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Antimicrobial Activity of Natural Products from Medicinal Plants

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Abstract—*World Health Organization (WHO) has estimated that at least 80% of the world population rely on traditional systems of medicine for their health needs. India is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. Due to indiscriminate use of antimicrobial drugs, many microorganisms have acquired resistance to specific antibiotic treatments (particularly hospital environment). Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to biologically active compounds isolated from plant species used in herbal medicine.*

Plants like Ocimum gratissimum and Eugenia uniflora contain upto 75% thymol which has antimicrobial effect and mainly used in the treatment of diarrhoea and ear infection in human beings. Methanol extracts of Phyllanthus niruri showed strongest antimicrobial activity as compared to commercially available antibiotics (Ciprofloxacin, Gentamycin) against Staphylococcus sp. and Pseudomonas sp. Malaria is a major public health problem in India. Every year around 2 million cases are reported from all over the country. Chloroquine is the most effective and widely used drug. However, the emergence of strains of Plasmodium falciparum resistant to chloroquine and many other drugs in succession has stimulated efforts to identify new antimalarial agents. Artemisinine the antipyretic principle of plant Artemisia annua has been shown conclusively to possess antimalarial activity.

Current social trends in healthcare show a definite movement towards use of natural remedies like medicinal plants. Hence, the prime need of the hour is to make use of medicinal plants for solving the health problems and major ailments of people. Only the research and development are the prime factor to reach the goal.

1. INTRODUCTION

Medicinal plants are the local heritage with global importance, World is endowed with a rich wealth of medicinal plants. Scientists realize that the effective life span of any antibiotic is limited, so plant sources are also being investigated. Secondly the public is becoming increasingly aware of the problems both in the Eastern and Western worlds, with the over prescription and misuse of traditional antibiotics. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects (Gonzalez *et al,* 1996) [19].

Herbal medicine is the oldest from of healthcare known to mankind. India has rich medicinal plants flora of more than 7500 species. Of these, 4635 species are used commercially on a fairly large scale. Over 50% of all modern clinical drugs are of natural product origin, play important role in drug development in the pharmaceutical industry. India is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In India thousands of species are known to have medicinal value, has beenin vogue since ancient times. India exports crude drugs mainly to developed countries, viz. USA, Germany, France, Switzerland, UK and Japan, who share between them 75 to 80 % of the export of crude drugs

In plants, therapeutic compounds are mostly secondary metabolites (alkaloids, steroids, tannins, and phenol), are synthesized and deposited in specific parts or in all parts of the plant. The alkaloids form the largest group, which includes morphine and *codein (poppy), strychnine and brucine (Nux vomica), quinine (cinchona), ergotamine (Ergot), hyocyamine (Belladona), scolapomine (Datura), cocaine (Coco), reserpine (Rauwolfia), aconitine (Aconite), santonin (Artemisia) etc*. Glycosides form another important group represented by *digoxin (Foxglove), stropanthin (Strophanthus), glycyrrhizin (Liquorice), barbolin (Aloe), sannocides (Senna)* etc. Corticosteroids have come into *sennocides (Senna*). Corticosteroids have come into prominence recently and *diosgenin (Dioscorea), solasodin (Solanum sp.),* etc. now command a large world demand.

The principal herbal drugs that have been findings a good market in foreign countries are Aconite, Aloe, Belladona, Acorus, Cinchona, Cassia tora, Dioscorea, Digitalis, Ephedra, Plantago, Cassia etc.

A. Medicinal Plants

(a). Anti-microbial

Clausenol, a carbazole alkaloid, isolated from an alcoholic extract of the stem bark of *Clausena anisata* was found to be active against gram positive and gram negative bacteria and fungi. Substantial anti-microbial, anti-fungal and moderate insecticidal, sporicidal and cytotoxic activities were observed

with the hexane extract of the stem bark of *Amona glabra* (Padmaja *et al*, 1995).

(b). Antibacterial

A carbazole alkaloid "clausenol" isolated from an alcoholic extract of the stem bark of *Clausena anisata* possesses antibacterial and antifungal activity (Chakraborty *et al*, 1995). 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*) (Padmaja *et al*, 1995) [39] .

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 (Nair and Bhinde, 1996) [35].

Agnihotri and Vaidya (1996) [1] have developed a novel approach to study the anti-bacterial property 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. Of the 4 plants selected, *Thymus vulgaris* had the most prominent anti-bacterial activity.

The acetone and alcoholic extracts of the leaves of Cassia alata showed significant in vitro antibacterial activity against Staphylococcus 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 cholera (Sakharkar et al, 1998)[43].

Table 1: Main groups of plant compounds with antimicrobial activity.

Class	Subclass	Examples	Mechanism
Phenolics	Simple phenols	Catechol	Substrate deprivation
		Epicatechin	Membrane disruption
	Quinones	Hypericin	Adhesin binding, complex with cell wall, enzyme inactivation
	Flavonoids	Chrysin	Adhesin binding
	Flavones	Abyssinone	Enzyme inactivation,HIV reverse transcriptase inhibition
	Tannins	Ellagitannin	Protein binding, Adhesin binding, Enzyme inhibition, Substrate deprivation, Complex with cell wall, Membrane disruption, Metal-ion complexation
	Coumarins	Warfarin	Interaction with eucaryotic DNA (antiviral activity)
Terpenoids, essential oils	-	Capsaicin	Membrane disruption
Alkaloids	-	Berberine, Piperine	Intercalation into cell wall and/or DNA
Lectins and polypeptides	-	Mannose-specific agglutinin	Block of viral fusion or adsorption
		Falxatin	Disulfide bridge formation

Source: Cowan (1999) [9]

(c). Antifungal

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 (Venugopal and Venugopal, 1994) [55]. Four Siddha drugs viz Nandhi mezhugh, Parangi pattai choornam, Erasa kenthi mezhugu and Vaan mezhugu (in order of efficacy) were found to have significant anti-fungal activity when tested against 14 strains of *Candida albicans* (Suresh *et. al.,* 1994) [51].

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%) (Rai, 1996) [41].

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 (Rana *et al*, 1997) [42].

Aqueous flower extract of *Cassia alata* can be used as a potential antifungal agent for aflatoxin fungi (*Aspergillus flavus* and *A. parasiticus*), plant pathogenic fungi (*Fusarium oxysporum* and *Helminthosporium oryzae*) and human pathogenic fungi (*Candita albicans* and *Microsporum audouinni*). *C. alata* leaf is also credited for the treatment of haemorrhoids, constipation, inguinal hernia, intestinal parasitosis, blennorrhagia, syphilis and diabetes (Kochar, 1981) [25]. The leaf extracts of the plant have been reported to possess medicinal properties against ringworm, scabies, ulcers, prurities, eczema and itching.

Essential oil obtained from the herb of *Santolina chamaecypar* showed significant anti-fungal activity both in vitro (against 13 strains of *Candida albicans*) (Suresh *et. al.,* 1997) [52]. The natural xanthones isolated from the fruit hulls of *Garcinia mangostana* showed good inhibitory activity against the three phytopathogenic fungi, *Fusarium vasinfectum, Alternaria tenuis* and *Dreschlera oryzae* (Gopalakrishnan *et. al*., 1997) [20]. The root of *Withania somnifera* was found to be effective in prolonging the survival of Balb/c mice infected intravenously with *Aspergillus fumigates.*

(d). Antiviral

Glycyrrhizin, a triterpenoid glycoside obtained from *Glycyrrhiza glabra* (Yasthimadhu) was tested against RNA viruses like the Chandripura virus, Measles virus, Polio vaccine Polio wild type viruses as well as DNA viruses like the viruses in vitro. It inhibited the DNA virus plaque formation at lower concentrations (0.608 mM) while the RNA viruses were inhibited at higher concentrations (Badam, 1994) [4].

Phyllanthus amarus was effective in inhibiting the secretion of HbsAg for 48 hrs thus proving its anti-hepatitis B virus property at the cellular level carried out in vitro screening of mangrove plant extracts for anti-immunodeficiency virus activity. HIV infected MT-4 cells were incubated with the extract and anti-viral 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 *Excoecaria agallocha, Ceriops decandra, Rhizophora apiculata and Rhizophora lamarckii*) completely inhibited the virus adsorption to the cells.

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.

(e). Antiprotozoal

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. 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 (Dua *et al*, 1995) [12]. Ethanolic and petroleum extracts of *Artemisia Japonica, Artemisia maritimia* and *Artemisia nilegarica* were tested for anti-malarial activity, both in vivo and in vitro (Valecha *et al*, 1994) [54].

Crude 50% ethanolic extract of *Parthenium hysteroporus* flowers exhibited trypanocidal activity against *Trypanosoma evansi* both in vitro and in vivo (Talakal *et al*, 1995) [53]. The methanolic extract of *Swertia chirata* was found to inhibit the catalytic activity of topoisomerase I enzyme of *Leishmania donovani.*

(f). Anthelminthic

Kumar *et al* (1995) [26] has studied the mechanism of action of palasonin, the active principle of *Butea frondosa* seeds on *Ascaridia galli*. Palasonin inhibited glucose uptake and depleted the glycogen content and thus the possible mechanism of its anthelminthic action may be related to inhibition of energy metabolism.

Both aqueous and alcoholic extracts of the leaves of *Sencio nudicaulis* Buch Ham were found to exert antifilarial activity when tested against *Setaria cervi* (Nematoda Filarioidea) (Sigh *et. al.*, 1996) [47].

The root tuber extract of *Flemingia vestita,* an indigenous medicinal plant in Meghalaya, exhibited antihelminthic activity in vitro, against 2 species of flukes, *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski* . It caused paralysis in both the species.

(g). Analgesics

Alcoholic extract of the roots of *Clerodendron serratum* showed significant anti-inflammatory activity (Narayanan *et. al.,* 1998) [36]. The aqueous extract of *Gymnema sylvestre* leaves showed significant anti-inflammatory activity (Diwan *et. al.,* 1995) [11].

The roots and seed of *Pongamia pinnata* showed significant analgesic (Singh *et. al.,* 1997) [48]. Alcoholic extract of the roots of *Clerodendron serratum* (Bharanji) showed significant analgesic activity (Narayanan *et. al.,* (1998) [36].

(h). Anti-inflammatory

The aqueous suspension of dried latex of *Calotropis procera* (Arka) showed anti-inflammatory property (Kumar and Basu, 1994) [27]. The roots and leaves of *Butea frondosa* (Palash) were evaluated for ocular anti-inflammatory activity in rabbits (Mengi and Deshapande, 1995) [32]. The oil of *Ocimum sanctum* and linolenic acid were found to possess significant anti-inflammatory activity. The anti-inflammatory activity of linolenic acid present in the fixed oil of *Ocimum sanctum* was probably due to blockade of both (Singh and Majumder, 1997) [49].

Alcoholic extract of *Ochna obtusata* stem bark demonstrated potent anti-inflammatory effects. All extracts of the root of *Pongamia pinnata* showed significant anti-inflammatory activity (Singh and Pandey, 1996) [46]. The methanolic extracts of the flowers of *Michelia champaca* Linn. (Champaka), *Ixora brachiata* Roxb (Rasna) and *Rhynchosia cana* Willd were found to possess significant anti-inflammatory activity.

(i). Antipyretics

The ethanolic extracts of *Ailanthus excels* (Mahanimba), *Toddalia asiatica* (Kanchana) and *Araucaria bidwilli* (Monkey puzzle) showed moderate to significant degree of antipyretic activity. (Suresh *et. al.*,1995) [50]. Alcoholic extract of the roots of *Clerodendron serratum* significant antipyretic activity following typhoid TAB vaccination in rabbits (Mukherjee *et.al.*, 1996) [34].

B. Phytochemicals

(a). Phenolics and Polyphenols

The common herbs tarragon and thyme both contain caffeic acid which is effective against viruses (Wild, 1994) [58], bacteria (Brantner and Grein, 1994) [7], and fungi (Duke, 1985) [13]. Catechol and pyrogallol both are hydroxylated phenols, shown to be toxic to micro organisms. (Geissman, 1963) [18].

The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds (Mason and Wasserman, 1987) [29].

(b). Quinones

Kazmi et al (1994) [24] deseribed an anthroquinone from *Cassia italica*, which was bacteriostatic for *Bacillus anthracis*, *Corynebacterium pseudodiphtherium* and *Pseudomonas aeruqinosa* and bactericidal for *Pseudomonas pseudomalliae*.

(c). Flavones, Flavonoids and Flavonols

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 grampositive bacteria as well as fungi and viruses (Meyer *et. al.*, 1997) [33]**.**

(d). Tannins

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 (Haslam, 1996) [22]. 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 (Ya *et al.*, 1998) [59]. According to a number of studies, tannins can be toxic to filamentous fungi, yeasts and bacteria (Scalbert, 1991) [44].

Table 2: Selected Indian medicinal used to treat various kinds of human diseases.

S. N.	Plant	Plant part used	Ayurvedic or Traditional Uses
1.	Acacia leucophloea	Bark	It is used as antimicrobial, antihelmentic, expectorant and blood purifier. It is also used to treat skin and used to treat skin diseases (leprosy), ulcer, gum bleeding, mouth ulcer, dry cough, dysentery, diabetes and fever.
2.	Acacia nilotica	Bark	It is used to treat cough, acute gonorrhoea dysentery, diarrhoea, cancers, syphilitic affections and genitourinary affections
3.	Aegle marmelos	Fruit	Fruits are used in diarrhoea and dysentery
4.	Chlorophytum borivilianum	Root	Roots are used to treat diarrhoea and dysentery and also used as demulcent and galactogogue
5.	Jatropha gossypifolia	Latex and Leaf	Root is used in diarrhoea and dysentery. Oil purgative and locally applies in skin disease and arthritis. Latex and leaf juice are used to treat ulcer, skin disease(leprosy) and gum infections
6.	Justicia zeylanica	Leaf	It is used in microbial infections, bronchitis, asthma, fever and arthritis
7.	Lantana camara	Leaf flower	Leaf juice is used as antimicrobial in skin diseases
8.	Phyllanthus emblica	Fruit and Seed	Fruits and seeds are used to treat asthma, bronchitis and Biliousness
9.	Phyllanthus urinaria	Whole plant	Plants is used to treat cough, bronchitis, skin disease, enlarged spleen and liver, jaundice, and fever
10.	Tamarindus indica	Whole plant	Plant is used to treat diarrhoea, lotions and pustules, sores, boils, asthma and amenorrhea
11.	Tephrosia purpurea	Whole plant Root	Plant is recommended in ulcers, spleenomegaly, liver dysfunction, anthelmentic, cough, cold, Skin disease(antimicrobial) and fever
12.	Woodfordia fruticosa	Flower	Flowers are used to treat ulcer, wounds, cough and small Pox.

Source: Dabur et. al., 2007[10].

Table 3: Plants containing antimicrobial activity.

Common name	Scientific name	Compound	Class	Activity	Reference(s)
Aloe	Aloe barbadensis, Aloe vera	Latex	Complex mixture	Corynebactrim, Salmonella, Streptococcus, S. aureus	Martinez et. al., 1996[28]

Ashwa gandha	Withani a somnifer um	Withafa rin A	Lactone	Bacteria, fungi	
Bael tree	Aegle marmelo s	Essenti al oil	Terpen oid	Fungi	Rana et. al., 1997[42]
Barberr y	Berberis vulgaris	Berberi ne	Alkaloi d	Bacteria, protozoa	McDevitt et.al., 1996[30]; Omuloki et. al., 1997[38]
Basil	Ocimum basilicu m	Essenti al oils	Terpen oid	Salmonella, bacteria	Wan et. al., 1998[57]
Bay	Laurus nobilis	Essenti al oils	Terpen oids	Bacteria, fungi	
Carawa y	Carum carvi		Coumar ins	Bacteria, fungi, viruses	Berkada, 1978[5]; Bose, 1958[6]; Humburger et. al., 1991[21]; Sheel, 1972[45]
Eucaly ptus	Eucalypt us globules	Tannin	Polyph enol Terpen oid	Bacteria, viruses	
Sweet flag, calamu s	Acorus calamus			Enteric bacteria	
Tarrago n	Artemisi a dracunc ulus	Caffeic acids,T annin	Terpen oid Polyph enols	Viruses, helminths	Wild, 1994[54]

Source: Cowan, 1999.

(e). Terpenoids and Essential Oils

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 (Vishwakarma,1990 [56];Cowan,1999 [9]). Terpenenes or terpenoids are active against bacteria (Amaral *et. al.,* 1998) [2], fungi (Ayafor *et. al.,* 1994) [3] and viruses (Fujioka and Kashiwada, 1994) [17]. 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 gramnagative bacteria as well as *Candida albicans* (Hufford *et. al*., 1993) [23].

(f). Alkaloids

Alkaloids are heterocyclic nitrogen compounds. The first medically useful alkaloid was morphine, isolated in 1805 from the opium poppy *Papaver somniferum*. Codeine and heroin are both derivatives of morphine (Fessenden and Fessenden, 1982) [15]. Diterpenoid alkaloids, commonly isolated from the plants of the Ranunculaceae family are commonly found to have antimicrobial properties (Omulokoli, 1997) [38].

Solamargine, a glycoalkaloid from the berries of *Solanum khasianum*, and other alkaloids may be useful against HIV infections (McMahon *et al*, 1995) [31] as well as intestinal infections associated with AIDS (McDevitt *et al*, 1996) [30]. Berberine is an important representative of the alkaloid group. It is potentially effective against trypanosomes (Freburghause *et al*, 1996) [16] and plasmodia. The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmane is attributed to their ability to intercalate with DNA (Phillipson and O'Neill, 1987) [40].

(g). Lectins and Polypeptides

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.

(h). Coumarins

Coumarins are phenolic substances made of fused benzene and alpha pyrone ring (O'Kennedy and Thornes, 1997) [37]. Warfarin is a particularly well-known coumarin which is used both as an oral anticoagulant and interestingly as a rodenticide. As a groupcoumarins have been found to stimulate macrophages (Casley-Smith, 1997) [8] 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 (Berkada, 1978) [5]. Hydroxycinnamic acids related to coumarins seem to be inhibitory to gram-positive bacteria (Fernandez *et. al.,* 1996) [14].

Conclusion

Development of resistance to chemotherapeutic agents shown by the microorganisms appears 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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