man and mammalians, as well as reptiles, fishes, amphibians are also susceptible to fungal infections (Campbell *et al.*, 2000; Alexopolous, 1979; Dube, 1990).

c. Antihelmintic activity of extracts of stem bark of *Bridelia retusa* with different solvents and standard agent.

In the present study, the extracts obtained using polar and non polar solvents were used for antihelmintic activity against earthworms. Using all extracts and standard of piperazine citrate of same concentration (50 mg/ml), it was clear that chloroform extract showed significantly better effect and hence higher antihelmintic activity in comparison to petroleum ether, chloroform and aqueous extract as well as that of
standard. The leave petroleum ether and chloroform extract of leaves of *Benincasa hispida* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 50 mg/ml, in shorter time as compared to reference drug piperazine citrate, alcoholic and aqueous extracts (Bhattacharjee *et al*., 2010) as also seen in the present study with best results for chloroform extract. Mali and Mahale (2008) reported the aqueous and ethanolic extracts of *Rhynchosia minima* not only demonstrated paralysis, but also caused death of worms especially at higher concentration, 100 mg/ml in shorter time as compared to reference drug piperazine citrate, petroleum ether and chloroform. The antihelmintic activity of *R. minima* may be attributed to the presence of active components such as flavonoids, tannins and terpenoids, the antihelmintic properties of which are well documented (Niezen *et al*., 1995). The ethanolic extract of the drupes of *Melia azedarach* L. was active against both the tapeworm and the earthworm tested and found to be comparatively more active than piperazine phosphate against *Taenia solium* (Szewczuk *et al*., 2003) as also seen in chloroform extract of the present study. The methanolic extract of *Indigofera tinctoria* displayed a significant antihelmintic property in a dose dependent manner giving shortest time of paralysis and death with 100 mg/ml concentrations, comparable with the standard drug (Balamurugan and Selvarajan, 2009) as seen at 50 mg for chloroform extract in the present study. From the results it is observed that *Moringa oleifera* showed potent antihelmintic activity along with *Vitex negundo* (Rastogi *et al*., 2000). It was concluded that active constituents responsible for antihelmintic activity was present in the ethyl acetate and petroleum ether extracts of seeds of *Pongamia glabra* comparable to standard drugs (Nirmal *et al*., 2007) which may be present in the chloroform extract of the present study. This indicates that the antihelmintic principles are non polar compounds as also seen in the present study. The results of antihelmintic activity revealed that petroleum ether, chloroform, ethanol and aqueous extracts exhibited varying degree of activity against the earthworms and caused paralysis followed by death which may be attributed to the presence of different phytoconstituents. The possible mechanism of the antihelmintic activity of *Asta churna* from previous results may be due to its effect on inhibition of glucose uptake in the parasites and depletion of its glycogen synthesis (Devi *et al*., 2009) which may be so in the present study as well.
Helminthiasis is among the most important animal diseases inflicting heavy production losses. The disease is highly prevalent particularly in third world countries (Dhar et al., 1982) due to poor management practices. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, increasing problems of development of resistance in helminths (Geert and Dorny, 1995; Coles, 1997) against antihelmintics have led to the proposal of screening medicinal plants for their antihelmintic activity. The plants are known to provide a rich source of botanical antihelmintics (Satyavati et al., 1976; Lewis and Elvin-Lewis, 1977). A number of medicinal plants have been used to treat parasitic infections in man and animals (Nadkarni, 1954; Chopra et al., 1956; Said, 1969; Akhtar et al., 2000). The antihelmintic activity of Nigella sativa, Ferula foetida, Cuminum cyminum, Piper longum, Piper nigrum have also been reported (Devi et al., 2009). The methanolic extract of bark of Terminalia arjuna showed antihelmentic activity against eggs, larvae and adult of Haemonchus contortus (Bachaya et al., 2009). It was reported that the extracts of Tetradenia riparia, Cassia occidentalis, Carica papaya, Momordica foetida, and Erythrina abyssinica showed antihelmintic properties (Peter and Deogracious, 2006). A study revealed a trypanocidal potential in methanolic extract of B. ferruginea stem bark which was attributed to the presence of alkaloids, tannins saponins, steroids and phlobatanins (Ekanem et al., 2008) which may be so in the present study as it belongs to Bridelia family. Oryema (1997) reported that substances like steroids, coumarins, tannins, and triterpoids and other chemical constituents of plants like alkaloids, glycosides, enzymes, anthraquinones, tannins, gums, fixed oils, fats, waxes, volatile oils, proteins and carbohydrates all have medicinal or pharmaceutical value, few of which were also tested present in the present study on B. retusa. Toxicity studies of the extracts should also be done to determine the safety indices of the extracts. Studies to determine the mechanisms of the action, compatibility with other drugs, side effects and other important parameters is also needed.

The phytochemical constituents in the plant extracts play an important role in antihelmentic activity. The antihelmintic effects of tannins may be attributed to its
capacity to bind free protein available in the tubes for larval nutrition and thus reduced nutrient availability could have resulted in larval starvation or decrease in gastrointestinal metabolism directly through inhibition of oxidative phosphorylation (Scalbert, 1991), causing larval death (Athanasiadou et al., 2001). Though, condensed tannins have been reported to exert direct antihelmintic effects, other phytochemicals like alkaloids, flavonoids and oleane type triterpenes (Anjaneyulu and Prasad, 1982; Gary and Kasera, 1983; Tripathi et al., 1992; Irobi et al., 1994; Brantner et al., 1996) Tannins were shown to produce antihelmintic activities (Niezen et al., 1995). Chemically tannins are polyphenolic compounds (Bate-Smith, 1962). Some synthetic phenolic antihelmintics e.g. niclosamide, oxyclosamide and bithinol are shown to interfere with the energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997). It is possible that tannins contained in the extracts in the present study also produced similar results. Another possible antihelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal (Athnasiadou et al., 1971) or glycoprotein on the cuticle of the parasite and cause death (Thompson and Geary, 1995). Results reported in the resent work constitute rational evidence and a scientific basis to justify and support the folklore claims of the potential antihelmintic activity of *B. retusa*. Further studies are required on phytochemical profiling as well as isolation and identification of bioactive component responsible for antihelmintic activity.

From earlier study it can be concluded that the petroleum ether, chloroform, alcoholic and aqueous extracts of leaves of *Hygrophila spinosa* were having both antihelmintic and antibacterial activities wherein alcoholic extract showed the highest activity (Patra et al., 2008). In the present study, the extract of *B. retusa* showed highest antimicrobial activity with ethanolic extract while antihelmentic by chloroform extract. It was quite apparent from the earlier studies that the steroid present in the ethanolic extract leaves of *Lagenaria siceraria* possesses significant antihelmintic activity and antimicrobial activity (Badmanaban and Patel, 2010) which may be so in the present study also. Among extracts of *Ramalina hossei* (Lichens) tested, methanol extract was found to produce marked inhibition of test fungi while potent antihelmintic activity was
observed in case of methanol extract followed by other extracts (Kumar et al., 2010). Thus the phytoconstituents present in the plant responsible for the antimicrobial and antihelmentic activity may be due to one or more constituent(s). Hence, scientists from divergent fields are investigating plants with an eye to evaluate their antimicrobial usefulness. A sense of urgency accompanies the search as the pace of species extinction continues. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types of organisms in vitro. More of these compounds should be subjected to animal and human studies to determine their effectiveness in whole-organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal microbiota. It would be advantageous to standardize methods of extraction and in vitro testing so that the search could be more systematic and interpretation of results would be facilitated. Also, alternative mechanisms of infection prevention and treatment should be included in initial activity screenings. Disruption of adhesion is one example of an anti-infection activity not commonly screened. Attention to these issues could usher in a badly needed new era of chemotherapeutic treatment of infection by using plant-derived principles (Cowan, 1999). Plant based products with enormous therapeutic potential as they can serve the purpose with lesser side effects, hence continued exploration is needed in the current scenario. The traditional medicines hold a great promise as a source of easily available effective biological agents to the people, particularly in developing countries, including India. Indigenous system of medicine reports a number of natural products for their biological efficacy. However, their scientific evaluation as compared to commercial agents is limited. Nowadays even there is risk of development of resistance by pathogens and parasites to the drugs (Kumar et al., 2010).
SUMMARY AND CONCLUSION

The petroleum ether, chloroform, ethanol and aqueous extracts obtained by sequential extraction from stem bark of Bridelia retusa were subjected to antimicrobial and antihelmintic screening against Bacillus subtilis, Staphylococcus aureus, Psuedomonas aeruginosa, Escherichia coli, Candida albicans, Cryptococcus luteolus Aspergillus niger and Fusarium equisetii and earthworms which were then compared with standard drugs.

1. Ampicillin showed antibacterial activity only against Gram positive bacteria while gentamicin and ceftriaxone inhibited both the bacteria. Flucanazole showed higher antifungal activity than amphotericin B. Amphotericin B showed inhibition against all organisms and similar zones against Aspergillus niger and Fusarium equesiti. Control of 1% DMSO which was used as solvent for the extract showed no inhibition zone.

2. Gram positive bacteria were inhibited by ethanol extract Bacillus subtilis and Staphylococcus aureus while amongst the Gram negative bacteria, only Pseudomonas aeruginosa gave antibacterial activity for all the extracts tested with maximum zones against ethanol extract though comparatively lesser than standard antibiotics.

3. Candida albicans and Cryptococcus luteolus was inhibited by only ethanol extracts which was lesser than standard antifungal agents. All the extracts had no effect against Aspergillus niger and Fusarium equesiti.

4. The antifungal activity was less compared to antibacterial activity. Ethanolic extract possessed both these activities which showed more effectiveness than other extracts used. The antimicrobial activity was observed to dose dependent effect but the inhibitory effect was lesser when compared to standard antimicrobial agents.
5. The antihelmintic activity was maximum in chloroform extract which was better than standard and in comparison to other extract followed by ethanol extract which showed similar effects to standard while the rest of the extract showed poor effects, all the extracts were compared with standard drug at 50 mg/ml. The control showed no mortality for 24 hrs in the present study. Earthworms were chosen in the present study due to their resemblance to human and animal gut parasites. The death of earthworms occurred within few minutes of their paralysis.

6. Terpenes, saponins, anthracenes and sterols present in free or in conjugation with glycosides, flavonoids, quinones, tannins and/or coumarins may be responsible for antimicrobial activity by acting on cell wall, enzymes, cell envelope and other proteins, prevention of adhesion, etc. The antihelmintic effect may be due to the phytoconstituents in the extract that interferes with the energy generation in helminth parasites by uncoupling oxidative phosphorylation or binding to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and finally causes death.

7. The overall antimicrobial and antihelmintic activity may be attributed to the presence of one or more phytochemicals which can have individual or combined effect. The antibacterial, bacteriostatic, antifungal activity may be due to influence on growth rate, lag time and maximum growth of microbes. The antihelmintic activity of the extract by paralysis and death of earthworms may be due to direct attack on earthworms or their food consumption or their attachment properties, etc.

In conclusion, stem bark of Bridelia retusa showed a broad range of antimicrobial and antihelmintic activity. But further studies need to be carried out to provide knowledge on the metabolites responsible for such therapeutic quality.
EXPLANATION TO PHOTOGRAPHS

1. The bacterial culture of *Bacillus subtilis* showed zones of growth inhibition marked a, b and c by standard antibiotics of ampicillin, ceftriaxone and gentamicin, respectively.

2. The bacterial culture of *Staphylococcus aureus* showed zones of growth inhibition marked a, b and c by standard antibiotics of ampicillin, ceftriaxone and gentamicin, respectively.

3. The bacterial culture of *Escherichia coli* showed zones of growth inhibition marked a, b and c, d by standard antibiotics of gentamicin and ceftriaxone, respectively.

4. The bacterial culture of *Pseudomonas aeruginosa* showed zones of growth inhibition marked a, b, c and d by standard antibiotics of gentamicin and ceftriaxone, respectively.
5. The fungal culture of *Candida albicans* showed zones of growth inhibition marked a, c and b, d by standard drugs of fluconazole and amphotericin, respectively.

6. The fungal culture of *Cryptococcus luteolus* showed zones of growth inhibition marked a, c and b, d by standard drugs of fluconazole and amphotericin, respectively.

7. The bacterial culture of *Fusarium equisetti* showed zones of growth inhibition marked b and d by amphotericin.

8. The bacterial culture of *Aspergillus niger* showed zones of growth inhibition marked b and d by amphotericin.
9. The bacterial culture of *Pseudomonas aeruginosa* showed zones of growth inhibition marked a, c, b and d by petroleum ether extract of *Bridelia retusa* at different concentration of 25, 50, 75 and 100 mg respectively.

10. The bacterial culture of *Pseudomonas aeruginosa* showed zones of growth inhibition marked a, c, b and d by chloroform extract of *Bridelia retusa* at different concentration of 25, 50, 75 and 100 mg respectively.

11. The bacterial culture of *Bacillus subtilis* showed zones of growth inhibition marked a, c, b and d by ethanol extract of *Bridelia retusa* at different concentration of 25, 50, 75 and 100 mg respectively.

12. The bacterial culture of *Staphylococcus aureus* showed zones of growth inhibition marked a, c, b and d by ethanol extract of *Bridelia retusa* at different concentration of 25, 50, 75 and 100 mg respectively.
13. The bacterial culture of *Escherichia coli* showed zones of growth inhibition marked d, c, a and b by ethanol extract of *Bridelia retusa* at different concentration of 25, 50, 75 and 100 mg respectively.

14. The bacterial culture of *Pseudomonas aeruginosa* showed zones of growth inhibition marked d, c, a and b by ethanol extract of *Bridelia retusa* at different concentration of 25, 50, 75 and 100 mg respectively.

15. The fungal culture of *Candida albicans* showed zones of growth inhibition marked a, c, d and b by ethanol extract of *Bridelia retusa* at different concentration of 25, 50, 75 and 100 mg respectively.

16. The fungal culture of *Cryptococcus luteolus* showed zones of growth inhibition marked d, c, a and b by ethanol extract of *Bridelia retusa* at different concentration of 25, 50, 75 and 100 mg respectively.

17. The bacterial culture of *Pseudomonas aeruginosa* showed zones of growth inhibition marked a, c, b and d by aqueous extract of *Bridelia retusa* at different concentration of 25, 50, 75 and 100 mg respectively.