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PLANT ANTIMICROBIAL PEPTIDES: A NOVEL APPROACH AGAINST DRUG RESISTANT MICROORGANISMS

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ABSTRACT: Antimicrobial peptides (AMPs) are the crucial factors, which plays an important role in host defense mechanisms. AMPs are ubiquitous and found in diverse organisms ranging from microorganisms to animals. Plants are the precious source of natural antimicrobial molecules including antimicrobial peptides known as plant antimicrobial peptides (PAMPs). PAMPs can be divided into different families based on their, molecular weight, activity against different microbes, structure, charge of molecules, content of disulphide bond and mechanism of action. Based on number of cysteine residues and disulfide bonds, PAMPs are categorized into six main families. These peptides may lead to degradation of nutrients that are specific or essential for microbes to grow, interfering with microbial membrane or by conflicting with their metabolism. PAMPs exert multiple antimicrobial activities which includes membrane permeabilization, interference with DNA, RNA and protein synthesis that might provide a suitable approach to prevent bacteria from developing resistance. This review provides an overview of all the major plant AMP families including their structure, function, mechanism of action and antimicrobial activity.

INTRODUCTION: The rapid emergence of resistant microorganisms is endangering the effectiveness of antibiotics, which have saved millions of lives through decades. Antibiotic resistance has been associated to misuse and overuse of medications, prolonged use of same chemical moieties as drugs, lack of new drug development by pharmaceutical industry, all leading to development of superbugs ¹⁻³.

As per WHO report 2012, around 170,000 people died globally because of multidrug resistant tuberculosis infection ⁴. The development of resistant human pathogenic fungal species is creating challenge for planning treatment strategies⁵. Therefore there is a need to develop a robust mechanism to combat the growing antibiotic resistant microorganisms. Antimicrobial peptides (AMPs) have been described as evolutionary ancient weapons against microbial infection. Antimicrobial peptides play a vital role in innate immunity of bacteria ⁶, insects ⁷, plants ⁸ and animals ⁹.

Plants have been a valuable source of natural products for maintaining human health by controlling infections ¹⁰. A vast number of medicinal plants have been recognized as major

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source of antimicrobial compounds, among these, plant AMPs play a vital role. PAMPs are one such compound. PAMPs can be source of revolution in the development of new antibiotics, which may be effective against Multi Drug Resistant (MDR) infections, currently difficult to treat, especially by inhibiting MDR pump. Due to its multifarious action on the microbes, PAMPs are potential candidate for developing new genre antibiotics with a remote chance of microorganisms getting resistant against these peptides¹¹ and can be an alternative approach. PAMPs are small, Positively charged peptides, comprising of 10 to 50 amino acids, having mass of around 2 to 9 kDa, cysteine-rich proteins with antimicrobial activities. These peptides mainly comprise of hydrophobic amino acid with helical structure¹².

The activities of PAMPs act primarily against microorganisms but some members of AMPs can act against herbivorous insects as targets¹³. The PAMPs are classified into various families based on amino acid sequences, identity, number of cysteine residues and their spacing^{12, 14}. They are broadly classified into anionic (AAMPs) and cationic peptides (CAMPs) depending on their electrical charges¹⁵. PAMPs comprises of six major families. These are thionins, defensins, lipid transfer proteins, cyclotides, snakins and hevein-like peptides¹².

Mode of Action: The exact mode of action for AMPs is still not well known. Several workers have proposed different hypotheses for the mechanism of antimicrobial peptide. Certain important features like amphipathicity, cationic charge and size of these peptides make them responsible for their antimicrobial property. The main hypothesis for the mechanism of action is the ability of AMP to cause membrane collapse by interacting with lipid molecules on bacterial surface and/ or thereby interacting with other targets in cytoplasm^{16 - 19}. Yeaman and Yount further proposed that charge affinity of AMPs towards cations is likely an important factor, conferring selectivity to AMPs¹⁹. Based on mode of action of AMPs, many various models were proposed, like "barrel-stave" (transmembrane pore formation), "carpet-like" (membrane destruction/solubilization) and toroidal (wormhole) (**Fig. 1**).

In the 'carpet-like' mechanism, peptide binds to acidic surface of the bacterial membrane thereby leading to membrane destruction²⁰. Antibacterial specificity of peptide depends upon the alignment of peptide within phospholipid membrane of bacteria. Matsuzaki *et al.*, suggested that magainin-2 peptide follows "barrel like" model, which binds laterally to the membrane surface causing translocation of phospholipid bilayer, leading to channel (pore) formation^{21,22}.

It was further suggested that peptide's antimicrobial activity depends not only on physicochemical properties of peptides but also on the phospholipids present in bacterial membrane^{23, 24}. According to the proposed "toroidal (or wormhole) model" for membrane disruption, AMPs aggregate on the membrane, leading to the formation of "bends" in the membrane, where phospholipids bilayer joins to form pore^{25,26}.

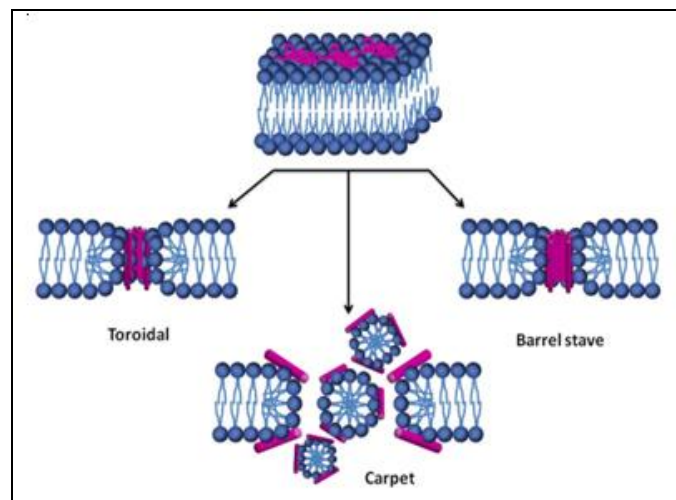


FIG. 1: DIFFERENT MODELS SHOWING ACTION OF ANTIMICROBIAL PEPTIDES (AMPs) CAUSING LIPID MEMBRANE PERMEABILIZATION

In the early stages, the peptide assimilates at the membrane surface leading to conformational changes in the peptide. Once a concentration of peptide reached to its threshold value, it will be pursued by membrane disruption by one of these three mechanisms. In Barrel- Stave model, AMP molecules embed themselves perpendicular into the membrane. In Carpet model, AMP molecules bind to region of the membrane having hydrophobic sides facing inward leaving orifice trailing in the membrane. In toroidal pore model, AMP molecules always encounter phospholipids head groups of the membrane^{27, 28}.

According to these hypotheses, when the concentration of peptides reaches threshold values it leads to pore formation in membrane. Formation of pores in the membrane causes leakage of ions and metabolites, disruption in the respiratory mechanism and synthesis of cell wall, ultimately leading to membrane collapse¹⁹.

The cationic peptides interact electrostatically with the negatively charged molecules such as phospholipids, lipopolysaccharides (LPS), teichoic acid, which are situated in the membrane of microorganisms. The cationic residues can also combine with membrane lipids using specific receptors present on the surface of the cell^{15, 17, 19}. Sometimes microorganisms may survive for extended periods even after membrane permeabilization, indicating that non-membranolytic mechanisms are responsible for cell death. These non-membranolytic mechanisms include, binding of PAMPs to certain factors like replicating enzymes, transcription factors, translation initiation factors, cofactors etc, which leads to termination of DNA synthesis, transcription and translation process ultimately to cell death.

Six Main Families of PAMPS:

Thionins: Thionins are positively charged with molecular weight about 5 kDa, rich in arginine, lysine, cysteine residues. Their structure comprises of two antiparallel α - helices and an antiparallel double-stranded β -sheet accompanying three or four disulfide linkages. The groove between the α -helices and β -sheets possess the Tyr 13 residue which may interact with lipid molecules of the membrane, leading to cell disruption and ultimately to cell lysis^{12, 29}. Thionins are subdivided into five classes- Type I, Type- II, Type- III, Type-IV and Type-V. Thionins show toxicity towards bacteria³⁰, fungi³¹, animal cell and insect larvae^{32, 33}. α/β thionins consist of eight or six conserved cystine residues which hereafter will be referred to as 8C or 6C thionins³⁴.

These peptides are secreted as preproteins and prothionin domain is bordered by conserved sequences of N- terminal signaling peptides and C-terminal acidic domain³⁵⁻³⁷. Thionins consisting of eight cystine residues, stabilized by four disulfide bonds between Cys I- Cys VIII linking β 1 to C-terminal coil, Cys II- CysVII linking the end of β 1

with beginning of β 2, Cys III-Cys VI linking α 1 and loop after α 2 and Cys IV- Cys V linking α 1 and α 2. Whereas, thionins having six cystine residues are stabilized by three disulfide bridges. In these peptides, Cys II – CysVII disulphide bond is absent^{34, 38 - 40}. Among all the AMPs isolated from the plants, the largest group belongs to families-thionin and defensin. The first basic polypeptides Purothionin was isolated from endosperm of wheat (*Triticum aestivum*) and other cereal species, which had ability to inhibit the growth of selected phytopathogens bacteria of genus *Pseudomonas*, *Xanthomonas* and *Corynebacterium*.

Using carboxy methylcellulose column chromatography, α and β purothionins were obtained from partially purified crude extract of purothionin³⁰. *Pyricularia* thionin was isolated from the nuts of *Pyricularia pubera*³². This peptide was basic in nature, comprises of 47 amino acids including two tyrosine residues and 4 disulfide bonds. This peptide was known for hemolytic, cytotoxic and neurotoxicity activity. In their study, they showed that iodination of *pyricularia* thionin leads to loss of hemolysis, cytotoxic activity and lethality in mice. This peptide causes membrane alteration by depolarization and a channel-mediated influx of Ca^{2+} ions, which in turn activates phospholipase A2 that results in loss of membrane integrity and ultimately cell death. This study indicated that iodination leads to inhibition of above mentioned all three cellular responses, which suggested the role of tyrosine either in preservation of peptide structure or in the association of peptide with cellular membrane.

Duvick and coworkers isolated MBP- 1 antimicrobial peptide belonging to thionin family of AMPs from maize kernels⁴¹. This peptide comprises of 33 amino acid residues (4127.08 Da). MBP-1 inhibits spore germination or hyphal elongation of *Fusarium moniliforme* Sheld. and *Fusarium graminearum*. These peptides also showed antibacterial activity against *Clauibacter michiganense* sp. *Nebraskense*. Novel thionin antimicrobial peptides, Tu-AMP1 and Tu-AMP2 of molecular weight 4,988Da and 5,006Da respectively were purified from *Tulipa gesneriana* L. bulbs using chitin affinity chromatography and reverse-phase high performance liquid chromatography (HPLC)⁴².

Chitin-binding peptides Pp-AMP 1 and Pp-AMP 2, which had antimicrobial activity against pathogenic bacteria and fungi, were purified from *Phyllostachys pubescens* (Japanese bamboo shoots) using a simple chitin affinity chromatography procedure. Pp-AMP1 and Pp-AMP2 showed homology with mistletoe toxins⁴³. Cytotoxic thionin peptides viscotoxin A1 and B1 were isolated and crystallized from Mistletoe and during formation process both the proteins form dimers. During dimer formation, Viscotoxin B2 (net charge +4) showed coordination with sulfate or phosphate anions whereas no correlation was found with Viscotoxin A1 (net charge +6)⁴⁴. Viscotoxins isolated from European mistletoe (*Viscum album*) was found to be active against phytopathogenic fungi and cancer cells⁴⁵.

Viscotoxin peptides were fused with 13 different proteins {His6 tag and His6-tagged versions of GB1 (protein G), ZZ tag ([glutamate/glutamine]² amino acid tag), Z tag (glutamate/glutamine amino acid tag), MBP (maltose binding protein), NusA (N utilisation substance protein A), glutathione S-transferase, thioredoxin, GFP (green fluorescent protein), along with periplasmic and cytosolic versions of DsbC (Disulfide bond C) and DsbA (disulfide-bond A oxidoreductase-like protein) as a fusion partners for their expression in *Escherichia coli* cells. Highest yield of soluble viscotoxin was observed in Viscotoxin fused with thioredoxin protein. Recombinant viscotoxins prepared using above method was found to be toxic against HeLa cells. Some of the other thionins are listed in **Table 1**.

TABLE 1: LIST OF THIONIN PEPTIDES SUBMITTED TO PHYTAMP DATABASE HAVING ANTIMICROBIAL ACTIVITY AGAINST TARGET ORGANISM

S. no.	Name	Organism	Activity	References
1	Viscotoxin-B (Viscotoxin-B2(VtB) Viscotoxin-a3 (Vta3)	<i>Viscum album</i>	Antifungal	46 - 48
2	Pp-AMP1 Pp-AMP2	<i>Phyllostachys pubescens</i>	Antibacterial Antifungal	43
3	Tu-AMP1	<i>Tulipa gesneriana</i>	Antibacterial Antifungal	42

Defensins: Defensins are small (ca. 5 kDa) ubiquitous, cationic peptide ranging from 45 to 54 amino acids. These peptides are expressed in various plant tissues, however they are present in ample amount in seeds. Defensin peptides stored in seeds protect them from fungal infection during seed germination and increase the seed survival rate^{49, 50}. Earlier defensins were classified as γ -thionins, γ 1/ γ 2 purothionins because of 32 - 36% sequence similarity with α/β thionins^{51, 52}. The three-dimensional structure of plant defensins shares similarities with those of insect defensins and scorpion toxins¹⁴.

A number of AMPs from the defensin family have been isolated from various plant sources. A 5kDa polypeptide, Pseudothionin-St1 (Pth-St1) was isolated from *Solanum tuberosum*. Pth-St1 peptide was found to be active against *Clavibacter michiganensis*, *Pseudomonas solanaceum* and *Fusarium solani*. Pseudothionin did not show any characteristic feature similar to true thionins. Now pseudothionin has been reclassified under defensins⁵³. In 1996, Thevissen and his co-workers isolated antifungal defensin Rs-AFP2 and Dm-AMP1 from

seeds of radish and dahlia respectively⁵⁴. When hyphae of *Neurospora crassa* were treated with Rs AFP2 and Dm-AMP1, rapid efflux of K⁺ ion, influx of Ca²⁺ ion, change in membrane potential of fungal cell and alkalization of medium took place, which led to busting of fungal cell. Rs-AFP2 leads to hyperpolarization whereas Dm-AMP1 causes depolarization in the membrane of *N. crassa*. An antifungal peptide Dm-AMP1 was isolated from seeds of *Dahlia merckii*, showing the IC₅₀ to be approximately 2 μ M against *Saccharomyces cerevisiae*⁵⁵. Antifungal peptides were radioactively labeled with t-butoxycarbonyl-[³⁵S]-L-methionine N-hydroxy-succinimidylester (Boc-Meth-NHS) to produce [³⁵S] Dm-AMP1. [³⁵S] Dm-AMP1 peptide showed antifungal activity similar to Dm-AMP1 peptide.

This indicated that addition of ³⁵ S to Dm-AMP1 did not cause any change in binding sites of the peptides. Dm-AMP1 binds to specific binding sites present on the plasma membrane of fungi, which leads to cell death. This work enables tracking and mode of action of defensin peptides in various organisms without hampering their activity.

Thevissen *et al.*, reported that Rs- AFP2 interact with glucosylceramides present in the membrane of fungi and yeast cell, which triggers membrane permeabilization and ultimately leads to cell death⁵⁶. *Petunia hybrida* defensin 1 (PhD1) was isolated from the flowers of *Petunia hybrida*, consisting of 47 cystine rich amino acid residues stabilized by five disulfides bonds. The structure of PhD1 was determined by ¹H NMR spectroscopy which reveals that it comprises of an α - helix, a triple stranded anti-parallel β - sheet and cystine stabilized $\alpha \beta$ motif folds⁵⁷. *Vigna radiata* (mung bean) defensin 1 (VrD1) was proclaimed as the first plant defensin showing insecticidal activity⁵⁸. The VrD1 structure consist of α -helix, triple-stranded antiparallel β -sheet and 3_{10} helix stabilized by four disulfide bridges which layout a characteristic cysteine-stabilized $\alpha \beta$ motif. VrD1 inhibits α -amylase activity in *Tenebrio molitor*. Production of α -amylase inhibitor 1 in transgenic pea also showed complete resistance against bruchids.

Plant antifungal peptides RsAFP2, HsAFP1 and PvD1 was isolated from *Raphanus sativus*, *Heuchera sanguinea* and *Phaseolus vulgaris* respectively had shown inhibition in the growth of *S. cerevisiae* and *Candida albicans*. Binding of peptide with glucosylceramides (GlcCer) present in the membrane of fungi and yeast cells causes

oxidative damage to cell, related to induction of reactive oxygen species (ROS) and Nitric oxide (NO) production⁵⁹⁻⁶¹.

NaD1 defensin from *Nicotiana alata* showed antifungal activity against *Candida albicans*⁶². NaD1 interact with fungal cell wall and cause membrane permeabilization. When NaD1 reaches cytoplasm, it leads to hyper-production of Reactive Oxygen Species (ROS) that induce oxidation damage. High osmolarity glycerol (HOG) pathway was identified which play an important role in protection of fungal cell against NaD1. This indicated that HOG pathway could be a suitable target site for increasing the effectiveness of the antimicrobial peptides against *Candida albicans*.

Putative defensin gene was isolated from *Vigna unguiculata* (cowpea). Sequence, amino acid arrangement, splicing analysis, secondary and tertiary structures of mature putative defensin peptide showed similarity towards a typical defensin peptide. Response of biotic stimuli on pathogen treated and untreated plants were examined by the expression level of defensin gene, using RT-PCR⁶³. A list of defensin peptides, their origin and action against specific microorganisms is listed in **Table 2**.

TABLE 2: LIST OF DEFENSIN PEPTIDES SUBMITTED TO PHYTAMP DATABASE HAVING ANTIMICROBIAL ACTIVITY AGAINST TARGET ORGANISM

S. no.	Name	Organism	Activity	Target organism	References
1	Floral defensin- like protein 1 (PhD1)	<i>Petunia hybrida</i>	Antifungal	<i>Fusarium</i> <i>Oxysporum</i> ,	64
	Floral defensin- like protein 2 (PhD2)			<i>Botrytis cinerea</i>	
2	VrD1	<i>Vigna radiata</i>	Antibacterial Antifungal	<i>Escherichia coli</i> <i>Rhizoctonia solani</i>	65
3	Defensin J1-1 Defensin J1-2	<i>Capsicum annuum</i>	Antifungal	<i>Fusarium oxysporim</i> , <i>Botrytis cinera</i>	66, 67
4	Antifungal protein AX 1 Antifungal protein AX2	<i>Beta vulgaris</i>	Antifungal	<i>Cercospora beticola</i> and other filamentous fungi	68
5	Fabatin-1 Fabatin- 2	<i>Vicia faba</i>	Antibacterial	<i>Bacillus subtilis</i> , <i>Enterococcus hirae</i> , <i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i>	69
6	Rs-AFP1 Rs-AFP2	<i>Raphanus sativus</i>	Antifungal	<i>Alternaria brassicola</i> , <i>Botrytis cinerea</i> , <i>Fusarium culmorum</i> , <i>Fusarium oxysporum</i> , <i>Pyricularia oryzae</i> , <i>Verticillium dahlia</i> ,	51, 70, 71
7	Flower specific gamma-thionin (Nad1)	<i>Nicotiana tabacum</i>	Antifungal	<i>Fusarium oxysporum</i> <i>Botrytis cinerea</i>	72, 73

Cyclotides: Cyclotides are largest family of circular proteins, comprising of 28 to 37 amino acid residues. These peptides contain cyclic cystine knot (CCK) motif created by six conserved cysteine residues. Apart from antimicrobial activity, cyclotides also shows insecticidal and anti-cancerous activity⁷⁴. First water soluble oxytocic polypeptide, kalata B1 of approximately 30 amino acids was isolated from the African plant *Oldenlandia affinis*⁷⁵. The natives used this plant extract to stimulate childbirth during labor, therefore kalata B1 was identified as the main uterotonic component of the plant. Another novel cyclotide 'kalata B8' was also isolated from *Oldenlandia affinis*⁷⁶. Kalata B8 displays anti-HIV activity but lacks haemolytic activity. This may be due to the hydrophilic nature of peptide. Two antimicrobial peptides Mj-AMP1 and Mj-AMP2 were isolated from the seeds of *Mirabilis jalapa* L. Mj-AMP1 and Mj-AMP2 peptides comprises of 37 and 36 residues respectively⁷⁷.

These are basic in nature and consist of three disulfide bridges. These peptides show broad-spectrum antifungal activity and anti gram-positive bacterial activity whereas no activity was observed against gram-negative bacteria and cultured human cell. Sequence of Mj-APMs showed similarity to μ -agatoxins, insecticidal neurotoxic peptide from spider venom and despite this homology Mj-AMPs did not affect pulse transmission in insect nerves even after many fold increase in the concentration. Four macrocyclic cystine-knot peptides, kalata, circulin A and B (CirA and CirB) and cyclopsychotride were extracted from coffee plants

⁷⁸. Kalata and CirA were found to be active against *Staphylococcus aureus* with MIC of 0.2 μ M.

However, kalata and Cir A were inactive against *Escherichia coli* and *Pseudomonas aeruginosa*. However, CirB and cyclopsychotride were found to be effective against both Gram-positive and Gram-negative bacteria. All the four cyclic peptides were slightly effective against *Candida kefyr* and *Candida tropicalis*, but were ineffective against *Candida albicans*.

Four novel cyclotides (macrocyclic knotted proteins) were isolated from *Viola hederaceae* (vhl)⁷⁹. vhl- 1 was leaf- specific cyclotide with 31-residue. EC50 for vhl-1 was found to be 0.87M against HIV- virus. Ribosome-inactivating peptide, Luffin P1 was isolated from the seeds of *Luffa cylindrical*⁸⁰. Luffin P1 was found to be effective against HIV 1 infected C8166T cell lines. Luffin P1 structure consists of helix-loop-helix motif and alpha helices held together by two-disulfide bond. Luffin P1 employed N-glycosidase activity to kill HIV infected cells. Trypsin inhibitor (BWI-2c) was isolated from the seeds of *Fagopyrum esculentum* (buckwheat)⁸¹.

BWI-2c peptide comprises of 41 amino acid residues with two disulfide bonds and shows structural similarity with VhT1 peptide isolated from *Veronica hederifolia*. Both these represents new family of protease inhibitors. **Table 3** depicts cyclotides peptides submitted to PhytAMP Database having antimicrobial activity against target organism.

TABLE 3: LIST OF CYCLOTIDES PEPTIDES SUBMITTED TO PHYTAMP DATABASE HAVING ANTIMICROBIAL ACTIVITY AGAINST TARGET ORGANISM

S. no.	Name	Organism	Activity	Target organism	References
1	Cycloviolacin H-2 Cycloviolacin H-3	<i>Viola hederaceae</i>	Antiviral	HIV virus	79
2	Kalata- B8	<i>Oldenlandia affinis</i>	Antiviral	HIV virus	76
3	Circulin – F (CIRF) Circulin – E (CIRE) Circulin – D(CIRD) Circulin – C(CIRC)	<i>Chassalia parviflora</i>	Antiviral	HIV virus	82
4	Cycloviolin- A Cycloviolin- B Cycloviolin- C Cycloviolin- D	<i>Leonis cymosa</i>	Antiviral	HIV-1 virus	83
5	Kalata- B2	<i>Oldenlandia affinis</i>	Insecticidal	<i>Helicoverpa armigera</i>	84, 85
6	Circulin-A Circulin-B	<i>Chassalia parviflora</i>	Antibacterial Antifungal	<i>Micrococcus luteus</i> , <i>Escherichia coli</i> , <i>Proteus vulgaris</i> , <i>Klebsiella oxytoca</i> <i>Candida albicans</i> , <i>Candida kefyr</i> , <i>Candida tropicalis</i>	86 - 89

Snakin: Snakins Protein (SN) represents a unique family of cysteine-rich plant antimicrobial peptides, present in both monocotyledonous and dicotyledonous species. Peptides belonging to this family have six potential disulfides bridges as compared to the other characterized families, which have two or four disulfide bridges. The amino acid sequence of snakin-1 peptide shows similarity towards hemotoxic desintegrin-like snake venoms⁹⁰. An antifungal basic 9kD peptide isolated from the seeds of *Raphanus sativus* (radish) having sequence similarity with nonspecific lipid transfer proteins isolated from other plant species. Later, this peptide was found to be the member of snakins family⁹¹.

Peptide showed antifungal activity against several fungi. Snakin-1 (StSN1) with 63 amino acid residues was isolated from the potato tubers⁹⁰. This was highly basic and had a short, central hydrophobic stretch of 25 to 30 amino acid residues, surrounded by highly polar, long N-terminal and C-terminal domains. This peptide was found to be active against bacterial and fungal plant pathogens. Sequence motifs of StSN1 showed similarity with hemotoxic 'kistrin' snake venom.

Similarly, an isolated snakin-2 (StSN2) peptide (7,025 Da) from potato (*Solanum tuberosum* cv Jaerla) tubers which was basic in nature and consist of 66 amino acid residues. StSN2 was effective against all the selected fungus and gram-positive bacterial pathogens for potato plant whereas it was observed to be ineffective against some of the Gram-negative bacteria⁹². cDNA of mature StSN2 peptide encoded for signal sequence which is preceded by a sequence of 15 acidic residues. St-PTH1 defensin and StSN1 snakin peptides accumulated at high concentrations together with StSN2 snakin peptide and were effective against bacterial and fungal pathogens for potato plants^{53, 90, 92}.

Snakin- Z peptide composed of 31 amino acid with molecular weight 3318.82Da from the fruit of *Zizyphus jujube* showed antimicrobial activity against bacteria and fungi whereas it was found to be ineffective against human red blood cells⁹³. This indicated that Snakin - Z could be a suitable candidate of antimicrobial peptides for therapeutic applications. Various other snakin peptides are listed in **Table 4**.

TABLE 4: LIST OF SNAKIN PEPTIDES SUBMITTED TO PHYTAMP DATABASE HAVING ANTIMICROBIAL ACTIVITY AGAINST TARGET ORGANISM

S. no.	Name	Organism	Activity	Target organism	References
1	Snakin-1 (StSN1)	<i>Solanum tuberosum</i>	Antibacterial	<i>Clavibacter michiganensis</i> , <i>Ralsotonia solanacearum</i>	90, 92
	Snakin-2 (StSN2)		Antifungal	<i>Botrytis cinerea</i> , <i>Fusarium solani</i> , <i>Fusarium culmorum</i> , <i>Fusarium oxysporum</i> f. sp. <i>Lycopersici</i> , <i>Plectosphaerella cucumeria</i> , <i>Collectotrichum graminicola</i> , <i>Aspergillus flavus</i>	

Hevein Like Peptides: Hevein like peptides are small 4.7 kDa (43 amino acid residues), monomeric, cysteine-rich and chitin-binding peptide. Hevein protein was first isolated and characterized from the latex of rubber tree (*Hevea brasiliensis*)⁹⁴. These peptides consist of 19.2% cystine amino acid residues. This protein inhibits the hyphal growth of fungi by binding to chitin⁹⁵. Avesin A peptide extracted from oat seeds has conserved domain containing a common structural motif of 37 amino acids with several cysteine and glycine residues and chitin binding domain. Avesin A showed mild antifungal activity on some selected fungal strains⁹⁶. Hevein like peptide Wj AMP-1 was isolated from leaves of *Wasabia japonica* L

⁹⁷. Wj AMP-1 showed antifungal as well as antibacterial activity. Wj AMP-1 showed 60% and 70 % sequence similarity towards hevein like protein isolated from *Hevea brasiliensis* and *Arabidopsis thaliana*. Two hevein homologues (Pn-AMP1 and Pn-AMP2) was extracted from *Pharbitis nil* with antifungal activities against both chitin and non-chitin containing fungi.

When cDNA of Pn-AMP2 was expressed in tomato plants under the regulation of CaMV35S promoter, the transgenic plants showed increased resistance towards non-chitinous fungus as well as chitin-containing fungus⁹⁸. This suggested that chitin might not be the only component for Pn-AMP's antifungal activity. Eucommia antifungal peptide2

(EAFP2) was obtained from *Eucommia ulmoides* olive, having chitin binding domain with a hydrophobic face and a characteristic disulfide bond between N- terminal and C- terminal residues⁹⁹. Novel antimicrobial peptides WAMP-1a and WAMP-1b were isolated from seeds of *Triticum kiharae*¹⁰⁰. WAMP-1a and WAMP-1b differ from each other by a single C- terminal amino acid residue. Recombinant WAMP-1a was produced in *Escherichia coli*, possessing high broad-spectrum inhibitory activity against varied chitin-containing and chitin-free pathogens. A chitin binding polypeptide WAMP-1a was isolated from *Triticum kiharae*, which showed similarity towards hevein like peptides¹⁰¹. WAMP-1a peptide showed antifungal and

antibacterial activity. WAMP-1a sequence features a substitution of conserved serine residue with glycine residue that decreases its carbohydrate binding efficiency. Six cysteine rich, hevein like peptides, aSG1-G3 and aSR1-R3, was isolated and characterized from various green and red varieties of *Alternanthera sessilis*¹⁰². Proteomic analysis revealed the presence of six cysteine, seven glycine, four proline residues and a conserved chitin-binding domain. Nuclear magnetic resonance (NMR) study showed that three disulphide bonds were present in a cysteine knot motif. **Table 5** showing some of the hevein like peptides having antimicrobial activity submitted to PhytAMP Database.

TABLE 5: LIST OF HEVEIN LIKE PEPTIDES SUBMITTED TO PHYTAMP DATABASE HAVING ANTIMICROBIAL ACTIVITY AGAINST TARGET ORGANISM

S. no.	Name	Organism	Activity	Target organism	References
1	AC-AMP1 AC-AMP2	<i>Amaranthus caudatus</i>	Antibacterial Antifungal	Gram positive bacteria: <i>Bacillus megaterium</i> , <i>Sarcina lutea</i> <i>Alternaria brassicola</i> , <i>Ascomyces pisi</i> , <i>Botrytis cinera</i> , <i>Fusarium culmorum</i>	103
2	Ee-CBP (Bark) Ee-CBP (leaves)	<i>Euonymus europaeus</i>	Antifungal	<i>Fusarium culmorum</i> , <i>Fusarium oxysporum</i> , <i>Mycosphaerella eumusae</i> , <i>Neurospora crassa</i>	104
3	Ar-AMP	<i>Amaranthus retroflexus</i>	Antifungal	<i>B. cinerrea</i> , <i>F. Culmorum</i> , <i>H. satium</i> , <i>A. consortiale</i>	105
4	EAFP2 EAFP1	<i>Eucommis ulmoides</i>	Antifungal	<i>Phytophthora infestans</i> , <i>Ascochyta lycopersici</i> , <i>Alternaria nicotiana</i> , <i>Fusarium moniliforme</i> , <i>Fusarium oxysporum</i>	99, 106, 107
5	PN-AMP-1 PN-AMP-2	<i>Ipomoea nil</i>	Antibacterial Antifungal	<i>Bacillus subtilis</i> <i>Botrytis cinerea</i> , <i>Fusarium oxysporum</i> , <i>Phytophthora parasitica</i> , <i>Pythium sp.</i>	108
6	Fa-AMP1 Fa-AMP2	<i>Fagopyrum esculentum</i>	Anti yeast Antibacterial Antifungal	<i>Saccharomyces cerevisiae</i> <i>Clavibacter michiganensis</i> , <i>Erwinia carotovora</i> , <i>Agrobacterium radiobacter</i> , <i>Agrobacterium rhizogenes</i> <i>Fusarium oxysporum</i> , <i>Geotrichum candidum</i>	109

Lipid transfer proteins (LTPs): The nonspecific lipid transfer proteins (ns-LTPs) are small proteins (approximately 8.7 KDa) composed of 90 amino acids preserved by four disulfide bonds accompanying central hydrophobic shaft. Due to small size of LTPs, they are able to penetrate through the fungal membrane and create pores that lead to efflux of intracellular ions and finally cell death¹¹⁰.

Tassin *et al.*, isolated antifungal *Ace*-AMP1 peptide from the seeds of onion¹¹¹. NMR spectroscopy revealed that *Ace*-AMP1 peptide comprises of 93 amino acid residues and four disulfide bonds. Structure of *Ace*-AMP1 peptide consist of four

helices connected by three loops and a C-terminal tail without secondary structures, except for 3₁₀-helix turn and a β turn.

LTPs of 9 kDa cysteine-rich cationic peptide was isolated from *Vigna unguiculata* seeds¹¹². These peptides play an important role in plant defense mechanism against microbial infection. Lin *et al.*, isolated a novel antifungal lipid transfer peptide from the seeds of *Brassica campestris*¹¹³. This peptide hinders the mycelial growth in *Fusarium oxysporum* and *Mycosphaerella arachidicola*. Similarly, lipid like protein of approx. 9 kDa was isolated from the seeds of chilli pepper¹¹⁴.

This peptide showed antifungal activity against *Fusarium oxysporum*, *Colletotrium lindemuthianum*, *Saccharomyces cerevisiae*, *Pichia membranifaciens*, *Candida tropicalis* and *Candida albicans*. This

peptide causes multiple changes in the morphology of *P. membranifaciens*. Some of the other lipid transfer peptides are listed in **Table 6**.

TABLE 6: LIST OF LIPID TRANSFER PEPTIDES SUBMITTED TO PHYTAMP DATABASE HAVING ANTIMICROBIAL ACTIVITY AGAINST TARGET ORGANISM

S. no.	Name	Organism	Activity	Target organism	References
1	La-LTP (LJAFP)	<i>Leonurus artemisia</i>	Antibacterial	<i>Bacillus subtilis</i> <i>Pseudomonas solanacearum</i> <i>Ralstonia solanacearum</i>	115
			Antifungal	<i>Alternaria alternate</i> , <i>Alternaria brassicae</i> , <i>Aspergillus niger</i> , <i>Bipolaris maydis</i> , <i>Botrytis cinerea</i> , <i>Cerospora personata</i> , <i>Colletotrichum gloeosporioides</i>	
2	Hv- LTP Cw-18 (PKG2316) Hv-LTP-1 (LTP4.1) (CW21)	<i>Hordeum vulgare</i>	Antifungal	<i>Fusarium solani</i> , <i>Pseudomonas solanacearum</i> , <i>Clavibacter michiganensis</i>	116
3	IWF1 (Bv-LTP), JWF2 (Bv-LTP2)	<i>Beta vulgaris</i>	Antifungal	<i>Cercospora beticola</i>	117
4	Ace-AMP1	<i>Allium cepa</i>	Antibacterial	<i>Bacillus megaterium</i> <i>Sarcina lutea</i>	118
			Antifungal	<i>Alternaria brassicicola</i> , <i>Ascochyta pisi</i> <i>Botrytis cinerea</i> , <i>Colletotrichum lindemuthianum</i> , <i>Fusarium culmorum</i> , <i>Pyricularia oryzae</i>	
5	Pa-LTP1	<i>Phaseolus aureus</i>	Antibacterial Antifungal	<i>Staphylococcus aureus</i> <i>Fusarium oxysporum</i> , <i>Pythium aphanidermatum</i> , <i>Sclerotium rolfsii</i>	119,120
6	Lc-LTP4, Lc-LTP8, Lc-LTP6, Lc-LTP5, Lc-LTP2	<i>Lens culinaris</i>	Antibacterial	<i>A.tumefaciens</i>	121

Some Other Classes of PAMPS: Some of the uncategorized AMPs have been isolated and characterized by various groups of scientists. Storage glycine-rich peptide (Pg-AMP1) of 6029.24Da was isolated from *Psidium guajava* (guava). PgAMPs1 showed antimicrobial activity against *Klebsiella* sp. and *Proteus* sp. However, no activity was observed against fungi. 3-D structure of PgAMP1 exhibits homology to enterotoxin, antibacterial proteins from *Escherichia coli* ¹²².

Cytotoxic peptide was partially purified from protein hydrolysate of *Euphorbia hirta* against gastric cancer. These bioactive peptides comprise of 2-10 amino acid residues. Protein hydrolysate of *Euphorbia hirta* was ultra filtrated to obtain low molecular weight (3KDa) peptides. Due to its small size, these peptides show immense biological effects and increase the probability to cross the intestinal barrier ¹²³. The chemically synthesized AMP is Shepherin I (Shep I), a glycine rich peptide

¹²⁴. It was synthesized by solid- Phase method at 60⁰C using conventional heating system. This peptide was effective against *Candida* species, but ineffective against bacteria and mycelia fungi. Truncation of the N- or C- terminal portion of Shep I decreases its antifungal activity. Carboxyamidation of *Shep I* did not affect the antifungal activity but increased activity against *Saccharomyces cerevisiae*.

A cysteine peptidase (Cg24-I) of 24,118 Da was absolved from the latex of *Cryptostegia grandiflora* using ion exchange and Reverse-phase high performance liquid chromatography (HPLC). At a dose of 28.1µg/ml Cg24-I inhibited growth of *Fusarium solani* ¹²⁵.

Mandal et al., isolated *Cn*-AMP1, *Cn*-AMP2 and *Cn*-AMP3 peptides with molecular weight less than 3kDa (858Da, 1249Da and 950 Da respectively) from green coconut (*Cocos nucifera* L.) water

using reversed phase-high performance liquid chromatography (HPLC)¹²⁶. *Cn*-AMP1 peptide was found to be more effective against both Gram-positive and Gram-negative bacteria when compared to *Cn*-AMP2 and *Cn*-AMP3 peptides. Antimicrobial peptides were extracted from six Indian medicinal plants (*Foeniculum vulgare*, *Cucumis sativus*, *Ammi majus*, *Allium ascolanicum*, *Cichorium intybus* and *Rumex vesicarius*). Protein extracts were prepared in sodium phosphate citrate buffer (pH 5.2, 5.8, 6.8, 7.4 and 7.8) and sodium acetate buffer (pH 6.5). Strong antimicrobial activity was observed in *Allium ascolanicum* seed extracts at pH (5.8) against *Proteus vulgaris*, *Escherichia coli* and *Staphylococcus aureus* with zone of inhibition 17 mm, 17 mm and 15 mm respectively. Antibacterial activity was also reported in seed extracts of *Rumex vesicarius* at pH (7.6), *Ammi majus* at pH (6.8), *Cichorium intybus* at pH (7.4), *Cucumis sativus* at pH (7.8) and *Foeniculum vulgare* at pH (6.5)¹²⁷.

Golla et al., screened 50 different seeds such as soya, barley, maize, jowar, paddy, millets, foxtail millets, red gram, green gram, black gram, groundnut, pea, field bean and wheat for the presence of small peptides having antimicrobial property¹²⁸. Protein was extracted from seeds using both liquid nitrogen and phosphate buffer (PBS) treatments. Antimicrobial activity was performed against four clinically important microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Protein extracts from germinated seeds of barley, soya, jowar, wheat and maize showed antimicrobial activity against both gram-positive and gram-negative bacteria.

Cationic bioactive peptide "Hispidalin" was extracted from the seeds of *Benincasa hispida*. This peptide was 5.7 kDa (49 amino acid residues), basic and amphipathic¹²⁹. It does not comprise of any sulphur containing amino acid, which suggested that it does not have any secondary structure. Hispidalin was different from plant defensin and found 66% similarity towards peptide isolated from *Aeromonas veronii*, a gram-negative bacteria. Hispidalin showed antimicrobial activity against various bacterial and fungal pathogens. Total 273 antimicrobial peptides have been characterized as per the PhytAMP database¹³⁰.

PhytAMP database is resource that encloses beneficial information (taxonomic, microbiological and physicochemical data) about plant antimicrobial peptides. This database comprises of 271 entries, secreted by various plant families predominantly by Violaceae and Brassicaceae. Out of total entries, only 102 peptides (37.63%) were reported with 3-D structure. Only 39.5% of reported plants AMPs were certified for biological activities. Most of the AMPs showed antifungal (51%) activity as compared to antibacterial (33%) and antiviral (10%) activities. These findings show the significance for isolation and characterization of novel plant AMPs with potency against microorganisms.

Indian plant biodiversity constitute 11.7% of the world flora, of which 28% plants are endemic and more than 6000 plant species (edible and non-edible) are known for their use in Indian System of Medicine¹³¹. Despite this rich biodiversity, few reports are available on isolation and characterization of PAMPs from Indian subcontinent.

CONCLUSION: The studies carried out until date unlocks the possibility for the use of edible and non-edible parts of plant for the extraction of antimicrobial peptides, which may be suitable candidates in the treatment of various human diseases. Since the various members of PAMPs belonging to different families have multifarious mechanism of action as well as different target molecules in organism, they may be chosen, used or modified against specific pathogenic microorganism, insects and pests. AMPs offer a favourable alternative for treating infections in relation to traditional antibiotics based on their spectrum activity and efficiency. Small genes with conserved sequences encode AMPs therefore, gene amplification and transgenesis are one of the feasible ways to increase the production and enhance specific activity of selected peptides.

Studying AMPs not only contributes to the evolution of new drugs by manufacturing analogs and by-products of natural antimicrobial peptides for medical purpose but also in genetic up-gradation of plants by increasing resistance towards pathogens. Increasing the pathogenic resistance, leads to decrease in the prerequisite of enlarge

quantities of pesticide used in agriculture. Therefore, AMPs present newer eco-friendly blueprint for natural antibiotics, as therapeutic use in healthcare, as alternative to their chemical counterparts and safeguards plants against pests and pathogens.

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