PART - B

CHAPTER - 5

Introduction about the medicinal plants

Nature always stands as a golden mark to exemplifly by the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all interdependent. The plants are indispensable to man for his life. The three important necessities of life - food, cloth and shelter and a host of other useful products are supplied to him by the plants kingdom. Nature has provided a complete store - house of remedies to cure all ailments of mankind. The human beings appear to be afflicated with more diseases than any other animal species. In the past, almost all the medicines used were from the plants, the plant being man's only chemist for ages. The history of herbal medicines is as that old as human civilization. The documents, many of which are of great antiquity, revealed that plants were used medicinally in China, India, Egypt and Greece long before the beginning of the Christian era. Most of the medicinally active substances identified in the nineteenth and the twentieth centuries were used in the form of crude extract. In China, many medicinal plants had been in use since 5000 B.C. A large portion of the Indian population even the present time, depends on the Indian System of Medicine, Ayurveda Ancient science of life. The well known treatises in Ayurveda are the Charak Samhita and the Sushrath Samhita.

Over years infectious organisms have developed resistance to synthesised drugs. Tetracycline and erythromycin have been used as treatment for *P.acne* since forty years, but recent reports have shown that these have now developed world wide antibiotic resistance.

Conventional herbal medicines offer safe, well tolerated remedies for chronic illnesses. It is experiencing a dramatic renaissance in Western countries, partly because no effective treatment as yet exists for many chronic illness. In addition concern over side effects of bio medicine is encouraging people to look for more gentle forms of treatment.

In recent years extensive research work is being carried out on medicinal plants possessing antibacterial and / or antifungal activities. Researchers always aim to bring out

cost - effective and efficacious medicines for benefit of mankind. Pure and isolated plant constituents have given various world's most useful drugs and are of great importance.

Medicinal plants have curative properties due to presence of various complex chemical substances of different composition, which are found as secondary plant metabolites in one or more parts of these plants. These plants metabolites, according to their composition are grouped as alkaloids glycosides, corticosteroids, essential oils etc. Among these alkaloids form the largest group.

Now a days research work in the area of medicinal plants is directed towards identification and collection of medicinal plants and evaluating antimicrobial and other activities of the crude extracts of different parts of medicinal plants. Further, importance is also given for separation, isolation and identification of bio-active molecules from these plants. Thus, enormous work is being carried in this area which has resulted in the accumulation of voluminous literature. However, keeping in view, the limitation of this thesis, only important and recent research findings have been described in the following pages.

The antibacterial properties of the ethanol and aqueous extracts of twenty eight common herbal remedies used in south Texas to treat wounds and infections were analysed. Disk diffusion susceptibility tests were carried out on three bacteria, *S. aureus, P.aeruginosa* and *E. coli.* Romeo *et al.*, found that most of the plants were potentially active against *S. aureus*[1].

Ethanol extracts of *Brunfelsia grandiflora, Caesalpinia spinosa, Dracontium loretense, Equisetum giganteum, Mayteus macrocarpa, Phyllanthus amarus, Piper aduncum, Terminalia catappa* and *Uncaria tomentosa* were tested for antimicrobiol activity

against nine strains of bacteria. Among these *Phyllanthus amarus* and *Terminalia catappa* showed most promising antibacterial activity[2] with minimum inhibitory concentrations (MICs) ranging from 0.25 to 16 mg/ mL.

The leaf, branch, stem, kernel, shell skins and seeds of *Pistaciavera* were screened against *E.coli, P.aeruginosa, Enterococcus faecalis, S.aureus, C.albicans* and *C.parapsilosis* by micro dilution method. The extracts were reported to possess little antibacterial activity but noticeable antifungal activity at 128-256 μg/mL concentration[3].

Ashwaganda (with *Ania somnifera*) was evaluated for its antibacterial activity[4]. Both the aqueous and alcoholic extracts of the root and leaves of the plant were found to be strong antibacterials.

A survey was done by Canales *et al.*, of the region covering San Rafael coxcatlan, Puebla, and sixteen plants were sequentially extracted with hexane, ethyl acetate and methanol. *Jatropha neopauciflora* and *Juliania adstringens* showed highest antibacterial activity[5].

The antioxidant, radical - scavenging, anti inflammatory, cytotoxic and antibacterial activities of methanolic extracts of seven hedyotis species were investigated. *H.capitellata* roots showed activity towards *B.subtilis and P.aeruginosa*[6].

Khattak *et al.*,[7] carried out antimicrobial activity of the ethanolic extracts of *Curcuma longa* and *Alpinia galanga.* The extracts were found to be phyto toxic against lemna minor and antifungal activity against *Trichophyton longifusus.*

Stafford *et al.*, assessed the *in vitro* biological activity of nine frequently used medicinal plants in South Africa. The plants *Alepidea amatymbica, Leonoties leonurus,*

Drimia robusta, Vernornia colorata, Merwilla natalensis, Eucomis autumnalis, Bowiea Volubilis, Helichrysum cymosum and *Siphonochilus aethiopicus* were investigated and all the plants had considerable antibacterial activity[8].

Six traditional medicinal plants, i.e. *Andredera cordifolia, Elaeodendron transvaalense, Elephantorrhiza burkei, Senna petersiana, Terminalia sericea* and *Rauvolfia ceffra* use to treat sexually transmitted diseases in folklore, were investigated for their antibacterial activity[9].

The results were encouraging and hence provided support to the ethnomedicinal uses of these plants.

Extracts of C*occinia adoensis, Cineraria grandiflora, Pavonia urens, Marattia fraxinea, Clutia abyssinica* and *Vangueria infausta* were made using ethyl acetate, methanol, cold water and boiling water. Upon testing these extracts against *C.albicans* and *S.aureus* considerable antimicrobial activity was observed [10].

Aqueous and methanolic extracts of *Urticaurens, Capparis tomentosa, Dicoma anomala, Leonotisleonorus, Xysmalobium undulatum, Helichrysum foetidum, Terminalia sericea* and *Gunnera perpensa* were investigated for antibacterial activity against *S.aureus, S.pyogenis, E.coli* and *P.*aerugenosa. Steenkamp and co-workers[11] found that *Terminalia sericea* and *Gunnera perpensa* extracts were more active against *S.pyogenes* and *S.aureus* compared to other extracts.

From the island Soqotra, twenty-five plants were selected by Ramzi *et al.*,[12]. All the plants were extracted with chloroform, methanol and hot water. The extracts were screened against Gram-positive and Gram-negative bacteria. The highest antibacterial activity was exhibited by the methanolic extracts of *Boswellia elongatta, Boswellia ameero, Buxus*

hildebrandtii, Jatropha unnicostata and *withania riebeckii.* Only methanolic extract of *Buxus hildebrandtii* displayed significant antifungal activity.

Mudassir *et al.*, from Balochistan, Pakistan found that the extracts of *Zygophyllum fabago* were highly effective against *C.albicans* and *E.coli.* The extract of *Vincetoxicum stocksii* was active against *C.albicans, B. subtilis* and *B.cereus. Hymenocrater sessilifolius* and *Grewia erythraea* showed activity only against *P.*aerugenosa[13].

Bonjar screened forty five species of plants against one or more bacterial species such as *S.qureus* and *S.epidermidis.* All plants under investigation showed activity[14].

Chemical and anti bacterial activity of mango varieties of Guinea was carried out by Keita *et al.*, Antibacterial activity was carried out using Cup- Plate method against *S.aurens and E.coli* - Different results were obtained depending upon the varieties of mango[15].

Voravuthikunchai *et al.*,[16] of Thailand tested fifty eight preparations of aqueous and ethanolic extracts of thirty eight medicinal plants for their antibacterial activity and demonstrated appreciable inhibition zone against *E. coli.*

The methanolic extracts of five tropical plants *B.frutescens, Glycyrrhiza glabra, Kaempferia pandurata, Physalis angulta* and *Quercus infectoria* exhibited antibacterial activity against carcinogenic bacterium *S. mutans*[17].

Kone *et al.*,[18] screened sixty seven crude ethanolic extracts from fifty plants against Gram negative *(E.coli and P.aerugenosa)* and Gram positive (*S.aureus, E. faealis, S.pyogenes and B.subtilis)* bacteria. Amongst the plants investigated, *Erythrina, Senegalensis, Ximenia americana, Khaya senegalensis, Lannea acida, Cissus populnea, Keetia hispida* and *Ficus thonningii* exhibited moderate activity.

The Nigerian plants, *Anthocleista djalonensis*, *Nauclea latifolia* and *Uvaria afzalii* were extracted by maceration in ethanol, cold and hot water. The extracts were tested against *S.aureus, E.coli, B. Subtilis, P.aerugenosa* by agar diffusion and macroboth dilution methods. Bacteriostatic to Gram positive and bactericidal to Gram negative strains was observed in case of *Anthocliesta djalonensis*. *Nauclea latifolia* extracts were bacteriostatic to both the strains, *Uvaria afzalii* showed bactericidal activity towards only Gram positive bacteria[19].

Dianthus coryophyuus was phytotoxic to Gram-positive and Gram-negative bacteria. *Myrtus communis* seeds were susceptible to *S.aureus, B.cereus and B. bronchiseptica*, where as *Terminalia chebula* ripe seeds were found to be active against *S. aureus*[20].

The leaves, bark and roots of seventy two Malaysian plants[21] were screened for antibacterial activity, out of which the following displayed maximum activity. *Peristrophe tinctoria, Polyalthia lateriflora, Knema malayana Solanum torvum, Celsosia argentea, Eclipta prostrata, Ancistroclodus tectorius, Dillenia suffruticosa, Piper stylosum* and *Rafflesia hasseltii.*

Chamundeeswari *et al.*,[22] have reported that the alcoholic extract, its chloroform soluble and aqueous fractions of the roots of *Trewia polycarpa* showed varying levels of antibacterial and antifungal activities against six bacterial and four fungal strains.

The hexane, chloroform and ethanol extracts of *Lippia graveolens, Lantana achyranthifolia, Turnera diffusa, Lippa oaxacana, Gymnolaena oaxacana, Cordia curassavicaa, Lantana camara* and *Acalypha hederacea* from Mexico were found to be effective against few Gram-positive and Gram-negative bacteria[23].

The Indonesian *ethnomedical* plants were extracted using methylene chloride and methanol. Extracts were tested for their antifungal and anti bacterial properties[24]. The results were appreciable and hence were found to be a new development in the field of pharmaceutical chemistry.

Twelve plants used traditionally in Kenya for treating infections and / or inflammatory diseases, were screened for antibacterial and antiinflammatory activity[25]. *Maytenus senegalensis, Plectranthus barbatus, Zanthoxylum chalybeum, Z. usambarense* and *Spiranthes mauritianum* were effective against *S. aureus.*

Methanol extracts of *Satureja hortensis* from Turkey were tested for antibacterial activity against fifty five bacterial strains, one yeast and four fungi using disk diffusion method. The extract showed both anti candidal and antibacterial effects[26]. Seven herbal drugs like, *Aristolochia trilobata, Syngonium podophyum*, etc. from central America were evaluated for their antibacterial properties against *E.coli, P.aeruginosa, S.aureus* etc. All the plant extracts were active against one or other micro organisms[27].

The antibacterial and antifungal activities of the ethanol extract of *Erigeron breviscapus* was evaluated and found that it showed moderate antibacterial activity but high antifungal activity[28].

Gnanamani and co workers[29] evaluated the antibacterial activity of crude alcohol extract of *Datura alba* and *Celosia argentea*, the former had more antibacterial activity in comparison with the latter.

The antimicrobial activity of an antioxidant ethanolic extract of *Arctostaphylos uva-ursi* leaves alone and in combination with nisin was determined against twenty - five food related bacteria by the spot - on lawn and the micro - dilution methods. The extracts alone

had no significant activity. It enhanced the antibacterial activity when used in combination with nisin[30].

Azadirachta indica, Cinnamomum cassia, Rumex nervosus, Ruta graveolens, Thymus serpyllum and *Z. officinale* were most sensitive against *B. cereus. Cinnamomum cassia* showed highest MIC with *E.coli* and *S.infantis* [31].

The ethanolic extracts of the leaves and flowering tops of *Acanthospermum hispidum* were tested against pathogenic fungi and it was found that the activity lies in the polar fractions of the ethanolic extract [32].

Getie *et al.*, tested crude extracts of the leaves of *Dodonaea viscosa, Rumex nervosus* and roots of *Rumex abyssinicus* against *S.pyogenes* and *S. aureus.* All the three plants were shown to possess good antibacterial activity[33].

The Bangladeshi medicinal plants *Toona ciliata* and *Amoora rohituka* along with siderin, a coumarin from *Toona ciliata* was demonstrated to exhibit significant anti bacterial activity[34].

Crude ethanol and water extracts of leaves and barks from *Cassia alata* were tested in vitro against two fungi *A. fumigotus* and *Microsporum canis*, one yeast-*C.albicans* and two bacteria-*S.auereus* and *E.coli.* Antifungal activity showed varying results depending upon the extract. *C.albicans* had concentration dependent susceptibility toward both ethanol and water extracts. The water extracts of the leaves showed significant antibacterial activity[35].

Antibacterial effect of *Terminalia macroptera* leaf extract was investigated on *Neisseria gonorrhoea* by Silva and co workers[36]. The plant was found to be active against the test organism with MIC between 100 and 200 mg/mL.

Crude methanolic extracts and fractions from the aerial parts of three species of *Pterocaulon - P. alopecuroides, P.balansae* and *P. polystachyum,* grown in southern Brazil were investigated for antifungal activity against a panel of standardized, clinical opportunistic pathogenic yeast and filamentous fungi including dermatophytes, by agar dilution method[37]. The crude methanolic extract of *P.alope curoides* was found to be most active. *P.balalsae lipophilic* fractions showed remarkable activity against dermatophytes.

Batawila and co workers[38] investigated five species of *Comretaceae* growing in Togo for their antifungal activity against twenty pathogenic fungi. Five hydroethanolic extracts of *Terminalia glaucescens* and *Anogeissus leiocarpus* with MIC from o.25 mg/mL to 4 mg/mL were termed to be most active.

The agueous extract of *Xanthosoma sagitti folium* from Brazil showed good anitfungal activity against yeast *Trichophyton rubrum.* Clear inhibition zone was observed in the case of aqueous extracts of *Schinus molle, Schlius terebinthifolics* against *C.albicans* and *Anacardium occidentale* against *C.neoformans*[39].

Prasad *et al.*, [40] reported that the extracts obtained from the seeds of *Psoralea corylifolia* showed several degrees of antifungal activity against *T.rubrum, T.mentagrophytes, E.floccosum* and M.*gypseum,* by disc fusion method. The methanol extracts of the seeds were found to be most effective. An active compound 4 - methoxy flavone was isolated form the active fraction.

The less polar fraction of the methanolic extract from the plant *E.peplis* exhibited interesting antifungal and antifuberculor activity. A complex mixture of four cerebrosides was found responsible for this activity[41].

The antimicrobial activity of 18 prenylated flavonoids from various medicinal plants was tested using micro dilution method against *C.albicans, S.cerevisiae, E.coli, S.typhimurium, S.epidermis and S.aureus.* Papyriflavonol A, Kuraridin, Sophoraflavanone D and Sophoraiso flavanone A. exhibited good antifungal activity with strong anti bacterial activity. Morusin, sanggenon B and D, kazinol B, kurarinone, kenusanone C and Isosophoranone were effective against Gram positive bacteria and broussochalcone A were effective against *C. albicans.* This supported the fact that prenylated flavonoids are very good antimicrobial agents[42]

Three known alkaloids cycleanine, cocsoline and N- desmethycycleanine were isolated from *Albertisia villosa. Cycleanine* revealed potent antibacterial, antifungal, anti plasmodial and cytotoxic activities[43]. Ravindranath *et al.*, isolated two deoxypreussomerins, palmarumycins along with palmaromycin from *Jatropa curcas*, The first two compounds showed remarkable antibacteiral activity[44].

Sanguinarine a benzophenanthridine alkaloid derived from the root of *Sanguinaria canadensis* was tested for its antibacterial properties. It exhibited good antibacterrial activity along with antiinflammatory and antiangiogenic role[45]. Simon Gibbons isolated isopimarane diterpenes from *Lycopus europaeus* and tested them for in vitro antibacterial activity, especially methicillin resistant *S.aureus*. It was found that the compounds had two - fold potentiation of activities[46].

The husk fiber of *Cocos nucifera* presents antibacterial and antiviral activities[47]. Propolis a natural product derived from plant resins collected by the honey bees is used for innumerable years in folk medicine. The extract contained amino acids, phenolic acids, phenolic acid esters, flavonoids, cinnamic acid, terpenes and caffeic acid. It had several biological activities like antiinflammatory, antiviral and anitbacterial[48]. *Momordica charantia* is used in various systems of traditional medicine for curing several ailments such as diabetes, abortion, helminthiasis, contraception, dysmenorrhoea, eczyma, emmenagogue, antimalarial, galactagogue, gout, jaundice, abdominal pain, kidneystone, laxative, leprosy, leucorrhoea, philes, pneumonia, psoriasis, purgative, rheumotism, fever and scabies[49].

Kar *et al.*, studied antimicrobial activity as well as wound healing activity of stem bark of *Toddalia siatica* Linn[50].

The leaves of *Bauhinia tomentosa* Linn, have been investigated for their antimicrobial activity of the chloroform extract. It has been found to possess considerable antimicrobial activity[51].

Amin *et al.*, in their research work on essential oils from plants observed that the oil obtained from the plant *Oliveria decumbens*, possessed promising antimicrobial activity[52].

Iranian scientists Arasi *et al.*, collected one medicinal plant available in and around Tehran and isolated few volatile constituents from these plants. Evaluation of antimicrobial activities indicated that many of these constituents had considerable antimicrobial activity in addition to their fragrance[53].

Ashok Kumar *et al.*, from Karnataka have published a paper describing antimicrobial activity of *Triphalachurnam*[54].

Some spice hydrosols have been investigated for their antifungal activity by scientist from Turkey[54].

Maxico is also known for its wealth of medicinal plants. This fact encouraged a few scientists of that area to take up research work of these medicinal plants. The plant *Alternanthera caracasana* has been studied and ethanol extract of aerial parts of this plant were found to possess significant antimicrobial activity[56]. Recently, Fukai *et al.*,[57] isolated 2-aryl-benzofurans from morous spicies and investigated that antimicrobial activity against methicilian resistant *Stephylococcus aureus*.

The aqueous extract of *Xanthosoma sagittifolium* from Brazil showed good antifungal activity against yeast *Trichophyton rubrum.* Clear inhibition zone was observed in the case of aqueous extracts of *Schinus molle, Schinus terebinthifolius* against *C.albicans* and *Anacardium occidentale* against *Cryptococcus neoformans*[58].

The aqueous extract of flowers of *Butea frondosa*, collected from Nagpur [Maharastra] were found to possess promising antibacterial activity[59].

Similarly, flowers of *Lucas aspera* collected from Porur [Tamil Nadu] on investigation for antimicrobial activity by cup-plate method were found to possess antibacterial and antifungal activity comparable to standards[60].

The Korean scientist Shin studied antifungal activities of essential oils obtained from *Glehnia littoralis* and also investigated their synergetic activity in combination with standard drug Ketoconazole[61].

Singh[62] from Gorekpur [Himachal Pradesh] investigated Mulluscicidal activity of some medicinal plants available in that area. Medicinal plants belonging to eastern Anatolia region of Turkey were evaluated for their possible antimicrobial activity by MIC-method[63].

Halstead *et al.*, isolated some alkoloids from the stem bark of *Zanthoxylum ovalifolium* and evaluated them for various activities including antimicrobial activity[64].

An *et al.*, could isolate a new monoterpene glycoside from *Paeonia suffruticosa*[65]. Phytochemical investigation and antimicrobial activity of *Centaurium pulchellum Druce* have been reported from Pakistan[66].

Bruni *et al.*, in their research work pertaining to medicinal plants evaluated the bark of *Maytenus krukovii* for antimutagenic, antioxidant and antimicrobial properties[67].

Salazar *et al.*, could isolate coupled hydroxyanthracenones from the plants of the *Genous karwinskia* and screened them for antifungal and antibacterial activity by using Cup-Plate method[68].

In vitro antioxidant and antibacterial activity of the stem of *Rhaphidophora pertusa* has been investigated by Sasikumar *et al.*, [69].

China is well known for its wealth of medicinal plants. Thus, many scientists from China have embarked their research work on investigation of medicinal plants. In this connection Xiang wei *et al.*, could find out chemical composition of the essential oils extracted from *Sagittaria trifolia*[70].

Medicinal plants from Mali [Germany] have been evaluated for in vitro and in vivo trypanocidal activity by Bizimana *et al* [71].,

Chemical composition of essential oils of *Triumfetta rhomboidea Jacq* has been established and their antimicrobial activity has been investigated by French scientists[72].

Some of the medicinal plants of South Africa are fully used in the treatment of benign prostatic hyperplasia and prostatitis.

This fact encouraged Steen Komp *et al.*, to study antibacterial, antiinflammatory and antioxidant activities of some of the medicinal plants[73].

Urzua *et al.*, isolated a new cleradone diterpenoids from the resinous exudate of *Haplopappus uncinatus* and found that this compound possessed considerable antibacterial activity when tested against both Gram positive and Gram negative bacteria by cup-plate method[74].

The plants *Ferula latisecta* and *Mozaffariania insignis* from Iran are known to contain some important essential oils. Habibi *et al.*, could isolate some compounds of these oils and assigned their structure on the basis of spectral studies. They also investigated antimicrobial activity of these oils[75].

Recently comparative study of Gimnosperms and Angiosperms plants on quality and antibacterial activity has been made by Afrouzan *et al.*,[76].

Sook and Shin *et al.*, studied synergic effect on antifungal activity against *candida* and *Trichophytol* species in combination with essential oils of cori and rum sativum antibiotics[77] and observed that there is remarkable enhancement of antifungal activity.

One new tri-terpenoid along with 4-tri-terpenoids from *Combretum imberbe*, have been isolated and evaluated for antibacterial, antifungal and anti-inflammatory activity[78].

Jain and Bohra carried out antibacterial screening of crude juices of some ornamental plants[79].

Mooan *et al.*,[80] took up major work in connection with antifungal activity of Australian grown Lavandula species against *Aspergillus nidulans*, *Trichophyton mentagrophytes*, *Leptosphaeria maculans* and *Sclerotinia sclerotiorum*.

All the above reports on various medicinal plants and their antimicrobial and other pharmacological activities prompted us to take up the present work. The work is directed towards identification of various medicinal plants in this area, their extraction, evaluation of phytochemical constituents present in them.

The screening of crude extracts for possible biological and pharmacological activities has been carried out. The active fractions are semi purified. Biomolecules are isolated by HPLC and characterised by spectral and elemental data.

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