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Perspective on Plant Products as Antimicrobials Agents: A Review

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ABSTRACT

Background: The use of plants for healing purposes predates recorded history and forms the origin of much of modern medicine. Many conventional drugs originated from plant sources. Since new and re-emerging infectious diseases are rising very rapidly, there is an urgent need to discover newer antimicrobial compounds having diverse chemical structures and novel mechanisms of action. The emergence of multiple drug resistant strains in human pathogenic organisms has further necessitated the search for new antimicrobial substances from natural sources including plants. This review deals with the phytomedicine usage, the emergence of resistance in the microorganisms, the chemistry and biological activity of the plant based antimicrobial compounds. **Results:** Medicinal plants have drawn the attention of the researchers worldwide and numerous publications have documented the therapeutic potential of phytochemicals to validate claims of their biological activity. Plants have limitless ability to synthesize aromatic substances, most of which are secondary metabolites including phenols, flavonoids, coumarins, alkaloids and terpenoids etc. These compounds serve as plant defense mechanisms against predation by herbivores, insects and microorganisms. Many plant products have ability to overcome resistance in organisms and thereby combat infectious diseases. This has led researchers to isolate active compounds, investigate their mechanisms of action and provide molecular mechanism of overcoming resistance in microorganisms. Antimicrobial agents are compounds capable of destroying or inhibiting micro-organisms even at low concentrations. They vary in their spectrum of activity and can be classified as antibacterial, antifungal or antiviral agents. **Conclusion:** Phytomedicines have shown great promise in the treatment of infectious diseases including opportunistic infections. The diversity of phytochemicals present in plants including secondary metabolites provide drug leads for the development of novel therapeutic antibacterial, antifungal and antiviral agents. The review also sheds light on the future development of combination therapy involving plant-derived agents which is indeed very promising.

Key words: Phytochemicals, infectious diseases, drug resistance, antibacterial, secondary metabolites, multi drug resistant pumps

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INTRODUCTION

Since dawn of the civilization, nature has been a treasure of remedies for providing relief from various ailments afflicting mankind. Plants usually contain many biologically active structurally diverse compounds which are useful as drugs, lead structures or raw materials and are used primarily for treating mild or chronic diseases (Baris *et al*., 2006; Gonzalez-Lamothe *et al*., 2009; Kumar and Pandey, 2013a, b). The earliest written records on Egyptian, Greek, Roman, Chinese and Indian traditional medicine have listed medicinal plants and prescriptions used in treating various ailments. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs (Cragg *et al*., 1997). Even in less developed societies, medicinal recipes from plants have been passed orally from generation to generation. According to World Health Organization (WHO), about 80% of the world population relies chiefly on the plant based traditional medicine especially for their primary healthcare needs (Nitha *et al*., 2012; Mishra *et al*., 2013a).

Medicinal plants are the richest bio-resource of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al*., 1999). It has been estimated that 14-28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethnomedicinal use of the plants. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, terpenoids and flavonoids etc. which possess antimicrobial properties and may serve as an

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alternative, effective, cheap and safe antimicrobial for the treatment of microbial infections. A number of interesting outcomes have been found with the use of a mixture of natural products to treat diseases, most notably the synergistic effects and polypharmacological application of plant extracts (Gibbons, 2003). Infectious diseases are responsible for large scale morbidity and mortality worldwide. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects (Mishra *et al*., 2011).The multi-drug resistant microbial strains are continuously increasing due to indiscriminate use of broad-spectrum antibiotics, intravenous catheters, immunosuppressive agents, organ transplantation and ongoing epidemics of Human Immunodeficiency Virus (HIV) infections (Dean and Burchard, 1996; Gonzalez *et al*., 1996). Plant based antimicrobial compounds have great therapeutic potential as they have lesser side effects as compared with synthetic drugs and also little chance of development of resistance. Therefore, there is a need to search for new antimicrobial substances from natural sources and to develop new infection fighting strategies to control microbial infections (Vaghasiya and Chanda, 2007). In this review an attempt has been made to summarize the current state of knowledge about antimicrobial phytochemicals.

HISTORY OF ETHNOMEDICINAL USES OF PLANTS

Plants have provided a source of inspiration for novel drug compounds. There is evidence that Neanderthals living 60,000 years ago in present-day Iraq used plants such as hollyhock which is still widely used in ethno medicine worldwide (Stockwell, 1988). In the late fifth century B.C., Hippocrates described about 300 to 400 plants having medicinal properties (Schultes, 1978). *De Materia Medica* (a medicinal plant catalog) written by Dioscorides in the first century A.D. became the prototype for modern pharmacopoeias. The Bible offered descriptions of approximately thirty medicinal plants. With the fall of ancient civilizations much of the documentation of plant pharmaceuticals was destroyed or lost which prevented Western advances in the understanding of medicinal plants (Stockwell, 1988). During the Dark Ages, the Arab world continued to excavate their own older works and to build upon them. Ofcourse, Asian cultures were also busy compiling their own pharmacopoeia. Among Europeans living in the New World, the use of botanicals was a reaction against invasive or toxic mainstream medicinal practices of the day (Cowan, 1999).

Literature is flooded with numerous examples of plant derived anti-infective drugs. The isoquinoline alkaloid emetine obtained from the underground part of *Cephaelis ipecacuanha* and related species, has been used for many years as an amoebicidal drug as well as for the treatment of liver abscesses due to the spread of *Entamoeba histolytica* infections. *Cinchona* bark alkaloid quinine is another important drug of plant origin with a long history of use. In addition to its continued usefulness in the treatment of malaria, it can be also used to relieve nocturnal leg cramps. Currently, the commonly prescribed drugs are analogs of quinine such as chloroquine. However some strains of malarial parasites have developed resistance to the quinines, therefore antimalarial drugs with novel mode of action are required. Higher plants have also made important contributions in the areas beyond anti-infectives, such as cancer therapies. Early examples include the antileukaemic alkaloids, vinblatine and vincristine, which were both obtained from the Madagascan periwinkle (*Catharanthus roseus* syn. *Vinca roseus*) (Nelson, 1982; Iwu *et al*., 1999; Ncube *et al*., 2008). Lastly, the rise of the Human Immunodeficiency Virus (HIV) has encouraged exhaustive exploration and investigation of the phytochemicals and their derivatives which might be effective, especially for use in underdeveloped countries with little access to expensive Western medicines (Clercq, 1995).

INFECTIOUS DISEASES IN HUMANS

Infectious diseases are the leading cause of death worldwide. There has been an increasing interest in medicinal plants as a natural alternative to synthetic drugs because clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow *et al*., 2003). Several members of enterobacteriaceae are responsible for causing severe infections (Fabio *et al*., 2007). *Klebsiella pneumoniae* and *Pseudomonas* spp. are emerging as an important cause of neonatal nosocomial infections. *Escherichia coli* causes septicemias, diarrhoea and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs especially in debilitate and immunodeficient patients. *Proteus mirabilis* causes wound infections and urinary tract infections in the elderly and young males often following catheterization or cystoscopy and it is a secondary invader of ulcers, pressure sores, etc. *Staphylococcus* and *Streptococcus* cause nosocomical infections, food poisoning, upper respiratory tract infections and other type of infections (Pandey, 2007; Mishra *et al*., 2011). Various diseases caused by different group of bacteria are given in Table 1.

DRUG RESISTANCE IN BACTERIA

The discovery of antibiotics in the 1940s during World War II era provided impetus for growth and development of effective antimicrobials with high

Table 1: Diseases caused by different groups of pathogenic bacteria

Gram positive bacteria		Gram negative bacteria	
Bacteria	Disease(s)	Bacteria	Disease(s)
Staphylococcus aureus	Food poisoning, wound infections, toxic shock syndrome	*Escherichia coli*	Gastroenteritis, urinary tract infections, neonatal meningitis
Streptococcus pyogenes (Group A strep)	Sore throat, scarlet fever, mastitis, necrotizing fasciitis,	*Salmonella enterica*	Gastroenteritis
Streptococcus pneumoniae	Pneumonia, otitis media, meningitis	*Salmonella typhi*	Typhoid fever
Streptococcus mutans	Dental caries	*Shigella dysenteriae*	Bacillary dysentery
Bacillus anthracis	Anthrax	*Yersina pestis*	Bubonic plague
Bacillus cereus	Food poisoning, gas gangrene, uterine infections	*Pseudomonas aeruginosa*	Opportunistic infections, swimmer's ear, hot tub itch, cellulitis, pneumonia
Clostridium botulinum	Botulism, infant botulism	*Vibrio cholera*	Cholera
Clostridium difficile	Antibiotic associated diarrhea	*Bordetella pertussis*	Whooping cough
Corynebacterium diphtheriae	Diphtheria	*Haemophilus influenzae*	Meningitis, pneumonia, sinusitis
Listeria monocytogenes	Listeriosis	*Helicobacter pylori*	Gastric and duodenal ulcers
Chlamydia trachomatis	Chlamydia, lymphogranuloma venereum, trachoma	*Campylobacter jejuni*	Gastroenteritis
Chlamydia pneumoniae	Pneumonia	*Neisseria gonorrhoeae*	Gonorrhea
Acid fast bacteria			
Mycobacterium tuberculosis	TB (tuberculosis)	*Treponema pallidum*	Syphilis
Mycobacterium leprae	Leprosy	*Borrelia burgdorferi*	Lyme disease
Mycoplasma pneumonia	Atypical pneumonia	*Rickettsias*	Typhus, RMSF

specificity for clinical use. This ultimately resulted in significantly lowered morbidity and mortality from bacterial infections. However extensive use and misuse of antibiotics has also led to the emergence of resistance in a variety of pathogenic bacteria. Microorganisms developed counter measures to deal with the lethal effects of antibiotics over the time resulting in worldwide treatment failures which consequently led to emergence of multiple drug resistant strains. Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the examples. This strain is resistant to a wide range of antimicrobial agents including beta-lactam antibiotics, macrolides, amino glycosides and fluroquinolones (Maple *et al*., 1989; Pandey, 2007).

Resistance in fungal pathogens is also increasing. Amphotericin B was the only treatment available for fungal infections for many years. In late 1980s fluconazole and itracozole were developed as additional therapeutic options (Ficker *et al*., 2003). Presently azole derivatives are the most widely used antifungal agents. However resistance against these drugs is emerging (Maple *et al*., 1989; Clercq, 1995; Groll *et al*., 1998; Ficker *et al*., 2003; Pandey, 2007; Mishra *et al*., 2011). All the available antifungal drugs used to date are not ideal in efficiency, safety and antifungal spectrum (Di Domenico, 1999). Combination antifungal therapy was also used to increase the efficiency but there is a real demand for a next generation of safer and more powerful antifungal agents (Bartroli *et al*., 1998; Mishra *et al*., 2009). In the last two decades, development of resistance to antibiotics by pathogenic organisms has become a serious problem. Knowing that modifying known antimicrobial compounds is increasingly difficult has created an urgent and very pressing need for isolation and identification of new bioactive chemicals from new sources including plants (Barker, 2006; Mishra *et al*., 2008; Talib, 2011) which can be used alone or in combination with other agents to control infectious diseases.

Mechanism of bacterial resistance against antibiotics: The mechanisms by which the microorganisms counteract the lethal effects of antibiotics can be divided into three main categories (Walsh, 2000; Alviano and Alviano, 2009):

- Direct destruction or inactivation of the antibiotic by modification of its active moiety
- The specific modification of the macromolecular target by mutation leading to prevention of interaction of the drug with target
- The prevention of antibiotics from reaching their targets through decreased uptake or more commonly, active removal of antibiotic by efflux pumps

Need of new antimicrobials to combat resistance: Bioactive natural compounds exhibiting antimicrobial activities have been isolated mainly from cultivable microbial strains. There is need to exploit the untapped natural resources of different origin including plants producing biologically active metabolites which still remains to be thoroughly investigated (Emma *et al*., 2001) to alleviate or help responding to current health care situations; such situations include but not limited to unmet clinical needs, increasing cost of chemotherapy, mycobacterial reemergence and the emergence of antibiotic resistant microbial strains such as MRSA (Alanis, 2005). Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes

that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are natural, relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Ghosh *et al*., 2008; Kumar and Pandey, 2012). Also plants derived agents may have different mechanisms than conventional drugs and could be of clinical importance in health care improvement (Eloff, 1998). There are two main classes of plant derived agents.

Phytoalexins: These are low molecular weight compounds produced in response to microbial, herbivorous, or environmental stimuli (Van Etten *et al*., 1994). Phytoalexins include simple phenylpropanoid derivatives, flavonoids, isoflavonoids, terpenes and polyketides (Grayer and Harborne, 1994).

Phytoanticipins: These are produced in plants before infection or from pre-existing compounds after infection (Van Etten *et al*., 1994). Phytoanticipins include glycosides, glucosinolates and saponins that are normally stored in the vacuoles of plant cells (Osbourn, 1996).

PLANT PRODUCTS AS MEDICINAL AGENTS

The development of any pharmaceutical agent begins with the identification of active principles, detailed biological assays and dosage formulations, followed by clinical studies to establish safety, efficacy and pharmacokinetic profile of the new drug (Iwu *et al*., 1999). The same principle is also applicable for the development of therapeutic agents from plants. Systematic and comprehensive biological evaluation of plant extracts is vital to ensure their efficacy and safety as drug. These factors are of importance if plant extracts are to be accepted as valid medicinal agents. Researchers especially ethnopharmacologists, botanists, microbiologists, biochemists and natural-product chemists world wide are continuously exploring the resources on the globe for novel phytochemicals or their derivatives which could be utilized for the treatment of infectious diseases (Tanaka *et al*., 2006; Maurya *et al*., 2012; Pandey *et al*., 2012) especially in light of the emergence of drug-resistant microorganisms and the need to produce more effective antimicrobial agents.

Though most of the clinically used antibiotics are produced by soil microorganisms or fungi, higher plants have also been a source of antibiotics. Examples of these are the bacteriostatic and antifungal properties of lichens, the antibiotic action of allinine in *Allium sativum* (garlic), or the antimicrobial action of berberines in goldenseal (*Hydrastis canadensis*). It is estimated that today, plant materials are present in, or have provided the models for 50% Western drugs (Ciocan and Bara, 2007).

Secondary metabolites produced by plants show wide ranging chemical, physical and biological activities which are the sources of natural bioactive substances for new drugs of plant origin. The process involves isolation, identification and characterization of secondary metabolites produced by plants and their use as active principles in medicinal preparations (Taylor *et al*., 2001; Kumar and Pandey, 2013b). Many of the plant secondary metabolites are constitutive, existing in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated in response to tissue damage or pathogen attack (Van Etten *et al*., 1994).

The bacterial resistance is conferred by multidrug resistance pumps (MDRs), membrane translocases that extrude structurally unrelated toxins from the cell. These protect microbial cells from both synthetic and natural antimicrobials (Stermitz *et al*., 2000). Secondary metabolites resemble endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to their recognition in potential target sites (Parekh *et al*., 2005). The use of plant extracts and phytochemicals can be of great significance in therapeutic applications and could help curb the problem of multi-drug resistant organisms. Cinnamon, clove, jambolan, pomegranate, thyme and lantana extracts have been shown to inhibit the growth of multi drug resistant *Pseudomonas* aeruginosa (Nascimento *et al*., 2000; Pandey *et al*., 2005; Mishra *et al*., 2010; Pandey *et al*., 2010). Menthol isolated from peppermint oil has been shown to eliminate the resistance plasmids of bacteria (Schelz *et al*., 2006) while carbazole alkaloids isolated from *Clausena anisata* stem bark have been reported to possess high antibacterial and antifungal activities (Chakrabarty, 2003).

A number of plant secondary metabolites have been used as anticancer agents. Flavonoid rich extracts from the mature roots of *Scutellaria baicalensis* have been shown to exhibit anti-proliferative effects on various cancer lines (Scheck *et al*., 2006). Taxol, a diterpene from the Pacific yew has been widely used as a drug for the treatment of ovarian and breast cancer (Iwu *et al*., 1999). Limonoids, a group of triterpenes, have been shown to be successful in treatments with *in-vitro* bioassays on human tumor cell lines, with limonin and isofraxinellone being the most active compounds (Ahn *et al*., 1994; Ncube *et al*., 2008). Phytochemicals present in *Parthenium hysterophorus* and *Tinospora cordifolia* have been shown to be promising antibacterial, anticancer, antioxidant and anti HIV agents (Pandey, 2007; Kumar *et al*., 2013b; Pandey and Kumar, 2013; Mishra *et al*., 2013b).

The synergistic effects of extracts having antimicrobial potential in association with antibiotics can provide effective therapy against drug resistant bacteria.

These synergistic combinations represent a largely untapped source of new pharmaceutical products with novel and multiple mechanisms of action that can overcome microbial resistance. Recent developments in molecular biology and plant biotechnology have created the tools to produce botanical mixtures at a level comparable to that of pure drug compounds (Gibbons, 2003) and through biosynthesis and bioengineering principles dependence on large amount of plant material is reduced and thereby limiting depletion of biogenetic resources in forests. These compounds, however, should be subjected to animal and human studies to determine their biological efficacy in whole-organism systems, including in particular toxicity studies along with evaluation of their effects on beneficial normal microbiota (Ncube *et al*., 2008). The method of extraction and *in vitro* testing should be standardized so that the search for new antimicrobial drugs from plants could be more systematic and it will facilitate proper interpretation of results (Cowan, 1999).

ANTIMICROBIAL SUSCEPTIBILITY TESTS

Successful discovery of novel natural antimicrobials has necessitated the development of new bioassay techniques which are sensitive enough to detect small amounts of biologically active chemicals (Lampinen, 2005). In ethnopharmacology research the Antimicrobial Susceptibility Test (AST) is used to determine the efficacy of potential antimicrobials from biological extracts against a number of different microbial species. AST methods are used to screen plant extracts for antimicrobial activity but are largely used to determine the usefulness of an antimicrobial in combating infections by determining its Minimum Inhibitory Concentration (MIC). In clinical research *in vitro* susceptibility tests are particularly important if an organism is suspected to belong to a species that has shown resistance to frequently used antimicrobial agents. They are also important in epidemiological studies of susceptibility and in comparisons of new and existing microbial agents (EUCAST, 2003).

AST standard tests can be conveniently divided into diffusion and dilution methods. Common diffusion tests include agar well diffusion, agar disk diffusion and bioautography, while dilution methods include agar dilution and broth micro/macrodilution (Ncube *et al*., 2008). The broth and agar based methods are the conventional reference methods for AST (Tenover *et al*., 1995). There are other commercial custom-prepared methods like the agar screen plate, Epsilometer test and the Vitek system which could be used in place of the standard reference methods but these are not common in routine AST (Joyce *et al*., 1992) and are not common for testing activity of plant extracts. The major problem in the diffusion and dilution based AST is one of availability of the active principles which is a function of the solubility of the test compound (Ncube *et al*., 2008).

MAJOR GROUPS OF PHYTOCHEMICALS ACTING AS ANTIMICROBIALS

Medicinal effects of plant derived materials typically emanate from the secondary metabolites such as alkaloids, steroids, tannins and phenolic compounds in isolation or in combination which are synthesized and deposited in specific parts or in all parts of the plant (Talib, 2011). The structural aspects and biological activities of the important phytochemical groups are described below:

Phenolics: Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenyl propane-derived compounds which are in the highest oxidation state. The common herbs tarragon and thyme both contain caffeic acid, which is effective against viruses, bacteria and fungi. Catechol and pyrogallol both are hydroxylated phenols, shown to be toxic to microorganisms. Catechol has two-OH groups and pyrogallol has three. The site(s) and 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. The mechanisms thought to be responsible for phenolic toxicity to microorganism include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins. Phenolic compounds possessing a C3 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. Eugenol is considered bacteriostatic against both fungi and bacteria (Cowan, 1999; Ciocan and Bara, 2007).

Phenolic compounds have diverse defensive functions in plants, such as cell wall strengthening and repair (lignin and suberin) and antimicrobial and antifungal activities (Furiga *et al*., 2009). Some polyphenols are phytoanticipins, compounds with a defensive role that are not synthesized in response to a pathogen attack but rather are constitutively present in plant cells. Phenolic constituents occur on the surface of plants or in the cytoplasmic fraction of the epidermal cells, where they act as a deterrent to pathogens. In contrast, phenolic phytoalexins are secreted by wounded plants or in response to incompatible pathogens (Gregoire *et al*., 2007). The induced defence response

includes cell death and the formation of a lesion that limits the growth of the pathogen. Cells around the lesion accumulate polyphenols and other antibiotic compounds (Grollier *et al*., 2009). Pholyphenols as catechin act on different bacterial strains belonging to different species (*Escherichia coli*, *Bordetella bronchiseptica*, *Serratia marcescens*, *Klebsiella pneumonie*, *Salmonella choleraesis*, *Pseudomonas aeruginosa*, *Staphilococcus aureus* and *Bacillus subtilis*) by generating hydrogen peroxide and by altering the permeability of the microbial membrane (Hattori *et al*., 1990; Haslam *et al*., 1992; Kumar *et al*., 2013a). Microbes stressed by exposure to polyphenols upregulate proteins related to defensive mechanisms, which protect cells while simultaneously downregulating various metabolic and biosynthetic proteins involved, for example, in amino acid and protein synthesis as well as phospholipid, carbon and energy metabolism (Hu *et al*., 2010). Moreover, polyphenols have been reported to interfere with bacterial quorum sensing, i.e., the production of small signal molecules by bacterial cells of *Escherichia coli*, *Pseudomonas putida* and *Burkholderia cepacia* that trigger the exponential growth of a bacterial population (Hubert *et al*., 2003; Ferrazzano *et al*., 2011).

Quinones: Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive. These compounds being colored are responsible for the browning reaction in cut or injured fruits and vegetables are an intermediate in the melanin synthesis pathway in human skin. Vitamin K is a complex naphthoquinone. Its antihemorrhagic activity may be related to its ease of oxidation in body tissues. In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins, 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. Hypericin, an anthraquinone from St. John's wort (*Hypericum perforatum*), has received much attention lately as an antidepressant and antimicrobial (Cowan, 1999; Ciocan and Bara, 2007).

Flavones, flavonoids and flavonols: Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. They are known to be synthesized by plants in response to microbial infection and have been shown in vitro to be effective antimicrobial substances against a wide array of microorganisms (Cowan, 1999; Kumar and Pandey, 2013b). Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as described above for quinones. More lipophilic flavonoids may also disrupt microbial membranes. Catechins, the most reduced form of the C3 unit in flavonoid compounds, deserve special mention. These flavonoids have been extensively researched due to their occurrence in oolong green teas. It was noticed some time ago that teas exerted antimicrobial activity and that they contain a mixture of catechin compounds. These compounds inhibited in vitro *Vibrio cholerae*, *Streptococcus mutans*, *Shigella* and other bacteria and microorganisms. Flavonoid compounds exhibit inhibitory effects against multiple viruses. Numerous studies have documented the effectiveness of flavonoids such as swertifrancheside, glycyrrhizin (from licorice) and chrysin against HIV (Critchfield *et al*., 1996; Ciocan and Bara, 2007; Kumar and Pandey, 2013b).

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 and divided into two groups, hydrolyzable and condensed tannins. Hydrolyzable tannins are based on gallic acid, usually as multiple esters with D-glucose, while the more numerous condensed tannins (often called proanthocyanidins) are derived from flavonoid monomers. Tannins may be formed by condensations of flavan derivatives which have been transported to woody tissues of plants. Alternatively, tannins may be formed by polymerization of quinone units (Ciocan and Bara, 2007; Karou *et al*., 2007).

Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity and a wide range of anti-infective actions, have been assigned to tannins. Thus, their mode of antimicrobial action, may be related to their ability to inactivate microbial adhesins, enzymes cell envelope transport proteins etc. Studies have shown that tannins can be toxic to filamentous fungi, yeasts and bacteria. Condensed tannins bind cell walls of ruminal bacteria; prevent growth and protease activity (Cowan, 1999).

Coumarins: Coumarins are phenolic substances made of fused benzene and a-pyrone rings. They are responsible for the characteristic odor of hay. They produce diverse biological activities such as antimicrobial, antithrombotic, anti-inflammatory and vasodilatory activities. Coumarin has been found in vitro to inhibit *Candida albicans*. Also, phytoalexins, which are hydroxylated derivatives of coumarins, are produced in

Table 2: Mechanism of anti-infective action of phytochemical groups

Phytochemicals	Activity	Mechanism of action
Quinones	Antimicrobial	Binds to adhesins, complex with cell wall, inactivates enzymes
Flavonoids	Antimicrobial	Complex with cell wall, binds to adhesins
	Antidiarrhoeal	Inhibits release of autocoids and prostaglandins, inhibits contractions caused by spasmogens, stimulates normalization of the deranged water transport across the mucosal cells, inhibits GI release of acetylcholine
Polyphenols and tannins	Antimicrobial	Binds to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption, metal ion complexation
	Antidiarrhoeal	Makes intestinal mucosa more resistant and reduces secretion, stimulates normalization of deranged water transport across the mucosal cells and reduction of the intestinal transit, blocks the binding of B subunit of heat-labile enterotoxin to GM1, resulting in the suppression of heat-labile enterotoxin-induced diarrhea, astringent action
	Anthelmintic	Increases supply of digestible proteins by animals by forming protein complexes in rumen, interferes with energy generation by uncoupling oxidative phosphorylation, causes a decrease in GI metabolism
Coumarins	Antiviral	Interaction with eucaryotic DNA
Terpenoids and essential oils	Antimicrobial	Membrane disruption
	Antidiarrhoeal	Inhibits release of autocoids and prostaglandins
Alkaloids	Antimicrobial	Intercalates into cell wall and DNA of parasites
	Antidiarrhoeal	Inhibits release of autocoids and prostaglandins
	Anthelmintic	Possess anti-oxidating effects, thus reduces nitrate generation which is useful for protein synthesis, suppresses transfer of sucrose from stomach to small intestine, diminishing the support of glucose to the helminthes, acts on CNS causing paralysis
Lectins and polypeptides	Antiviral	Blocks viral fusion or adsorption, forms disulfide bridges
Glycosides	Antidiarrhoeal	Inhibits release of autocoids and prostaglandins
Saponins	Antidiarrhoeal	Inhibits histamine release *in vitro*
	Anticancer	Possesses membrane permeabilizing properties
	Anthelmintic	Leads to vacuolization and disintegration of teguments
Steroids	Antidiarrhoeal	Enhance intestinal absorption of Na^+ and water

carrots in response to fungal infection and can be presumed to have antifungal activity. Coumarins may be responsible for antimicrobial activity in woodruff (*Galium odoratum*) extracts (Ciocan and Bara, 2007).

Terpenoids and essential oils: The fragrance of plants is carried in the 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 $C_{10}H_{16}$ 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. Terpenoids are synthesized from acetate units and as such they share their origins with fatty acids. They differ from fatty acids in that they contain extensive branching and are cyclized. Examples of common terpenoids are menthol and camphor (monoterpenes) and farnesol and artemisin (sesquiterpenoids). Terpenenes or terpenoids are active against bacteria, fungi, viruses and protozoa. The ethanol-soluble fraction of purple prairie clover yields a terpenoid called petalostemumol, which produces significant activity against *Bacillus subtilis* and *Staphylococcus aureus* while lesser activity against gram-negative bacteria as well as *Candida* albicans (Cowan, 1999; Ciocan and Bara, 2007).

Lectins and polypeptides: Peptides which are inhibitory to microorganisms were first reported in 1942. Recent interest has been focused mostly on studying anti-HIV peptides and lectins. The inhibition of bacteria and fungi by these macromolecules from the herbaceous *Amaranthus* has long been known. Thionins are peptides commonly found in barley and wheat and consist of 47 amino acid residues. They are toxic to yeasts as well as gram-negative and gram-positive bacteria (Ciocan and Bara, 2007; Karou *et al*., 2007). Fabatin, a newly identified 47- residue peptide from fava beans, appears to be structurally related to g-thionins from grains and inhibits *E. coli*, *P. aeruginosa* and *Enterococcus hirae* but not *Candida* or *Saccharomyces*.

Alkaloids: Alkaloids rank among the most efficient and therapeutically significant plant substances. They are chemically very diverse group of organic nitrogen compounds. Generally they are extremely toxic though they do have a marked therapeutic effect in minute quantities. For this reason plants containing alkaloids were not often used in folk medicine and then for external application only. Pure, isolated plant alkaloids and their synthetic derivates are used as basic medicinal agents all over the world for their analgesic, antispasmodic and bactericidal effects (Stary, 1996).

Other compounds: Several other compounds also show antimicrobial properties. These include polyamines

(in particular spermidine), isothiocyanates, thiosulfinates and glucosides. Polyacetylenes deserve special mention. Acetylene compounds and flavonoids from plants are traditionally used in Brazil for treatment of malaria fever and liver disorders. Historically, women have been told to drink the juice in order to prevent and even cure urinary tract infections. In the early 1990s, researchers found that the monosaccharide fructose present in cranberry and blueberry juices competitively inhibited the adsorption of pathogenic *E. coli* to urinary tract epithelial cells, acting as an analogue for mannose (Cowan, 1999). Clinical studies have borne out the protective effects of cranberry juice. Researchers are now seeking a second active compound from cranberry juice which contributes to the antimicrobial properties of this juice (Zafriri *et al*., 1989).

MECHANISM OF ANTIMICROBIAL ACTION OF PHYTOCHEMICALS

Plant products including polyphenols that show health benefits may act via similar or different mechanisms in humans as those functional in plants. Phytochemicals may also modulate transcription factors, redox-sensitive transcription factors, redox signaling and inflammation (Doughari *et al*., 2009). As an example, Nitric oxide (NO), a signaling molecule of importance in inflammation, is modulated by plant polyphenols and other botanical extracts. The mechanism of anti-infective action of phytochemicals (Tiwari *et al*., 2011) are summarised in Table 2.

FUTURE PROSPECTS

Sanguinarine, an alkaloid derived from rhizomes of *Sanguinaria canadensis* L. was shown to possess anti-protozoal activity, antibacterial activity against Gram-positive bacteria (including those resistant to penicillin), Gram-negative bacteria and pathogenic fungi (Vichkanova *et al*., 1996). Because of its systemic toxicity, it has only been used in oral health products such as mouth rinses or toothpastes (Godowski, 1989). Recent studies have indicated that phytochemicals may act as antibiotics as well as antibiotic potentiators. Studies have shown that inhibiting or disabling Multi Drug Resistant Pumps (MDRs) by adding MDR inhibitors increased the penetration of several plant antimicrobial agents into bacterial cells (Stermitz *et al*., 2000; Tegos *et al*., 2002). The medicinal plant *Berberis* produces both the antibacterial alkaloid berberine as well as the NorA efflux pump inhibitor 5'-methoxyhydnocarpin (Stermitz *et al*., 2000). The observed synergy between berberine and 5'-methoxyhydnocarpin and the elucidation of its mode of action toward NorA has triggered the development of screens for identification of plant products that have antibiotic activities against Gram negative bacteria.

Utilization of known synthetic inhibitors of Gram negative MDRs has revealed the potential broad spectrum antibacterial activity of many phytochemicals such as rhein, plumbagin, resveratrol, gossypol, coumestrol and berberine (Tegos *et al*., 2002). The antimicrobial agent, rhein, isolated from rhubarb was only slightly effective against *E. coli*. The addition of two MDR inhibitors increased the activity significantly by 2000-folds demonstrating the potential of rhein as an effective antimicrobial agent against Gram negative bacteria. Even *P. aeruginosa*, which is known to be resistant to almost all antimicrobial agents and antibiotics due to its outer membrane and MDRs, was also rendered susceptible to rhein. The study proved that plant antimicrobial agents are potentially as effective as antibiotics if they are delivered appropriately into the cell (Onawunmi, 2006).

Efficacy of β-lactam antibiotics can be improved by combination with antimicrobials of plant origin such as baicalin, corilagin and epigallocatechin gallate, to name a few. Baicalin, a flavone isolated from the herb *Scutellaria amoena* C.H. Wright used in Chinese traditional medicine, produces moderate activity against MRSA and other strains resistant to other β-lactams. However when the β-lactams such as ampicillin, amoxycillin, benzylpenicillin and methicillin were combined with baicalin at a concentration of 16ìg/ml their activities got potentiated (Liu *et al*., 2000).

Corilagin, a polyphenol isolated from *Acalypha* species alone showed weak anti-MRSA activity. However low concentrations of corilagin (16 μg mL^{-1}) markedly decreased the MIC of oxacillin and other β-lactams against MRSA strains. Corilagin also caused some reduction in the MICs of streptomycin and tetracycline against some strains of MRSA (Shimizu *et al*., 2000; Adesina *et al*., 2000). Similarly combinations of carbapens and epigallocatechin gallate, a main constituent of the catechins extracted from tea (*Camellia sinensis*), have produced potent synergy against several clinical isolates of MRSA (Hu *et al*., 2002). This indicates that plant based antimicrobials have the potential to restore the effectiveness of β-lactam antibiotics against resistant strains of *Staphylococcus aureus*. These agents may have potential for development as adjuncts to β-lactam treatments against resistant strains of bacteria such as MRSA. N-trans-feruloyl 4'-*O*-methyldopamine isolated from the methanolic extract of *Mirabilis jalapa* has ability to block NorA and thus it significantly improves the activity of norfloxacin against *S. aureus* (Michalet *et al*., 2007; Gonzalez-Lamothe *et al*., 2009). An attractive and highly cost effective and preventive approach is the use of vaccines. Newer developments in immunology, molecular biology and biotechnology allow us know to realistically approach the diseases for which vaccines

were not feasible. Recently plants have emerged as alternative production system for subunit vaccine production (Pandey and Sharma, 2011).

Future studies may provide other novel or innovative ways of achieving this, hence there is need to continue the search for antimicrobial agents from plants. The studies predict that future development of combination therapy involving such plant-derived agents is indeed very promising.

CONCLUSION

The potential isolation and use of new and novel bioactive products from plant origins is still very productive playground for the development of new drugs to improve health care in certain medical fields. It is essential to emphasize that extensive *in vitro* and *in vivo* tests must be conducted to assure the selection of active and nontoxic antimicrobial phytochemicals. It would be advantageous to standardize methods of extraction and in vitro testing so that the search could be more systematic and interpretation of results would be facilitated. There is a need to exploit the potential synergistic effects of plant-derived agents with known antibiotics/antimicrobial agents in chemotherapy.

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