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Review Article

Anti-Microbial Peptides from Medicinal Plants as an Alternative against Multi Drug Resistance

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Abstract

From its discovery to today, antibiotics have revolutionized medicine, and various antibiotics have been studied, discovered and put to significant application and it continues to be helpful in controlling infections. Nonetheless over application of these antibiotics have given rise to Antibiotic Resistance (AR) and Multidrug Resistant pathogens (MDR) against the various antibiotic's agents. Anti-microbial peptides are being explored as an alternative against the prevalent issue of MDR and AR. Anti-microbial peptides are the part of host's first line of defense mechanism of innate immune response, are small peptides its molecular weight is 2-10kDa, it holds amphiphillic properties, and is usually positively charged at neutral pH value. The advantages posed by anti-microbial peptides are many like broad antimicrobial spectrum, rapid action, and lower risk of resistance, low toxicity and high selectivity. It poses many therapeutic like anti-cancerous, anti-inflammatory, anti-bacterial, anti-fungal, anti-viral, and immunomodulator properties as well. Plants are good source of anti-microbial peptides. A variety of applications can be achieved with plant derived antimicrobial peptides, including antibacterial, insecticide, and infection control, including the control of cellular infection by viruses. AMPs exist in different molecular forms like Cyclotides, cyclic cysteine knot, defensin, thionin, snakin-Like, hevein-like, knottin like peptides etc. It is expected that anti-microbial peptides will have a positive impact on medicine, food, industries as antifouling agents and agriculture. The major objective of this review articleis to explore and identify important antimicrobial peptides in medicinal plants like *Ocimum sanctum* and *Santalum album*.

Keywords: Anti-microbial peptides; Multi-drug resistance; *Ocimum sanctum*; *Santalum album*

Abbreviations

AMPs: Antimicrobial Peptide; MOA: Mechanism of Action; MDR: Multi-Drug Resistance; AR: Antibiotics Resistance; MRSA: *Methicillin- Resistant Staphylococcus Aureus*

Introduction

The use of medicinal plants dates back to Vedic period (3500–1600 B.C) when books on Ayurvedic medicine were written and the use and practice of medicinal plant was described which formed the basis of medical sciences [1]. Over 90% of traditional medicine remedies contain medicinal plants and continues to be used to aid certain diseases and enhance immune system [2]. Tulsi (*Ocimum sanctum)* is an aromatic herb which has been very widely used for centuries due to its healing properties and is known as the 'Queen of herbs' [3]. It belongs to the *Lamiaceae* family and is usually found in the tropical and subtropical regions [4]. It is an erect, sub shrub with purple and green leaves and possesses definite therapeutic properties [1]. There are certain studies that pointed out towards the anti-microbial activity of tulsi which exhibits anti-bacterial, anti-fungal, anti-viral activity, and anti-cancer activity [5], *O. sanctum* L. essential oil is shown to have antibacterial activity against several pathogenic microorganism such as *Staphylococcus aureus, Bacillus pumilus* and *Pseudomonas aeruginosa* [6]. The leaves of tulsi are also shown to have anti-fungal properties against the *Aspergillus* species. *Santalum album* on the other hand is an aromatic wood, which has been esteemed since primeval times commonly known as Sandalwood; it belongs to the *Santalaceae* family and is usually found in the dry regions of India and in China, Indonesia and the Philippines. It is an evergreen, semi parasitic plant, the mostly used part of sandalwood is heartwood which appears yellowish brown when fresh and gradually turn dark in color upon exposure, the heartwood is scented and possess diuretic, disinfectant anti-pyretic haemostatic and many such properties [3]. The leaf extract of *S. album* is shown to have anti-microbial activity against *E.coli, Staphylococcus aureus* and *Pseudomonas* [7], its aqueous extract is shown to have strongest inhibition against *S. aureus* [8]. Now with the help of advancement in genome wide research and whole genome sequence available in public database like NCBI, the scientific and actual secret behind the numerous advance properties of this medicinal plant can be explore. Moreover, the gene or cluster of gene can be identified which decode the full or partial peptide which own the AMPs activity. Anti-microbial peptides are part of host's first line of defense mechanism of innate immune response, they are generally small peptides with molecular weight is upto 2-10kDa. These tiny peptides hold amphiphillic properties and is usually positively charged at neutral pH value. These are small proteins or part of protein with potent anti-bacterial, anti-viral and anti-fungal activity and are ubiquitous in nature [9]. AMPs have a wide range of activity, including the ability to kill bacteria, fungus,

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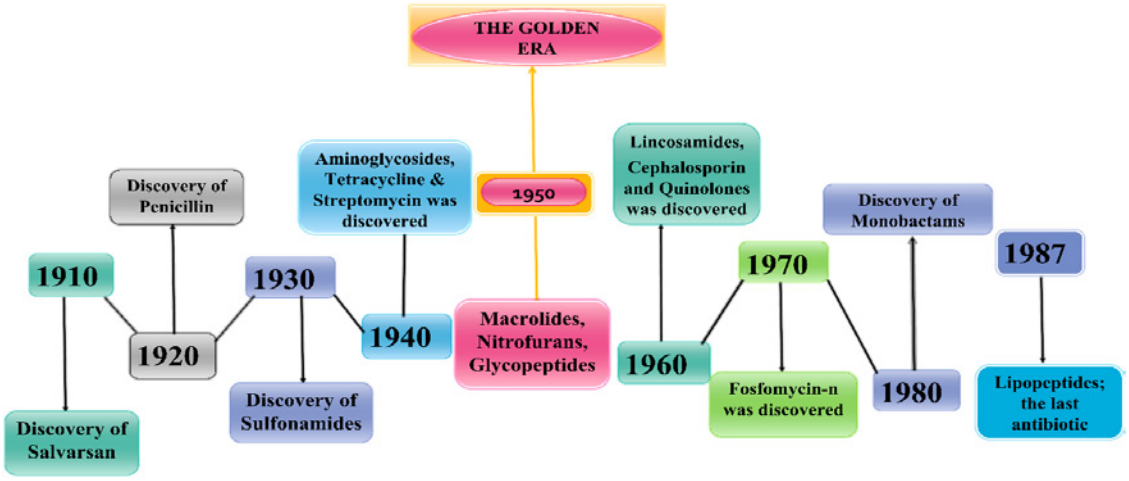

Figure 1: Milestone timeline in the discovery of various antibiotics.

yeasts, and cancer cells, viruses either directly or indirectly. It is also known as host defense peptides because of its immune modulatory activities which make it unique in nature. These peptides are mostly used by plants and insects as an antibiotic to lookout against potentially dangerous microorganisms [10]. The plant kingdom is adapted to hold diverse types of peptides to protect against microbes since they are short of specialized immune system like animals [11]. Anti-microbial peptides are quite diversified and are classified on the basis of; source, activity, structural characteristics and amino acid rich species [12]. There are several features of AMPs that makes it important like AMPs target the lipopolysaccharide layer of the microorganism unlike the antibiotics which targets specific cellular activities [13], and second is its rapid killing effect [14]. AMPs have been studied as an alternative microbial agent as its mode of action of killing bacteria is discrete than the Mechanism of Action (MOA) of antibiotics [15]. Its mode of action to kill the pathogen or bug are usually depends upon their interaction with bacterial cell membranes or cell walls [10]. They kill bacteria using two extensive mechanisms of action; first mechanism is membrane disruption induced by AMPs which leads to cell lysis and death [16]. The second mechanism of APMs is through entering the cell without any membrane disruption and binding to nucleic acids or intracellular proteins to inhibit all the essential intracellular functions [17]. AMPs enter into the well-defined membrane bilayers and form pores in different way like 'barrel-stave', 'carpet' or 'toroidal-pore' mechanisms [16]. AMPs can be employed in various areas like medicine as they can recruit cells, promote wound healing, stimulate the proliferation of cells, kills cancer cells, alter gene expression, as well as regulate pro-inflammatory reactions [18]. In food industry, as it inhibits bacteria and fungi and many AMPs are also resistant to high temperatures, alkalis and acids and can be hydrolyzed byproteases easily and therefore it is a potential alternative as food preservatives [19], in aquaculture, poultry and animal husbandry to enhance production performance [20,21]. Also, the AMPs not only applicable in above-mentioned sector but also, they are having potential application in agriculture industry as AMPs have the potential to control the pathogenic infection of plants by bacteria and fungi. Due to its physio-chemical properties, activity towards wide spectrum of bacteria and different mode of action from the current in use antibiotics it is a striking alternative to traditional antibiotics [22].

Table 1: Classification of antimicrobial peptide based on its various properties.

S.No	Basis of classification	Classes of anti-microbial peptides
1.	Based on their electrical charge	Cationic and Anionic peptides
2.	Based on their sequence similarity	Lipid transfer proteins
3.	Based on the presence of cysteine motifs	Snakins, Defensins, Cyclotides
4.	Based on the presence of tertiary structures	Thionine, Defensin, Knottin

History of Antibiotics

Discovery of antibiotics certainly revolutionized the whole world by significantly contributing to controlling infections by either killing the bacteria or inhibiting its growth. The antibiotic era started with the very first antibiotic 'Penicillin' in 1928 which led to the production and commercialization of various other antibiotics. Though penicillin was not the first antibiotic, it was first Salvarsan developed in 1909-10 by Paul Ehlrich and Sahachiro Hata to treat syphilis [23], which was later on replaced by penicillin in the 1940s. Several new antibiotics have been discovered and use, important types of antibiotics which still playing remarkable role include penicillin, tetracycline, cephalosporin, quinolones, linomycins, macrolides, sulfonamides, glycopeptides, aminoglycosides, and carbapenems. It has been widely used by the health practitioners to treat and control infections and also has been used in agriculture, aquaculture and horticulture [24] (Figure 1).

Evolution of Multi Drug Resistance (MDR)

In recent times, the biggest public health challenge has been the case of rising antibiotics resistance, the pathogens developing resistance and multi-drug resistance has worsened the whole scenario limiting therapeutic options for treating or controlling infections. The emergence of dangerous, resistant strains of bacteria has occurred frequently with a disturbing regularity within the past twenty years, although the phenomenon has been documented almost since the dawn of the antibiotic era. Generally, Multidrug Resistance (MDR) is the insensitivity or resistance of a microorganism to antimicrobial

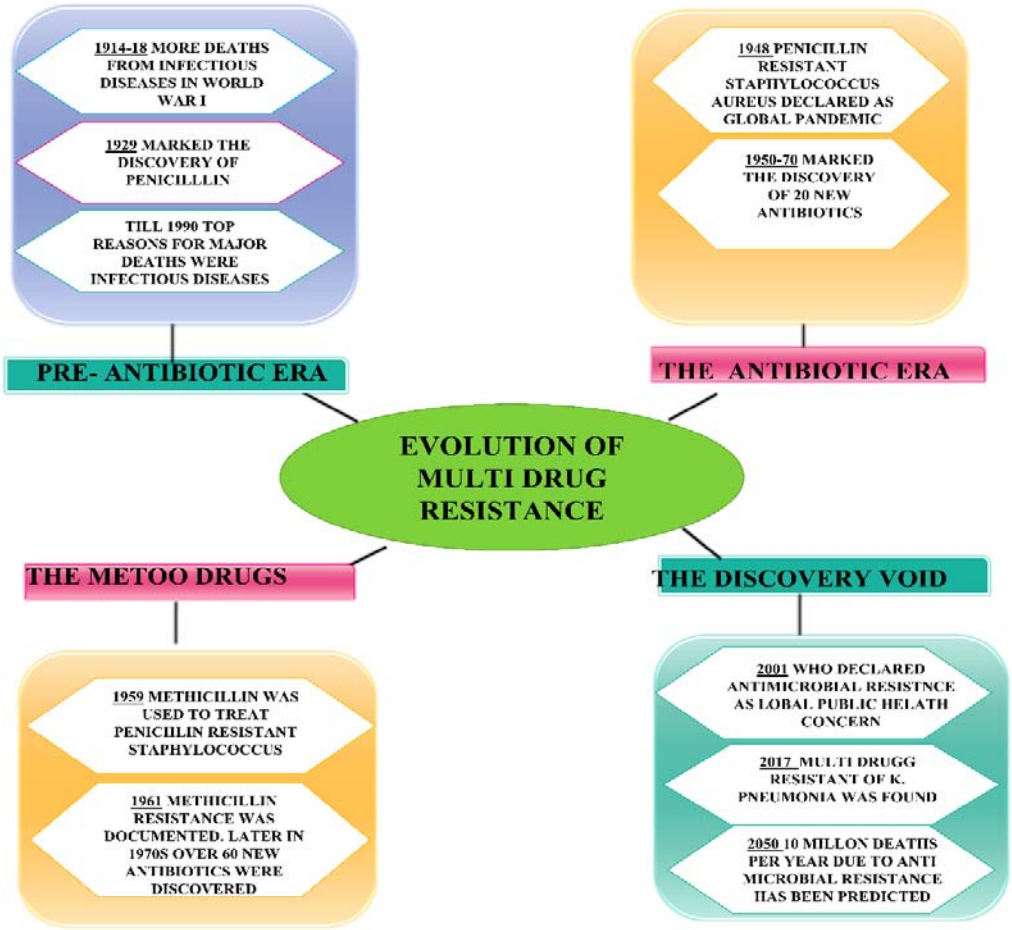

Figure 2: Illustration of evolution of multi-drug resistant.

drugs (which have different molecular targets and are structurally dissimilar) [25]. Bacteria that cause common or severe infections have developed resistance to almost every new antibiotic. There are several ways the resistance is being spread, extensive use of antibiotics for human therapy or in fishes for aquaculture or in farm animals have very efficiently contributed to pathogenic bacteria developing resistance against multiple drugs [26]. (Figure 2) demonstrated the spread of multi-drug resistance via various modes. It dates back to 1930s with emergence of sulfonamide (an active agent of Protonsil) which was used to treat infections that were caused by gram negative and gram-positive cocci, soon after that streptococcus pyogenes were reported to have had developed resistant against sulfonamide [27]. In 1950s the case of multi drug resistance was reported in *E.coli* and *shigellae* against sulfonamides, tetracycline, chloramphenicol, and streptomycin [28]. Multi- Drug Resistant (MDR) strains are coming as a great threat to life as these organisms become resistant to certain antibiotics resulting as no effect of antibiotics for cure of bacterial diseases. MRSA (*Methicillin- resistant Staphylococcus aureus*) is one such MDR strains that are difficult to treat because of resistance to Methicillin, aminoglycosides, macrolides, tetracycline, chloramphenicol, and lincosamides [26]. The reason given for multiple resistances in pathogens was acquirement of transferable DNA molecules also called R factors or R plasmids and resistance to each antibiotic or drugs were encoded in these plasmids by separate genes [29]. Many resistant pathogenic bacteria strains of a number of species like penicillin resistant *Haemophilus Influenzae* [30], *Pneumococci* [31] were reported back in the 1970-80s.

In general, antimicrobial drugs operate in one of two ways: either by competing with the substrate of enzymes or by inhibiting a metabolic pathway such as nucleotide synthesis [32]. The main reason of multiple drugs resistance is use of antibiotics with transformation of resistance conferring genes amid bacteria [33]. Microorganisms usually develop resistance by chromosomal resistance or exchanging elements of extra chromosomal DNA by transformation or conjugation which can affect the structural composition of the cell membrane and affect permeability and drug uptake in the cell [34]. The major cause of food borne disease is reported to be *Salmonella* which contaminates food products and there are studies which reports *Salmonella* multidrug resistant strain that is resistant to streptomycin, ampicilin, sulfamethoxazole, tetracycline and chloramphenicol, and additional resistance to cephalothin, amoxicillin-clavulanic acid, cefotoxin, and ceftiofur due to the strain acquiring the CMY-2 AmpC-like gene [35]. Multidrug resistance has led us to a point where the diseases which were curable has become untreatable because of the causative agent developing resistance against the drug or antimicrobial agent, for instance pneumonia which is caused by *Streptococcus pneumonia* is found to be resistant to cephalosporin and carbapenems [36],

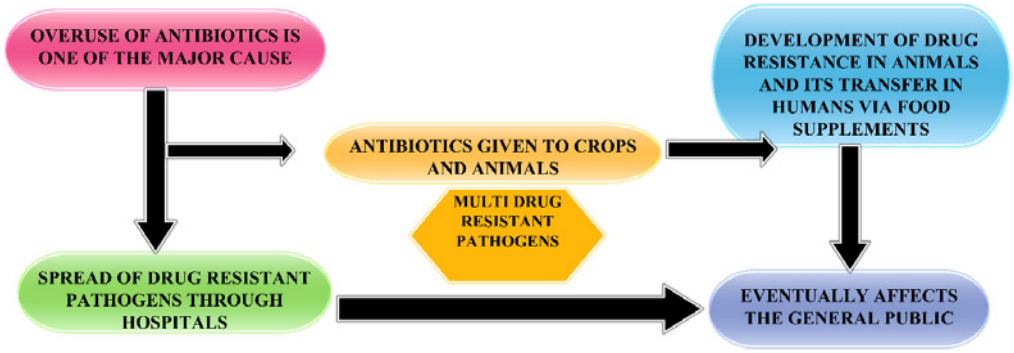

Figure 3: Mode of spread of multidrug resistance via overuse of antibiotics, food supplement, pathogen from hospital.

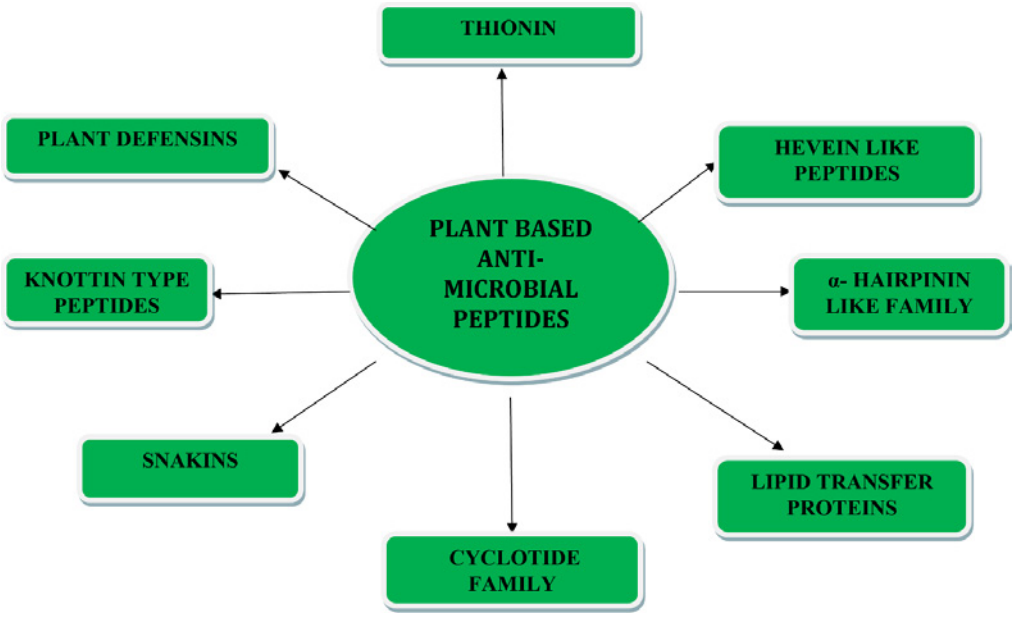

Figure 4: Major classes of antimicrobial peptide from plants.

malaria's causative agent is also found to be resistant to chloroquine, pyrimethamine and artemisin in [37]. Multidrug resistance poses a serious health threat due to the problem it poses, which includes potential for therapeutic failure, high mortality rates, prolonged illness, vulnerability to immuno-compromised conditions, and decreased effectiveness of drugs [34]. In addition, it increases the duration of treatment thus also contributing in high medical costs which leads towards economy burden hence, discovery of new antibiotics or anti-microbial agents or drugs are urgently required as an alternative. The discovery and commercialization of antibiotics take very long, so alternatives must be considered, the potential of antimicrobial peptides of medicinal plants to be used as therapeutic agents needs to explore further. Thus, in this review, the major plant-based Anti-Microbial Peptides (AMPs) has been discussed.

Plant Based Anti-Microbial Peptides (AMPs)

Medicinal plants like Tulsi (Ocimumtenuiflorum)and Sandalwood (Santulum album) parts have been time and again studied for its antimicrobial activity, there are various studies which reveal that plant extracts and derivatives display anti -microbial, anti-inflammatory, anti-cancer and anti-fungal activity (Figure 5). However, a detailed study on the plant based antimicrobial peptides, genes responsible for coding these particular peptides/protein or compounds that leads to target activity and the underlying mechanism of these AMPs in Tulsi and Sandalwood have not studied yet. As plants produce these peptides as a defense to protect themselves from pathogens. Due to the rapid increase in antibiotic resistance, plant derived AMPs have been found to be a great alternative solution. Plant derived AMPs possess high stability and are highly antimicrobial and are usually positively charges at normal pH with molecular weight of 2-10 kDa, the greater part of plant-based AMPs is Cystine-rich [38], As a result, multiple disulfide bonds (usually two to six) form, which makes the molecule more compact structurally, resistant to chemical degradation, and more rigid [39]. At present, the total number of AMPs isolated from a very limited range of plants already exceeds a thousand, and this number is likely to grow in the future. The first antibacterial peptide was purothion in isolated from wheat flour and had the ability to inhibit the growth of phytopathogens [40]. There is a

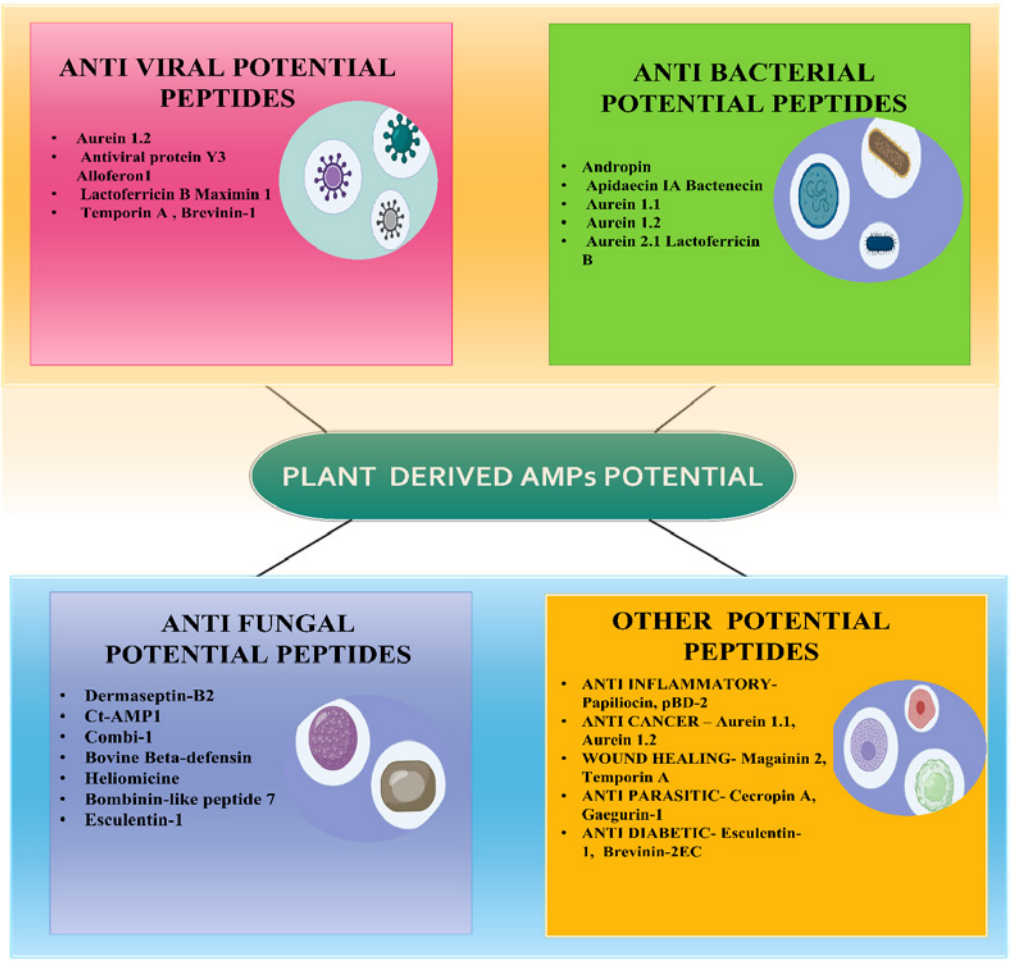

Figure 5: Action potential of plant derived antimicrobial peptide.

great deal of complexity in plant, plants can contain an array of AMPs [41]. Plant based AMPs are majorly classified on the basis of their electrical charge, sequence similarity, presence of tertiary structures and presence of cysteine motifs, an important criterion for classifying plant AMP family members is a Conserved Cysteine Motif (CCM). The classification of AMPs based on their characteristic's properties are illustrated in (Table 1); This motif exhibits a characteristic Cys pattern with a defined number of non-cysteine residues between two adjacent Cys [40]. The study aims to provide a general overview of the major families of plant AMPs., (Table 1).

Some major families of plant based anti-microbial peptides are Thionin, plant Defensin, Knottin type peptides, α-hairpinin like family, lipid transfer proteins, snakins, cyclotide family and Hevein like peptides [42]. Thionins are small peptides with 6-8 cysteine residues and 3-4 disulfide bonds [43] as a result of adisulfide bond connecting the N-and C-termini, they could be classified as cyclic peptides, they have antimicrobial activity against bacteria, fungi, nematodes [43-45]. Plant Defensin is a large family that exists widely in the plant kingdom i.e., are found in almost all plants as it has highly conserved scaffolds, [46], even though their structures are highly conserved, and their amino acid sequences are highly variable, with the exception of the cysteine that form stable disulfide bonds and some other conserved residues. Furthermore, there are many biological functions exhibit by these compounds which are capable of, including inhibiting microbial growth, inhibiting, amylase and trypsin activity, mediating abiotic stress, acting as epigenetic factors, and altering ascorbic acid redox state [47-52]. Knottin is type of peptides which is found as smallest plant AMPs. Many knottins are composed of conserved disulfide bonds forming cysteine knots, these show not only antimicrobial but alsoexhibit cytotoxic, insecticidal, and HIV-inhibitory properties [53-55]. There are a variety of biological activities undertaken by the α- hairpin like AMP family, including antimicrobial, trypsin-inactivating, and ribosome-inactivating activities [44,56]. Lipid transfer proteins are cationic peptides with low amino acid sequence similarity [57]. Snakins are cysteine rich family with 6 disulfide bonds [58]. Cyclotide family consists of anionic peptides they are high resistance towards thermal and chemical denaturation, as well as proteolytic degradation, which makes them potential therapeutic agents [59]. Hevein-like antimicrobial peptides are alkaline peptides and are effective at inhibiting the growth of chitin-containing fungi as well as protecting plants from fungal pathogens [60] (Figure 4).

Future Prospects

There is a huge investment of time, effort, research, and money

involved in the development of new drugs, and with rising threat of antibiotics resistance and MDR, alternatives need to be considered. The use of traditional medicinal plants to treat infections have a long history while the anti- microbial peptides have been studied enough to understand its potential to be used as an alternative therapeutic agent. To combat the prevailing problem of multidrug resistance new AMPs from medicinal plants such as tulsi, sandalwood etc., can be identified *via* genome mining and can also be studied for it synthesis through plants. The work is currently under progress in our laboratory as the genomic sequences are available in public database for exploration and it can serve as a great alternative to be used as a next generation peptide based novel drugs or antibiotics.

Conclusion

We are currently facing a silent pandemic with rising threat of antibiotics resistance and multi-drug resistance and therefore alternatives need to be considered. AMPs have been proved to be the next generation peptide based antimicrobial drug. Identification of AMPs in existent eminent medicinal plants like tulsi, sandalwood would lead to production of plant based antimicrobial drug which would be safe for human consumption as well as safe from multi drug resistance.

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