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Antimicrobials in higher plants: classification, mode of action and bioactivities

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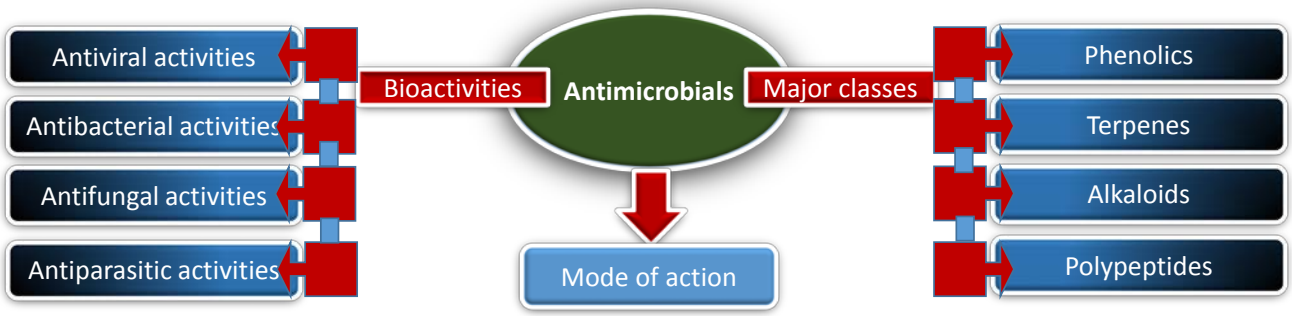
ABSTRACT



Plants produce an enormous number of phytochemicals as a part of their primary as well as secondary metabolism of which many could possess therapeutic value especially antimicrobial properties. Majority of the isolated antimicrobials pertain to chemical groups namely, phenolics, terpenes, alkaloids and polypeptides, and exhibit multiple mechanisms of action against disease causing microorganisms. The target sites of different antimicrobials range from molecular to organism level. During their antimicrobial action at molecular level these phytochemicals interact covalently and non-covalently with macromolecules and render them non-functional. At cellular level antimicrobials disrupt the functioning of various cell components such as capsule, cell wall, cell membrane and mitochondria leading to the death of pathogens. Various antimicrobial activities like antiviral, antibacterial, antifungal and antiparasitic activities of different plants and their isolated chemicals have also been discussed.

Keywords: Secondary metabolites, Phytochemicals, Classification, Mode of action, Antimicrobial activities

INTRODUCTION

Humans have been relying on plants and their products for fulfilling their day to day needs since antiquity. The use of plants in treatment of various ailments was very common in many human civilizations and some of the systems of medicine like Indian (Ayurveda) and Chinese systems, still in practice, are chiefly herbal based. Plants and their products as a part of traditional and complementary medicines (T&CM) are still widely used as curing agents in most of the developing countries where modern medicine is not accessible to the people, especially those living in remote places. Now, T&CM practices are being chosen as an alternative/additional system of health-care world over for various reasons such as, a) an increased demand for all kinds of health services, b) an increased awareness about availability of options of treatment for various health problems, c) an increasing dissatisfaction with existing health-care services, and d) a rekindled interest in "whole person care" and disease prevention, which are more often associated with T&CM.[1,2] WHO has also recognized the role and importance of T&CM and promoting their integration

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into world health-care system in order to attain the target of universal health coverage.[3]

Currently in USA one quarter to one half of all the pharmaceuticals used are of higher plant origin.[4] However, a very few phytochemicals are used as antimicrobials and majority of the antimicrobials available in market are of microbial origin.[5] The discovery of antibiotics from actinomycetes and some of the fungi and their initial success in curtailing infectious diseases discouraged the researchers to look for alternative sources of antimicrobials. However within few decades, because of the frequent reports of antibiotic resistance in majority of pathogens due to excessive use of antibiotics, it was realized that rather than considering the era of infectious diseases to be over, it would be more appropriate to consider it to be an ongoing battle where upper hand can be maintained only by continuously adding new antimicrobials in the arsenal of antimicrobial drugs.[6-8] Therefore, the pace of research in direction of new antimicrobials has again increased in recent years and new sources including higher plants are being investigated. Already a multitude of plant products whose purity is often doubtful is readily available over the counter and self-medication with these products is commonplace.[3] This review work is intended to explore the literature published in recent past years and gather information related to various aspects such as chemistry, mode of action and nature of antimicrobial activity of antimicrobials of plant origin so that, a general account of knowledge available till date may be provided for future studies.

GROUPS OF NATURAL ANTIMICROBIALS

It is estimated that there are 250,000 to 500,000 species of plants on Earth of which only one to ten percent are consumed as food by humans and other animal species.[9] However, the scope of using plants as source of the medicine is much bigger because plants produce enormous number of highly diversified metabolites as a part of their defense mechanism. Majority of plant compounds exhibiting antimicrobial properties discovered so far are secondary metabolites and in comparison to this only few are primary metabolites. The exact function of secondary metabolites in plant system is still unknown but it is speculated that such compounds play important roles as a part of plant defense mechanism against predators, pathogens and provide an edge of survival against competitors as well. Some of them also provide odour, flavour and colour to plants.[4] The phytochemicals having antimicrobial properties can be divided into following four major categories:

1. Phenolics
2. Terpenes
3. Alkaloids
4. Antimicrobial Peptides

Phenolics: This is the largest class of secondary metabolites which are found widely distributed in the kingdom, Plantae. Various phenolic compounds contain one or more hydroxyl groups (OH) that remain directly attached to an aromatic hydrocarbon chain. While classifying different phenolic compounds various parameters such as chemical composition, number of hydroxyl groups and substitutes present in carbon skeleton are used.[10] On the basis of number of aromatic rings and carbon atoms in the side chain present, various classes of phenolic compounds have been given in Table 1.[11] Some of the groups of phenolics are:

Phenolic compounds containing one aromatic ring: This includes some of the simplest natural forms such as phenol (C_6H_5OH) which consist only of a six carbon atoms (C_6) aromatic ring. Besides these simple forms, compounds may have a single aromatic ring (C_6) attached either to one carbon atom (e.g. salicylic acid) or two carbon atoms (e.g. apocynin) or three carbon atoms (phenylpropanoids, e.g. caffeic acid) or four carbon atoms (naphthoquinones) (Figure 1). Phenolic acids are the phenols containing one carboxylic acid functional group (COOH).

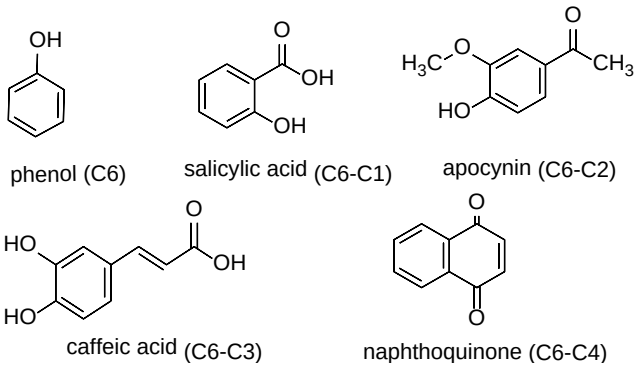

Figure 1. Examples of phenolics containing one aromatic ring attached to variable number of carbons.

Phenolics containing two aromatic rings: These compounds contain two (C_6) aromatic rings that have been further divided on the basis of number of carbon atoms linking two rings into xanthones (where one carbon atom connects two aromatic rings), stilbenes (where two carbon atoms connect two rings) and flavonoids (where three carbon atoms connect two aromatic rings) (Figure 2).

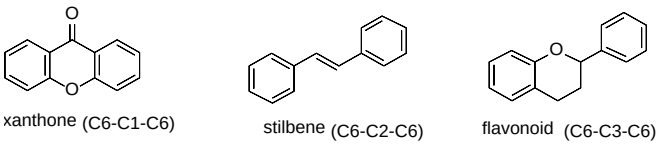

Figure 2. Phenolics containing two aromatic rings attached to variable number of carbons.

Flavonoids are water soluble polyphenols, containing more than one OH groups in aromatic ring. These are widely distributed in plants and have been reported to be associated with pigmentation in plants, UV (ultraviolet) protection, disease resistance and symbiotic nitrogen fixation.[12] The main subclasses of flavonoids are, flavones, flavonols, flavan-3-ols, isoflavones, flavanones and anthocyanidins; other flavonoid groups, which comparatively are minor components of the diet

quantitatively, are dihydroflavonols, flavan-3, 4-diols, coumarins, chalcones, dihydrochalcones and aurones.[13]

Table 1. Major classes of phenolics on the basis of aromatic ring present.

Basic carbon skeleton	Total carbon atoms	Class
C6 (aromatic ring)	6	Simple phenols, Benzoquinones
C6-C1	7	Phenolic acids
C6-C2	8	Acetophenones, Tyrosine derivatives
C6-C3	9	Hydroxycinnamic acid, Coumarins
C6-C4	10	Naphthoquinones
C6- C1-C6	13	Xanthones
C6- C2-C6	14	Stilbenes
C6- C3-C6	15	Flavonoids
(C6- C3)2	18	Lignans
(C6- C3-C6)2	30	Bioflavonoids
(C6- C3-C6)n	N	Condensed tannins

Tannins although difficult to define accurately are polyphenolic compounds of high molecular weight ranging from 500 Da to more than 3000 Da which, are usually water soluble. They have ability to bind with proteins and form insoluble or soluble tannin-protein complexes.[14] Besides proteins tannins are also known to form complexes with polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids and minerals.[11,15] They have been closely associated with plant defense mechanisms towards mammalian herbivores and insect. According to their chemical structure and properties, tannins are divided into two main groups, hydrolysable (HT) and condensed tannins (CT). Hydrolysable tannins are usually found in lower concentrations in plants than CTs. Two groups differ in their molecular weights, chemical structure and different effects they produce after ingestion on the herbivorous animals especially on ruminant.[14] Structurally, HTs (gallotannins and ellagitannins) contain a carbohydrate, generally D-glucose, as a central core.[16] The hydrolysable groups of these carbohydrates are esterified with phenolic groups, such as gallic acid or ellagic acid.[17,18]

Hydrolysable tannins are subdivided into taragallotannins (gallic and quinic acid) and caffetannins (caffeic and quinic acid).[17] When catechin unit binds glycosidically to a gallotannin or an ellagitannin unit, tannins are called complex tannins.[11] CTs (proanthocyanidins) have a variety of chemical structures affecting their physical and biological properties[16] and consist of flavanoid units (flavan-3-ol) linked by carbon-carbon bonds.

Terpenes: These are the polymeric isoprene derivatives whose synthesis take place by mevalonic acid pathway from acetate as the starting unit. The classification of various terpenes is based on the number of isoprene units (C_5) they contain. Terpenes are divided into monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes(C_{25}), triterpenes (C_{30}), tetraterpenes (C_{40}) and polyterpenes ($C_{>40}$).[10] When terpenes contain some extra elements usually oxygen they are called terpenoids. Some of the examples of different classes of terpenes have been given in Figure 3.

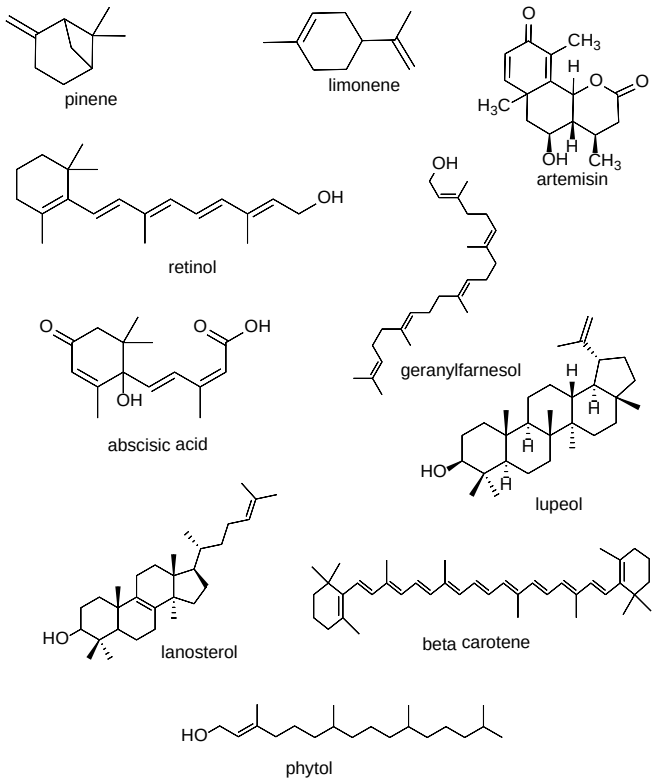

Figure 3. Examples of terpenes - pinene & limonene (monoterpenes), abscisic acid & artemisin (sesquiterpenes), retinol & phytol (diterpenes), geranylfarnesol (sesterterpene), lanosterol & lupeol (triterpenes) and beta carotene (tetraterpene).

Alkaloids: These are the nitrogen containing secondary metabolites of basic nature. Besides carbon, hydrogen and nitrogen some of the alkaloids may also contain oxygen, sulfur and rarely some other elements like chlorine, bromine and phosphorus.[19] There is no clear-cut distinction between alkaloids and other nitrogen containing natural substances such as amino acids, nucleotides and amines.[20] In contrast to other classes of secondary metabolites, alkaloids are structurally more diversified and therefore, there is no any uniform classification for these.[21] The alkaloids depending on the precursors and the final structure have been divided into three major classes, true alkaloids, pseudoalkaloids and protoalkaloids.[22] The true alkaloids are derived from amino acids and contain nitrogen in a heterocyclic ring, e.g. nicotine and atropine. The pseudoalkaloids are not synthesized from amino acids, e.g. caffeine and solanidine. The protoalkaloids are derived from amino acids but the nitrogen is not present in a heterocyclic ring, e.g. the phenylethylamine-derived alkaloids such as

mescaline (Figure 4). The various classes of alkaloids as per the type of heterocyclic ring (Figure 5) they contain are – (1) Pyrrolidine alkaloids, e.g. hygrine; (2) Pyridine alkaloids, e.g. piperine; (3) Pyridine-piperidine alkaloids, e.g. nabasine; (4) Pyrrolidine-pyridine alkaloids, e.g. nicotine; (5) Quinoline alkaloids, e.g. quinine; (6) Isoquinoline alkaloids, e.g. Opium alkaloids such as papaverine, morphine, codeine, and heroine; (7) Reduced isoquinoline alkaloids, e.g. baldine; (8) Quinolizidine alkaloids, e.g. spartine and lupanine; and (9) Indole alkaloids, e.g. aspidospermine and vinblasine.[11,23]

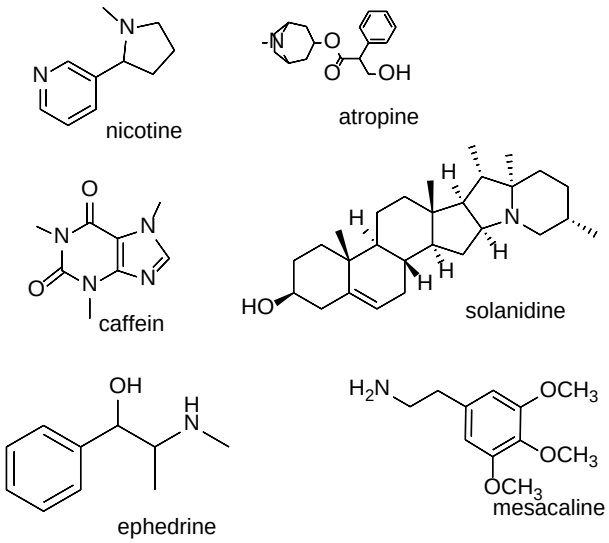

Figure 4. Examples of various classes of alkaloids - nicotine & atropine (true alkaloid), caffein & solanidine (pseudoalkaloid) and ephedrine & mesacaline(protoalkaloid).

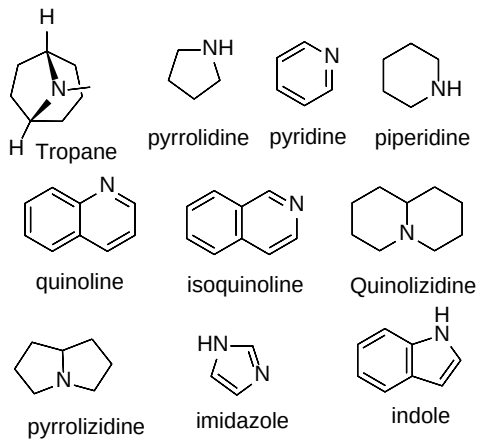

Figure 5. Various types of heterocyclic rings present in different alkaloids.

Antimicrobial Polypeptides: Plant antimicrobial peptides (AMPs) are a group of small proteins produced by different plants as a part of their defense mechanism. Majority of AMPs are 10-60 amino acids containing polypeptides whose molecular weight range from 2 to13 kDa. These are often positively charged proteins with helical structure.[24] Biological properties of AMPs have been reported against a variety of microorganisms but most of the studies have been conducted against different bacterial and fungal species.[24,25-27] Based on chemical structure different AMPs have been classified[24] (Table 2) into following families:

1. Thionins: these are antimicrobial peptides of molecular size around 5 kDa which, remain positively charged at pH 7. The tertiary structure of these AMPs contain two antiparallel α-helices and an antiparallel β-sheet with three or four conserved disulfide linkages. Thionins have further been subdivided into five types, a) type I thionins (purothionins) – polypeptide contains 45 amino acids of which eight are cysteine that form four disulfide bridges; these are highly basic in nature, b) type II thionins – the number of amino acids varies from 46 to 47; contain four disulfide bridges and, are less basic in nature in comparison to type I, c) type III thionins – polypeptide contains 45 to 46 amino acids and three disulfide bonds; as basic as type II; examples, viscotoxins and ligatoxins A, d) type IV thionins (crambins) – polypeptide comprises of 46 amino acids and three disulfide bonds and carry zero charge at pH 7 and e) type V thionins – these are the truncated forms of thionins.
2. Defensins: these were initially named as γ-thionins, and are cysteine-rich polypeptides containing 45 to 54 amino acids and molecular weight of 5 kDa. These are basic in nature and their tertiary structure comprise of a triple-stranded β-sheet arranged in parallel to an α-helix that is stabilized by four disulfide bridges, e.g. γ- hordothionins.
3. Lipid transfer proteins: they share a common structural architecture where a hydrophobic cavity is formed by four α-helices held by four disulfide bonds.[28]
4. Puroindolines: are tryptophan-rich basic proteins of size 13 kDa. The chemical structure is comprised of four α-helices separated by loops of variable length and held together by five disulfide bridges.[29]
5. Snakins: these are basic AMPs of 6.9 kDa size. The polypeptide contains 63 amino acids of which, 12 are cysteine residues having conserved positions and form six disulfide bonds.[30,31]
6. Cyclotides: these are plant cyclotides containing 28-37 amino acids and three disulfide bonds. There are six backbone loops with different degrees of sequence diversity present between the conserved cysteine residues.[32] On the basis of structural similarity there are two subfamilies, Mobius (containing cis-proline) and bracelet (lacking cis-proline).[33] Kalata B8, a hybrid between Mobius and bracelet has also been reported.[34]
7. Hevein-like proteins: these are small, cysteine-rich chitin binding peptides of size, 4.7kDa, which contain 43 amino acid residues. The number of disulfide bonds present in these varies however, four disulfide bonds formed by eight cysteine residues are most common.[35]

Besides these different forms, some other AMPs such as knottin-type peptides, Ib-AMPs and 2S albumin proteins have also been reported.[24,36,37]

Table 2. Major families of plant antimicrobial peptides.

Family	Subfamily	Example	Ref.
Thionins	Type I	Wα1, Wα2, Bα, Bβ	38
	Type II	BLa, BLb, BLc	38
	Type III	Viscotoxin A1, ligatoxin A	39, 40
	Type IV	Crambin	41
	Type V	Hellothionin D	42
Defensins		γ- hordothionins, PhD1, VrD1	43
Lipid transfer Proteins		Ace-AMP1, Cw 18	44, 45
Puroindolins		PINA, PINB	46
Snakins		StSN1, StSN2	30
Cyclotides	Mobius	Kalata B1	47
	Bracelet	CycloviolacinO2	47
Hevin-like proteins		WAMP-1a, WAMP-1b	48

MODE OF ACTION

Because of huge chemical diversity present in phytochemicals the action mechanisms of all these compounds are not fully known. On the basis of various studies conducted it seems that different phytochemicals target various levels of organization ranging from molecular level to organism level[49,50] and community level, as well in certain cases such as biofilms.[51] The diversity of action mechanisms exhibited by phytochemicals appears to be very promising in tackling the problem of antibiotic resistance often observed in pathogens causing infectious diseases.

At molecular level, various antimicrobial-phytochemicals react with different biomolecules present at site of action and consequently modify them chemically and physically to the extent that they lose their bio functionality either partially or completely. During such reactions phytochemicals or their bioactive products bind to various biomolecules like protein and nucleic acid by covalent or noncovalent bonds. Several of these active compounds contain some very reactive groups such as aldehyde and SH groups, epoxides, double bonds with enon configuration, and triple bond in their structure which can form covalent bonds with proteins and sometime DNA of microorganisms.[51,52] For example, under physiological conditions aldehyde functional group of these compounds can establish a Schiff's base with amino/imino groups occurring in amino acid residues and nucleotide bases of proteins and DNA, respectively. Exocyclic methylene groups occurring in phenylpropanoids, allicin and sesquiterpene lactone can bind to SH groups in proteins and glutathione.[49] Proteins are the functional units of the cell and play important cellular roles such as enzymes, receptors, transcription factors, ion channels, transporters, or cytoskeleton proteins. Therefore, any alteration in the structure of cellular proteins by any of these antimicrobial plant compounds will definitely have an adverse impact on the survival of the organism in which changes have taken place. Similar to covalent interaction, noncovalent interactions may also alter the protein structure, for example, phenolic compounds that contain one to many hydroxyl groups can form hydrogen bonds with electronegative atoms in proteins. Further, phenolics can partially dissociate to negatively charged phenolate ions under normal physiological conditions and form ionic bonds with positively charged amino acid residues of proteins, and affect the structural and functional properties of the proteins.[54] Some of the secondary metabolites such as pyrrolizidine alkaloids in Boraginaceae and Asteraceae, aristolochic acid in *Aristolochia*, furanocoumarins in Apiaceae can intercalate or alkylate DNA and hence, may have mutagenic as well as carcinogenic properties.[49,55]

Reactive oxygen species (ROS) are a group of highly reactive and unstable chemical forms of oxygen that react with a range of biomolecules and if not curbed at early stage of synthesis may cause several disorders and induction of apoptosis as well. A number of phytochemicals such as phenolic compounds on one hand have the capability to reduce the production of ROS through their higher antioxidant properties, on the other hand some of phytochemicals induce the generation of ROS.[56-58] Exposure of *Candida albicans* to berberine (Figure 6) increases the intracellular level of ROS in the fungus.[59] ROS appear to play a very important role in induction of programmed cell death. During which O_2^- generated in mitochondria via aerobic respiration is converted to H_2O_2 by superoxide dismutase[60] which in turn reacts with ferrous ions and gives rise to very reactive OH-radicals. OH-radicals react indiscriminately with various macromolecules like unsaturated fatty acids, proteins, and DNA and as a consequence cause induction of apoptosis.[61] Several apoptosis–inducing phytochemicals have been reported to produce a significant amount of OH-radicals during apoptosis.[62-65]

At cellular or subcellular level, phytochemicals have been reported to exert antimicrobial activities against various pathogens by affecting different cell components.

Polysaccharide rich capsule is an important structure associated with pathogenicity in many bacteria such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Bacillus anthracis*.[66,67] Further, it protects the bacteria from phagocytosis and enhances bacterial adhesion and biofilm formation.[56] Salicylic acid (Figure 1) and its derivatives have been reported to be effective in reducing capsule production.[69,70]

Cell wall is an outermost dynamic structure in some of the microorganisms like bacteria and fungi that protects the organism from external osmotic shocks, and is also responsible for distinct morphologies of different organisms. Any change induced by an antimicrobial causing the organizational or functional disruption of the cell wall would result in the death of pathogen. The mechanism of cell wall disintegration is well understood in case of antibiotics of microbial origin like

penicillin that inhibit cell well synthesis. The phytochemicals like eugenol (Figure 6) have also been reported to affect cell wall integrity which is followed by cell wall disruption and cell lysis.[71] Eugenol, an active component of clove oil, is a phenylpropanoid which exhibits antimicrobial properties in addition to various pharmacological activities. All these biological properties of eugenol have been attributed to the presence of phenolic groups.[71] The studies conducted on *C. albicans* related to effect of polyphenols such as catechins and flavins on ultrastructural changes have shown the features of cellular degradation like extravasation of cellular content and shriveled and deflated cells. Some yeast cells also showed mulberry-like surface features indicating probably a transitional phase between normal state and total collapse of cell wall due to the effect of polyphenols.[72]

Cell membrane is an important cell component which is composed of phospholipid bilayer in which extrinsic and intrinsic proteins that play roles of enzymes, signal proteins and transport proteins, are found embedded. Various phytochemicals may cause membrane disruption due to their either lipophilic nature or binding to some specific membrane component resulting in the loss of membrane integrity and functionality. Borges et al. (2013)[73] studied the mechanism of action of phenolics, gallic acid and ferulic acid against various bacterial species and showed that both chemicals led to irreversible changes in membrane properties in terms of charge, intra and extracellular permeability and physicochemical properties causing pore formation in cell membranes and ultimately leakage of intracellular constituents. Similar findings have also been reported about phytochemicals, protocatechuic acid, chlorogenic acid and shikimic acid which exhibit antibacterial, antifungal and antiviral activities.[74-77] Antimicrobial activities of essential oils have been suggested because of the presence of their hydrophobic-components like thymol, carvacrol, eugenol and isoeuginol (Figure 6) as they interact with phospholipids of bio membrane and increase its permeability that leads to cell lysis.[78,79] Hyperforin, a phloroglucinol derivative obtained from *Hypericum perforatum* activates TRPC6, a Ca^{2+} - conducting channel in plasma membrane that changes the membrane fluidity.[80]

Mitochondria are the basic energy generating units of a eukaryotic cell, therefore any condition leading to malfunctioning of mitochondria would result into collapse of whole cellular system. The mitochondrial malfunctioning can be assessed in terms of either depolarization of the mitochondrial membrane potential or the release of cytochrome C, an essential component of electron transport chain in mitochondrial inner membrane, from mitochondria to cytosol.[50] Both, along with stimulation of ROS production are considered to be the characteristics of an early stage of apoptosis.[81-83] A phytol-rich hexane fraction from the leaves of *Lacistema pubesscens* was effective against *Leishmania amazonensis* as it caused depolarization of mitochondrial membrane potential followed by the increase of ROS levels in the organism.[84] Similarly, ascardole, a major component of essential oil of *Chenopodium ambrosioides* exhibiting activity against *Leishmania* also causes breakdown of mitochondrial potential.[85]

Biofilms are the surface associated microbial communities enclosed in a self-generated exopolysaccharide matrix.[86] These are known to possess certain unique developmental characteristics over free-floating planktonic cells.[87] Trans-cinnamaldehyde inhibits biofilm formation by *Cronobactor sakazakii* and *E. coli*,[88,89] and chlorogenic acid by *Stenotrophomonas maltophila*.[90] Biofilms of *C. albicans* are reported to be inhibited by carvacrol.[91] Polymicrobial biofilms are inhibited by condensed tannin extracted from astringent persimmon and effect have been reported to be dose-dependent.[92]

Although the mechanism of action for the antiviral efficacy of the natural components is not fully understood, but studies conducted[93,94] so far indicate that phytochemicals prevent the viral attachment to host cells by causing blockage/damage either on the viral capsids or the receptors on the cell membranes. For example, the saponins present in *Quillaja* extracts change the cell membrane receptors which block the rotavirus (RoV) infection by inhibiting virus-host attachment[95] however, polysaccharides occurring in *Stevia rebaudiana* bind to VP7, a glycoprotein of capsid of RoV and blocks its binding to cell membrane receptors by steric hindrance, which results in the blockade of the virus attachment to cells.[96] The inhibition of HIV-1 infection by epigallocatechin gallate takes place due to the binding of catechin (Figure 6) with the CD4 receptor on the cell surface.[97,98]

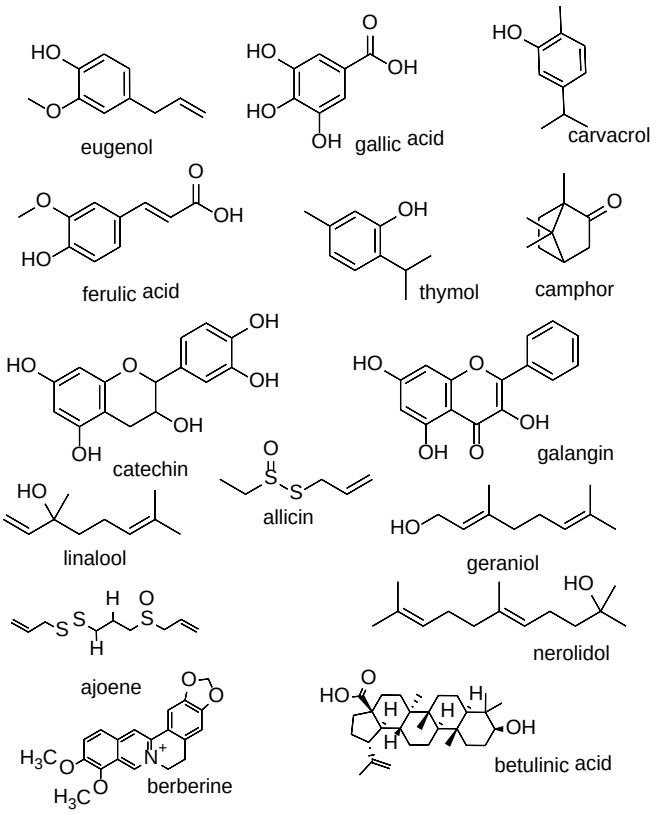

Figure 6. Chemical structures of some of the phytochemicals possessing antimicrobial properties.

ANTIMICROBIAL ACTIVITIES OF PHYTOCHEMICALS

Some of the important categories of antimicrobial activities where plants and their isolated compounds especially secondary metabolites have been found effective are following.

Antiviral activity

Most of the plant antimicrobials isolated either as pure compounds or as crude extracts, have been screened against bacteria, yeast and molds[99] and reports of the antiviral effects of natural biochemical substances are rather limited.[100] The research on the antiviral effect of the natural bioactive substances is still at a preliminary stage. Research strategies should be improved in the future considering first of all, standards should be established for sample preparation, toxicity test or antiviral effect evaluation. Secondly, most of the antiviral studies performed in tissue culture medium or sterile buffer solutions cannot simulate the physiological conditions as bio-components such as proteins and fat may have a protective effect on the viral particles from various physical and chemical inactivation.[101,102] Lastly, the mechanism of action for the antiviral efficacy of the natural components is not fully understood therefore time of addition experiments and in-depth exploration on the molecular mechanism for the antiviral effect would be of great help in the safety evaluation and practical application in the long run.[103] Over all, the exploration and use of natural antiviral compounds with low toxicity and economical cost are well noteworthy due to the low infection dose, the high genomic variability and the broad range of reservoir of viruses

Amongst various categories of viruses, non-enveloped viruses are generally less sensitive to the tested biochemical compounds as compared to the enveloped viruses such as human immunodeficiency virus (HIV), herpes simplex virus (HSV).[103,104] Cranberries (*Vaccinium macrocarpon*) are known to have several bioactive phytochemicals such as flavonoids, phenolic acid derivatives, hydroxycinnamic acid derivatives, organic acids, and isoprenoids (including ursolic acid and lutein).[105] Cranberries also have high content of oligomeric and polymeric pigments, proanthocyanidins (CTs) consisting primarily of epicatechin tetramers and pentamers with at least one A-type linkage.[106,107] The virucidal effect of cranberry juice has been reported against bacteriophages (T4, MS2 and φX-174), rotavirus (RoV), murine norovirus (MNV-1) and feline calicivirus (FCV).[108, 109] Cranberry proanthocyanidins inhibit the absorption of bacteriophage T4 to its bacterial host cells and replication of RoV in its host cells, and induce structural changes in viral capsid of FCV.[108,110] Similarly, antiviral activity of proanthocyanidin extracted from grape seeds was seen against FCV and coxsachievirus (Cox) A7 strain by Iwasawa et al. (2009).[106] Kwon et al. (2010)[111] evaluated the ability of six polyphenols isolated from the roots of *Glycyrrhiza uralensis* to inactivate RoVs, and different polyphenols were found to inhibit either virus binding to host cell or viral replication. Antiviral effect of flavonoids, myricetin, L-epicatechin, tangeretin, and naringenin was studied against human norovirus (HNoV) surrogates namely, FCV-F9 and MNV-1. The tested concentrations of flavonoids were found effective against FCV-F9 but ineffective against MNV-1 suggesting that the antiviral effects of the tested flavonoids were dependent on the flavonoid concentration, virus type, and virus titer.[112] The infectivity of FCV and MNV-1 is also reduced by the essential oils of clove, oregano and zataria.[113] Ueda et al. (2013)[114] investigated the antiviral effects of tannins obtained from persimmon (condensed tannin), green tea (hydrolysable tannin), acacia (condensed tannin), coffee (caffenol, a pseudo tannin) and gallnuts (pentagalloyl glucose, a hydrolysable tannin) on 12 different viruses including both enveloped viruses namely, influenza viruses H3N2 and H5N3, HSV-1, vesicular stomatitis virus, sendai virus and newcastle disease virus and non-enveloped viruses namely, poliovirus, Cox, adenovirus, RoV, FCV and MNV. Extracts from persimmon (*Diospyros kaki*) showed potent antiviral effects against all of the viruses tested and hence found effective against a broad range of viruses. Other tannins derived from green tea, acacia and gallnuts were effective against some of the viruses, while the coffee extracts were not found effective against any of the virus tested. They also studied the mechanism of the antiviral effects of persimmon extracts that inhibited attachment of the virus to cells, and protein aggregation seems to be a fundamental mechanism underlying the antiviral effect of persimmon tannin as viral proteins formed aggregates when purified virions were treated with the persimmon extracts, and the antiviral effect was competitively inhibited by a non-specific protein, bovine serum albumin.[114] Catechins, tannins from green tea or black tea, were reported to inhibit pathogenic viruses such as influenza virus, HSV- 1 and HIV.[115-120] Abd-Kadir et al. (2013)[121] reviewed potential anti-dengue activities from plants distributed around the world and on the basis of sixty-nine studies conducted between periods, 1997 to 2012 described 31 different plants species belonging to 24 families to possess anti-dengue properties and anti-dengue phytochemicals have been described in another study.[122]

Antibacterial activity

The antibacterial effect of plant extracts and phytochemicals has been studied by a large number of researchers and a lot of literature is available especially related to screening of different plant extracts for antimicrobial activities that has also been reviewed.[4,9,123-129] However, in contrast to preliminary investigations related to report of antimicrobial properties of plants, antimicrobial activities of specific phytochemicals are relatively few.

Kazmi et al. (1994)[130] described an anthraquinone from *Cassia italica*, to be bacteriostatic for *Bacillus anthracis*, *Orynebacterium pseudodiphthericum* and *Pseudomonas aeruginosa*, and bactericidal for *Pseudomonas pseudomalliae*. The exracts of *Zingiber zerumbet* rich in flavonoids - catechin, quercetin, rutin, leteolin, myricetin and kaempferol and phenolic acids - gallic acid, ferulic acid, caffeic acid and cinnamic acid were investigated for their antibacterial activities against Gram positive and negative bacteria (Figures 2, 6); amongst test strains *Staphylococcus aureus* was found to be most sensitive.[131,132] Antibacterial properties of catechins have also

been reported against bacteria, *Vibrio cholera*,[133] *Streptococcus mutans*,[134] *Shigella*,[135] and other bacteria and microorganisms. Chemical composition and antibacterial activity of aqueous (ethanolic and methanolic) extracts from herbs often used in Polish cuisine and traditional herbal medicine including thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), peppermint (*Mentha piperita*) and sage (*Salvia officinalis*) were compared by Kozłowska et al. (2015).[136] Different species of bacteria exhibited different sensitivities to different plant, *S. aureus* strains were found to be the most sensitive bacteria to aqueous (ethanolic and methanolic) rosemary and sage extracts and aqueous methanolic thyme extract. *Klebsiella pneumoniae* ATCC 13883 and *Proteus vulgaris* NCTC 4635 were more susceptible to the aqueous methanolic thyme extract. However, *Listeria monocytogenes* 1043S was the most sensitive to the aqueous ethanolic rosemary extract. Gram-positive bacteria in general were more sensitive to the tested extracts than Gram-negative ones.[136, 137] Rosmarinic acid and caffeic acid were present in all the plants; other important compounds identified in extracts from thyme, oregano and rosemary were thymol, carvacrol, camphor, borneol and α-terpineol. Terpene compounds such as eugenol, geraniol, thymol and carvacrol derived from essential oils have also been found inhibitory to methicillin-resistant *Staphylococcus aureus* (MRSA).[138] Freires et al. (2015)[51] reviewed the literature related to antibacterial activities of essential oils and their isolated constituents against cariogenic bacteria and reported plants, *Achillea ligustica*, *Baccharis dracunculifolia*, *Croton cajucara*, *Cryptomeria japonica*, *Coriandrum sativum*, *Eugenia caryophyllata*, *Lippia sidoides*, *Ocimum americanum*, and *Rosmarinus officinalis* to be most promising against such bacteria. Further, among isolated constituents, menthol and eugenol were considered outstanding compounds demonstrating an antibacterial potential against streptococci and lactobacilli. Other promising compounds in essential oils with antibacterial activities were 1, 8 cineole, camphor, caryophyllene oxide, linalool, pulegone, sabinene, terpinen-4-ol, verbenone, viridiflorol, α-pinene, α-terpeneol, β-caryophyllene, β-myrcene, β-pinene and γ-terpinene. However, on the basis of literature survey they concluded that most of the knowledge was based on *in vitro* studies and only a limited number of clinical trial based reports were available. Hence, suggesting the need of more stringent measures in order to make knowledge further useful. In another study, the volatile oils of black pepper (*Piper nigrum*), clove (*Syzygium aromaticum*), geranium (*Pelargonium graveolens*), nutmeg (*Myristica fragrans*), oregano (*Origanum vulgare* ssp. *hirtum*) and thyme (*Thymus vulgaris*) were assessed for antibacterial activity against 25 different genera of bacteria.[139] The volatile oils exhibited considerable inhibitory effects against all the tested organisms while their major components demonstrated various degrees of growth inhibition.The thymol exhibited the widest spectrum of antibacterial activity followed by carvacrol, α-terpineol, terpinen-4-ol, eugenol, (±)-linalool, (–)-thujone, δ-3-carene, *cis*-hex-3-an-1-ol, geranyl acetate, (*cis* + *trans*) citral, nerol, geraniol, menthone, β-pinene, *R*(+)-limonene, α-pinene, α-terpinene, borneol,(+)-sabinene, γ-terpinene, citronellal-terpinolene, 1,8-cineole, bornyl acetate, carvacrol methyl ether, myrcene, β-caryophyllene, α-bisabolol, α-phellandrene, α-humulene, bocimene, aromadendrene, *p*-cymene, in decreasing order.[139] The antibacterial properties of saponins,[140] alkaloids[23] and polypeptides[24] have also been published.

Antifungal activity

The fungal infections are relatively difficult to cure in contrast to bacterial infections as antibiotics available to curtail such infections are limited. Moreover, the pace of development of new antifungal drugs has also been slow. Nosocomial infections caused by fungi have increased greatly in recent years, mainly due to the rising number of immunocompromised patients. Therefore, the search for alternative drugs with low resistance rates and fewer side effects remains a major challenge. Plants produce a variety of medicinal components that can inhibit growth of pathogens including pathogenic fungi. A considerable number of studies of medicinal plants and alternative compounds, such as secondary metabolites, phenolic compounds, essential oils and extracts, have been performed considering various parameters such as sustainability, affordability, and antimicrobial property.[141]

Author, in one of the investigations screened aqueous and organic solution (OS) extracts of 50 plants belonging to 27 families of seed plants for antifungal activities against *Aspergillus flavus* and *A. niger* using agar-well diffusion method. OS extracts of 23 plants showed antifungal activities, of those *Trachyspermum ammi*, *Allium sativum*, *Syzygium aromaticum* and *Plectranthus rugosus* were found most effective.[142] Further, chloroform extracts of *T. ammi*, *S. aromaticum* and *Zingiber officinale* were also found to be antiaflatoxigenic in property.[143] Polyphenoles are known to possess antifungal activities along with other antimicrobial activities.[144] Among tea catechins, epigallocatechin gallate exhibited variable time-dependent and concentration-dependent fungicidal activities against several fungi, including *C. albicans*, suggesting that flavan-3-ols may be useful in the treatment of severe infections of the oral cavities, intestine, and vagina caused by *C. albicans*, which may result from an excessive use of antibiotics.[145] Investigations also pointed out the fungicidal activity of propolis flavonols such as galangin, izalpinin, and rhamoncitrin against *Microsporum gypseum*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*.[146] Ellagitannins isolated from *Ocotea odorifera*, a medicinal plant commonly used in Brazil, have potent activity against *Candida parapsilosis* at a concentration level of 1.6 mM.[147] Antimicrobial activities of flavonoids (5,7-dimethoxyflavanone-4′-*O*-β-D-glucopyranoside, 5,7-dimethoxyflavanone-4′-*O*-[2″-*O*-(5‴-*O*-trans-cinnamoyl)-β-D-apiofuranosyl]-β-D-lucopyranoside, 5,7,3′-trihydroxy-flavanone-4′-*O*-β-D-glucopyranoside, naringenin-7-*O*-β-D-glucopyranoside, rutin, and nicotiflorin) isolated from three medicinal plants namely, *Galium fissurense*, *Viscum album* ssp. *album*, and *Cirsium hypoleucum* were found effective against *C. albicans* and *C. krusei*.[148] *Chloranthus*, a genus of the family Chloranthaceae,

has been reported to possess antitumor, antifungal, and anti-inflammatory activities. The various species of this genus contain 82, sesquiterpenoids; 50, dimeric sesquiterpenoids; 15, diterpenoids; one coumarin, and five other secondary metabolites.[149] Among them, dimeric sesquiterpenoids (chloramultilide B and CHE-23C) and eudesmane sesquiterpene (CJ-01) have attracted considerable attention due to their antifungal activities against various human and phytopathogenic fungi.[150-152] Gozubuyuk et al. (2014)[153] investigated antifungal activity of *Lawsonia inermis* (henna) against 70 clinical isolates of dermatophytes representing six different species namely, *Trichophyton rubrum*, *T. mentagrophytes*, *T. tonsurans*, *T. violaceum*, *Microsporum canis* and *Epidermophyton floccosum* by agar diffusion method and henna paste showed high antifungal activity against all tested dermatophytes. Similar results were also reported by Berenji et al. (2010).[154] Phytochemical investigations of henna have shown the presence of the β-sitosterolglucosides, flavonoids, quinoids, naphthalene derivatives, luteolin, betulin, lupeol, gallic acid, coumarins, xanthones, and phenolic glycosides.[155] Antifungal proteins and peptides have been isolated from a variety of organisms including plants.[24,156] Brassiparin, a peptide of molecular mass of 5716 Da was isolated from the seeds of *Brassica parachinensis* that potently inhibited mycelial growth in a number of fungal species including *Fusarium oxysporum*, *Helminthosporium maydis*, *Mycosphaerella arachidicola* and *Valsa mali*.[157]

Antiparasitic activities

Eukaryotic parasites, protozoans and helminths, in particular are known to cause severe infections such as malaria, leishmaniasis, trypanosomiasis, giardiasis and helminthic infections in different animals including human beings. Plants, *Allium sativum* (garlic), *Melissa officinalis* (lemon balm), *Origanum vulgare* (oregano), *Thymus vulgaris* (thyme), *Cinnamomum zeylanicum* (cinnamon), *Melaleuca alternifolia* (tea tree), *Citrus limon* (lemon) have been reported to be effective against a wide range of parasites.[158-166] For instance, garlic oil that contains allicin and its chemically stable transformed products such as diallyl trisulphide (DAT) and ajoene (Figure 6) inhibits the growth of various protozoan parasites, including *Leishmania major*, *Leptomonas colosoma*, *Crithidia fasciculate*,[158] *Cryptosporidium baileyi*,[160] *Tetratrichomonas gallinarum*, *Histomonas meleagridis*,[161] *Giardia duodenalis*,[162] *Plasmodium berghei*,[163] *Trypanosoma brucei brucei*, *Trypanosoma brucei rhodesiense*, *Trypanosoma bruceigambiense*, *Trypanosoma brucei congolense*, *Trypanosoma evansi*, *Trypanosoma equiperdum*[164] and *Trypanosoma cruzi*.[165]

Leishmania is a unicellular protozoan parasite that causes several human diseases, ranging from localized self-healing cutaneous lesions to deadly visceral infections. The effect of allicin on the growth of *L. major* promastigotes was evaluated under *in vitro* conditions and 50 μM concentration of allicin was found inhibitory to the growth of promastigotes. Moreover, topical application of allicin-cream reduced cutaneous leishmanial lesion size without any adverse side effect in mice.[168] Nerolidol (Figure 6), a sesquiterpene inhibited the growth of *Leishmania amazonensis*, *L. braziliensis*, and *L. chagasi* promastigotes and *L. amazonensis* amastigotes with *in vitro* IC_{50} of 85, 74, 75, and 67 μM, respectively. The treatment of *L. amazonensis*-infected macrophages with 100 μM nerolidol resulted in 95% reduction in infection rates. The inhibitory effect of nerolidol can be attributed to the blockage of an early step in the mevalonic acid pathway. Both, intraperitoneal and topical applications of nerolidol in *L. amazonensis*-infected BALB/c mice showed significant reduction of lesion sizes.[169] Linalool and ascardole present in the essential oils of *Croton cajucara* and *Chenopodium ambrosioides*, respectively showed inhibitory effect on various species of *Leishmania* including *L.amazonensis.*[170,85] Similarly, phytol-rich hexane fractions from the leaves of *Lacistema pubesscens* and extract of *Artemisia absinthium* were also found effective against *L. amazonensis* and *L. infantum*, respectively.[171,172, 84]

Chagas disease, an endemic disease of Latin American countries that affects 8-10 million people is caused by another protozoan parasite *Trypanosoma cruzi*.[173,174] Betulinic acid, a triterpenoid has been tested for trypanocidal activities in addition to antiplasmodial, and antileishmanial activities[175,176] (Figure 6). Meira et al. (2010)[177] investigated trypanocidal effects of betulinic acid and its semi-synthetic amide derivatives against *T. cruzi*. The betulinic acid caused membrane blebling, flagella retraction, atypical cytoplasmic and Golgi cisternae dilatation in trypanomastigotes at cellular level and ultimately led to the death of parasite due to necrosis. Ajoene and allicin have also been reported to have inhibitory effects against various species of *Trypanosoma* including *T. cruzi*.[164] The essential oils of lemon balm (balmint), peppermint, thyme and tea tree were tested against *Trypanosoma brucei*, and tea tree oil that contains an active compound, terpinen-4-ol was found most potent followed by thyme, peppermint and lemon balm.[178] The alkaloid, berberine isolated from *Mahonia aquifolia* is highly effective against trypanosomes.[4] *Artemisia absinthium* extracts have been reported to possess antiprotozoal activity against *T. brucei* and *T. cruzi*.[171,172]

Artemisin (Figure 3), a sesquiterpene from *Artemisia annua*, has been developed into a potent antimalarial drug (artesunate) that is used in combination with modern synthetic chloroquine-derived compounds against *Plasmodium falciparum*, and it is very effective in the treatment of falciparum malaria especially when the parasite is resistant to both chloroquine and sulfadoxinepyrimethamine.[179] The increasing multidrug resistance of *P. falciparum* has broadened the search for natural plant products as sources of novel drugs. Garlic compounds, ajoene and allicin have been reported to possess antimalarial properties.[163,180] A single dose of ajoene (50 mg/kg), given on the day of infection, inhibited parasitaemia in murine (*P. berghei*) malaria, with no observable toxic side effects. When given in conjunction with 4.5 mg/kg chloroquine, complete suppression of parasitaemia occurred, and further investigations indicated that ajoene potentiated the effect of chloroquine.[163] Similarly, allicin was also effective in curtailing malaria at

various stages as it prevented the infection by inhibiting a parasite-derived cysteine protease required for the processing of the circumsporozoite protein, a major surface protein of *Plasmodium* sporozoites associated with sporozoite invasion of host cells.[180] The essential oils isolated from the Cameroonian plants, *Hexalobus crispiflorus*, *Pachypodanthium confine*, *Xylopia aethiopica*, *X. phloidora* and *Antidesma laciniatum* were exhibited antiplasmodial activity against the W2 strain of *P. falciparum* activity however, the oil of *H. crispiflorus* showed maximum activity with an IC_{50} of 2 μg/ml.[181] All the tested oils contained terpenoids as one of the major components, in which compounds, α-copaene, γ-cadinene, δ-cadinene, α-cadinol, spathulenol and caryophyllene oxide were most common. The essential oil of *H. crispiflorus* also contains high content of nerolidol, which inhibits protein glucosylation by competing with the biosynthesis of isoprenoid derivatives in *P. falciparum*, *in vitro*, in addition to decreasing the ability of the intraerythrocytic parasite to synthesize coenzyme Q.[181] The oil of *Cochlospermum planchonii*, an African plant used as an antidote of malaria whose major constituents are β-caryophyllene, (E,E)–α–farnesene and tetradecan-3- one, has been reported to inhibit *P. falciparum* proliferation under both *in vivo* and *in vitro* conditions.[182,183] The crude extract of *Antrocaryon klaineanum* and the isolated ergostane steroids were evaluated for their ability to inhibit the 3D7 strain of *P. falciparum*. Compounds, 6α-methoxy-4,24(28)-ergostadiene-7α,20*S*-diol and 6α-methoxy-4,24(28)-ergostadien-7α-ol showed potent antiplasmodial activity.[184] The ethanolic extracts of neem leaves and seeds were found effective against chloroquine-sensitive as well as chloroquine-resistant strains of the malaria parasite and suppressed the growth of parasites within 72 hours.[185] A limonoid compound- gedunin, isolated from neem is said to be as effective as quinine on malaria-infected cell cultures.[186] The leaf extract of *Lawsonia innermis* has also been reported to be effective against *P. falciparum*.[187]

In Northeast India *Trifolium repens*, *Houttuynia cordata* and *Lasia spinose* are used as remedy for helminthic infection. *In vitro* anthelmintic testing of phytochemicals, biochanin A, ursolic acid, betulinic acid and beta-sitosterol isolated from these plants, was undertaken against *Hymenolepis diminuta*, a zoonotic tapeworm, and their efficacy was compared with a reference drug, praziquantel. The results revealed that all the tested compounds except beta-sitosterol, possessed high anthelmintic activity. Amongst the tested phytochemicals, betulinic acid (1 mg/ml) showed the best anthelmintic effect.[188] Similarly, tobacco has also been reported to exhibit anthelmintic properties. Anthelmintic effect of aqueous and alcoholic extracts of tobacco in reference to drug, levamisole was studied against parasitic nematode, *Marshallagia marshalli*. Both the extracts of tobacco possessed considerable anthelminthic activity however, highest concentration (75 mg/ml) was more effective.[189] The oil from the seeds of *Nigella sativa* (black cumin), when given to mice infected by *Schistosoma mansoni*, reduced parasite egg burden by augmenting the host protective immune response and reduced parasite-induced hepatic damage.[190] Essential oils of *Ocimum gratissimum* and *O. sanctum* also exhibit anthelmintic activities.[191,192] Anthelmintic activity of *Trachyspermum ammi* (ajwain) was studied against *Haemonchus contortus* in sheep and *Ascaris lumbricoides* in humans, and positive results were observed. The antiparasitic property of ajwain was due to the increased ATPase activity that caused depletion of energy reserves of parasites. Ajwain has also been reported to exhibit cholinergic activity with peristaltic movements of gut that may help in expulsion of intestinal parasites and could be a contributory factor to its anthelmintic activity.[193,194] Ajwain was also evaluated for its nematicidal activity against pinewood nematode, *Bursaphelenchus xylophilus*.[195] Nematicidal activity of ajwain was mainly attributed to the thymol and carvacrol.[196]

CONCLUSIONS

Plants produce a multitude of antimicrobial secondary metabolites and majority of these are phenolic and terpenoid compounds. Although the modes of action in most of the cases of secondary metabolites possessing antimicrobial properties are still not completely known but appear to be multi-target oriented. This suggests their potential application in handling the infections caused by multidrug-resistant pathogens whose frequent incidents have alarmed the entire therapeutic industry. Herbal therapy which, is still used in many countries world over, if supported by a scientific base could be a cost-effective and low-risk alternative to synthetic drugs which often show a wide range of severe side effects. However, in order to establish phytochemicals as safe compounds/drugs a long distance has to be covered as bulks of the reports are *in vitro* based and other essential studies such as toxicity tests and clinical trials involving animal system and humans are lacking.

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