2. REVIEW OF LITERATURE
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2.1 INDIAN MEDICINAL PLANTS AND TRADITIONAL SYSTEM OF MEDICINES

Since the time immemorial, man has made use of plants in the treatment of diseases. The pharmacopoeias of many countries include a large number of drugs of plant origin. Although it is true that synthetic chemical compounds are extensively being employed in clinical practice, the interest in the examination of plants as potential source of new drugs has never been waned. The history of medicinal plants dates back to Rigveda; perhaps the oldest repository of human knowledge which was written around 4500 - 1600 B. C. The Ayurveda, developed around 2500 B.C. contains a detailed account of many drugs in use even today. Ancient Ayurveda, however, includes the comprehensive works of Charaka [1000 B.C.] and that of Sushruta (800 B.C.) and provides a base for the materia medica through its detailed descriptions. The work of Atreya, Jivaka and Kashyap [about 600 B.C.], Vyadi [≈ 500 B.C.], Patanjali [≈ 200 B.C.] and Nagarjun [≈ 500 B.C.] as well as "Bower script" [≈ 300 - 400 A.D.] added to knowledge of herbal medicines. The Mohammedan culture enriched the vegetable materia medica which was further improved by those in Greece, Arabia and Persia [Arora, 1965].

Little attention was paid to the medicinal plants of India till the Asiatic Society of Bengal was established by Sir William Jones [Jones, 1799]. According to him, hundreds of plants were not well understood by European botanists grew wild on plains in the forests of India.

In the beginning of the nineteenth century, John Flemming contributed a monograph of great value. The scattered information on the subject was collected and placed before the medical professionals [Flemming, 1910]. According to Kiritikar and Basu [1975], "Pharmacographica India" is a most authentic and useful compilation containing information on the use of Indigenous materia medica. In this series of publications, the most illustrious work is "A Dictionary of Economic Products of India" published in 1889 - 1904 by Sir George Watt. This monumental work gives a summary of the previous work on the medicinal plants as well as provides the information on the use of different parts of the medicinal plants. A dramatic decline in the popularity of medicinal plants as therapeutic agents has been observed with the emergence and use of sulphadireugs, antibiotics and synthetic drugs. As the success of any health system depends on the ready availability and use of suitable drugs on a sustainable basis, the medicinal plants have always played a key role in world health. W.H.O. has taken
a serious initiative to create awareness in the traditional medicines to the world's population and to promote increased national exploitation of safe and effective practices, including the use of medicinal plants as therapeutic agents. [Olayiwola Akerle, 1993]. The fortunate factor, herbal medicaments do not produce many side effects commonly seen after long term administration of synthetic drugs, has also resulted in the revival of interests in their use in both developing as well as developed countries [Jain & Nagrajan, 1990]. Also, a considerable growth has occurred officially to popularize the use of natural products as therapeutic agents.

Out of the 250,000 to 500,000 plant species on the planet, more than 90% are yet to be investigated for their medicinal properties. The flora of India comprises about of 45,000 plant species, from unicellular blue green algae to the flowering plants. These comprise approximately 11% of the total species of flora of the world, out of which about 4% are the flowering plants. The biogeographic position of India is unique as we have diverse ecosystems, ranging from the humid tropics of western Ghats to the alpine zone of the Himalayas and from the dry deserts of Rajasthan to the tidal mangroves of the Sunderbans and, hence, India is endowed with a rich flora [Dahanukar, 1995].

Ayurveda uses about 800 plants, while the siddha system of medicine makes use of about 600 plants in their various formulations. Amchi and Unani systems together make use of about 700 plants in their different preparations. The commercial value of drug products derived directly from higher plants is fairly high. According to a recent estimate by Council of Scientific and Industrial Research (India), the total herbal production in India is expected to reach Rs. 4,000 crores by the year 2000 A.D. from the Rs. 1000 crores in 1997 [Pushpangadan & George, 1997]. This means a three fold increase in production and sale of herbal drugs in India alone. The same is the scenario in other parts of the world e.g., American consumer paid about 8 billion US dollars for prescription drugs derived solely from higher plants in 1980. Whereas the community pharmacies in the United States dispensed 25% drugs derived from higher plants in their prescription from 1959 to 1980, excluding non prescription drug products or drugs used exclusively in hospitals. Plants derived drugs, thus, represent stable market upon which both physicians and patients can rely upon. In addition, the world wide market in plants derived drugs, may undoubtedly amount to many additional billion dollars although it is difficult to estimate [Farnsworth, 1973; Farnsworth and Soejarto, 1985].

2.2 ANTIMICROBIAL PROPERTIES OF MEDICINAL PLANTS

Research on medicinal plants has continued to be an area of major interest and
priority for pharmacologists, microbiologists & drug developing industries/institutions in India and abroad for the last several decades. The work on antimicrobial activity of medicinal plants in India and other parts of world has been reported by several workers [Bhawasar, 1965; Radhakrishnan, 1976; Iyenger, 1985; Chopra, 1992; Behl, 1993; Shah and Qadry, 1993; Zafar, 1994; Dahanukar and Hazra, 1995; Rastogi and Mehrotra, 1995; Ahmad et al, 1995; Chaudhri, 1996; Phillipson, 1997; Mehmood et al, 1997 and Ahmad et al, 1998].

2.2.1 In Indian Context

The reports on antimicrobial activity of indigenous plants from many regions have been published, however, a systematic study on large number of medicinal plants is largely not done. Therefore, it is desirable to screen various medicinal plants commonly used in Indian system of medicine for various biological activities and to develop a scientific basis for their use.

or more of the 4 microorganisms tested viz. *Staphylococcus aureus, Bacillus anthracis, Escherichia coli* and *Pseudomonas aeruginosa*.

Strong antimicrobial activity of methanol extract of *Butea monosperma* was reported by Zafar *et al*, [1989]. Eswaramurthy *et al*, [1989] observed the inhibitory effects of *Acacia arabica, Ipomoea carnea* and *Prosopis juliflora* extracts on *Sarocladium oryzae* and *Fusarium oxysporum*. Lakshmanan *et al*, [1990] tested aqueous extracts of *Allium sativum, Bougainvillea spectabilis* and *Azadirachta indica* which significantly inhibited mycelial growth and sclerotial germination of *Thanatephorus cucumeris in vitro*. Mishra [1990] observed antifungal activity of the volatile oil from rhizomes of *Zingiber officinale* against *Fusarium moniliforme*.

Ethanolic and methanolic extracts of *Momordica charantia* seeds and ethanolic extracts of bark and heart wood of *Pterocarpus marsupium* were screened for their antimicrobial activity against eight gram+ve and three gram-ve bacteria and twelve strains of fungi. All the extracts showed considerable antimicrobial activity in vitro. The overall antifungal activity was more pronounced with the ethanolic extract of heart wood of *Pterocarpus marsupium* followed by methanolic extracts and then ethanolic fractions of *Momordica charantia* [Peddanna *et al*, 1990]. Upadhyay and Rai [1990] examined leaf extracts of thirteen medicinal plants, viz., *Argemone mexicana, Azadirachta indica, Caesalpinia bonducella, Cassia fistula, Cassia tora, Catharanthus roseus, Clerodendron serratum, Eucalyptus globulus, Jatropha curcas, Lawsonia inermis, Ocimum sanctum, Saraca indica*, and *Vitex negundo* against *Curvularia tuberculata* responsible for die-back disease. The extracts showed a wide range in their fungitoxic activity. The highest activity was exhibited by the extracts of *Eucalyptus globulus*, and *Catharanthes roseus* against *Curvularia tuberculata*, followed by the extracts of *Ocimum sanctum, L. inermis, Cassia tora* and *Azadirachta indica*. Palanichamy and Nagarajan [1990] also reported antifungal activity of *Cassia alata* leaf extract. Qiao *et al*, [1990] isolated twelve compounds from the roots of *Rubia cordifolia* L., only three of them exhibited mild antibacterial activity.

In a report from Iyer and Williamson [1991], it is mentioned that the extracts of *Allium sativum, Ocimum sanctum, Catharanthes roseus* and *Azadirachta indica*, showed high phenol content and inhibited the protease activity of *Trichophyton* sp. completely.

The antifungal activity of saponins isolated as byproduct from the defatted cake of *Madhuca butyraceae* oil seed is reported by Lalitha and Venkataraman
The inhibitory concentrations against plant pathogenic fungi ranged from 500-2000 ppm. Maximum sensitivity to saponins was shown by *P. expansum*, *C. acrimonium*, *H. oryzae* and *T. viridae*. The saponins perforate the cell wall causing leakage of protoplasm. Scalbert [1991] reported that the tannins also possess antimicrobial properties. Singh and Pandey [1992] tested bark extracts of thirty plants against *Microsporum gypseum* and *Trichophyton mentagrophytes*. Only *Lawsonia inermis* exhibited fair antifungal activity with fungistatic activity at a maximum inhibiting dilution of 1:30 [w/v] whereas fungicidal activity at a dilution of 1:10 [w/v] against both the test pathogens. The *Lawsonia inermis* extract showed a broad fungitoxic spectrum when tested against thirteen ringworm fungi. The antifungal activity of the extract remained unaltered even after autoclaving and long storage periods. Mahajan *et al.*, [1991] found that aqueous extract of *Camellia sinensis* is more effective inhibitor of bacterial growth than black tea. They also reported that gram+ve bacteria are more sensitive than gram-ve. Suresh and Chauhan, [1992] observed that *Staphylococcus albus* was completely resistant to the leaf extract of *Calotropis procera* whereas other tested fungal sp. and bacterial isolates were fairly inhibited. Dobhal and Joshi [1992] found that ethanolic and aqueous extracts of *Berberis chitria* roots gave very good response in eye infections caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Gnanaguru *et al.*, [1992] tested aqueous, ethylacetate and methanol extracts of *Funaria hygrometrica* against *E. coli*, *H. influenza*, *Klebsiella pneumoniae*, *P. mirabilis*, *S. typhi*, *Shigella boydii*, *X. oryzae*, *X. campestris* and *Fusarium oxysporum*. Antimicrobial activities were observed with ethylacetate extract against all the bacterial and fungal isolates. Methanol extracts also inhibited all the bacterial isolates except *P. mirabilis* and *Shigella boydii* while aq. extracts did not show any activity against either organism. Partially purified compound was found to be phenolic in nature.

Jain *et al.*, [1992] investigated the seed and callus extracts of *Cuminum cyminum* for antibacterial, antifungal, antiviral and antitumour activities. The extracts inhibited the growth of bacterial, fungal as well as poliomyelitis and Coxsakie viruses.

Venkatanarayana *et al.*, [1992] reported the antibacterial activities of ethanolic extracts from dried peels of *Citrus aurantium* and *Citrus lemon*, dried roots of *Hedychium spicatum* and dried rhizomes of *Curcuma longa*. Chandel *et al.*, [1992] observed significant antibacterial activity in alcohol and water extracts of *Acacia*
arabica bark against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Klebsiella* sp. Both the extracts showed better inhibitory response against gram+ve organisms than with gram-ve organisms.

Ganguli and Bhatt [1993] explained the mode of action of active principles from stem bark of *Albizia lebbeck*. Isolated principles were found to be active against test cultures selected for the study. Mode of action of the active principles against aerobes, showed that the glycosides from the extract caused leakage of cytoplasmic constituents of microbial cells. Electron micrographs of *Staphylococcus aureus* cells treated with MIC of anthraquinones from the extract, revealed the coarse granulation of the cytoplasmic matrix, vacuolation of cells and in a few cases disruption of the cell surface. Singh and Tripathi [1993] reported that strong fungicidal activity of leaf extracts of *Artabotrys hexapetalous*, *Aegle marmelos*, *Croton roxburghii* and *Physalis peruviana*.

Triphala, a combined formulation fruits of three plants *Emblica officinalis*, *Terminalia chebula* and *Terminalia belerica* is frequently used in preparation of one or more medicines in Unani and Ayurveda as antidiarrhoeal, antidysenteric, as blood purifier and a purgative [D'souza, 1993]. The preliminary scientific evaluation of this formulation indicated strong antimicrobial activity against *Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholerae* and *Klebsiella pneumoniae* [Mehta et al, 1993 and El. Mekkaway et al, 1995].

Nair and Bhide [1996] tested antimicrobial properties of water, alcoholic and oil extracts of commercially available dry nuts of *Semecarpus anacardium* against gram-ve and gram+ve bacterial strains. Alcoholic extract was found to be the most effective and showed strong bactericidal activity against gram-ve *Escherichia coli* *Salmonella typhi* and *Proteus vulgaris* as well as gram+ve *Staphylococcus aureus* and *Corynebacterium diphtheriae*. The alcoholic extracts of other parts of the tree such as twigs, leaves, green fruits showed antibacterial activity against only *Corynebacterium diphtheriae* whereas alcoholic leaf extract showed bactericidal activity against all above mentioned bacterial strains. Chukwarthi [1997] reported that the ethanol extract of *Holarrhena floribunda* stem bark exhibited antibacterial and antifungal activities against tested microorganisms.

### 2.2.2. In World context

As a large segment of the world population both in developing as well as in developed countries depends on the traditional system of medicine for a variety
of diseases. Several hundred genera are being used as source materials for potent herbal drugs which have stood the test of time and the modern chemistry could not replace most of them [Vashist, 1994]. The emerging relevance of herbal drugs as antimicrobial therapeutic agents has prompted many workers over the world to divert their efforts toward this field and the subject has been widely reviewed. There will always remain a growing need for the substances which are cheap, easily available, with no side effects, and with antimicrobial activity since the treatment of microbial infections by modern medicines, is often unsatisfactory.

Investigations for antibacterial agents were conducted by Osborn, 1943 in England and Atkinson, 1946 in Australia on non-sterile aqueous extracts of plants against gram+ve *Staphylococcus aureus*, gram-ve *Escherichia coli* and *Salmonella typhosa*.

Atkinson's group reported the screening of 1500 species of plants for their antimicrobial activity. Fifty of them showed fair activity and four of them inhibited both gram+ve and gram-ve bacteria. These four plants, *Drosera whittakeri* and the berries of three species of *Persoonia* (the former is known to contain two naphthoquinone derivatives, viz. droserone and hydroxydroserone), are being further investigated [Atkinson et al., 1946]. Screening results indicated that the seasonal variations, the part of the plants tested and treatment accorded may alter the nature of the results obtained, besides the species to which a particular plant belongs. Luckner and Luckner [1970] reported the antibacterial activity of a Naphthoquinone - Plumbagin, in *Drosera rotundifolia*. Mukherjee and Kundu [1973] observed antifungal activity of some phenolics and related compounds in which tannic acid, pentachlorophenol, picric acid and pyrogallol were proved to be the most promising inhibitors of *Helminthosporium oryzae*, *Alternaria solani* and *Curvularia lunata*. Increased antibacterial and astrigent properties were exhibited by *Psidium guajava* with the increase in the tannin content in the plant parts. Hence, the bark (30% tannins) showed highest, leaves (10% tannins) showed medium and root (low tannin) showed minimum activity [Ekabua and Eka-ou, 1978]. Clark, [1981] also observed antimicrobial activities of phenolic constituents of *Magnolia grandiflora* L.

Details of seventeen plants extracts on the inhibitory effects of the pathogenic *Candida* and *Aspergillus* sps. are tabulated by Simeray et al., [1982]. The extract of *Cocculus laurifolius* showed a wide spectrum of activity and its properties were attributed to the presence of condensed tannins. Shirata and Takahashi [1982]
detected the production of antimicrobial substances in the leaves of *Morus alba* and other plants of *Moraceae* family. Phytochemical screening of the aerial parts of *Thymus carnosus* Boiss revealed the presence of essential oil, tannins, flavonoids, phenolic acids, sterols and triterpenes. The phenolic compounds caffeic, vanillic, p-coumeric, p-hydroxy-benzoic and syringic acids showed antibacterial activity against several human pathogenic gram-ve and gram+ve bacteria [Marhuenda, 1987].

Hasan *et al.*, [1989] examined antibacterial activity of *Nigella sativa* extract against gram+ve and gram-ve bacteria. The lowest minimum inhibitory concentration of 5 µg/disc was observed against *Bacillus polymyxa*.

Kambu *et al.*, [1990] conducted a screening study on thirty eight plant extracts used as traditional medicines in a village, Kinshasa [Zaire], against diarrhoea. Thirty five of them showed significant bactericidal activity, thirty of them inhibited the growth of all test bacteria and five of them inhibited only some of the bacteria. The bactericidal activity has been found to be due to the presence of polyphenols, flavonoids, catechins, tannins, saponins and alkaloids in the extracts.

Brantner and Brantner [1991] tested the activity of flavonoids against gram+ve as well gram-ve human pathogenic bacteria and yeast. Flavonoid compounds like flavones, flavonols, flavonones and glycosides were dissolved in DMSO and tween 80 in a concentration range from 50 to 800 µg/ml nutrient medium. Bioassay guided fractionation of methanol extract of *Impatiens balsamina* known as "Bong Sun Hwa" in Korea, gave a napthoquinone derivative, 2- methoxy-1, 4-naphthoquinone which showed strong antifungal activity against *Candida albicans*, *Aspergillus niger*, *Cryptococcus neoformans* and *Epidermophyton floccosum*. This compound (MIC 5µg/ml) showed a comparable potency as that of nystatin. Strong antibacterial activity of 2 - methoxy - 1, 4 naphthoquinole against gram+ve bacteria *Bacillus subtilis* as well as gram-ve bacteria *Salmonella typhimurium* was also reported by Kang and Moon, 1992.

Gebre Mariam *et al.*, [1993] found that the aqueous extracts of the stem bark and wood of seven mostly chewed plant sticks namely *Clausena anisata*, *Liccrustum vulgare*, *Olea africana*, *Phoenix reclinata*, *Rhamnus prinoids*, *Salix subserrata* and *Sterospermum kunthianum* were found to have significant activity against *Streptococcus viridans*. Chemical analysis of the extracts showed the presence of alkaloids, polyphenols, and saponins in all the plants, cardiac glycosides in two plants and anthraquinone in one of the plants. The antimicrobial activity shown by plant extracts may be attributed to the presence of the polyphenols.
Belachew Desta [1993] tested a total of 315 extracts/fractions from sixty three traditionally used Ethiopian plants including Achyranthes aspera L., Berberis holstii Engl., Calotropis procera [Ait]., Chenopodium ambrosioides, Combretum paniculatum vent., Commelina benghalensis L., Commiphora Sp. Cucumics prophetarum; Datura stramonium L.; Lepidium sativum L., Nigella sativa, Ocimum lamifolium, Plantago lanceolata L., Plumbago zeylanica L., Punica granatum L., Solanum tuberosum L. for their antimicrobial activity using known strains of Staphylococcus aureus, Salmonella gallinarum, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumoniae and Candida albicans by agar plate well diffusion method with sample concentration of 1000 μg/ml. All the plants showed activity against one or more of the microorganisms. Direct aqueous extracts of some of these plants were found to be active against all the test organisms supporting the traditional therapeutic use of these herbs in aqueous dosage forms. The relative susceptibility of the test organisms to the some of extracts/fractions indicated a decreasing rank of order of Staphylococcus aureus> Pseudomonas aeruginosa> Candida albicans > Salmonella gallinarum > Escherichia coli > Klebsiella pneumoniae > Proteus vulgaris.

Khan et al, [1993] found that the growth of Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Aspergillus niger was markedly inhibited by the extracts and purified compounds obtained from the extracts of the fruits of Solanum nigrum and Solanum xanthocarpum. The extract and one of the purified compounds obtained from Withania somnifera fruits showed little inhibitory activity against the test organisms, whereas, another compound from Withania somnifera showed inhibitory activity against Bacillus subitilis, Staphylococcus aureus and Escherichia coli. Didry et al, [1993] investigated the antimicrobial properties of protoanemonin isolated from the aerial parts of Ranunculus bulbosus. Due to its marked inhibitory effects and broad spectrum activity against aerobes and anaerobes including multiresistant pathogenic strains, protoanemonin has been reported as a promising antimicrobial natural compound.

Combinations of Protoanemonin and prevalent antibiotics were also investigated. Out of twenty two antibiotic- protoanemonin combinations, twenty showed partial and one showed a complete synergism against aerobic bacteria. The best was the protoanemonin-cefamandole combination against a pathogenic strain of Staphylococcus aureus. Olukoya et al, [1993] studied the antibacterial activity of following plants used by local populace of Nigeria in the treatment of
various infections viz. *Anthocleista nogellii, Asparagus africanus, Boerhavia diffusa, Combretum bracteatum, Combretum racemosum, Crewia carpinifolia, Emilia cocinea, Ipomoea involucrata, Lannea melwitschii, Phyllanthus discoideus*. Most of them showed definite antimicrobial activity.

Brantner and Green [1994] screened plants selected on the basis of medicinal folklore reports and literature data from twenty eight families. The results indicated that about 60% of the aqueous extracts exhibited some level of antibacterial action and the following plants seems to be the most promising for further studies, leaves of *Betula pendula, Eucalyptus globulus, Hamamelis virginiana, Rubus fruiticose, Salvia officinalis* and *Vaccinium myrtillus*, aerial parts of *Drosera rotundifolia, Hedera helix, Origanum majorana, Origanum creticum, Pulmonaria officinalis* and *Sanicula europaea*, flower of *Tilia cordata*, seeds of *Quercus robur*, barks of *Hamamelis virginiana* and *Quercus robur*, roots of *Arctium lappa* and rhizomes of *Potentilla erecta*.

Saxena *et al*, [1994] evaluated the antimicrobial activity of the methanol extract and isolated constituents of *Rhus glabra* (Anacardaceae) a sp. used in folk medicine by North American native people against eleven microorganisms including gram+ve and gram-ve bacteria. The extract was subsequently fractionated and monitored by biossay leading to isolation of 3 antibacterial compounds, the methylester of 3,4,5 - trihydroxybenzoic acid [methyl gallate] [MIC 12.5 μg/ml], 4- methoxy-3,5- dihydroxybenzoic acid [MIC 25 μg/ml] and gallic acid [ MIC> 1000 μg/ml]. Tanira *et al*, [1994] reported the antimicrobial and phytochemical screening of twenty one medicinal plants of the United Arab Emirates including *Nigella sativa, Cyperus rotundus, Calotropis procera, Cistanche tubulosa, Heliotropium kotschyi* and *Teucium stocksianum*. About 50% of the plant extracts showed antimicrobial activity against one or another type of microorganisms such as *Candida albicans* and *Klebsiella pneumoniae* .

Kandil *et al*, [1994] reported a preliminary phytochemical screening of the plant *Thymus capifatus* which exhibited the presence of saponins, resins, flavonoids, essential and fixed oils. Aqueous and ethanolic extracts (10 - 200 mg/ml) as well as saponins, resins and essential oil of the plants ( 10-5000 μg/ml ) inhibited the growth of several bacteria and fungi. Perez and Anesini [1994] screened aqueous extracts of 132 samples from fifty four plants families, commonly used in Argentine folk medicine for their antibacterial activity against *Salmonella typhi* by agar well diffusion method. A reference concentration response curve for ampicillin was used
to estimate the apparent activity of the samples. Twenty four species showed antibacterial activity, in which the extracts of *Foeniculum vulgare*, *Citrus sinensis*, *Glycrrhiza astragalina*, and *Rosmarinus officinalis* *Cassia occidentalis* roots, *Hemila salicifolia* aerial parts, *Punica granatum* fruit pericarp and *Rosabohnana* flowers were found to be more active. In view of the multiple drug resistance of *Salmonella typhi*, these findings could be useful in the search of new clinically useful antimicrobials.

Sotohy *et al.*, [1995] tested *in vitro* effect of tannin containing Egyptian plants and their extracts on the survival of pathogenic bacteria and reported antibacterial activity of soluble polyphenols and condensed tannins. The antimicrobial effect was only observed on *Clostridium perfringens*. It is suggested that plants containing tannins could be used as a forage supplement (at low dose of 0.5%) to reduce animal losses by enterotoxaemic diseases such as pulpy kidney disease in sheep. Amphawan *et al.*, [1995] studied antifungal properties of turmeric oil and curcumin, isolated from *Curcuma longa*, against fifteen isolates of dermatophytes, four isolates of pathogenic molds and six isolates of yeasts. The inhibitory activity of turmeric oil was tested in *Trichophyton* induced dermatophytosis in guinea pigs. The results showed that all fifteen isolates of dermatophytes could be inhibited by turmeric oil at dilution of 1:40-1:320, however, curcumin did not show any activity. Isolates of pathogenic fungi were inhibited by turmeric oil at dilution of 1:40-1:80 but not inhibited by curcumin. All the six isolates of yeasts proved to be insensitive to both turmeric oil and curcumin in an other experiment. The dermal application of turmeric oil (1:80 dilution) on the 7th day following dermatophytosis induction with *Trichophyton rubrum* in the experimental animals, an improvement in lesions was observed in 2-5 days and the lesions disappeared in 6-7 days after the application. Alonsopaz *et al.*, [1995] screened Uruguayan medicinal plants for their antimicrobial activity including *Apium leptophyllum*, *Andredera cordifolia*, *Commelina erecta*, *Equisetum giganteum* specially against *Escherichia coli* and *Pseudomonas aeruginosa*. It was observed that gram-ve organism *Pseudomonas aeruginosa*, was inhibited by plant extracts although it is more resistant to antibiotics than other gram-ve bacteria [Stickler and King, 1992].

John *et al.*, [1996] reported activity of bromhexine and ambroxol, semisynthetic derivatives of vasicine from the Indian shrub *Adhatoda vasica* against *Mycobacterium tuberculosis* *in vitro*. Morina [1996] tested aqueous extract of

David Mirelman [1997] investigated the mechanism of fighting infection by allicin, the main biologically active component in Allium sativum. Allicin disables dysentery causing amoebae by blocking two enzymes, cysteine proteinases and alcohol dehydrogenases. The cysteinproteinase enzymes provide infectious organism a way to damaging and invading into tissues while alcohol dehydrogenases help the harmful organism to metabolise and survive. As these enzymes are found in many different harmful organisms, the allicin could be used as as broad-spectrum antimicrobial drug that can fight a wide range of infections.

Zhu et al, [1997] observed the biological activity in polyphenols obtained from some plant extracts, whereas the biological activity was missing in the remaining extract. It was suggested that the loss of biological activity was due to the removal of active compounds from the extract.

Akande and Hayashi [1998] tested two strains of enteropathogenic gram+ve bacteria (Staphylococcus aureus and Staphylococcus auricularis) for their resistance to extracts from tropical chewing stick species. The Result showed that only Terminalia glaucesens showed appreciable broad antibiotic effect against Staphylococcus aureus and Staphylococcus auricularis. Intense antibiotic activity against Staphylococcus aureus occured when using a 2.0 g/l extract concentration and a thirty hours incubation. Terminalia glaucesens also showed strong activity against Staphylococcus auricularia at 2.0 g/l concentration at a thirty hours incubation when all other extracts had lost their potency. Azadirachta indica is, however, most effective against Staphylococcus aureus, showing appreciable antibiotic activity at 0.4 g/l concentration at a thirty hours incubation. Zanthoxylum gillettei has no antibiotic activity against any of the test bacteria.

2.2.3. Selected Indian Medicinal plants

The Indian subcontinent is enormously endowed with one of the most
indigenous knowledge base of medicinal plants and herbs that has played a vital role in traditional health care system. Recently attention is being paid to the possible use of the plants as another source compounds with antibiotic activity. The Indian folk medicines comprise of numerous herbal prescriptions for various therapeutic purpose. The plants were selected on the basis of (a) medicinal folklore reports and (b) some preliminary reports on antimicrobial properties of medicinal plants.

2.2.3.1. Emblica officinalis (Euphorbiaceae) is locally known as "Amla". The fruit extract of the plant showed several biological activities such as antiulcer, antmutagenic, hepatoprotective, hypolipidaemic, anti-inflammatory and antipyretic [Chawla et al, 1982; Thakur and Mandal, 1984; Asmawi, 1993; Rani et al, 1995; Gulati et al, 1995]. The scientific evaluation of Emblica officinalis revealed their antibacterial activity against Staphylococcus aureus and Escherichia coli and antimicrobial substance phylllemblin was isolated from stem gall callus of the plant [Khanna et al, 1973; Jain, 1974]. However, detailed study on antibacterial and antifungal activities of this plant has not yet been reported.

2.2.3.2. Holarrhena antidysenterica (Apocynaceae) is commonly known as "Kurchi". The crude extract of this plant is widely known for its antiprotozoal, anthelmintic, antiamoebic and hypotensive activity [Singh et al, 1980; Shah & Qadry, 1993 and Siddiqui et al, 1995]. The in vitro inhibitory action of active alkaloid conessine, on the growth of Mycobacterium tuberculosis was observed by Lambir and Bernard [1953]. In India, Dey & Das [1988] reported antibacterial activity against Shigella sp. However, no detailed study were reported for their antibacterial and antifungal activity.

2.2.3.3. Plumbago zeylanica (Plumbaginaceae) is popularly known as "Chita". Screening of the root extract demonstrated several types of bioactivity including antiulcer, antileprosy, antitumour, anticancer and hypolipidaemic activity [Chopra et al, 1956; Dahanukar & Hazra, 1995; Kaviman et al, 1996]. Ethnopharmacological data available on this plant indicated promising antimicrobial activity against Staphylococcus aureus, Staphylococcus citreus, Klebsiella pneumoniae and Neisseria gonorrhoe [Krishnaswamy, 1980; Gundidza & Manwa, 1990]. It was interesting to note that the active principle of this plant, a napthoquinone, plumbagin also exhibited antifungal activity against Rhizopus nigricans and Penicillium notatum [Purushothaman, 1985] This further prompted us to study the antimicrobial
activity of this plant against several pathogenic fungi and bacteria.

2.2.3.4. *Terminalia chebula and Terminalia belerica* (Combretaceae) are commonly known as "Harir and Bahera" respectively. The crude extract of *Terminalia chebula* is widely known for its anticancer, antiasthmatic and antimutagenic property [Azeem *et al.*, 1992; Grover & Bala, 1992; Tokura *et al.*, 1993] while the extract of *Terminalia belerica* for its antiseptic and antiasthmatic activities [Trivedi *et al.*, 1982; Bakhru, 1995]. The antibacterial activity of *Terminalia chebula* fruit extract against *Clostridium tetani* was reported by Kulkarni *et al.*, [1995] while as Nandy *et al.*, [1997] observed the antimicrobial activities of fruit extract of *Terminalia belerica*.

2.3. CYTOTOXICITY OF PLANT EXTRACTS

Cytotoxicity testing of indigenous drugs is another essential part of preclinical studies when the drugs are to be used in clinical practice. All active principle of plants and natural products should be subjected to the some cytotoxicity studies as for synthetic drugs. Because there is a large gap between potential antimicrobial extracts and therapeutically potential extract. This may be due to the many reasons including the toxicity of plant extract itself. Several in vitro and in vivo methods are available for toxicity screening of medicinal plants. In the recent years many in vitro method have been developed which includes cytotoxicity studies by crown gall tumor inhibition, cytotoxicity to sheep erythrocytes, plant cell culture and animal cell culture and DNA intercalation prescreen etc. as described by [Martin-cordero *et al.*, 1995; Gupta *et al.*, 1996]. Of these, cytotoxicity to sheep erythrocytes assay is more valuable in determining the primary toxicity of crude plant extracts.

Kusumoto [1992] found that the plant extracts of Indonesian plants *Loranthus parasiticus*, *Helicteres isora*, *Strobilanthes crispus* and *woodfordia floribunda* having inhibitory effects on reverse transcriptase of an RNA tumor virus-I showed no appreciable cytotoxicity at concentrations where more then 90% of reverse transcriptase activity was inhibited.

Maria [1994] reported the cytotoxic screening of selected terpenoids from Asteraceae species and twelve pure compounds were subjected to cytotoxic screening. Three different cell lines in culture (KB, KB-VI and P 388) were used in cytotoxicity assay. The significant cytotoxic activity was exhibited by five sesquiterpene lactones and moderate cytotoxicity by eudesmane.

Xian-guoHe [1994] examined the cytotoxicity of the extract of *Solanum nigrescens* against sheep erythrocytes and observed hemolysis of the erythrocytes
at all dilutions of the extract ranging from 1:1 to 1:1000 to a similar degree as in a positive control of tap water leads to be an obstacle to its systemic use.

Vijaya et al, [1995] performed cytotoxicity test of the extracts of *Camellia sinensis* and *Euphorbia hirta* L. using the cell line. These extracts were found to be non-cytotoxic and effective antibacterial agents. Martin-Cordero et al, [1995] evaluated cytotoxic activity of an aqueous extract obtained from *Retama sphaerocarpa* against an animal cell culture (Help-2 Cell line) and brine shrimp toxicity test. It was concluded that this extract inhibited the cell growth in animal cell cultures and induced brine shrimp lethality.

Gupta et al, [1996] tested the cytotoxicity activity of crude extracts from 20 Panamanian plants used in traditional medicine and observed cytotoxicity in the clonogenic assay in three plant extracts, *Cyperus luzulae, Piper auritum and Psychotria correae*. Desmarchelier et al, [1996] performed the brine shrimp cytotoxicity bioassay to determine the toxicity of thirty nine extracts used traditionally as medicines by Ese'ejas, Amerindians who occupy the south-west Amazon rain forest, where only six plants showed strong cytotoxicity.

2.4. PHYTOCHEMISTRY OF MEDICINAL PLANTS

The post independence period has witnessed a spurt in the chemical studies on Indian medicinal plants and natural products isolated from them showing pronounced antimicrobial activity for their economic production and utilization in chemotherapy. Nevertheless, natural products of plant origin remain one of the few *de novo* sources of drug discovery [Dhawan, 1986]. Primary metabolites such as proteins, nucleic acid are the substances that are widely distributed virtually in all organisms occurring in one form or the other. They are needed for general growth and physiological development and they play an important role in basic processes of metabolism [Applezweig, 1980]. Primary metabolites can not be used as intermediates in the manufacture of high value semi-synthetic pharmaceutical products [Aharonowitz and Cohen, 1981]. Secondary metabolites often play ecologically significant role in how plants deal with their environment and, therefore, they are important in their ultimate survival. Secondary metabolites in plants may serve as a pollinator attractants. They help in chemical adaptations to environmental stresses and serve as defensive, protective or offensive chemicals against microorganisms, insects and higher herbivorous, predators and even against higher plants. [Wallace and Mansell, 1976; Mann, 1978; Rosenthal and Janzen, 1979; Bell and Charlwood, 1980].
Secondary metabolites are, by definition, biologically active compounds, frequently present in small quantities compared to primary metabolites [Farnsworth and Morris, 1976]. Although, large quantities of secondary metabolites are not usually required due to their very strong biological activity and their selection by the external pressure during evolution, they are accumulated in plants due to continuous stimulation. They are biosynthesised in special type of cell and at distinct developmental stages as shown in Flow chart 1 making their isolation and purification difficult [Farnsworth and Bingel, 1977]. As a result, the secondary metabolites used commercially as biologically active compounds such as in pharmaceuticals, flavour, fragrances and pesticides etc. are generally high value-low volume products. Thus, many bioactive secondary metabolites can be considered as speciality chemicals. As secondary metabolites often have highly complex stereostructures with many chiral centres for their biological activity, the conception that the most plant derived bioactive compounds are now produced synthetically seems to be unjustified [Nakanish, 1982; Klayman, 1985]. Biologically active secondary metabolites have found medicinal application as drug entities or as model compounds (templates) for synthetic and semi-synthetic drugs [Bohonos & Piersma, 1966]. Some of the important secondary metabolites found in higher plants are alkaloids, flavonoids, glycosides, saponins, tannins, volatile oils, gums and resins etc. [Iyenger, 1985].

2.4.1. Alkaloids: Alkaloids are naturally occuring, nitrogenous compounds. They are mostly basic in character and exist in plants, in the form of salts of inorganic or organic acids [Chatwal, 1990]. The nitrogen, they contain, may be a part of the open chain or a part of the heterocyclic ring system [Deven & Scott, 1975]. Alkaloids are, more or less toxic substances which act primarily on the central nervous system [Shah and Qadry, 1993]. According to Iyenger [1985] alkaloids may be classified into following groups: Tropanes (Belladonna herb, Hyoscyamus and Datura), Indoles (Rauwolfia, Ergot etc.), Quinoline (Cinchona), Isoquinolines (Ipecac), Steroids (Holarrhena antidyssenterica), Phenanthrenes (Opium), Pyrazole (Withania), and Diterpenoids (Aconite). Alkaloids are good analgesic, antipyretic, antispasmodic, stimulant, narcotic, and sedative, [Iyenger, 1985].

2.4.2. Glycosides: Glycosides are naturally occuring compounds which yield a sugar portion (glycone) and a non-sugar portion (aglycone) on hydrolysis [Chatwal, 1990]. The linkage between the reducing group of sugars and the phenolic hydroxyl or alcoholic hydroxyl groups of aglycone is called a "hemiacetal linkage."
FLOW CHART 1

Biosynthetic origin of some commercially important plant derived compounds (major groups of secondary metabolites are indicated by boxes)

\[ \text{Co}_2 + \text{H}_2\text{O} \xrightarrow{\text{hv}} \text{CHLOROPHYLL} \]

CELLULOSE PECTIN
GUMS STARCH MUCILAGES

PHOTOSYNTHESIS

SUGARS

TERPENOIDS

DIGITOIN
STEVIOSIDE

NAPHTHOQUINONES

SHIKONIN

AMINO ACID

ACETYL - CoA

MALONYL - CoA

POLYKETIDES

ALKALOIDS

TERPENOIDS

FLAVONOIDS

PYRUVIC ACID

CYANOCENIC GLYCOSIDE

NON - PROTEIN AMINO ACID AND DERIVATIVES

PHENOLS
POLYPHENOLS
TANNINS
VANILLIN

Menthol
Rose oil
Peppermint oil
Myrrh
Terpine Sterol, Steroids
Caralone

Reserpine
Codiene
Morphine
Callithraethes Alkaloids
Atropine
Cocaine
Nicotine

Source: Biotechnology in agriculture and forestry Medicinal and aromatic plants lst edited by X P S Bajaj, Springer - Verlag Berlin Heideby (New York)
Glycosides of this type are referred to as O-glycosides which easily undergo hydrolysis. Besides these, there are other types as well, like C-glycosides which do not undergo hydrolysis under normal conditions, N-and S-glycosides etc. [Iyenger, 1985]. According to the basic structure of the aglycone portion, glycosides are further grouped as under:

2.4.2.1. Steroidal (cardiac) glycosides: Their aglycones have a steroidal structure and are responsible for their specific actions, for example, they increase the intensity and decrease the rate of heart beat [Iyenger, 1985]. Based on the lactone ring, cardiac glycosides are divided into two groups; if 5 membered lactone ring, then cardenolides e.g. *Digitalis*, and if 6 membered lactone ring, then Bufadienolides e.g. *Urginea* [Chatwal, 1990]. Slight disturbance in the lactone ring causes the loss of activity of the compound. Cardiac glycosides contain a special sugar called digitoxose and they may carry other sugars like glucose and rhamnose as well. Very often diuretic action is associated with steroidal glycosides as they promote improved circulation of blood through kidneys [Shah and Qadry, 1993].

2.4.2.2. Saponin glycosides: The aglycones which result after hydrolysis of the glycosides are called sapogenins. Sapogenins could either be steroidal or triterpenoid type [Lalitha and Venkataraman, 1991]. They are of medicinal value and are employed, for instance, as expectorant, emetic and diuretic etc. A common example is Licorice. [Iyenger, 1985].

2.4.2.3. Anthracene glycosides: These are very good laxative [Dhawan, 1986]. They irritate the mucosa of the gut mainly of the large intestine. Chemically they are derivatives of anthracene and occur usually in two forms: the oxidized form e.g. anthraquinones and reduced forms e.g. anthrones, anthranols etc. [Devon and Scott, 1975].

2.4.2.4. Cyanogenetic glycosides: They yield hydrocyanic acid as one of the products on hydrolysis. Wild cherry bark contains this type of glycoside [Iyenger, 1985].

2.4.2.5. Flavonoid glycosides: These are mostly yellow pigments present in plants and licorice is one of the examples. They are phenolic in nature and are derivatives of 2-phenylbenzopyrones. A large number of physiological activities have been attributed to them. Some flavones may act as cardiac stimulant, some strengthen weak capillary blood vessels, some are good diuretic and some proved to be
extremely good against liver damage [Havsteen, 1983].

2.4.2.6. Resinous glycosides: Some cathartic drugs, such as Ipomoea, owe their purgative action to the resin part of the glycosides [Iyenger, 1985]. The aglycone portion of the glycosides is responsible for the attributed therapeutic activity. It is very essential that sugars should also be present for promoting the activity [Shah and Qadry, 1993].

2.4.3. Tannins: Tannins, e.g. Catechu, are naturally occurring complex organic compounds possessing nitrogen free polyphenols of high molecular weight. They precipitate proteins and alkaloids. The astringent nature of tannins is due to their capability to precipitate proteins and to render them resistant to enzymatic attack. When applied on injury or a wound, tannins form a protective coating so as to prevent external irritation and thus promote healing [Sawabe et al, 1996].

2.4.4. Volatile oils: Volatile oils, such as lemongrass and cinnamon oils, are volatile principles obtained from plant materials by steam distillation. They possess characteristic odour. The active constituents present in volatile oils, are terpenes in association with alcohols, phenols, ketone, aldehyde or esters [Chatwal, 1990]. They are largely carminatives in action. Some are good appetizers as they can stimulate the secretion of gastric juice. Some volatile oils are good expectorant, diuretic, antiseptic, anthelmintic and antiparasitic [Iyenger, 1985].

2.4.5. Gums: These are plant exudates, chemically they are amorphous polysaccharides. They have protecting and soothing action. Some are good laxatives while others are used in pharmaceutical preparation as emulsifying or suspending agents [Shah and Qadry, 1993].

2.4.6. Resins: Resins are naturally occurring complex organic compounds. Like gums these are also exudates from the tree bark. They are oxidation products of essential oils. Resins are used in wound dressing and for filling the cavities of teeth caries as they melt [Leung, 1980]

2.5. PHYTOCHEMICAL SCREENING OF SELECTED PLANTS

2.5.1. Emblica officinalis: Chopra et al, [1980] reported that Emblica officinalis fruit contains phyllembic acid (6.3%), lipids (6%), and gallic acid (5%). Phyllembin from fruit pulp was identified as ethylgallate. Fruit of the Emblica officinalis is a rich source of vitamin C, pectin and tannins such as gallic acid, trigalloylglucose, terchebin, corilagin, phyllembic acid, phyllembelin and ellagic acid [Iyenger, 1985; Chopra, 1992; Shah & Qadry, 1993; Rastogi & Mehrotra 1995].

2.5.3. *Terminalia belerica*: The fruits of *Terminalia belerica* contain 17% tannins such as gallic acid, ellagic acid, ethyl gallate, galloylgucose and chebulagic acid. It also contains glucose, galactose, mannotol, fructose and rhamnose [Iyenger, 1985]. Rastogi and Mehrotra [1995] isolated a new cardiac glycoside, bellericanin, which yielded glucose and galactose (2:1). Nandy *et al.*, [1997] reported triterpenoids such as methylester of arjungenin, methylester of tomentosic acid, bellericagenin-B, bellericaside-B, belleric acid, arjunglucoside-I, and bellericoside responsible for antimicrobial properties of the fruits of *Terminalia belerica*.

2.5.4. *Plumbago zeylanica*: Purushothaman *et al.*, [1985] reported a naphthoquinone, plumbagin, in the roots of *Plumbago zeylanica*. Behl *et al.*, [1993] as well as Rastogi & Mehrotra [1993 & 1995] isolated a number of naphthoquinone derivatives such as plumbagin, 5- chloroplumbagin, 3, 3-biplumbagin, elliptinone, isozeylinone, chitanone, zeylinone, drosenerone, plumbagic acid and plumbazeylenone from the roots. The roots of *Plumbago zeylanica* also yielded three new compounds viz. nonylnonanoate, nonyl-8-methyl-dodec-7-enoate and benzyl-2,5-dihydroxy-6-methoxy benzoate. The compounds such as 2,2-dimethyl-5-hydroxy-6-acetyl chromene, stigmasterol acetate, lupeol acetate, friedelinol, lupeol, luponane, sitosterone and stigmasterol were also isolated [Gupta *et al.*, 1995].

2.5.5. *Holarrhena antidysenterica*: Iyenger [1985] isolated steroidal alkaloids (2-4%) such as conessine, norconessine, isoconessine, kurchicine, holarrhimine and holarrhenine from the stem bark of *Holarrhena antidysenterica*. Chopra, [1992] and Shah and Qadry [1993] reported that stem bark of *Holarrhena antidysenterica*
contains 1.5% to 3% alkaloids in which 20-30% is conessine, the active alkaloid. Conessine is non-oxygenated and in addition to steroid structure, it contains 5-membered nitrogen containing ring and a dimethyl amino group at 3 position. Other minor alkaloids are conessimine and isoconessimine. A second group of oxygenated tetracyclic alkaloids like holarrhimine, kurchicine and hollarrhidine are also present.