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Role of natural metabolties in plant disease management

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ABSTRACT

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Ethno-pharmacologists, botanists, microbiologists, and natural-products chemists' arecombing the Earth for phytochemicals and "leads" which could bedeveloped for treatment of infectious diseases. Many pathogenic microbes have capability to develop resistance against synthetic formulation. Synthetic formulation is very toxic and destroys the soil fertility and ecological balance. Plant based formulation are least toxic and better for environment balance so it can be replace by synthetic formulation. Antimicrobial activity of plants is mainly due to the presence of secondary metabolites. Plants are rich in wide varietyof secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobialproperties. This review attempts to summarize the current statusof botanical screening efforts, as well as *in vivo* studies of their effectiveness and toxicity. The structure and antimicrobial properties of phytochemicals are also addressed.

Introduction

Plant diseases create challenging problems in commercial agriculture and pose real economic threats to both conventional and organic farming systems. Disease management is complicated by the presence of multiple types of pathogens. For any one crop the grower must deal with a variety of fungi, bacteria, viruses and nematodes. This situation is even more complicated for organic vegetable growers because they usually produce a wide array of vegetable crops and are prohibited from applying conventional synthetic fungicides. The world market continues to be extremely competitive and continues to require that growers supply high-quality disease free product with an acceptable shelf life. Disease management is therefore a critical consideration in organic vegetable production (Koike *et al.*, 2000).

Bavistin, mancozeb and thiram are the most commonly used plant fungicides. Such synthetic fungicides bring about the inhibition of pathogens by either destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogens and hence are extremely effective. The flip side of this is that synthetic chemicals are harmful for human as well as soil health. Chemical fungicides are known to pollute the environment; soil and water besides causing deleterious effects on human health and biosphere. Inappropriate use of agrochemicals especially fungicides not only imposes adverse effects on ecosystems, it also possess a possible carcinogenic risk higher than that of insecticides and herbicides put together (Cameron and Julian 1984; Osman and Al-Rehiayam 2003; Masuduzzaman *et al.*, 2008; Siva *et al.*, 2008). Moreover, resistance by pathogens to fungicides has rendered certain fungicides ineffective (Zhonghua and Michailides, 2005).Hence there is a need to search for an environmentally safe and economically viable strategy for the control of diseases and to reduce the dependence on the synthetic agrochemicals.

Recent trends favour the use of alternative substances derived from natural plant extracts to control pests (Lale and Abdulrahman, 1999; Xan *et al.*, 2003; Islam *et al.*, 2004). The use of natural products for the control of fungal diseases in plants is considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment (Cao and Forrer, 2001b).

These natural products or plant extracts can be exploited either as leads for chemical synthesis of new agrochemicals, or as commercial products in their own right, or as a source of inspiration to biochemists for the development of new bioassays capable of detecting other, structurally simpler, compounds with the same mode of action (Lange *et al.*, 1993).Plant product preparations and bioagents do not leave any toxic residues and therefore can effectively replace synthetic fungicides.The use of medicinal plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against these microorganisms (Munoz-Mingarro *et al*., 2003 and Coelho de Souza *et al*., 2004).

Reviewing some important methods

Plant preparations have been used for centuries in medicine and pest control. Farmers in India use neem leaves to protect their stored grain from insects.

Herbs and spices, such as basil and clove, have been used by many cultures to protect food from spoilage, as both have antimicrobial properties (Arora and Kaur, 1999; Manohar *et al.*, 2001). Antifungal activity of plant extracts and their essential oils against a wide range of fungi has been reported (Kurita *et al.*, 1981; Grane and Ahmed, 1988; Cowan, 1999; Wilson *et al.*, 1997; Abd-Alla *et al.*, 2001).

In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period. Dahanukar *et al*., (2000) have reviewed the research on plant based antifungal compound as a scientific approach and innovative scientific tool from 1994-1998.

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Antimicrobial screening of plant extracts is usually done with crude alcohol or aqueous extracts prepared either by cold or hot extraction methods. Crude or alcohol extract of several plants have been screened for their possible antimicrobial activities against pathogenic virus, bacteria, fungi and protozoa (Mahmoud, 1999; Digrak *et al*., 1999; Bowers and Locke, 2000; Eksteen *et al*., 2001; Hol and Van-veen, 2002; Magama *et al*., 2003; Gulluce *et al*., 2003; Afolayan, 2003; Meena, *et al*., 2003; Shamin *et al*., 2004; Nair and Chanda, 2005; de Oliveira *et al*., 2005; Rahman *et al*., 2005; Phongpaichit *et al*., 2005; Tasdemir *et al*., 2005; Likhitwitaywuid *et al*., 2005; Shittu *et al*., 2006; Pujol *et al*., 2006; Khosravi and Behzadi, 2006;Abere *et al*., 2007; Ayandele and Abebiyi, 2007; Harlapur*et al*., 2007; Fawzi,*et al*., 2009; Shanmugavalli *et al*., 2009).Pretorius *etal*., (2002) tested crude extracts from thirty nine plant species for their antifungal potential against seven economically important plant pathogenic fungi.

All the active principles present in plants are usually aromatic or saturated organic compounds so they get extracted in ethanol or methanol (Cowan, 1999). Some proteins and glucosides etc. are soluble in water hence antimicrobial assay of anti microbial principle is usually done with aqueous, 50% alcohol or 100% alcohol extracts. Mughal *et al*., (1996) observed that aqueous leaf extracts of *Allium sativum*, *Datura alba*and *Withania somnifera* inhibited the growth of *Alternaria alternata, A. brassicola* and *Myrothecium roridum*. According to Khan *et al*., (1998) aqueous extract of *Allium cepa* exhibited antifungal activityagainst *Helminthosporium turcicum* and *Ascochyta rabiei* and that of *Calotropis procera* against *Alternaria redicina*. Saha *et al.*, (2005) studied the antifungal activity of some plant extracts against fungal pathogens of *Camellia sinensis.* Rahman *et al*. (2005) reported the antimicrobial activity of crude extract of stem bark of *Barringtonia acutangula*. Bajwa*et al.*, (2004) reported the antifungal activity of aqueous extract of *Parthenium hysterophorous*, a herb, against *Drechslera hawaiiensis, Alternaria alternata* and *Fusarium monilifrome*. Antimicrobial potential of crude ethanolic extract of certain medicinal plants has been studied by Maniet *al*. (2005). Crude methanolic leaf extract of leaves of *Newbouldia* was screened against some bacteria and fungi by Usman and Osuji (2007). Crude aqueous pod extract of *Lecanioidiscus cupanioides* showed potent anticandidal activity (Okore *et al*., 2007).Hussin*et al.* (2009) reported the antifungal activity of methanolic, ethanolic and boiling water extracts of *Barringtonia racemosa* leaves, sticks and barks.

Initial antimicrobial screening with crude extract is followed by screening of extracts prepared in various organic solvents. These extracts are studied to search for various phytochemicals, responsible for antimicrobial activity.

Shukla *et al*. (2000) reported antibacterial activity of methanol, n-hexane, ethyl acetate and n-butanol fraction of root extract of *Oenothera biennis* against some bacteria. Souza *et al*. (2003) evaluated essential oil of *Hyptis ovalifolia* for its antimicrobial activity towards dermatophytes. Tatli and Akdemir (2005) reported antibacterial potential of methanolic extract of Turkish *Verbascum spp* against *Candida albicans, Cryptococcus neoformans, Staphylococcus aureus, S. aureus, Pseudomonas aeruginosa, Aspergillus fumigatus and Mycobacterium intracellulare.*Momeni *et al*. (2005) investigated antimicrobial activity of different fractions of stem bark extracts of *Ricidodendron heudelotii* (Euphorbiaceae). Saadabi (2007) studied the antimicrobial activity of aqueous, chloroform and methanol extract of *Lawsonia inermis* against six fungal pathogens (*Epidermophyton floccosum, Microsporum odouinii, Trichophyton rubrum, Trichophyton concentricum, Trichophyton tonsurans* and *Candida albicans*) and four human pathogenic bacteria (*Staphylococcus aureus, Bacillus subtilis, E. coli* and *Pseudomonas aeruginosa*).

Seetharam *et al*. (2003) reported antibacterial activity of *Saraca asoca* bark. Chloroform, ethyl acetate and aqueous extracts of husk of *Cocos nucifera* showed antibacterial activity against several bacteria Srinivas *et al*. (2003).Versha *et al*. (2003) reported that petroleum ether, chloroform, ethyl acetate and methanol extracts of leaf powder of *Alstonia scholaris* show antimicrobial activity against certain bacteria and fungi. Karthikumar *et al*. (2007) reported inhibitory activity of hexane, ethyl acetate, ethanol and water extract of all aerial parts of *Eclipta prostrata* (L.) against *E. coli, K. pneumoniae, Shigella dysenteriae, S. typhi* and *P. aeruginosa*.Jayaraman *et al*. (2008) studied antimicrobial activity of ethyl acetate, acetone, chloroform and water extract of *Stevia rebaudiana* leaves against *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Bacillus subtilis, Aeromonas hydrophila, Vibrio cholerae* and *Candida albicans, Cryptococcus neoformans, Trichophyton mentagrophytes, Epidermophyton species.*

Antifungal activity of petroleum ether, chloroform and acetone and ethanol extracts of *Calendula officinalis* against *A. fumigatus, Rhizopus japonicum, C. albicans, C. tropicalis* etc. has been investigated (Kasiram *et al*., 2000).Jayaprakash *et al*. (2001) evaluated turmeric oil for its antifungal activity against *A. flavus, A. parasiticus, Fusarium moniliforme* and *Penicillium digitatum*.Obafemi *et al*. (2006) reported that hexane, ethyl acetate and methanol extracts of *Tithonia diversifolia* exhibit antimicrobial activity against *Staphylococcus aureus, Bacillus cereus* and others. Aqil and Ahmad (2007) reported antibacterial properties of ethyl acetate, acetone and methanol extract of traditionally used Indian medicinal plants. Nguyen *et al.* (2009) studied antimycotic potential of *Cinnamon* extract against *R. solani.*

Plant extracts also show potent antimicrobial activity against plant pathogenic fungi. Sharma and Bohra (2003) studied antifungal activity of *Boerhavia diffusa, Salvadora persica* and *Leptadenia pyrotechnica* against *Fusarium oxysporum* and found that leaf extract of *Boerhaavia* showed maximum inhibition. Essential oils of ten medicinal plants were assayed for inhibitory activity against *Rhizoctonia solani* by Dhaliwal *et al*. (2003).Pandey (2003) reported antifungal activity of essential oil of *Mentha arvensis* against *Fusarium oxysporum* and *Trichophyton mentagrophytes*. Chapagain *et al.* (2007) reported antifungal potential of saponin rich-extracts from *Balanites aegyptiaca* fruit mesocarp, *Quillja saponaria* bark and *Yucca schidigera* against common phytopathogenic fungi (*Pythium ultimum, Fusarium oxysporum, Alternaria solani, Colletotrichum coccodes* and *Verticillium dahliae*).Bobbarala *et al*. (2009) reported antifungal activity of some medicinal plants against phytopathogenic fungi *Aspergillus niger*. Essential oil of *Luvunga scandens, Curcuma longa* and *Citrus sinensis* showed potent antifungal activity against human pathogens (Garg and Jain, 1999;Rath*et al*., 2001;Patra *et al*., 2003).

Plant extracts also exhibit antiviral, trypanocidal, leishmanicidal and antimalarial activity. Li *et al*. (2004) reported antiviral activities of aqueous extracts of medicinal herbs traditionally used in Southern Mainland China. Antiviral activities of some Ethiopian medicinal plants used for the

treatment of dermatological disorders have been reported (Gebre-Mariam *et al*. 2006).Filho *et al.* (2008) investigated the antiviral activity of sorghum bicolor against HSV-1, Bovine herpes virus 1 (BHV-1) and Polio vaccine virus.

Goncalves *et al*. (2005) screened some medicinal plants for *in vitro* anti-rotavirus activity against diarrhea. Awasthi *et al*. (2005) reported that root extract of *Boerhavia diffusa* exhibits antiviral activity against Cucumber *mosaic virus*.

Weniger *et al*. (2004) reported antiplasmodial activity of nine Benin medicinal plants against *Plasmodium falciparum*. Antiplasmodial activity of crude extracts of 19 species of *Strychnos* was assayed *in vitro* by Philippe *et al*. (2005). Osorio *et al.* (2007) reported antiprotozoal activity of extract prepared from *Annona muricata*, *Rollinia exsucca*, *Rollinia pittieri, Xylopia aromatic,Desmopsis panamensis* and *Pseudomalmea boyacana* against three *Leishmania* species, epimastigotes of *Trypanosoma cruzi* and both chloroquine sensitive (F32) and resistant (W2) *Plasmodium falciparum.* Antimalarial activity of plant extracts has been assayed by several workers. (Waako *et al*., 2005; Kanokmedhakul *et al*., 2005; Mbatchi *et al*., 2006 ; Hilou *et al*., 2006).

Plant extracts have also been described for their anticancerous and antimutagenic activity. Extracts prepared from *Gymnocladus dioicus, Holodiscus discolor, Stephanandra tanakae, Ligustrum delavayanum, Ligustrum vulgare* and *Staphylea pinnata* investigated for their cytotoxic activity against HeLa cell lines Jantova *et al.* (2001). Jayaraman *et al*. (2008) evaluated antitumour activity of *Stevia Rebaudiana* extractusing Human laryngeal epithiloma cell line (HEp2) via MTT assay.

Several reports are also available on pesticidal activity of plant extracts. Tewary *et al.* (2005) reported pestcidal activity of *Berberis lycium*, *Hedera nepalensis*, *Acorus calamus*, *Zanthoxylum armatum* and *Valeriana jatamansi* against pests (*Aphis cracccivora*, *Tetranychus urticae* and larvae of *Spodoptera litura*, *Plutella xylostella* and *Helicoverpa armigera*). Kouninki*e al.* (2007) studied the toxicity of some terpenoids of essential oils of *Xylopia aethiopica* from Cameroon against pest *Sitophilus zeamais Motschulsky*. Pavela *et al*. (2007) reported insecticidal properties of neem oil from seeds of *Azadirachta indica*, pongam oil from *Pongamia pinnata* and essential oils from some aromatic plants against *Trialeurodes vaporariorum*, *Tetranychus urticae* and aphids and caterpillars. Mondal *et al*. (2008) described the toxicity of chloroform extracts of *Derris indica* Bennet against *Callosobruchus maculates.*

Medicinal plants have generated the interest of man for therapeutic values chiefly because of the presence of secondary metabolites. The antimicrobial properties of plant extracts are a result of presence of secondary metabolites such as alkaloids, phenols, Flavonoids, terpenoids, essential oils etc. (Harborne, 1984). Several workers have reported antimicrobial activity of secondary metabolites of plants (Kishore *et al*., 2000; Sartoratto *et al*., 2004; Solis *et al*., 2004; Azabaze *et al*., 2004; Chebli *et al*., 2004; Masika *et al*., 2004;De Campos *et al*., 2005; de Leon *et al*., 2005; Deng and Nicholson, 2005 ; Satya *et al*., 2005; Ragasa *et al*., 2005; Deachathai *et al*., 2006; Chapagain*et al*., 2007 ; Bakar *et al*., 2009; Benn *et al.* , 2009).

Eloff (1998) reported that tannins, saponins, polypeptides and reducing sugars are soluble in water whereas terpenoids, flavonoids, alkaloids, and fatty acids are soluble in organic solvents. Similar findings have been reported by several workers (Scalbert, 1991; Zhang and Lewis, 1997; Mendoza *et al.*,1997). Tannins and reducing sugars are soluble in both water as well as organic solvents but their solubility is more in organic solvents as compared to water. Harborne (1984) and Kokate *et al.* (1990) suggested that extraction of secondary metabolites from plant material by hot extraction with petroleum ether separates sterols, waxes and fatty acids leaving behind residue containing the defatted plant materials. Subsequently extraction of this residue with benzene separates out sterols and flavonoids. Terpenoids and flavonoids get extracted with chloroform. The last solvents i.e. alcohol removes alkaloids, flavonoids, polyphenols, tannins and reducing sugar from residue. Finally extraction with water yields remaining water-soluble metabolites such as anthocyanin, starch, tannins, saponins reducing sugar and polypeptides (Zhang and Lewis, 1997; Scalbert, 1991). All the active principles present in plants are saturated organic compound so they get extracted in ethanol or methanol (Cowan, 1999).

Flavonoids are low molecular weight, polyphenolic compounds available in practically all dietary plants (Ren *et al*., 2003). Flavonoids represent a widespread group of water-soluble phenolic derivatives, which are mostly, brightly coloured. Over 4000 structurally unique flavonoids have been identified in plants. As polyphenols, phenolic acids and flavonoids are powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasiodilatory actions (Sharma *et al.,* 2009; Galeotti *et al.,* 2008; Mattila and Hellstrom, 2007). The common feature of these compounds is phenylbenzopyrone skeleton (C_6-C_3-C_6). They are mainly classified into flavanols, flavanonoles, flavanones, flavones and isoflavones on the basis of saturation level and opening of the central ring (Ren *et al*., 2003; Ebadi, 2002; Harborne and Williams, 2000). Flavonoids have existed since one billion years and survived in vascular plants throughout evolution indicating their importance in nature. The association between plant flavonoids and various animal species, a wide range of biological activities of flavonoids has been reported (Ebadi, 2002). They are known to be synthesized by plants in response to microbial infection. The inhibitory activity is due to formation of complexes with extracellular and soluble proteins and bacterial cell wall and disruption of microbial membranes (Tim Batchelder, 2004).Canales-Martinez *et al*. (2005) isolated coumarin from *Alternanthera caracasana* and reported its antimicrobial activity. Several workers have reported antimicrobial activity of flavonoids (Masika *et al*., 2004; de Campos *et al*., 2005; Han *et al*., 2005; Komguem *et al*., 2005).

Phenols and polyphenol group of compounds consist of thousands of diverse molecules with heterogenous structure with common feature of having one or more phenol ring. They are synthesized in plants by shikimic acid pathway. The site and numbers of hydroxyl groups on the phenol ring is related to their toxicity to microorganisms hence increased hydroxylation results in increased toxicity (Geissman, 1963). Several workers have reported that phenolic compounds such as caffeic acid, cinnamic acid, catechol, pyrogallol, eugenol, coumarins etc. show antimicrobial activity against virus, bacteria and fungi (Taguri *et al*., 2004; Saify *et al*., 2005; Satya *et al*., 2005; Deachathai *et al*., 2006; Romero *et al*., 2007).

Alkaloids are heterocyclic nitrogen compounds. Alkaloids are synthesized by decarboxylation of amino acids. Cinchona alkaloids present in the bark of *Cinchona* sp. have quinine as their main constituent, which is known since 1630 for its antimalarial activity. Diterpenoid alkaloids isolated from the

family Ranunculaceae are commonly found to have antimicrobial properties (Omulokoli *et al*., 1997). Antibacterial activity of alkaloid was also reported by Nagavalli *et al*. (2006). Amarbellisine, a lycorine type alkaloid isolated from *Amaryllis belladonna* was found to be antimicrobial in nature (Evidento *et al*., 2004). Bahceevli *et al*. (2005) reported antifungal activity of beta-carboline, tryptamine and phenylethylamine derived alkaloids isolated from the extract of arial parts and roots of C*yathobasis fruticulosa* (Chenopodiaceae). Indolizium alkaloid isolated from *Aniba panurensis* was found to be active against C*andida albicans* (Klausmeyer *et al*., 2004). Slobodnikova *et al*. (2004) reported antimicrobial activity of two protoberberine alkaloids namely berberine and jatrorrhizine isolated from crude extract of *Mahonia aquifolium*. Rahman and Grey (2005) isolated a benzoisofuranone derivative, a dimeric carbazole alkaloid, six carbazole alkaloids and three steroids from stem bark of *Murraya koenigii*, which showed inhibitory activity against microorganisms. Garcia *et al*. (2005) and Morel *et al*. (2005) reported antibacterial activity of pentacyclic oxindol alkaloid isolated from *Uncaria tomentosa* and cyclopeptide alkaloid isolated from *Scutia buxifolia* towards gram positive and gram negative bacteria and yeasts. Minor alkaloids were isolated from *Guatteria dumetorum* and showed inhibitory activity against *Leishmania mexicana* (Correa *et al*., 2006). Two alkaloids oriciacridone A and B isolated from stem bark of *Oriciopsis glabersima* were found to be active against a wide range of microorganisms (Wansi *et al*., 2006).

Tannin refers to polymeric phenolic substances capable of taning leather or precipitating gelatin from solution, known as astringency. Tannins are divided in two groups: hydrolysable and condensed tannins. Condensed tannins, which are generally known as proanthocyanidins are derived from flavonoid monomers (Tim Batchelder, 2004). Their molecular weight ranges from 500 to 3000 (Haslam, 1996) and they are found in almost every plant part: bark, wood, leaf, fruit and root (Scalbert, 1991). Tannins work by stimulation of phagocytic cells, host-mediated tumor activity and a range of anti-infective actions as well as to complexed with proteins (Haslam, 1996). Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes cell envelop transport proteins etc. They also complex with polysaccharide (Ya *et al*., 1988). Scalbert (1991) reported the antimicrobial properties of tannins. According to his studies, tannins can be toxic to filamentous fungi; yeasts and bacteria. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity (Jones *et al*., 1994). Methanol and hexane extract of *Punica granatum* showed a wide range of antimicrobial activity due to tannin (Duraipandiyan*et al*., 2006). Several workers have reported antimicrobial activity of tannins (Reddy *et al*., 2007; Ho *et al*., 2001; Smith, 2003; Cheng *et al*., 2004; Hori *et al*., 2006).

Catechins are more reduced form of the C_3 unit of flavonoid compounds. Catechins are known to possess antimicrobial activity (Toda *et al*., 1989) and they contain a mixture of catechin compounds. *In vitro* antimicrobial activity of catechin compound against *Vibrio cholerae* 01, *Streptococcus mutans*, *Shigella* and other bacteria and fungi has been reported by several workers (Batista *et al*., 1994; Borris, 1996; Sakanaka *et al*., 1998; Sakanaka *et al*., 1992; Tsuchiya *et al*., 1994; Vijaya *et al*., 1995). Veluri *et al*. (2004) evaluated antimicrobial and phytotoxic activities of catechin derivatives against some bacteria and fungi.

Effect of green tea catechin on the antifungal activity of antimycotics against *Candida albicans* was studied by Hirasawa and Takada (2004). Antimicrobial activity of leaf extract of green tea has been reported against *Staphylococcus aureus*. Rauha *et al*. (2000) screened thirteen phenolic substances and twenty-nine extracts of Finnish plant against selected fungi and bacteria and found that flavone, quercitin and naringenin were most effective in inhibiting growth of microbes. Isoflavones isolated from *Andira inermis* showed potent antiplasmodial activity (Kraft *et al*., 2000). Pegnyemb *et al*. (2005) isolated a biflavonoid, sulcatone from aerial parts of *Quratea sulcata* and reported its antimicrobial activity against range of microorganisms.

Quinones are aromatic rings with two ketone substitutions that are ubiquitous in nature and highly reactive. They are responsible for the browning reaction in injured fruits and vegetable (Cowan, 1999). They form complexes with nucleophilic amino acids in proteins, leading to inactivation of the protein and loss of function (Stern *et al*., 1996).

Eyong *et al*. (2005) isolated new naphthoquinone-coupled pigments from *Newbouldia laevis* and assayed its antibacterial activity against *Bacillus megaterium*. Manojlovic *et al*. (2005) assayed antifungal activity of methanol extracts and major anthraquinones like aglycones, alizarin and emodin of *Rubia tinctorum* (Rutaceae) and *Rhamnusfrangula* (Rhamnaceae). Naphthoquinones isolated from the roots of *Euclea natalensis* (Ebenaceae) were evaluated for their inhibitory action against *Mycobacterium tuberculosis* (Lall *et al*., 2005). Maurya and Jadhav (2005) isolated anthraquinones from *Sonneratia apetela* and they were found to be inhibitory against gram-positive and gram-negative bacteria. Weighnand *et al*. (2004) reported antibacterial activity of naphthaquinones and triterpenoids isolated from ethanolic extract of roots of *Euclea natalensis*. Eyong *et al*. (2006) isolated anthraquinone ether coupled pigment and it was found to be inhibitory against a wide range of microorganisms.

Saponins are naturally occurring surface-active glycosides. They are mainly produced by plants, but lower marine animals and some bacteria are also known to produce these compounds (Riguera, 1997; Yoshiki *et al.* 1998). Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone (sapogenin) which may be triterpenoid in nature. A large number of the biological effects of saponins have been ascribed to their action on membranes.

The steroidal alkaloids, tomatine and solanine are found in solanaceous plants, especially in *Lycopersicon* and *Solanum* spp. and produce a broad range of deleterious effects in mammals, insects and plant pathogens through disruption of membranes (Roddick and Drysdale, 1984; Roddick, 1986*)*, protein synthesis *(*Wink and Twardowski, 1992) and other effects. A variety of plant triterpenoid saponins and defensive antifungal proteins can directly interact with phospholipids or act by interfering with cell membrane structure, integrity and permeability (Polya, 2003).

The fragrance of plant is due to presence of essential oil fractions. These oils are highly enriched secondary metabolites that are based on isoprene units. They are also called as terpenes. Their general chemical structure is $C_{10}H_{16}$ and they occur as diterpenes, triterpenes and tetraterpenes (C_{20}, C_{30} and C_{40}) as well as hemiterpenes (C_5) and sesquiterpenes (C_{15}). When the compound contain additional elements usually oxygen, they are termed as terpenoids (Cowan, 1999). Triterpenes functions by

weakening the membranous tissue, which results in dissolving the cell wall of microorganisms so that they can be more efficiently eliminated (Dutta and Basu 1967; Bisignano *et al*., 1999).

Antimicrobial terpenoid were isolated from *Pterocarpus indicus* by Ragasa *et al*., (2005). Ten sesquiterpenes and six diterpenes were isolated and screened for antimicrobial activity against S*taphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Rhizoctonia solani* etc. (Solis *et al*., 2004). Several workers have reported antimicrobial activity of terpenes or essential oil. (Mamtha *et al*., 2004; Garcia Vallejo *et al*., 2006; de Souza *et al*., 2005; Ragasa *et al*., 2005; Saroglou *et al*., 2005; Abdelwahed *et al*., 2006; Kurkcouglu *al*., 2006).

One new (1) and four known (2-5) ursane triterpene with potent inhibition of the bacterial biofilm *Pseudomonas aeruginosa* PA 01 were obtained from *Diospyros dendo* (Hu *et al*., 2006). da Silva *et al*. (2004) reported antimalarial activity of terpenes isolated from *Humiria balsamifera*. Espindola *et al*., (2004) isolated a new diterpene from *Casaeria sylvestris* var. lingula and it was found to be inhibitory against *Trypanosoma cruzi*. Two new monoterpene glycosides and trypanocidal terpenoids were isolated from *Dracocephalumkotschyi* by Saeidnia *et al*. (2004). Mandal *et al*., (2006) reported antileishmanial activity of saponin triterpenoid isolated from the leaves of *Careya arborea*. New bioactive clerodane diterpenoids isolated from the bark of C*asearia*g*rewiifolia* show antimalarial and antimycobacterial activities (Kanokmedhakul *et al*., 2005).

There are several methods available to assay antimicrobial sensitivity, but Disc or Agar well diffusion method (Collee *et al*., 1996) is commonly used to determine antimicrobial sensitivity test. This method depends on the inhibition of bacterial growth as an indication of activity and is measured as a function of the diameter of inhibition zone. The activity of extract is always compared with that of the currently used antibiotic in parallel line assay. Garud *et al*. (2004) evaluated formulation of disinfectant from plant *Tridax procumbens* by cup plate method. Aboaba *et al*., (2006) investigated the antibacterial effect of edible plant extract on *E. coli* 0157: H7. Leite *et al*. (2006) reported antimicrobial activity of *Indigofera suffructicosa* by agar well diffusion method. Sensitivity of microbes against plant extracts by agar well method has been studied by several workers (Natarajan *et al*., 2007; Erturk *et al*., 2006; Okore *et al*., 2007; Abere *et al*., 2007; Shittu *et al*., 2006). Antimicrobial activity by disc diffusion method has also been studied by several workers (Vadlapudi and Naidu, 2009; Ayandele and Abebiyi, 2007; Usman and Osuji, 2007; Usmanet *al*., 2007; Khosravi and Behzadi 2006; Satya *et al*., 2005; Erturk *et al*., 2006).

Although the diffusion method is commonly used in preliminary susceptibility testing but it is not always an accurate method to assay antimicrobial activity because there is a high degree of interference with this method, arising from drug diffusion problems. A more generally accurate method of assessment is the broth dilution technique (Collee *et al*., 1996). Therefore the broth dilution method was used to determine antimicrobial activity measured as MIC. In the diffusion methods there is the limited diffusion of the less polar active compound in solid media, which shows the lack of inhibition zone while in the broth dilution method the compounds in solution come in direct contact with the organisms (Rios *et al*., 1988; Silva *et al*., 1996).

Okore *et al*. (2007) assayed anticandidal activity of crude aqueous pod extract of *Lecaniodiscus cupanoides* by broth dilution technique. Antimicrobial sensitivity by broth dilution technique has been reported by several workers (Usman *et al*., 2007; Aboaba *et al*., 2006; Khosravi and Behzadi 2006; Rath *et al*., 2001; Banso and Adeyemo, 2006; Obafemi *et al*., 2006; Wilson *et al*., 2005).

The biological and molecular action of secondary metabolites induces various morphological and cytological changes in microorganisms. These changes can be studied at microscopic as well as macroscopic level. Macroscopic changes include change in colony colour, shape, size etc. Changes in cell number, cell size, cell shape, number of reproductive structure can be observed at microscopic level. Effect of extract on cytomorphology i.e. cell size; cell shape and cell number of different organisms has been studied by several workers. Burt and Reinders (2003) reported that oregano essential oil show extensive morphological changes to treated cells. Zeylastral and demethylzeylastral, two phenolic compounds isolated from the roots of *Maytenus blepharodes* (Celastraceae), showed inhibition of synthesis of DNA, RNA, protein and cell wall (de Leon *et al*., 2005). Complete inhibition in the incorporation of the N-aceylyl-D-I-14C glucosamine suggests that the phenolic compounds compromise the cell wall synthesis and/or cytoplasmic membrane. Zhang *et al*. (2006) isolated steroid saponin from *Tribulus terrestris* L. and these steroid saponins showed significant *in vitro* and *in vivo* antifungal activity, weakening the virulence of *Candida albicans* and killing fungi through destroying the cell membrane.

Apart from this, plant extracts also have the ability to affect the protein, carbohydrate and lipid content of plasma membrane as well as their permeability. Plasma membrane of fungi consists of bilayer of protein and lipids. Inhibition of synthesis of DNA, RNA, protein, lipid and carbohydrate may be due to presence of secondary metabolites. These secondary metabolites are target specific and their biochemical and molecular targets are mainly proteins such as receptors, enzymes and polynucleotides like DNA and RNA (Pathipati *et al.*, 2006).

Some plant extracts have ability to inhibit secretion of extracellular enzymes. Inhibition of α-amylase, β-galactosidase, α-mannosidase, alkaline phosphatase, acetylcholine esterase, trypsin, chyemotrypsin, papain and α-cheymotrypsin by methanol extract of several plants has been reported by Kellam *et al*. (1992). Eldeen *et al*. (2006) studied the effect of root extract of *Terminalia serice* bacterial cyclogenase on enzyme (COX-1 and COX-2). Several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as pectolytic macerating enzymes etc. used by plant pathogens (Aboaba and Efuwape, 2001).

Conclusion

This review suggested that medicinal plant parts extract can used for development of several drugs due to its antimicrobial nature. Antimicrobial nature of plant extracts is mainly due to the presence of secondary metabolites. However these extracts do not possess any side effects and residual affects but need to be analysed for toxicity and quality assurance.

Upcoming scenerio will be on the plant based medicine due to comparable efficacy of purified preparation with chemical one. In addition, microbes have also developed multi drug resistance against existing medication and in this case require the replacement with safe and ecofriendly drugs i.e. plant based drugs.

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