REVIEW ARTICLE

ANTIMICROBIAL ACTIVITY OF NATURAL PRODUCTS FROM MEDICINAL PLANTS

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INTRODUCTION

In the recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of Medicinal Plants used in various traditional, complementary and alternate systems of treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids, etc, which have been found in vitro to have antimicrobial properties.[1,2]

Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by the physicians; several are already being tested in humans. Scientists realize that the effective life span of any antibiotic is limited, so new sources especially plant sources are also being investigated. Second the public is becoming increasingly aware of the problems with the over prescription and misuse of traditional antibiotics. In addition many people are interested in having more autonomy over their medical care. A multitude of plants compounds (often of unreliable purity) is readily available over the counter from herbal suppliers and national food stores and the self medication with these substances is a common practice to certain extent.[2]

Section A highlights the various Medicinal plants which have shown antibacterial, antifungal, antiviral and antiprotozoal properties whereas in section B the main focus is on the different groups of phytochemicals exhibiting medicinal properties.

A. MEDICINAL PLANTS

ANTIBACTERIAL

In a study carried out by Chakraborty and coworker it has been observed that a carbazole alkaloid "clausenol" isolated from an alcoholic extract of the stem bark of clausena anisata possesses antibacterial and antifungal activity.[3] The acetone and alcoholic extracts of the leaves of Cassia alata showed significant invitro antibacterial activity against Staphylococcus aureus, coagulase positive Staplylococcus aureus, Bacillus subtilis, Bacillus stearothermophillus, Escherichia coli, Salmonella typhi and Salmonella dysentriae. Further, alcoholic extracts also inhibited the growth of Klebsiella pneumoniae where as the acetone extract inhibited the growth of Vibrio cholerae.[4]

The Alcoholic extract of dry nuts of Semecarpus anacardium (Bhallatak) showed bactericidal activity in vitro against 3 gram negative strains (Escherichia coli, Salmonella typhi and Proteus vulgaris) and 2 gram positive strains (Staphylococcus aureus and Corynebacterium diphtheriae). Subsequent studies have shown that the alcoholic extracts of different parts of the plant (leaves, twigs, green fruits) also possess antibacterial properties especially the leaf extract.[5]

Substantial antibacterial, antifungal and moderate insecticidal, sporicidal and cytotoxic activities were observed with the hexane extract of the stem bark of Amona glabra. Chromatographic fractionation of the stem led to the isolation of kaur-16-en, 19-oic acid, which was found to be largely responsible for its biological activities.[6]

Keeping in view the difficulties encountered with aromatic oils due to lack of ideal diffusion and evaporation in disc diffusion methods, Agnihotri and Vaidya developed a novel approach for studying antibacterial properties of certain plants like Eugenia caryophyllus, Thymus vulgaris, Cinnamonum zeylanium and Cuminum cyminum. Volatile components of the hexane extracts of these plants were tested against standard gram positive and gram negative bacteria grown on agar slants and the results were expressed as percentage inhibition of the area of the slants. Of the 4 plants selected, Thymus vulgaris had the most prominent antibacterial activity.[7]

ANTIFUNGAL

Four siddha drugs viz Nandhi mezhugh, Parangi pattai choornam, Erasa kenthi mezhugu and Vaan mezhugu (in order of efficacy) were found to have significant antifungal activity when tested against 14 strains of Candida albicans.[8] The aqueous and ethanolic extracts of Azadirachta indica

leaves have been shown to have anti-dermatophytic activity when tested in vitro against 88 clinical isolates of dermatophytes using the agar dilution technique. The activity was more prominent in ethanolic extract as compared to that in aqueous extract.[9]

Rai observed antimycotic activity against the test pathogen Pestalotiopsis mangiferae in 14 medicinal plants. Maximum anti-mycotic activity was shown by Eucalyptus globulus (88%) and Catharanthus roseus (88%) followed by Ocimum sanctum 85.50%, Azadirachta indica (84.66%). Ricinus communis (75%) and Lawsonia inermis (74.33%) while the minimum activity was exhibited by Jatropha curcas (10%).[10]

Essential oil obtained from the herb of Santolina chamaecyparissus showed significant antifungal activity both in vitro (against 13 strains of Candida albicans) and in vivo (experimentally induced vaginal and systemic candidiasis in mice).[11] It also showed activity against experimentally induced superficial cutaneous mycosis in guinea pigs by the hair root invasion test.[12]

The essential oil isolated from the leaves of Aegle marmelos exhibited significant antifungal activity against different fungal isolates and 100% inhibition of spore germination of all the tested fungi when evaluated using the spore germination assay. Kinetic studies showed that the inhibition was both concentration as well as time dependent.[13] The petroleum ether, chloroform, acetone and ethanol (95%) extracts of the leaves of the Cassia alata also showed significant in vitro antifungal activity against various fungi viz. Aspergillus niger, R. Japonicum, Candida albicans, C. tropiathis and R. glutinis.[14]

The natural xanthones isolated from the fruit hulls of Garcinia mangostana showed good inhibitory activity against the three phytopathogenic fungi, Fusaium vasinfectum, Alternaria tenuis and Dreschlera oryzae.[15] The root of Withania somnifera was found to be effective in prolonging the survival of Bulb/C mice infected intravenously with Aspargillus fumigatus. This protective activity is probably due to the observed increase in phagocytosis and intracellar killing capacity of peritoneal maccrophages induced by treatment with Withania somnifera, thus suggesting that the plant has potential to activate macrophage function in infectious states.[16]

ANTIVIRAL

Four compounds have been isolated from an extract prepared from the fruit rind of Terminalia belerica viz termilignan, thannilignan, 7 hydroxy 3, 4 (Methylenedioxy) flavone and anolignan B. All possessed demonstrable anti HIV-1, antimalarial and antifungal activity in vitro.[17] In an in vitro study, the aquous extracts of Phyllanthus amarus was incubated with the Alexander cell line, a human hepatocellular carcinoma derived cell line which has the property of secreting the Hepatitis B surface antigen (HbsAg) in the supernatant. The results demonstrated that Phyllanthus amarus was effective in inhibiting the secretion of HbsAg for 48 hours thus proving its anti hepatitis B virus property at the cellular level.[18]

Glycyrrhizin, a triterpenoid glycoside obtained from Glycyrrhiza glabra was tested against RNA viruses like the Chandripura virus, Measles virus, Polio vaccine viruses type 1,2 and 3, Polio wild type viruses 1,2 and 3 as well as DNA viruses like the Herpes type 1 and 2 viruses in vitro. It inhibited the DNA viruses plaque formation at lower concentrations (0.608mM) while the RNA viruses were inhibited at higher concentrations (1.216 mM).[19]

Premanathan et al[20] carried out an in vitro screening of mangrove plant extracts for anti immunodeficiency virus activity. HIV infected MT-4 cells were incubated with the extract and antiviral activity was detected using tetrazolium-based colorimetric assay. Seven extracts were found to be effective, five of which (bark of Rhizophora mucronata and leaves of Exoecaria agallocha, Ceriops decandra, Rhizophora apiculata, Rhizophora lamarckii) completely in habited the virus adsorption to the cells.

ANTIPROTOZOAL

Antimalarial: Ball shaped wood scrapings soaked in 5% Neem oil (Azadirachta indica) diluted in acetone and placed in water storage over head tanks controlled the breeding of anopheles stephensi and aedes aegypti in 45 days.[21] Similarly application of a cream of Azadirachta indica on exposed parts of the body at the rate of 2.0 gm/person significantly protected against Aedes, Culex and Anopheles mosquito bites.[22]

Ethanolic and petroleum extracts of Artemisia Japonica, Artemisia maritimia and Artemisia Nilegarica were tested for anti-malarial activity, both in vivo and in vitro. In vivo studies were carried out in Balb/c mice using the Rane test wherein all the compounds prolonged the survival time of the mice. In vitro all 3 compounds inhibited schizont maturation in chloroquine sensitive strains of plasmodium falciparum.[23]

Antileishmanial: The methanolic extract of Swertia charata was found to inhibit the catalytic activity of topoisomerase I enzyme of Leishmania donovani. On subjecting the extract to fractionation, it yielded 3 secoiridoids glycosides: Amarogentin, amaroswerin and sweroside. Of

these amarogentin was found to be a potent inhibitor of topoisomerase 1 and exerted its effect by interacting with the enzyme thus preventing binary complex formation.[24]

Antitrypanosomial: Crude 50% ethanolic extract of Parthenium hysteroporus flowers exhibited trypanocidal activity against Trypanosoma evansi both in vitro and in vivo.[25]

There is growing interest in correlating phytochemicals of a plant with its pharmacological activity.[26] The following section describes the major groups of antimicrobial compounds from various plants.

B. PHYTOCHEMICALS

PHENOLICS AND POLYPHENOLS

Some of the simplest bioactive phytochemicals consist of single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane-derived compounds which are in the highest oxidation state. The common herbs tarragon and thyme both contain caffeic acid which is effective against viruses,[27] bacteria,[28] and fungi.[29]

Catechol and pyrogallol both are hydroxylated phenols, shown to be toxic to micro organisms. Catechol has two OH groups and pyrogallol has three. The site and the number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity.[30] The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interaction--- with proteins.[31]

Phenolic compounds possessing a C_3 side chain at a lower level of oxidation and containing no oxygen are classified as essential oils and often cited as antimicrobial as well. Eugenol is a well-characterized representative found in clove oil. It is considered to possess bactariostatic[32] and antifungal properties.[29]

QUINONES

Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive. The switch between diphenol (or hydroquinone) and diketone (or quinone) occurs easily through redox reactions. In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in protein often leading to inactivation of the protein and loss of function. For that reason the potential range of quinone antimicrobial effects is great. Probable targets in the microbial cell are surface-exposed adhesins, cell wall polypeptides and membrane bound enzymes. Quinones may also render substrates unavailable to the microorganism.[33]

Kazmi et al[34] deseribed an anthroquinone from Cassia italica a Pakistani tree, which was bacteriostatic for Bacillus anthracis, Corynebacterium pseudodiphtherium and Pseudomonas aeruqinosa and bactericidal for Pseudomonas pseudomalliae.

FLAVONES, FLAVONOIDS AND FLAVONOLS

Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). The addition of a 3-hydroxyl group yields a flavonol.[35] Flavonoids are also hydroxylated phenolic substances but occur as a C6- C3 unit linked to an aromatic ring. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes.[36]

Catechins, the most reduced form of the C3 unit in flavonoid compounds, deserve special mention. These compounds inhibit in vitro Vibrio cholerae,[37] Streptococcus mutans[38] and Shigella.[39] Flavonoid compounds exhibit inhibitory effects against multiple viruses. Numerous studies have documented the effectiveness of flavonoids such as swertifrancheside,[40] Glycyrrhizin (from licorice),[41] and chrysin[42] against HIV.

Kaul et al[46] provided a summary of the activities and mode of action of quercetin, naringin, hesperetin and catechin in in-vitro cell culture monolayers. While naringin was not inhibitory to herpes simplex virus type 1 (HSV-1), poliovirus type 1, parainfluenza virus type 3, or respiratory syncytial virus, the other three flavonoids were effective in various ways. Hesperetin reduced intracellular replication of all four viruses; catechin inhibited infectivity but not intracellular replication of RSV and HSV-1; and quercetin was universally effective in reducing infectivity.

Galangin (3,5,7 trihydroxyflavone) derived from the perennial herb Helichrysum aureonitens, seems to be a particularly useful compound, since it has shown activity against a wide range of gram-positive bacteria as well as fungi[44] and viruses, in particular HSV-1 and coxsackie B virus type 1.[45]

TANNINS

Tannin is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. They are found in almost every plant part: bark, wood,

leaves, fruits and roots.[46] Tannins may be formed by condensations of flavan derivatives or by the polymerization of quinine units.[30]

Many human physiological activities, such as stimulation of phagocytic cell, host mediated tumor activity and a wide range of anti infective actions, have been assigned to tannins.[47] Their mode of antimicrobial action as described for quinone, may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope, transport-proteins etc. They also complex with polysaccharide.[48] According to a number of studies, tannins can be toxic to filamentous fungi, yeasts and bacteria.[46] At lease two studies have shown tannin to be inhibitory to viral reverse transcriptase.[43,49]

COUMARINS

Coumarins are phenolic substances made of fused benzene and alpha pyrone ring.[50] Warfarin is a particularly well-known coumarin which is used both as an oral anticoagulant and interestingly as a rodenticide. Since coumarins are highly toxic in rodents they should be treated with caution by the medical community.[51] It may also have antiviral effects.[52] As a group coumarins have been found to stimulate macrophages[53] which could have an indirect negative effect on infections. More specifically coumarin has been used to prevent recurrences of cold sores caused by HSV-1 in humans.[52] Hydroxycinnamic acids related to coumarins seem to be inhibitory to gram-positive bacteria.[54]

TERPENOIDS AND ESSENTIAL OILS

The fragrance of plants is carried in the so called quinta essentia, or essential oil fraction. These oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure. They are called terpenes, their general chemical structure is C10 H16 and they occur as diterpenes, triterpenes and tetraterpenes (C20, C30 and C40) as well as hemiterpenes (C5) and sesquiterpenes (C15). When the compounds contain additional elements, usually oxygen, they are termed terpenoids. Example of common terppenoids are menthol and camphor (monoterpenes) and farnesol and artemisin (sesquiterpenoids). Artemisin and its derivative alpha arteether, also known by the name qinghaosu, find current use as antimalarials.[2,55]

Terpenenes or terpenoids are active against bacteria,[56] fungi[57] and viruses.[58] The ethanol-soluble fraction of purple prairie clover yields a terpenoid called petalostemumol, which showed excellent activity against Bacillus subtilis and Staphylococcus aureus and lesser activity against gram-nagative bacteria as well as Candida albicans.[59] Kadota et al found that trichorabdal A, a diterpene from a Japanese herb, could directly inhibit H.pylori.[60]

ALKALOIDS

Alkaloids are heterocyclic nitrogen compounds. The first medically useful alkaloid was morphine (the name morphine comes from the greek Morpheus, god of dreams), isolated in 1805 from the opium poppy papaver somniferum. Codeine and heroin are both derivatives of morphine.[35] Diterpenoid alkaloids, commonly isolated from the plants of the Ranunculaceae or buttercup family are commonly found to have antimicrobial properties.[61]

Solamargine, a glycoalkaloid from the berries of Solanum khasianum, and other alkaloids may be useful against HIV infections[62] as well as intestinal infections associated with AIDS.[63] Berberine is an important representative of the alkaloid group. It is potentially effective against trypanosomes[64] and plasmodia.[61] The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmane is attributed to their ability to intercalate with DNA.[65]

LECTINS AND POLYPEPTIDES

Peptides which are inhibitory to microorganisms are often positively charged and contain disulfide bonds. Their mechanism of action may be the formation of ion channels in the microbial membrane[66] or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors.[67]

Recent interest has been focused mostly on studying anti-HIV peptides and lections, but the inhibition of bacteria and fungi by these macromolecules such as that from the herbaceous, Amaranthus, has long been known.[68]

Thionins are peptides commonly found in barley and wheat are toxic to yeasts and gram-negative and gram-positive bacteria.[69] Fabatin, a newly identified peptide from fava beans appears to be structurally related to Gamma thionins from grains and inhibits E.coli, P.aeruginosa and Enterococcus hirae.[66]

The larger lectin molecules, which include mannose-specific lectins from several plants eg. MAP 30 from bitter melon,[70] GAP 31 from Gelonium multiflorum[71] and jacalin are inhibitory to viral proliferation (HIV cytomegalovirus) probably by inhibiting viral interaction with critical host cell components.[72]

CONCLUSION

Development of resistance to chemotherapeutic agents shown by the microorganisms ap-

pears to be a continuous process since the time antibiotics were discovered. So every antibiotic has certain life span regarding its efficacy. Scientists have realized an immense potential in natural products from medicinal plants to serve as alternate source of combating infections in human beings which may also be of lower cost and lesser toxicity. Further work on isolation and characterization of active principles from medicinal plants and their pharmacodynamic study using latest techniques would be highly beneficial to human beings.

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