Chapter II

Review of Literature
REVIEW OF LITERATURE

One of the major challenges of twenty first century lies in the field of healthcare, especially in finding out new remedies for the treatment of recently emerged infectious viral diseases like AIDS, SARS etc., along with their secondary infections caused by bacteria, fungi and protozoa and different types of cancers due to immunodeficiency. Bacterial, fungal and protozoan diseases can be treated with various commercially available antibacterial, antifungal and antiprotozoan drugs even though these are very costly now a days. But viral diseases, in general are not treated using such agents because of the serious side effects produced by antiviral agents available now. This is because of the fact that highly specific agents are required for the destruction of viruses, without affecting the host genome. Specific antiviral drugs are very few and are not recommended routinely. Other antimicrobial agents also are toxic and have the risk of development of drug resistance in bacteria more easily.

Developed countries utilize various synthetic preparations which are very costly and with much toxicity. Developing countries like India are facing an alarming situation because of the huge
number of patients infected by different types of pathogens and the rapid spreading of diseases without much control. Here comes the importance of herbal medicines which are not causing much financial expenses. More over, lesser toxicity is expected in herbal preparations.

Another advantage of using natural herbal preparations is that no problem of drug resistance are reported yet towards these agents. This makes the herbal medicines more acceptable. Many countries across the world, including developed countries are interested in such natural healing products because of the lesser toxicity and so are encouraging the use and development of natural curing products.

The search for new herbal drugs are going on in different countries including India. A newly developed discipline called Ethnopharmacology deals with various such drugs, as the secondary metabolites from plants like alkaloids, terpenoids, tannins, flavanoids etc., have various pharmacological activities. India is actually the ancient centre of herbal therapy and Ayurveda, the indigenous medicinal system of the country, utilize a wide variety of plants. A number of treatment measures using various plant parts are reported in Ayurvedic literature. These traditional systems are
getting more public interest and relevance in synergistic treatment. The traditional healers use crude extracts which possess different pharmacological activities for the treatment of various diseases including infectious diseases. Some of these plants routinely used are aromatic, like basil, coleus, pepper, clove and other spices etc., whose volatile oil has antimicrobial properties also. For some ailments, fruits like papaya, pomegranate, grapes, mango etc., are used which also have antimicrobial properties.

A review of literature on the various medicinal properties especially the antimicrobial activities of plants used to treat a wide range of diseases are described in the following pages.

The references regarding the curative properties of some herbs in Rigveda seem to be the earliest records of use of plants in treatment. Palash, pipal etc., are very briefly referred in Rigveda, but in Atharvaveda, a more detailed account of plants are available. Later Charaka Samhita and Susruta Samhita recorded many drugs which are herbal in origin, but some plants are now not seen in India. This may be because of the geographical and environmental changes occurred later, which affected the biodiversity.
The voluminous literature on Indian medicinal plants illustrates the antibacterial values and utility of herbs in the treatment of a variety of infectious diseases [Kritikar and Basu, (1935); Nadkarni (1954); Chopra et al., (1956); Ogale, (1986); Sawant, (1974); Deshpande et al., (1989)]. Out of the two thousand Indian drugs, about one thousand and five hundred are of plant origin and the rest only are animal or mineral origin. When considering our plant variety and country area, this number is not very large. Medicinal plants have been used for centuries to treat a wide range of diseases and many are still in use today (Whistler, 1992).

*Ocimum sanctum*, the sacred Tulsi is found to have antibacterial property (Phadke and Kulkarni, 1989). In addition, it posses anti-inflammatory (Singh, 1988), antioxidant (Maulik et al., 1997), anti ulcer (Sairam et al., 2000), antifertility (Kasinathan et al., 1972), antispermatogenic (Seth et al., 1981), immunoregulatory (Mediratta et al., 1988), hypoglycemic (Chattopadhyay, 1993) and anti stress activities (Bhargava and Singh, 1981). The leaves of the plant have been used as an expectorant, diaphoretic, anticancer, antihelmintic, antiseptic, analgesic and tonic rejuvenator. [Kritikar and Basu, (1975); Chopra et al., (1958)]. The antibacterial activity of ether extract of the leaves against *Escherichia coli, Staphylococcus*
aureus and Mycobacterium tuberculosis have been reported [Joshi and Magar, (1952); Gupta and Vishwanathan, (1955)]. The Essential oils of O. sanctum possess antifungal activity against Aspergillus niger, Rhizopus stolonifer and Penicillium digitatum (Grover and Rao, 1977). It was reported that the aqueous extract is a better inhibitor for enteric bacteria, Staphylococcus aureus and Candida albicans rather than alcoholic extract; while alcoholic extract showed more activity against Vibrio cholerae (Geeta et al., 2001). The alcoholic and aqueous extract of O. sanctum have shown adaptogenic action against antigenic challenge of Salmonella typhi (Godhwani et al., 1987).

Alcoholic extract of root of Aegle marmelos was found to have antibacterial properties against both Gram positive and Gram negative bacteria, and Gram positive bacteria are more inhibited in lesser concentrations (Valsaraj et al., 1997). The antifungal and antibacterial activity of alcoholic and aqueous extract of leaves of Aegle marmelos in high concentration was reported by Sasidharan et al., (1998). Wound healing activity of it was reported by Jaswanth et al., (2001).
Lawsonia alba is traditionally used against fungal infections in many countries including India. The leaves in the form of a paste (henna) has been regarded as excellent topical remedy for ringworm in indigenous medicine (Kritikar and Basu, 1935). The antidermatophytic activities of ethanol, ethyl acetate and hexane extracts of L. alba were reported (Natarajan, et al., 2000). The active compound was identified as lawsone [Dixit et. al., (1980); Natarajan and Lalithakumar, (1987)]. The antibacterial activity of methanolic extract of L. alba leaves against Escherichia coli, Staphylococcus aureus and Staphylococcus epidermidis was also reported (E Thomas, et al., 1999).

Medicinal properties of Camelia sinensis were known to mankind since antiquity. The antibacterial property of tea was first reported in Japan by using Japanese tea against various diarrhoeal pathogens. (Shimamura et al., 1989). The bactericidal activity of tea against bacterial isolates from infected root canal provide enough evidences to support the antimicrobial activity of tea. (Horiba et al., 1991). MRSA (methicillin resistant Staphylococcus aureus) were considerably inhibited by low concentration present even in ordinarily brewed tea according to Toda et al., (1991). The alcoholic extract of Camelia sinensis inhibited Salmonella serotypes causing
enteric fever. (Ciraj et al., 2001). The antiviral activity of tea also was reported (John and Mukundan, 1979).

*Alstonia scholaris* is a plant of interest as it is mythologically important in Hinduism. Its root bark was found to have antibacterial activity against both Gram positive and Gram negative bacteria (Valsaraj et al., 1997).

In a study conducted by Valsaraj et al., (1997) the seeds of *Carica papaya* was found to inhibit Gram positive bacteria like *Staphylococcus aureus*.

*Leucas aspera* is an aromatic plant and is used for various medicinal purposes. It is widely used in India as a mosquito repellent from ancient times. Still now it is practiced as a home remedy. The methanolic extract of *Leucas aspera* was found to have antistaphylococcal activity (E Thomas et al., 1999). Its activity against Gram positive bacteria was reported by Valsaraj et al., (1997).

*Glycosmis species* belongs to Rutaceae family and posses sulphur containing amides like sinharine which exhibit antifungal activity against Cladosporium. (Greger et al., 1993).

*Eupatorium odoratum* is used in Kerala for various skin infections. The antibacterial and antifungal activity of alcoholic
extract of this plant was tested and found to be promising (Sasidharan, *et al.*, 1998). Its activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* was also reported. (R Rana *et al.*, 1998).

Members of Aristolochiaceae family are having many medicinal effects and various alkaloids and terpenes were isolated from different species. Among the four hundred species, only five are seen in India. *Aristolochia indica* and *Aristolochia tagala* are commonly seen and are used to treat stomach ache, snake bite and rheumatism in different countries including India. It is used as an antifertility agent also [Nadkarni, (1976); K K Sundari *et al.*, (2001)]. The antiviral activity of *Aristolochia elegans* was reported by Berghe, *et al.*, (1978). *Aristolochia cucurbitifolia* is used as an expectorant, analgesic and antiasthmatic agent and also for the treatment of snake bite and lung inflammation. (Tian-Shung *et al.*, 1998). *Arstilochia debilis* contains an aristolane sesquiterpene aldehyde in its root, which showed moderate antibacterial activity (Rodriguez, *et al.*, 1995). *Thottea siliquosa*, one of the members seen in Kerala is found to have antibacterial activity against both Gram positive and Gram negative bacteria and Gram positive were found more susceptible to it (Valsaraj *et al.*, 1997). But the chronic use of these plants which
contain aristolochic acids produces nephrotoxicity and so prohibition of their use is recommended. (Hashimoto et al., 1999).

The antimicrobial effect of *Tamarindus indica* in high concentration (both aqueous and alcoholic) was reported by Sasidharan et al., (1998). It was found active against bacteria like *Escherichia coli* and *Staphylococcus aureus* and also against fungi like *Aspergillus niger* (Sasidharan et al., 1998). The antibacterial effects were reported by Melendez and Capriles, (2006) against *Staphylococcus aureus* and *Escherichia coli*.

*Aloe vera* is traditionally used as a herbal remedy for various cosmetic and medical problems in many countries. (Morton, 1961). The anti inflammatory activity of the gel from *Aloe vera* was reported in 1996 by Beatriz et al. This effect was suggested due to the inhibitory action on the arachidonic acid pathway via cyclooxygenase. *Aloe vera* is used for topical treatment of skin burns and wounds also (Rovatti and Brennan, 1959). Wound healing property of *Aloe vera* was reported by Chitra et al., (1998). The antibacterial activity against *Staphylococcus* by *Aloe vera* gel was reported recently and the effect was attributed to the anthraquinones present in it (Agarry et al., 2005).
Medicinal effects of Allium species especially *A. cepa* and *A. sativum* are well known for centuries and many researchers conducted various experiments on the isolation and characterization of the active principles involved. The anti bacterial effect of *Allium sativum* (garlic) was tested against human pathogenic bacteria like *Staphylococcus aureus*, *Salmonella typhimurium* and *Yersinia enterocolitica* by Elizabeth (2001) and found that the MICs and MBCs are 7.5, 8 and 10 mg per ml and 22.5, 24 and 40 mg per ml respectively for the three pathogens. It also showed activity against bacterial isolates like *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and also against the yeast like fungi, *Candida albicans*. These results suggest that it can be used as a broad spectrum antimicrobial agent. (Elizabeth, 2001). *Bacillus cereus*, a Gram positive bacillus which can cause food poisoning was also inhibited by garlic. (Zahira et al., 1982).

Antibacterial effect of garlic on various pathogens including food poisoning micro organisms were tested by various scientists [Al-Delaimy and Ali, (1970); De Wit et al., (1979); Mantis et al., (1978); Tynecka and Gos, (1973); Walton, et al., (1936); Woo, (1973)]. Its antifungal effects also were reported [Barone and Tansey, (1977); Moore and Atkins, (1977)]. Appleton and Tansey,
African Journal of Microbiology Research

Review of Literature

(1975) reported that aqueous extract inhibited many species of zoopathogenic fungi. Amonkar and Banerji, (1971) reported its larvicidal principle. Oil of garlic and onion in very small concentrations was found to be inhibitory to both food spoilage yeast and industrially important yeasts. (Conner and Beuchat, 1984). Dankert et al., (1979) reported that crude juice of garlic inhibited both Gram positive and Gram negative bacteria and yeast, while onion juice inhibited only Gram positive bacteria. Similar observations were obtained by Elnima et al., (1983). The antimicrobial property was attributed to ajoene (a sulphur containing compound with a double bond) and MICs for different pathogens were determined by Naganawa et al., (1996).

*Helicobacter pylori*, a Gram negative bacterium which is now proved to be the causative agent of gastric ulcer was found inhibited by aqueous garlic extract and MIC was found to be 40 mcg thiosulfinate per ml present in it (Sivam et al., 1997).

Aspergillus, the commonest cause of otomycosis was also inhibited by aqueous garlic extract and concentrated garlic oil. (Pai and Platt, 1995). In a comparative study, it was revealed that raw garlic was more potent than tetracycline hydrochloride in equal
concentrations towards caecal microflora. (Shashikanth et al., 1984).

Allicin, an active component from garlic was isolated and characterized (Adetumbi and Lau, 1983). It was reported that the anti candidal effect of garlic is due to the changes exerted by allicin on the structure and integrity of the outer surface of the yeast cells. (Mahmoud, 1988).

Many spices are proved to have a number of medicinal properties and are used in all continents as flavouring agents in various food items and also for the treatment of many infections. It was reported that essential oils from spices inhibit food poisoning bacteria [Aktug and Karapinar, (1986); Beuchat, (1976); Dabbah et al., (1970); Gnan and Sheriha, (1986); Huhtanen, (1980); Martini, (1980); Raccach and Henningsen, (1984); Saleem and Al-Delaimy, (1982); Sampurna and Nigam, (1979 and 1980); Tharib et al., (1983)].

Peppermint (Mentha piperita) is used as a stimulant, carminative and for curing local pains. (Singh and Singh, 1996). When compared with other spices, the inhibitory effect on yeast produced by mint was negligible according to Conner and Beuchat (1984). Pimenta dioica (all spice) was tested by many workers and were found to have antifungal effects against toxin producing fungi [Hitokoto et al., (1980); Azzouz and Bullerman, (1982)].
Oil from Pimenta was found to have high antibacterial activity against both Gram positive and Gram negative bacteria (Deans and Ritchie, 1987). Pimenta is a well known herbal remedy used in the treatment of high blood pressure, high blood sugar, obesity and irregular digestion (Vargas, 1990). The leaves posses tannins and essential oils (Dominguez et al., 1962). The antibacterial property against *Aeromonas hydrophila* and *Listeria monocytogenes* also were reported. (Yun-Yun Hao et al., 1998). Strong antilisteric activity of Pimenta was reported by Hefnaway et al., (1993).

*Murraya koenigii* (curry leaf) is a spice commonly used in Kerala in almost all curries. It was reported to posses antibacterial and antifungal activities. [Valsaraj et al., (1997); Sasidharan et al., (1998); Das et al., (1965)]. *Klebsiella pneumoniae*, a highly resistant Gram negative bacterium and *Staphylococcus epidermidis* were found to be inhibited by *M.koenigii* (E Thomas et al., 1999). The antiulcer activity of ether and aqueous extracts was found to be significant by Aswatha Ram et al., (2002).

*Azadiracta indica* (neem) is well known to have many medicinal properties. Its antibacterial, antifungal and even antiviral properties are reported. The antiviral properties were known to
common people from ancient times and because of this, they used the leaves as a bed during chicken pox and small pox attacks. It was found that a 10% aqueous extract of the leaves prevented plaque formation of vaccinia virus in tissue culture. The experiment on the skin of rabbits and monkeys showed the antiviral effects of the leaf extract (Rao et al., 1969). The antibacterial effect of A.indica was reported by Srivastava, (1986), E Thomas et al., (1999) and E Thomas, (2002). Antimalarial activity of methanolic extract of its leaves was also reported (Badam et al., 1988). The antifungal effect of neem and preparations containing neem was reported by Govindachari et al., (2000).

The antibacterial, antifungal and antiviral properties of Moringa oleifera were studied by different researchers. In a study, the methanolic extract of stem bark of it was found to have considerable inhibitory effect on Staphylococcus aureus and Bacillus subtilis while it was found ineffective against Escherichia coli at the concentrations tested. (Valsaraj et al., 1997). The 50% methanol extract of root and bark showed antiviral activity against vaccinia virus (Dhar et al., 1968). Babbar et al., (1970) studied the root extract of the plant and reported its antiviral action against vaccinia. Alcoholic extract of the plant at 5% concentration inhibited
Aspergillus niger, Staphylococcus aureus and Escherichia coli and aqueous extract at same concentration was found to be less effective (Sasidharan et al., 1998).

Psidium guajava (guava) showed antibacterial effect towards Gram positive bacteria rather than Gram negative bacteria and methanolic extract was found to be more effective than aqueous extract, but no effect was found against Klebsiella pneumoniae (Rabe and Staden, 1997). It has known antibacterial action with the leaf yielding three active substances, namely quercetin, avicularin and guaijaverin. [Khadem and Mohammed, (1958); Watt and Breyer-Brandwijk, (1962); Oliver-Bever, (1986)]. Lutterodt (1989] suggested inhibition of gastrointestinal release of acetyl choline by quercetin as a possible mode of action of P.guajava leaf extracts in the treatment of acute diarrhoeal disease. Quercetin has been reported for its antiviral activity against HSV-1 in vero cells. (Amoros et al., 1992). The effect of aqueous extract of the leaf of this plant was studied by Gnan and Demello, (1999) and found that it was highly active against different strains of Staphylococcus aureus, a potential human pathogen. This justifies the use of its decoction for the treatment of boils (Gelfland et al., 1985). Gnan and Demello, (1999) observed a complete inhibition of all the strains of
Chapter II

*S. aureus* (causing food poisoning and pyogenic infections) tested, at a concentration of 6.5 mg per ml. This indicates that the extract could be an important source of food preservative and a new source of an antimicrobial agent against *S. aureus*, which is notorious in drug resistance. In Brazil, the bark of the plant is used for common and infantile diarrhoea, leucorrhoea, cholera and external ulcers; root and leaf for diarrhoea; flowers and bud for diarrhoea, stomach and skin diseases and for bloody dysentery (Gnan and Demello, 1999).

According to Lima *et al.*, (1993) *Cymbopogon citratus* leaves have antifungal activity, which may be due to the oils and derivatives. Its antibacterial activity also was reported by Sasidharan *et al.*, (1998).

*Tinospora cordifolia* is reported to have antibacterial activity against Gram positive and Gram negative bacteria. Methanolic extract of leaf and stem were found to be effective against bacteria. (Valsaraj *et al.*, 1997). The different solvent extracts of the stem of Tinospora produced inhibitory effects on different types of bacteria. Ethyl acetate extract showed inhibition to *Salmonella gallinarum*; aqueous, ethyl acetate and methanol extracts inhibited *Staphylococcus aureus*. *E.coli* was inhibited by methanol and ethyl acetate extract. (Rajurkar and Vadlamudi, 2001–2002). Ethyl
acetate extract of it was found to be effective against *S. aureus*, *S. epidermidis* and *Escherichia coli* (E Thomas, 2002).

*Holarrhena antidysenterica* is used in traditional Ayurvedic medicine to treat dysentery, especially amoebic dysentery (Bhutani, 1984). It possess antihelmintic, appetizing, antidiarrhoeal and astringent properties (Chopra *et al.*, 1982). It was reported to be used as an immunomodulatory agent (Atal *et al.*, 1986), larval growth inhibitor (Thappa *et al.*, 1989) and as an agent against malaria and vaginitis (Hagers Handbuch, 1976). Antibacterial studies of leaves and callus culture also were reported [Dohmal *et al.*, (1990); Rahman *et al.*, (1997)]. It is used to treat haemorrhoids, jaundice, gonorrhoea and fever and the latex is used to wash wounds (Neuwinger, 1996). Asima and Brantner, (1999) found that the methanolic extract of stem bark was active against bacteria like *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus faecalis, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*. Further studies by them indicated that the total alkaloids had remarkable activity against *S. aureus* with MIC 95 mcg per ml.
Cinnamomum verum is one of the most important tree spices in India. Cinnamon is reported to have antibacterial, antifungal and antiviral properties. Yousef and Tawil, (1980) found that cinnamon oil has the highest activity among various volatile oil from twenty two plants against bacteria and fungi. Many oral bacteria were inhibited by cinnamon bark oil (Saeki et al., 1989). Smith – Palmer et al., (1998) found out that cinnamon oil was active against certain food borne pathogens.

The fungicidal activity of cinnamon was reported by various researchers. Jaiswal, (1990) reported the fungitoxicant effect of vapour of cinnamon bark oil in comparatively lower doses. Cinnamaldehyde, the major component of cinnamon oil was inhibitory to the toxin production by Aspergillus parasiticus at a concentration of 150 ppm and the essential oil of cinnamon at a concentration of 200 – 250 ppm inhibited the growth and subsequent production of toxin by the fungi (Bullerman et al., 1977). The antidermatophyte effect of volatile oils of cinnamon was studied by Manimaran et al., (2002) and Singh et al., (1995). According to Sasidharan et al., (1998) the aqueous and alcoholic extracts of leaves of Cinnamomum verum were inhibitory to bacteria and fungi in comparatively small concentrations.
Cassia fistula (Kanikonna) flowers are traditionally used in decorating ‘vishukkani’ in the Hindu families of Kerala during the celebrations of ‘Vishu’, the festival of crops. Many medicinal properties were reported for it. The ethanolic extract of its seeds were found to have antibacterial properties (Valsaraj et al., 1997). According to Dhar et al., (1968), the alcoholic extract of the pods and stem bark are having antiviral activity against Vaccinia virus.

The antimicrobial properties of Calotropes procera was studied by many scientists. The antibacterial and antifungal activities were reported by Desta et al., (1993); Kumar and Chanhan, (1992) and Jain et al., (1996). The different parts of the plant like flower, leaf, stem, root bark etc., possess antibacterial activity in alcohol, ethyl acetate, ether and chloroform extract. Whole plant extracts with solvents also were reported to have antibacterial activity. (Almagboul et al., 1985). Calotropes procera is used traditionally as an antimalarial drug. The plant is worshiped by Hindus and is usually planted near temple of Lord Shiva. In the traditional Indian medicinal system, different parts of the plant have been advocated for a variety of diseased conditions (Warrier et al., 1994).
Latex of the plant also was found to be active against bacteria, fungi and yeasts. [Shukla and Krisnamurti, (1961); Tanira et al., (1994); Nawazisht et al., (1979) and Larhsini et al., (1997)]. It is given in malarial fever (Khory and Katrak, 1981). In a study conducted by Sharma and Sharma, (1999) using the crude fractions of flower, bud and root, proved that these extracts can inhibit the maturation of schizonts of *Plasmodium falciparum*. The antimalarial activity was reported by Mishra et al., (1991) also. The wound healing property of the latex of calotropes was reported by Rasik et al., (1999). The anticancer activity of latex was studied by Hussain Ayoub and Kingston, (1981).

*Sida retusa* (Kurumthotti) is traditionally used in Ayurveda for treating rheumatism. According to Valsaraj et al., (1997). *Sida rhombifolia* inhibited *Escherichia coli*, Bacillus and *Staphylococcus aureus*.

In a study conducted by Bhakuni et al., (1964) *Mimosa pudica* (Thottalvadi) was found to have antiviral activity against Vaccinia virus in 50% ethanolic extract.

*Myristica fragrans* (nutmeg) is used as a condiment and also as a medicine in many countries. It is a stimulant, carminative,
antiseptic, astringent and antiparasitic agent. It is used in tonics and forms a constituent of preparations prescribed for dysentery, stomach ache, flatulence, nausea, vomiting, malaria, rheumatism and the early stages of leprosy (Singh and Singh, 1996). Various medicinal properties were reported for nutmeg like curing of gastrointestinal and urinary disorders, dental caries etc. by Usmanghani et al., (1997); Gurudat et al., (1996); Pruthi, (1998) Shidore et al., (1985) and Hada et al., (1988). Sherry et al., (1982) and Rasheed et al., (1984) also observed various effects of myristica. The oil from nutmeg has been used in many preparations like soaps, tooth pastes, hair lotions and in perfumes. It has weedicidal activity and is toxic to houseflies and cockroaches. (Pruthi, 1998).

The antibacterial activities of nutmeg was reported by various researchers. De et al., (1999) observed that it was effective against some enteric bacteria. The inhibitory effect on bacteria was observed by Dorman and Deans, (2000) also. Alcoholic extract of nutmeg was found inhibitory to Micrococcus (Pruthi 1998). The antifungal activities against dermatophytes like Microsporum canis and against Pseudoallescheria boydii, the fungi causing subcutaueous infection like mycetoma, were reported by Rahman et al., (1999). Dorman and
Deans, (2000) observed that volatile oil from nutmeg was equally effective against Gram positive and Gram negative bacteria. The antimicrobial properties of orange and lemon were studied by Subba et al., (1967) and found that the oils from these were inhibitory to a wide variety of food spoilage organisms, notably yeasts. Antimicrotical action of citrus fruit oils on food borne bacteria was reported by Dabbah et al., (1970). Piacentini, (1948) measured the phenol coefficient of orange and lemon essences and found them to have the value 0.44. It was also found that these extracts were more effective than phenol in inhibiting the growth of various spore forming bacteria. According to Deans and Ritchie, (1987) undiluted oil from orange inhibited Bacillus subtilis, Brocothrix thermosphacta, Flavobacterium suaveolens and Leuconostoc cremoris and that of lemon inhibited Aeromonas hydrophila, Brocothrix thermosphacta, Brevibacterium linens, Erwinia carotovora, Flavobacterium suaveolens, Leuconostoc cremoris, Salmonella pullorum and Streptococcus faecalis which include plant and animal pathogens and saprophytes. Alderman and Marth, (1976) reported that citrus essential oils inhibited the growth of Aspergillus parasiticus.
The bark and leaf tinctures of *Syzygium cumini* (black plum) possess antibiotic activity against *Escherichia coli* and *Micrococcus pyogenes* (Dutta et al., 2000). Dutta et al., reported the antifungal effects of this plant against dermatophytes, *Trichophyton tonsurans*, *T.rubrum*, *Trichosporon beigellii*, *Microsporum fulvum* and *M.gypseum* at 5% concentration for bark and 15% concentration for leaves. But these were found ineffective against *Candida albicans*, in their study.

*Emlica officinalis* (Indian gooseberry) is antiscorbutic, acrid, cooling and refrigerant. It is used as diuretic, as laxative and to treat anaemia, cough, dyspepsia and jaundice. The seeds are also used in treatment of asthma and bronchitis (Chopra et al., 1956). Dutta et al., (2000) observed that the ethanolic extract of bark of Emblica at a concentration of 10% was effective against dermatophytes.

*Curcuma longa* (turmeric) is widely used as a spice and coloring agent and is well known for its medicinal properties (Luthra et al., 2001). A number of biological activities were reported for *Curcuma longa* like antioxidant (Srnivas et al., 1992), anti-inflammatory (Ghatak et al., 1991), wound healing (Chang and Bni, 1987), anticancerous (Surh, 1999) and antiproliferative (Han et al.,
Various sesquiterpenes and curcuminoids have been isolated from the rhizome and these are attributed to the various biological activities of *Curcuma longa* [Oshiro *et al.*, (1990); Hegnauer, (1963)]. Curcumin, the main constituent was reported to possess antibacterial activity (Kokate, 1994).

The antibacterial properties of *Curcuma longa* were reported by Shankaranarayanan and Jolly, (1994). E Thomas *et al.*, (1996) reported that the ether and ethyl acetate extract of *C. longa* showed much significant antibacterial activity among the plants tested against multidrug resistant strains of Gram positive and Gram negative bacteria. In a comparative study with standard antibiotics and rhizome extracts of *C. longa*, R Sing *et al.*, (2002) observed significant antibacterial activity at very low concentration of the essential oil fraction against pathogenic bacteria like *Staphylococcus aureus*. Moreover, a higher activity was observed towards Gram positive bacteria rather than Gram negative bacteria. They also reported that, curcuma extracts were found effective even against the drug resistant strains.

The essential oil from *Curcuma longa* was tested against ten bacterial and fungal species each using the oil as such and with and five different dilutions prepared in ethylene glycol by Anup Banerjee
and Nigam, (1978). They found that it responded well against almost all the selected organisms even at higher dilutions. The plant was claimed to be used as blood purifier, as a preventive against common cold and as antiparasitic agent for many skin infections [Chopra et al., (1956) and Satyavati et al., (1976)].

Other species of curcuma like C. aromatica (Kasthurimanjal), C. decipiens (Kuzhikoova) and C. caesia (Kali haldi) were also tested for antimicrobial properties and found to be much effective [E Thomas et al., (1996); E Thomas, (2002); Garg and Jain, (1998)].

*Cyperus rotundus* (Nut grass or muthanga) is used in many preparations in Ayurvedic Medicine. It is added as an ingredient in various ‘Kashaya’ used for intestinal problems. This may be due to the antimicrobial effect of the plant on enteric bacteria. The inhibition of enteric bacteria by nut grass was reported by Puratchikody et al., (2001). The ether and ethyl acetate extract of the rhizome was found active against *Pseudomonas aeruginosa* (E Thomas, 2002). Its antibacterial property against Gram negative bacteria was demonstrated by Valsaraj et al., (1997). Anti malarial activity [Thebiaranonth et al., (1995); Weenen et al., (1990)] and diuretic
activity (Sripandikulchai et al., 2001) of *Cyperus rotundus* also were reported.

According to Meena and Sethi, (1994), *Zingiber officinale* (ginger) was found to be less effective when compared with other spices in antifungal and antibacterial activity. But E Thomas et al., (1996) reported that ethyl acetate extract of it exhibited a remarkable inhibition zone (18mm) diameter against *Pseudomonas aeruguosa*. The active principles were reported as gingerols and zingiberene. [Solladie and Ziani, (1993); Bhonsle et al., (1994)]. *Kaempferia galanga* (Kacholam), another member of zingberaceae family also was reported to possess moderate antibacterial activity (E Thomas, 2002).

*Cyclea peltata* (Padakkizhangu) and *Hemidesmus indicus* (Naruneendi) were tested for antibacterial activity by Valsaraj et al., (1997) and found that they were ineffective against *Escherichia coli* but inhibit *Staphylococcus aureus*. Hemidesmus was found ineffective against other bacteria tested but Cyclea was found to inhibit other bacteria like Bacillus and Pseudomonas.

*Crocus sativus* (Saffron) cures urinary, digestive and uterine troubles. (Singh and Singh, 1996). The antimicrobial effect was not
yet reported. The flower is used to remove marks of small pox, chicken pox, measles etc., and also as a fairness agent.

*Eugenia caryophyllata* (clove) is the second most important spice of the world. The commercial product is the air dried, unopened bud. It is widely used in Asian herbal medicines. It is used for dental infections, viral hepatitis, bacterial colitis, cholera, amoebic dysentery, infectious acnes, sinusitis, bronchitis, flu, tuberculosis etc., and to treat hypertension, thyroid dysfunction, gout etc., in many countries. It is antibacterial, antifungal, anti inflammatory, antiseptic, antispasmodic, antiparasitic, antitumoral and antiviral. It is a carminative, and can prevent nausea. Clove is used as a topical anaesthetic agent for toothache. Eugenol is the main content of clove and is used in dental problems to numb the gums. Clove oil compounds are inhibitory to plaque and gum disease causing bacteria. So it is widely used in tooth pastes and mouth washes. It is also used in soaps, perfumes and cleansers in histological work etc. (Singh and Singh, 1996).

Many reports are available regarding the antibacterial, antifungal and antiparasitic effects of clove. Flowers of clove were found to have antimicrobial activity by agar well diffusion method,
with cephazolin as a standard antibiotic, by Perez and Anesini, (1994). Its activity against *Helicobacter pylori* was demonstrated by Bae *et al.*, (1998). Clove exhibits a broad spectrum antibacterial activity against enteropathogens and food spoilage bacteria (Ramanoelina *et al.*, 1987). Even multidrug resistant bacteria was found sensitive to clove extracts (Arora and Kaur, 1999).

The anticandidal effect of essential oil of clove was studied by J Briozzo *et al.*, (1989). The mycelial growth and aflatoxin production by fungi was found inhibited by clove [Mabrouk and El- Shayeb, (1980); Hasan and Mahmoud, (1993)]. Bullerman *et al.*, (1977) proved that clove oil at 250 ppm inhibited mycelial growth and toxin production by Aspergillus. It was also demonstrated by them that eugenol, the main ingredient of clove oil inhibited Aspergillus at 125 ppm. Ground cloves have also been reported to be highly active towards *Staphylococcus aureus* (Hargreaves *et al.*, 1975). Yun-Yun Hao *et al.*, (1998) reported the inhibitory effect of eugenol on two psychrophilic bacterial pathogens namely *Aeromonas hydrophila* and *Listeria monocytogenes* in refrigerated cooked beef.

*Santalum album* (sandalwood) bark and root are used against dermatitis, sinusitis, vomiting, piles, urinary tract infections, dysuria and leucorrhoea. It is a cooling agent. *Pterocarpus santalinus* (Red
sandal) bark is used to treat abscesses, eye infections, piles, dysentery etc. It is used as a fairness agent and also for wound healing (Nesamony, 1998). It is anti inflammatory and cooling. Reports on in vitro antimicrobial studies are not available.

*Trichosanthes cucumerina* is used for wound healing, dermatitis, leprosy and fever. *Achyrantes aspera* is used to cure ear infections, piles and diarrhoea. (Nesamony, 1998). Its leaf and stem posses inhibitory effect against Gram positive bacteria but not against Gram negative bacteria (Valsaraj *et al.*, 1997).

*Phyllanthus niruri* (Kizhukanelli) is used in folk medicine and Ayurveda. Its significant antibacterial effect towards *Escherichia coli* and *Staphylococcus aureus* was reported earlier [E Thomas *et al.*, (1999); E Thomas, (2002)].

*Plumbago zeylanica* (Kotuveli) is a plant reported to have antibacterial activity. According to Ahmed *et al.*, (1998), it was one of the plants which showed potential activity against bacteria tested, during screening of eighty two Indian medicinal plants traditionally practiced. Gram positive and Gram negative bacteria were inhibited by plumbago and it is due to the main antimicrobial principle plumbagin [Durga *et al.*, (1990), M. Krishnaswamy and
Purushothaman, (1980)]. According to E Thomas (2002), among the fifty three plants screened for antibacterial properties, \textit{P. zeylanica} manifested the broadest and highest activity against the tested microbes except \textit{Pseudomonas aeruginosa} and \textit{Escherichia coli}.

**Antimicrobial principles from plants**

Various antimicrobial principles have been isolated from many plants. The important ones from the plants screened are listed in Table I.

### Table I: Antimicrobial principles from plants

<table>
<thead>
<tr>
<th>Name of the plant</th>
<th>Part used</th>
<th>Active compound or extract</th>
<th>Micro organism inhibited</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Glycosmis cochinensis}</td>
<td>Not specified</td>
<td>Sinharine</td>
<td>Cladosporium</td>
<td>Greger \textit{et al.}, (1993)</td>
</tr>
<tr>
<td>\textit{Aristolochiaceae members}</td>
<td>Leaf, root, stem</td>
<td>Sesquiterpenes</td>
<td>Bacteria</td>
<td>Rodriguez \textit{et al.}, (1995)</td>
</tr>
<tr>
<td>\textit{Aloe vera}</td>
<td>Leaf</td>
<td>Anthraquinones</td>
<td>Staphylococcus</td>
<td>Agarry \textit{et al.}, (2005)</td>
</tr>
</tbody>
</table>
### Review of Literature

<table>
<thead>
<tr>
<th>Species</th>
<th>Part Used</th>
<th>Active Constituents</th>
<th>Modes of Action</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Psidium guajava</em></td>
<td>Leaf</td>
<td>Quercetin, Avicularin, Guaijaverin</td>
<td>Bacteria</td>
<td>Gnan and Demello, (1999)</td>
</tr>
<tr>
<td><em>Holarrhena antidysenterica</em></td>
<td>Stem bark</td>
<td>Conessine, Kurchine, Kurchimine</td>
<td>Bacteria</td>
<td>Radt, (1965)</td>
</tr>
<tr>
<td><em>Cinnamomum verum</em></td>
<td>Stem bark</td>
<td>o-methoxy, cinnamaldehyde, Eugenol</td>
<td>Fungi, bacteria</td>
<td>Morozumi, (1978)</td>
</tr>
<tr>
<td><em>Calotropes procera</em></td>
<td>Root, flower, bud</td>
<td>Tannins, Saponins, Flavanoids</td>
<td>Bacteria, fungi, protozoa (Plasmodium)</td>
<td>Sharma and Sharma, (1999)</td>
</tr>
</tbody>
</table>
In the present study, maximum antibacterial property was shown by the alcoholic and aqueous extracts of *Punica granatum* fruit peel. The antimicrobial activity of it seems to be comparable with that of commercial antibiotics and is very promising against multidrug resistant bacterial strains. So a detailed study was conducted on the antimicrobial properties of *Punica granatum*.

**Punica granatum (Pomegranate)**

*Punica granatum* is a medicinal plant seen in India, Afghanistan, Arabia, Iran, Iraq etc. It belongs to the family Punicaceae. In India it is cultivated in house premises and also in gardens on commercial basis. In Sanskrit it is known as Dadimum. Its fruit (pomegranate) is edible and is also known as Carthaginian apple, Punica apple etc. It grows well in warm climates of the South East Asian countries.

Punica plant is a small shrub which can grow up to 5m height. Branches can be seen from the beginning of stem itself. Most of the branches end in a thorn like structure. The stem bark is greenish ash in colour. Leaves are distributed opposite and at nodes leaves are seen bushy. The surface of leaf is smooth and shiny.
Plate 1: *Punica granatum* – Young Plant

One to five flowers are seen at the tip of small branches. Flowers are large, red, attractive and almost odorless and are bisexual. The length of the flower ranges from four to five cm. Beneath the flowers there are small sepals. Number of petals ranges from five to eight. The petals are thick and fleshy and are arranged alternately.

Plate 2: *Punica granatum*-flower  
Plate 3: Pomegranate (fruit)
Fruits have an attractive brownish red colour with a size of five to eight cm. At the top of the fruit, there may be outer petal remaining. The pericarp of the fruit is thick. Seeds are many and are covered with juicy pulp. This juicy pulp is the edible part of the fruit. It is sweet with slight bitterness.

The fruit contains proteins, lipids, carbohydrates, magnesium, calcium, sulfur, thiamin, nicotinic acid, vitamin C, pectin and tannin. The juice contains acids, pectin, sugars and tannin. The root bark contains many alkaloids and tannin. Stem bark and root bark contain piperidin products like pelletirin, methyl pelletirin etc. (Nesamony, 1998).

**Medicinal uses**

Pomegranate is a fruit with exceptionally rich ethnomedical applications. The peel is well regarded for its astringent properties, the seed for stimulating beauty and fertility (Aslam *et al.*, 2006).

In Ayurveda and in folk medicine it is used in many preparations. It is good for regulation of ‘vatha’, ‘pitha’ and ‘kapha’ according to Ayurveda. It can kill intestinal worms. The ‘kashaya’ (an aqueous preparation) prepared using the root helps to expel
tape worm. The fruit helps digestion. It removes fatigue and thirst and increases semen production (Nesamony, 1998).

Almost all parts of this plant are used in Indian traditional medicine for various ailments. Bark and rind of the fruit are used for curing dysentery, diarrhoea, piles, bronchitis, and helminth infections. The flower bud is used in chronic diarrhoea and dysentery in children [Kritikar and Basu, (1975); Nadkarni, (1976)]. Rural tribal people of Barisal, Bangladesh use seed of this plant to treat diarrhoea effectively (Das et al., 1999).

The stem bark of *P.granatum* is used as astringent and antihelmintic for treating tapeworm infections. Rind of fruit is used combined with aromatics like cloves in diarrhoea and dysentery cases (Chopra et al., 1956). Male abortive flowers are used to treat diabetes mellitus in Unani medicine (Jafri et al., 2000).

The root bark, stem bark, fruit, seed, leaf, flower etc., are used for medicinal purposes. It is used as antidysenteric agent and to cure malaria, diarrhoea, vomiting, menstrual disorders, leucoderma, acidity, stomach and intestinal ulcers etc. It is also used to increase the uterine musculature. Punica is used generally as an energy restoring fruit after many intestinal diseases, malaria,
fatigue and hallucinations after excess liquor consumption. ‘Dadimaghrutham’, ‘Dadimashtaka choornam’ etc., are some preparations in Ayurveda which contain punica as the major ingredient. Eventhough many parts are used, the fruit rind, fruit and leaves of punica have been commonly employed in traditional medicines for diarrhoea [Perez and Anesini, (1994); Anesini and Perez, (1993); Sudheesh and Vijayalakshmi, (2005); Mahmoud et al., (1994)]. The effect of seed as an antidiarrhoeal agent also was reported (Das et al., 1999).

**Antibacterial effects**

Only limited number of reports are available regarding the antibacterial effects of *Punica granatum*. The inhibitory effects of active compounds from *Punica granatum* pericarp on verocytotoxin production by enterohaemorrhagic *Escherichia coli* was reported by Voravuthikunchai et al., (2005).

Antibacterial properties of *Punica granatum* was studied by Prashanth et al., (2001) and observed that various extracts had inhibitory activity against *Escherichia coli*. According to Machado et al., (2003) methicillin resistant *Staphylococcus aureus* (MRSA) strains were susceptible to extracts of *P.granatum* and they suggested it as
a potential candidate for the development of new strategies to treat MRSA infections.

In a screening study of various extracts of medicinal plants, E Thomas, (2002) observed that ether and ethyl acetate extract of *P.granatum* fruit was inhibitory to Gram positive bacteria like *Staphylococcus aureus* and *S.epidermidis* but not against Gram negative bacteria like Klebsiella, Pseudomas and *Escherichia coli*.

Elizabeth, (2001) reported that *P.granatum* extract produced no inhibitory effects on *S.aureus*, *Salmonella typhimurium* and *Yersinia enterocolitica* at the concentration tested.

**Antifungal properties**

A few reports are available regarding the antifungal activities of *P.granatum*. Dutta *et al.*, (2000) reported the effect of ethanolic extract of the bark against dermatophytes and found out the MIC as 5%. The leaf extract also was tested and found to inhibit all the dermatophytes tested with MIC at 10%. In their work, *Candida albicans* was found to be less susceptible at the concentrations tested. Another attractive finding in their work was that, a complete inhibition of the dermatophyte occurred at the concentrations tested, even after 21 days.
Chapter II

Anticandidal effect of *Punica granatum* was reported by Vasconcelos *et al.* (2003). They conducted a comparative study for the effectiveness of *P.granatum* gel with miconazole; and it was found that the gel can be used as a topical antifungal agent for the treatment of candidosis associated with denture stomatitis.

The antidermatophytic activity of the aqueous extract of *P.granatum* was reported by Dutta *et al.* (1998). M A Azzouz and Bullerman, (1982) reported that pomegranate peel was a good inhibitor against four *Penicillium* species, that is *P.citrinum, P.patulum, Penicillium species* and *P.roquefortii* and also against *Aspergillus ochraceus*.

**Antiprotozoan effects**

*P.granatum* was reported to have antiprotozoan effect towards *Entamoeba histolytica* and *E.invadens* (Segura *et al.*, 1990). Calzada *et al.*, (2006) observed that the methanolic extract of pomegranate was most effective against *E.histolytica*. They reported its effects against *Giardia lamblia* also.

The moderate inhibitory action of fruit bark and root bark aqueous crude extract from pomegranate against protozoa was reported by Yang *et al.*, (1996).
Antihelmintic effects

Pomegranate has antihelmintic property also. Raj, (1975) reported that rind of it possess moderate in vitro antihelmintic activity. Mudzhiri, (1954) suggested pomegranate bark extract as a therapeutic agent for tapeworm infections.

Antiviral properties

Antiviral effects of pomegranate also were reported. Neurath et al., (2005) observed that the juice of P.granatum fruit provides an HIV-1 entry inhibitor which adsorb on to corn starch; and this HIV-1 entry inhibitor prevents the binding of HIV to CD4 cells. The anti HSV-1 (Herpes virus type 1) activity was reported by Li et al., (2004). Zhang et al., (1995) reported the antiviral activity of the tannin from the pericarp of pomegranate against genital Herpes virus.

Other Biological activities

Apart from antimicrobial effects, Punica granatum was reported to have a number of other properties like antioxidant, [Gil et al., (2000); Murthy et al., (2002); Singh et al., (2002); Schubert et al., (1999); Ricci et al., (2006); Kaur et al., (2006)], anticancerous [Lansky and Newman, (2007); Malik and Mukhtar, (2006); Adams et al., (2006); Malik et al., (2005); Lansky and
Mehta, (2004); Lansky et al., (2005); Kim et al., (2002)], anti-diabetic [Huang et al., (2005); Das et al., (2001); Jafri et al., (2000)], immunomodulatory (Ross et al., 2001), wound healing (Murthy et al., 2004), cosmeceutical (Aslam et al., 2006), anti-diarrhoeal (Das et al., 1999), anti-ulcer (Alkofahi and Atta, 1999) and gastroprotective [Gharzouli et al., (1999); Khennouf et al., (1999)] effects.

**Active principles from *P.granatum***

*P.granatum* was reported to have many biological effects due to its alkaloids, tannins, terpenes, fatty acids, flavanoids etc. Table II shows some identified compounds present in it. The antimicrobial property is attributed to the various tannins present in the plant. Tannins are reported to have broad spectrum antimicrobial activity [Burapadaja and Bunchoo, (1995); Dold and Knapp, (1948)].

Tannins are phenolic metabolites seen in plants. There are two different groups of tannins, hydrolysable tannins with a central carbohydrate core and condensed tannins with a flavan derivative. Tannins damage bacterial cytoplasmic membrane. Chelating properties of tannins coupled with their ability for denaturing bacterial proteins contribute the antibacterial activity (Shimamura et al., 1990).
Epigallocatechin gallate, epigallo catechin and epicatechin gallate are tannins which inhibit *Staphylococcus aureus*, *Vibrio cholerae* O 1 classical Inaba and el Tor Inaba strains (Ikigai et al., 1993). The bactericidal action of *P.granatum*, which is rich in tannins, against *V. cholerae* was reported by Guevara et al., (1994) also.
Table II: Compounds isolated from *Punica granatum*

<table>
<thead>
<tr>
<th>Part used</th>
<th>Compound</th>
<th>Chemical name</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit</td>
<td>Punicalagin</td>
<td>-</td>
<td>Gil <em>et al.</em>, (2000)</td>
</tr>
<tr>
<td>Leaf</td>
<td>Gallotannins</td>
<td>1, 2, 4-tri-o-galloyl-beta glucopyranose and 1, 3, 4-tri-o-galloyl-beta glucopyranose</td>
<td>Hussein <em>et al.</em>, (1997)</td>
</tr>
<tr>
<td></td>
<td>Ellagitannins</td>
<td>1, 4-di-o-galloyl 1-3, 6-(R)-hexahydroxydiphenyl-betaglucopyranose and Brevifolincarboxylic acid 10-monopotassium sulphate</td>
<td></td>
</tr>
<tr>
<td>Seed</td>
<td>Triglyceride</td>
<td>di-o-punicyl-o-octadeca 8z, 11z 13-E-trienyl glycerol</td>
<td>Yusuph and Mann (1997)</td>
</tr>
<tr>
<td>Seed oil</td>
<td>Monoacyl glycerol</td>
<td>1-o-trans, cis-trans-9, 11, 13-octadeca trienoyl glycerol; 1-o-isopentyl-3-o- octadec-2-enoyl glycerol and cis-9-octadecenoic, octadecanoic and eicosanoic acid.</td>
<td>Fatope <em>et al.</em>, (2002).</td>
</tr>
<tr>
<td>Part</td>
<td>Compound Type</td>
<td>Constituents</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------</td>
<td>------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Heart wood</td>
<td>Ellagic acid rhamnosides</td>
<td>3-o-methyl ellagic acid, 4-o-alpha-L-rhamnopyranoside, 3,4'-o-dimethyl ellagic acid, Brevifolin carboxylic acid, 3-o-methyl ellagic acid and 4,4'-o-dimethyl ellagic acid.</td>
<td>El Toumy and Rauwald, (2003)</td>
</tr>
<tr>
<td>Heart wood</td>
<td>Ellagittannins</td>
<td>Diellagic acid rhamnosyl (1→4) glucopyranoside and 5-o-galloyl punicacortein D</td>
<td>El Toumy and Rauwald, (2002)</td>
</tr>
<tr>
<td>Seed oil</td>
<td>Fatty acids</td>
<td>Punicic acid, Palmitic acid, Stearic acid, Oleic acid, Linoleic acid</td>
<td>Schubert et al., (1999)</td>
</tr>
</tbody>
</table>