# Antimicrobial agents from terrestrial Plants

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The ever growing problem of resistance to antibiotics is one of the most serious threats to global public health because antibiotic resistance has been reported against all classes of antibiotics prevalent in clinical practices. To overcome drug resistance, the present scenario warrants an extensive search for new drugs with elucidation of their structure and mechanism of action. Natural plant products have served as an important and effective alternative source of antimicrobial products. A majority of these antimicrobial compounds are secondary metabolites and are produced as a result of reciprocal interactions between plants, microbes and animals. Plant derived antimicrobials generally involve flavonoids, quinones, tannins, coumarins, terpenoids, lectin and polypeptides. The biggest challenge in the present scenario is the characterization of their mode of action which is essential to support their use as lead molecule for drug-development programs. In this chapter, we will review recent works on naturally isolated antimicrobial terrestrial plant products with main focus centered on their function and mode of action.

**Key words:** Antibiotic resistance, antimicrobial agents, plant metabolites

## 1. Ethanomedicinal Perspective of Plants:

Ancient civilizations all across the globe had the empirical knowledge of medicinal applications of plant based drugs. The first written records on medicinal plants date back to 2600 BC with the existence of a highly advanced medicinal system in Mesopotamia comprising of about 1000 plant based drugs [1]. The discovery of penicillin in 1928 led to an era which witnessed a decline in the therapeutic use of extracts and partly purified plant products as the pharmaceutical industry shifted its focus towards synthetic compounds libraries and high throughput screening [HTS] in search of new drugs [2]. In last few decades, combinatorial chemistry and HTS has been primarily used to screen out new drugs. However, the output has not been satisfactory which is quite evident by the decline in the number of new drugs reaching the market [3]. This has rekindled the interest in plant based drug discovery, despite complexity associated with it as it requires broad interdisciplinary approach [4]. One notable feature in drug discovery from plants is that often traditional knowledge of the compound help to understand its therapeutic potential [5]. This idea is further strengthen by the fact that in an analysis of 122 plant derived compounds identified as drugs across the globe, 80% of them originated from plants having well documented ethanomedicinal use [6]. Furthermore, the natural products used for development of drugs have a very high chance of being used traditionally even if it was not known at the time of drug discovery [4].These multiple advantages have ensured that although HTS approaches with synthetic compound libraries still remains the primary focus of pharmaceutical industry, natural compounds are still a valuable source of drug discovery [7]. The rejuvenation of plant based drug discovery is in sync with the rapid advancement in the field of science and technology. The better understanding of diseases and the associated mechanisms, newer and robust screening methods, advanced analytical methods and better optimization of newly synthesized drugs has further stimulated research in this regard [8].

## 2. Major Phytochemical groups exhibiting antimicrobial properties and their mode of action:

Bacterial resistance to antibiotics is increasing rapidly and is one of the most serious challenges to global public health, as drug resistance has been reported for all clinically significant classes of antibiotics [9]. Moreover, not many new antibiotics have been discovered in last few decades further compounding the problem. In general, natural plant products have emerged as important alternative sources of new antimicrobial compounds. The antimicrobial activity of many plant derived products has been reported [7] and different classes of compounds have been identified [10]. Most antimicrobial agents derived from plants are secondary metabolites. Secondary metabolites and other phytochemicals can be classified according to their chemical structure and the major groups are phenolic and polyphenols, alkaloids, terpenoids, lectins and antimicrobial peptides (AMPs) [11]. Attributes like significant antimicrobial effect, no visible side-effects and availability of these compounds has contributed to their growing use as effective antimicrobials and disinfectants in the food industry, components of herbal medicine and source of development of novel antibiotics in pharmaceutics. Some of the antimicrobial phytochemicals are listed in Table 1.

**Table 1**: Some terrestrial plants, their antimicrobial compounds and susceptible organisms

| **Plant source** | **Antimicrobial Compound/s** | **Susceptible organism/s** |
|---|---|---|
| *Abrus schimperi* | Quinones | *Leishmania donovani* |
| *Allium sepa* | Flavonoids, polyphenols | MDR *P.aeruginosa*, *S.typhi*, *E.coli* |
| *Aloysia triphylla* | Terpenoids | *M. tuberculosis*, *S. aureus* |
| *Angelica lucida* | Coumarins | *S. viridians*, *S. mutans* |
| *Artemisia dracunculus* | Terpenoids and Polyphenols | Viruses |
| *Cassia angustifolia* | Anthraquinones | *S. aureus* |
| *Centella asiatica* | Terpenoids | *M. leprae* |
| *Cinnamomum* spp. | Cinnamaldehyde | MDR *E. coli*, *C. albicans* |
| *Cirsium hypoleucum* | Flavones | MDR *K. pneumoniae* |
| *Hypericum perforatum* | Hypericin | MRSA |
| *Lawsonia inermis* | Quinones | MDR *P. aeruginosa* |
| *Mahonia aquifolia* | Alkaloids | *Plasmodium* |
| *Medicago sativa* | Saponins, Canavanine | *Enterococcus faecium*, *S. aureus* |
| *Mentha longifoilia* | Essential oil | MDR *S. aureus* |
| *Ocimum basilicum* | Terpenoids | *Salmonella* and other bacteria |
| *Onobrychis sativa* | AMPs | *E. faecium*, *S. aureus* |
| *Piper longum* | Piperine, Saponin, alkaloids | MDR *B. subtilis*, *Shigella sonnei* |
| *Raphanus sativum* | RsAFP2 (Antifungal peptide) | *C. albicans* |
| *Ranunculus scleratus* | Flavonoids | *Salmonella*, *Agrobacterium* |
| *Rhazy astricta* | Alkaloids and Non alkaloids | MDR *E. coli* |
| *Santolinachamae cyparissus* | Essential oils | *Candida*, *Schistosoma* |
| *Sorghum* spp. | Tannins | *S.aureus*, *S. typhimurium* |

2.1 Quinones:

Quinones are aromatic compounds with two ketone substitutions. Quinones with antibacterial activity are anthraquinone, beznoquinone, napthaloquinone, plastoquinone, and pyrrloquinoline. Two quinones namely hypocrellins A and B have been evaluated for *in vitro* antimicrobial and antileishmanial activities. Hypocrellin A exhibits good activity against *Candida albicans* and less activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Pseudomonas aeruginosa* and *Mycobacterium intracellulare*. Hypocrellin A also has potent antileishmanial activity. Hypocrellin B has weak antimicrobial as well as antileishmanial activity [12].

Antimicrobial photodynamic therapy is a therapeutic option that combines a non-toxic photosensitizer with harmless visible light to inhibit microbes. In another study antimicrobial activity of hypocrellin A has been examined against Gram-positive (*S. aureus*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Salmonella typhimurium*). The results vindicates that HA has a photodynamic antimicrobial activity against both Gram-positive and Gram-negative bacteria [13]. Anthraquinone have shown antimicrobial potential against *Bacillus anthracis*, *Corynebacterium pseudodiphthericum* and *Pseudomonas aeruginosa* [14]. The prominent targets of quinones in a microbial cell are adhesion proteins present on the cell surface, polypeptides present in the cell wall and membrane-bound enzymes [15].

2.2 Coumarins:

Coumarins are phenolic substances found in plants, bacteria and fungi. They consist of fused benzene and α-pyrone rings. Coumarins possess many pharmacological activities such as anti-inflammatory, anticoagulant, antibacterial, antiviral, antihypersensitive, antioxidant, antihyperglycemic and antiadipogenic [16]. Coumarins and its derivatives exhibit good antifungal activity as comparable to fluconazole [17]. *In vitro* antimicrobial activity of a synthetic coumarin has been screened against *S. aureus*, *Bacillus megaterium*, *E. coli* and *Proteus vulgaris* and efficacy has been found to be more in comparison to standard drugs like ampicillin, amoxicillin, ciprofloxacin and erythromycin [18]. Novel 8-ethoxycoumarins has been screened for antimicrobial activity against a variety of organisms such as *E. coli*, *S. aureus*, *Candida albicans* and *Saccharomyces cerevisiae* [19]. Coumarins also act as antimicrobial agents by targeting

bacterial vitamin K epoxide reductase [VKOR], homologue of humanVKOR, an enzyme involved in activation of blood clotting factors which could pave way for anticoagulants like warfarin [20].

### 2.3 Flavonoids:

Flavonoids are hydroxylated polyphenols compounds which are synthesized by plants in response to a microbial infection. Flavonoids are found in nature as a glycones, glycosides and methylated derivatives. They are effective against a wide array of microorganisms under *in vitro* condition. Prominent classes of flavonoids showing antibacterial properties are falvone, flavanone, falvonol, dihydroflavonol, chalcone, aurone, isoflavanone and bioflavonoids [21]. Among the flavonoids, catechins have been extensively studied because of their antimicrobial potential. Catechins show antibacterial activity against pathogenic bacteria such as *S. aureus*, methicillin resistant *S. aureus* (MRSA), *E.coli* and *Helicobacter pylori* [22]. Flavones extracted from medicinal plants showed strong antibacterial activities against *E. coli*, *S. typhimurium*, *S. epidermis* and *S. aureus* [23]. Naringenin and sophoraflavonone G have exhibited antibacterial activity against MRSA as well as streptococci [24]. Licochalcones A and C isolated from the roots of *Glycyrrihzza inflata* shows antibacterial activity against *S. aureus* and *Micrococcus luteus* [25].

Direct antibacterial activity of flavonoids involves three different mechanisms [26]: 1.Inhibition of nucleic acid synthesis (DNA and RNA) as observed after addition of genistein to a culture of *Vibrio harveyi* [27]. 2. Inhibition of cytoplasmic membrane as evident in a study involving galangin. The flavonoid exhibits its antibacterial activity against *S. aureus* by causing rapid efflux of $K^+$ ions from the cytoplamsic membrane [26]. 3. Inhibition of energy metabolism caused by apigenin and naringenin in Enterobacteriaceae [28].

### 2.4 Tannins:

Tannins are secondary metabolites of plants, non-nitrogenous, phenolic in nature. Their molecular weight ranges from 500 to 3,000 [29], and they are found in nearly all plant parts like bark, wood, leaves, fruits, and roots [30]. They are divided into three major classes, hydrolysable, condensed and pseudo 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. Pseudo tannins are low molecular weight compounds associated with other compounds. Tannin is produced by plants in adverse environmental conditions, being responsible for their protection against herbivores and pathogenic diseases and is essential for growth and reproduction of the plants [31]. Plant derived hydrolysable tannins have antibacterial effects against *H. pylori*. It shows dose dependent membrane damaging activity [32]. Different studies have suggested that tannins interact with viral proteins of some animal viruses resulting in the inhibition of viral attachment and penetration [33].

### 2.5 Terpenoids:

A large number of terpenoid compounds found in nature. Indeed, the structural diversity associated with at least 40,000 compounds makes the class of terpenoids one of the most impressive examples in the divergent evolution of plant chemicals. The evolutionary success of this compound class is in part based on the simplicity of constructing different size molecules. Terpenoids are terpenes that undergo biochemical modifications *via* enzymes that add oxygen molecules and move or remove methyl groups [34]. Terpenes are composed of isoprene units and are generally represented by the chemical formula $(C_5H_8)_n$. Terpenes can be acyclic, monocyclic, bicyclic, or tricyclic [35]. Owing to the diversity in their chemical structures, terpenes are classified into several groups such as monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), and triterpenes ($C_{30}H_{40}$). The major component of bioactive essential oils is constituted of monoterpenes (70–90%) [36].The inhibitory activity of terpenoids on bacteria [36], viruses [37] and protozoa [38] has been reported.

### 2.6 Essential oils:

Essential oils (Os) which are produced by the secondary metabolism of herbs and/or spices and their constituents have uses in human consumption as functional food (nutraceuticals, biopolymers), food additives (flavourings, antioxidant and antimicrobial), medicines (pharmaceuticals, therapeutic products), nutritional supplements (dietary supplements, culinary) and the manufacture of cosmetics (perfume/fragrances, aromatherapy, hair and skin care). Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties [39]. Essential oils obtained from plants are aromatic in nature and is a mixture of diverse group of chemical substances that belong to different chemical families, including terpenes, aldehydes, alcohols, esters, phenolic, ethers, and ketones [40]. Essential oils are hydrophobic and thus only slightly soluble in water. Essential oils have the ability to hamper the growth of a diverse range of pathogens and bioactivity of an essential oil depends on its chemical constituents. They are stored in plants in special brittle secretory structures, such as glands, secretory hairs, secretory ducts, secretory cavities or resin ducts [41]. Essential oil compounds maybe classified into three main categories: terpenes (monoterpene hydrocarbons and sesquiterpene hydrocarbons), terpenoids (oxygenated monoterpenes and oxygenated sesquiterpenes) and phenylpropanoids [42]. The chemical constituents of plant essential oils differ between species. Some factors that can

affect these constituents include the geographical location, environment, and stage of maturity [43].This chemical difference is directly related to differences in antimicrobial activities against various pathogenic microorganisms [44]. Different modes of action are involved in the antimicrobial activity of essential oils and extracts. Generally, there are six possible mechanisms of antimicrobial action, which include: (1) disintegration of cytoplasmic membrane, (2) interaction with membrane proteins (ATPases and others), (3) disturbance of the outer membrane of Gram negative bacteria with the release of lipopolysaccharides, (4) destabilization of the proton motive force with leakage of ions, (5) coagulation of the cell content and (6) inhibition of enzyme synthesis [45]. Essential oils components (eugenol, terpineol and terpinene) have a bactericidal effect against the both Gram positive and Gram negative bacteria by disrupting their membrane systems

The antifungal actions of essential oils are similar to that of previously explained antibacterial mechanisms. Generally, exposure of essential oils leads to the coagulation of the cellular components because of irreversible cell membrane damage. In yeast cells, essential oils establish a membrane potential across the cell membrane and disrupt the production of ATP, which leads to cell membrane damage [46]. The essential oils have the ability to penetrate and disrupt the fungal cell wall and cytoplasmic membranes through a permeabilization process, which leads to the disintegration of mitochondrial membranes. This is caused by alterations in the flow of electrons inside the electron transport system (ETS) pathway. At present, various essential oils may be a promising alternative against viral infections [47]. Essential oils might interfere with virion development, designed for entry into host cells. For instance, the sesquiterpene triptofordin C-2 suppressed the synthesis of viral proteins and inhibit the early gene expression process of the HSV-1 virus [48].

### 2.7 Lectins:

"Lectin" comes from the Latin word "legere", which means "to select". Lectins are oligomeric carbohydrate-binding proteins that are involved in various biological recognition processes [49]. Lectins are ubiquitous in nature, found in all kinds of organisms, from virus to humans [50]. They are a large and diverse group of proteins that have the ability to bind reversibly to monosaccharide and oligosaccharides, which can be defined as a class of structurally diverse proteins or glycoproteins [51]. Nowadays, proteins that can agglutinate red blood cells with known sugar specificity are referred to as "lectins". Lectins are sometimes called "agglutinin" [52]. Putative role of lectins in plant defence mechanisms is being discussed since the time, when it was discovered that lectins are capable to specifically bind with sugars, located on surfaces of microorganisms, causing their agglutination [53]. The lectins that bind to accessible carbohydrate residues from the cell wall or cell membrane trigger a cascade of biological responses. The capacity for identifying and binding glycoconjugates from the microorganism's surface is exclusive to lectins. Consequently, they are capable of inhibiting the motility and multiplication of microorganisms [54]. The interaction between carbohydrates and lectins act in many biological processes, including bacterial and fungal growth inhibition [55]. Generally, lectins are classified into five groups on the basis of their affinity for (i) Glucose/Mannose (ii) Galactose and N-acetyl-D-galactosamine (iii) N-acetylglucosamine (iv) L-fucose and (v) Sialic acid [56]. Some lectins contain more than one type of acting site or one activity in single molecules so that they can bind to carbohydrate and can exhibit other behaviours such as enzymatic activity (which make this lectin called "lectzyme"), mitogenic activity, and transportation activity in the same time. From these phenomena, the lectins can be classified into three types according to their acting sites as "merolecins" (the lectins with only single carbohydrate binding domain, usually small single peptides), "hololectins" (the lectins with two resemble carbohydrate binding domains), "chimerolectins" (the lectins contains both carbohydrate binding domain and other well-defined biological active domains which act dependently of previous domain). Most lectins are present in seed cotyledons of the plant (but also found in any other parts such as roots, stems, rhizomes, and leaves in lesser amounts). In such tissues, most lectins are located within cytoplasm or protein bodies inside the cells [57]. Several lectins such as Concanavalin A and wheat germ agglutinin (WGA) are toxic to mammalian cells, but relatively low compared with other toxic substances such as approximately 1000 times lower than ricin (an toxic albumin from Caster bean). It is believed that production and accumulation of toxic lectins in some plants are a kind of defending mechanisms, which plants develop for protecting them from certain plant eating organisms such as insects and mammals [58] and plant pathogens. Aside from defense mechanisms, the lectins also have their essential roles in plant-microorganism symbiosis, cell differentiation, pollen recognition, cell wall elongation, and as a reserved protein [59]. Some plant lectins react with viral surface glycoprotein and are hoped to use in controlling many diseases originated from viruses. Lectins manifest a diversity of activities including antitumor, immunomodulatory, antifungal, HIV-1 reverse transcriptase inhibitor and anti-insect activities, which may find practical applications. Recombinant lectins have been expressed in heterologous systems [60]. The compact globular structures, molecular aggregation and glycosylation in general result in high structural stability of lectins [61].Combination of chemotherapeutic agents with naturally occurring tumour active compounds such as polyphenols would be better able to overcome mechanisms of drug resistance. Excessive ROS levels in normal cells has been reported to be involved in various aspects of carcinogenesis by altering cellular signalling and gene expression pattern[62]. Plant derived dietary compounds play anticancer role either by increasing ROS level in cancer cells, resulting induction of programmed cell death or counter-regulating elevated ROS level in normal cells by antioxidant mechanism [63].

### 2.8 Alkaloids:

Alkaloids are a large structurally heterogeneous group of natural products found in bacteria, fungi, plants and animals. The number of nitrogen atom is variable with one being most common. Nitrogen is present in the form of different amines (primary ($RNH_2$), secondary ($R_2NH$) and tertiary ($R_3N$)). The research concerned with antibacterial potential of alkaloids date back to 1940s. Recently, more in depth studies have been conducted as many potent antibacterial monomers have been characterized in several classes such as indole [64]. Alkaloids extracted from *Tamarindus indica* showed antibacterial activity against pathogenic organisms like *E. coli*, *S. aureus* and *P. aeruginosa* [65]. Plant aqueous extract rich in alkaloid content isolated from *Zapoteca portoricensis* exhibits a large halo zone of inhibition against *P.aeruginosa* and hence can be used in treatment of skin infection [66]. Studies have reported that methanolic extracts rich in alkaloid content of *Scadoxus multiflorus* shows antibacterial activity against *S. aureus* and *S. typhi* while that of *Acacia nilotica* shows antimicrobial potential against *S. Aureus* and *P. aeruginosa* [67].

The mechanism of action of the alkaloids such as indolizidine, isoquinoline, quinoline, agelasine and polyamine has been studied. The alkaloids such as perguarinine and tylophorinidine of class indolizidine act by inhibiting nucleic acid synthesis [68]. Natural, semisynthetic and synthetic alkaloids have been reported to increase the efficacy of antibiotics. Studies involving synergistic combinations of alkaloids and antibiotics resulted in many fold reduction in antibiotic minimum inhibitory concentration MIC [69]. Most alkaloids enhance the antibiotic activity by inhibiting efflux pump [70].

### 2.9 Anti-microbial Peptides [AMPs]:

Plants produce a large number of toxic molecules, including antimicrobial peptides (AMPs) that kill microorganisms. Antimicrobial peptides are either non-ribosomally synthesized (NRAMPs) [71] or ribosomally synthesized (RAMPs) [72]. AMPs possess antiviral, antiparasitic, antineoplstic and immunomodulatory activity. AMPs act as a part of the host's nonspecific defense system and are also active against wide range of microorganisms [73]. Plant antimicrobial peptides have been isolated from a wide variety of plant species. These peptides have shown efficacy against phytopathogens as well as human pathogens [74]. Plants produce an extremely diverse array of AMPs and based on amino acid homology, AMPs can be grouped into six main families, namely, defensins, thionins, lipid transfer proteins, cyclotides, snakins and heveins-like proteins. Bioinformatics studies have revealed that in spite of significant differences in amino acid sequences between the families; they share many similarities in tertiary structures [75]. AMPs have high content of cysteine and/or glycine. AMPs also possess disulphide bridges which play a vital role in enhancing structural stability under stress conditions. Antimicrobial peptides act through a variety of mechanisms such as formation of membrane pores, depolarization, inhibition of respiratory processes and cell death [76]. AMPs interact with the phospholipids mainly through amphipathic structure. Peptides accumulate on the membrane surface due to the electrostatic interactions between the cationic residues and the negatively charged molecules such as lipopolysaccharides and teichoic acids and when peptide concentration reaches a threshold level collapse begins.

Among main families of AMPs, thionins are low molecular weight (about 5kDa). They are rich in arginine, lysine and cysteine residues. They are made of two antiparallel α-helices and an antiparallel double stranded β-sheet with conserved disulphide linkages. Thionins are toxic against bacteria, fungi and yeast. Thionins have shown antimicrobial properties against *Pseudomonas solanacearum*, *Xanthomonas phaesoli*, C*orynebacterium fascians*, *Pseudomonas syringae* [77], *Fusarium solani*, *Scelerospora graminicola* [78,79]. Plant defensins are low molecular weight molecules [about 5 kDa]. They are AMPs that range from 18 to 48 amino acids. All of them contain several conserved cysteinyl residues that form disulphide bridges contributing to stability. Plant defensins can be divided into two groups: (1) plant defensins that alter the morphology of fungal hyphae and thus inhibiting fungal growth and (2) plant defensins that inhibit fungal growth without morphological distortions. Plant defensins have exhibited antimicrobial potential against a broad range of plant pathogens such as bacteria, yeast and necrotrophic pathogens [80]. The mode of action of plant defensins is still unclear. Plant defensins probably use glycosylceramides as receptors for fungal cell membrane insertion. Repulsion of defensins into cell membrane by their positive charges leads to membrane disruption and destabilization leading to ion influx and fungal cell death [81]. Plant defensins showing antifungal activity against *Aspergillus flavus* and *Candida albicans* have been successfully isolated [82]. Non-specific lipid transfer proteins [ns-LTPs], found in many plants are capable of exchanging lipids in between membranes. It can be subdivided into two families namely LTP1s and LTP2s having relative molecular mass of 9 kDa and 7 kDa, respectively. LTP1s show a greater homology in N-terminal amino terminal sequence across dicots and monocots. Non-specific LTPs have been reported to show antimicrobial activity against organisms such as *C. albicans* and *C. tropicalis* [83]. Snakins, isolated from potato are small peptides comprising of 63 amino acids and having molecular weight 6.9 kDa. Snakins are rich in cystine residues which form disulphide bridges contributing the overall stability. These peptides exhibit a high degree of sequence homology and possess identical antimicrobial properties against a wide range of bacterial and fungal pathogens [84]. Cylotides are circular proteins found in bacteria, plants and animals [85]. They seem to possess a high sequence similarities and structural identity. Plant cyclotides consists of about 28-37 amino acids with a cyclised backbone and three intramolecular disulphide bonds which are arranged as cyclic cysteine knot CCK. This knot is responsible for the remarkable stability of cyclotides. Many cylcotides showing antimicrobial properties have been

isolated such as Kalata B1 and B2 from *Oldenlandia affinis* [86]. Heveins are chitin-binding small peptide having a molecular weight 4.7 kDa. All known chitin binding proteins possess a carbohydrate domain with conserved cysteine and glycine residues. Heveins-like AMPs have shown antifungal activity against both chitin and non-chitin containing fungi. These proteins inhibit fungal growth by rapidly penetrating into fungal hyphae and by disrupting fungal membrane [87]. Antifungal heveins like proteins have been isolated from leaves of paper mulberry [88].

## 3. Role of "omics" technologies in screening of plant-derived antimicrobials and elucidation of their mechanism of action:

Great advancement has been made in the development of analytical tools for classification and isolation of plant based antimicrobials. Identification of a possible drug target relies on the combined application of several biochemical and genetic assays. However these techniques require large amount of the compound involved and is a time consuming process [89]. Breakthrough in genome sequencing, bioinformatics and tools such as chromatography and mass spectrometry has galvanized "omics" technologies (genomics, transcriptomics, proteomics and metabolomics). Omics technologies have enabled critical analysis as well as understanding of process related to bacterial metabolism, pathogenesis and mechanism of action of antimicrobial compounds. Efficient genetic sequencing technologies have enabled decoding of microbial genomes. This has led to identification of genes linked to vital metabolic processes involved in bacterial growth, pathogenesis and mutagenesis. These genes and their metabolic products are studied for their potential as new antimicrobial targets [90].

Genome-wide mutant library has been utilized to generate and select mutants with altered sensitivity to the compounds. The molecular pathway involved in the action of mutants is elucidated. This approach has been successfully employed to reveal the mechanism of action of alfalfa snakin-1 (MsSN1), an antimicrobial peptide isolated from *Medicago sativa*. This peptide was produced against *Pseuodmonas fluroscens* Pf-5 and works by altering the adhesive properties of the microorganism [91]. Sequencing methods are also used to select mutants generated when a wild type strain is cultured in presence of a new antimicrobial compound by the process of natural selection. This strategy has been successfully employed in elucidating the mode of antimicrobial action of a new compound SPI1031 against *P. aeruginosa* [92].

Research has reported that the mechanism of action of an antibiotic relies on the interaction of multiple pathways [93] and hence transcriptional analysis has been widely used to understand the action mechanism of an antibiotic derived from plants. For large scale studies of gene expression, DNA Microarray is the most widely used technique. Microarray has been used to understand the mechanism of action of many phytochemicals. These studies have open new frontiers with regard to the modulation of various pathways caused by an antimicrobial compound [94, 95]. Proteomics studies have also been used to understand antibacterial mechanism of many plant derived compounds. MALDI-TOF and 2-DE was used to study the effect of tea polyphenols (TP) on protein expression in *P.aeruginosa* [96]. Proteomics studies have been used to study biochemical details involved in mechanism of action of phytochemicals such as plumbagin on *B. subtilis* [97], *Papaver rhoeas* against *E. coli* [98].

## 4. Future perspective:

Plant derived antimicrobials are now well recognized for their pivotal role in the development of effective therapeutics against pathogenic organisms either alone or in synergy with conventional antibiotics. Major challenges in this regard are to find compounds with lower minimum inhibitory concentrations [MIC], low toxicity and high bioavailability.

Omics based technologies have elucidated the antimicrobial mechanisms of plant derived antimicrobials. Phytochemicals comprise a large and highly heterogenous group of compounds so modern technologies are essential in identification of new compounds with better and efficient modes of action. It has also been envisioned that understanding the details of molecular mechanisms of action of these compounds would lead to selection of compounds that does not inhibit the host. However some barriers still remain such as operating cost and complex structural features of plant antimicrobials. Future of the war against pathogenic organisms requires an integrated study using different technologies.

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