IJPSR (2018), Vol. 9, Issue 1 (Review Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 May, 2017; received in revised form, 31 August, 2017; accepted, 17 September, 2017; published 01 January, 2018

PLANT ANTIMICROBIAL PEPTIDES: A NOVEL APPROACH AGAINST DRUG RESISTANT MICROORGANISMS

Samriti [1], Rajesh Biswas [2] and Kakoli Biswas[*1]

Department of Biotechnology [1], DAV College, Sector- 10, Chandigarh - 160011, Punjab, India.
Department of Zoology [2], Government Home Science College, Sector- 10, Chandigarh - 160011, Punjab, India.

Keywords:

Plant Antimicrobial peptide, Thionins, Defensins, Haveins, Lipid transfer protein, Snakins

Correspondence to Author:
Dr. Kakoli Biswas

Assistant Professor,
Head of Biotechnology Department, DAV College, Sector - 10, Chandigarh - 160011, Punjab, India.

E-mail: kakolibiswas14@gmail.com

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 permeabillization, 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].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(1).1-15

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(1).1-15

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 strategies[5]. 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

source of antimicrobial compounds, among these, plant AMPs play a vital (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 peptides [11] 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 structure [12].

The activities of PAMPs act primarily against microorganisms but some members of AMPs can act against herbivorous insects as targets [13]. 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 charges [15]. PAMPs comprises of six major families. These are thionins, defensins, lipid transfer proteins, cyclotides, snakins and hevein-like peptides [12].

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 AMPs [19]. 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 destruction [20]. 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].

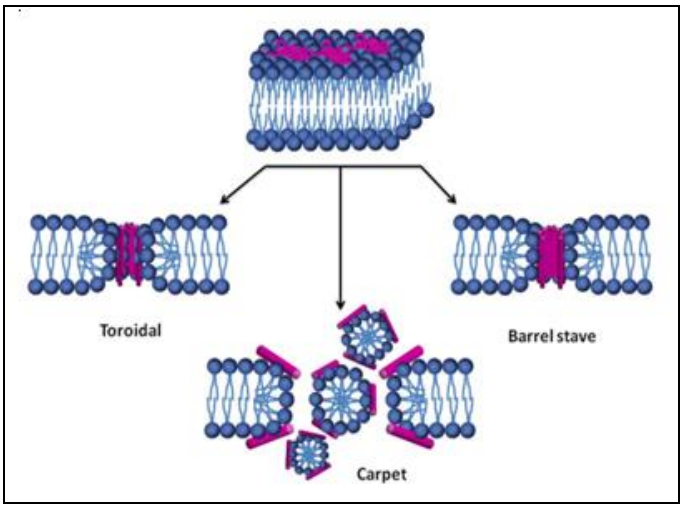

FIG. 1: DIFFERENT MODELS SHOWING ACTION OF ANTIMICROBIAL PEPTIDES (AMPs) CAUSING LIPID MEMBRANE PERMEABILIZATION

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 collapse [19].

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 permeablization, indicating that non-membranolytic mechanisms are responsible for cell death. These non- membranolytic mechanism 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 posses 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 bacteria [30], fungi [31], 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 thionins [34].

These peptides are secreted as preproteins and prothionin domain is bordered by conserved sequences of N- terminal signaling peptides and C-terminal acidic domain [35-37]. Thionins consisting of eight cystine residues, stabilized by four disulfide bonds between Cys I- Cys VIII linking β1 to C-terminal coil, Cys II- CysVII linking the end of β1 with beginning of β2, Cys III-Cys VI linking α1 and loop after α2 and Cys IV- Cys V linking α1 and α2. 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 defensisn. 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 Pseudo-monas, Xanthomonas and Corynebacterium.

Using carboxy methylcellulose column chromatography, α and β purothionins were obtained from partially purified crude extract of purothionin [30]. Pyrularia thionin was isolated from the nuts of *Pyrularia 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 pyrularia 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 kernels [41]. 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 toxins [43]. 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 cells [45].

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 Viocotoxin 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)	*Viscum album*	Antifungal	46 - 48
2	Pp-AMP1 Pp-AMP2	*Phyllostachys pubescens*	Antibacterial Antifungal	43
3	Tu-AMP1	*Tulipa gesneriana*	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 ϒ-thionins, ϒ1/ ϒ2 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 toxins [14].

A number of AMPs from the defensin family have been isolated from various plant sources. A 5kDa polypeptide, Pseudothionin-St1 (Pth-Stl) was isolated from *Solanum tuberosum*. Pth-St1 peptide was found to be active against *Clavibacter michiganensis*, *Pseudomonas solanacerum* and *Fusarium solani*. Pseudothionin did not show any characteristic feature similar to true thionins. Now pseudothionin has been reclassified under defensins [53]. In 1996, Thevissen and his co-workers isolated antifungal defensin Rs-AFP2 and Dm-AMP1 from seeds of radish and dahlia respectively [54]. When hyphae of *Neurospora cassa* were treated with Rs AFP2 and Dm-AMP1, rapid efflux of K^+ ion, influx of Ca^{2+} 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 wheras Dm-AMP1 causes depolarization in the membrane of *N. crassa*. An antifungal peptide Dm-AMP1 was isolated from seeds of *Dahlia merckii*, showing the IC_{50} to be approximately 2 µM against *Saccharomyces cerevisiae* [55]. Antifungal peptides were radioactively labeled with t-butoxyczrbonyl-[^{35}S]-L-methionine N-hydroxy-succinimidylester (Boc-Meth-NHS) to produce [^{35}S] Dm-AMP1. [^{35}S] 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 death [56]. *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 ^{1}H NMR spectroscopy which reveals that it comprises of an α- helix, a triple stranded anti-parallel β- sheet and cystine stabilized α β motif folds [57]. *Vigna radiata* (mung bean) defensin 1 (VrD1) was proclaimed as the first plant defensin showing insecticidal activity [58]. 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 α β 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) production [59 - 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 uniguiculata* (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-PCR [63]. 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) Floral defensin- like protein 2 (PhD2)	*Petunia hybrida*	Antifungal	*Fusarium Oxysporum, Botryris cinerea*	64
2	VrD1	*Vigna radiata*	Antibacterial Antifungal	*Escherichia coli* *Rhizoctonia solani*	65
3	Defensin J1-1 Defensin J1-2	*Capsicum annuum*	Antifungal	*Fusarium oxysporim, Botrytis cinera*	66, 67
4	Antifungal protein AX 1 Antifungal protein AX2	*Beta vulgaris*	Antifungal	*Cercospora beticola* and other filamentous fungi	68
5	Fabatin-1 Fabatin- 2	*Vicia faba*	Antibacterial	*Bacillus subtilis, Enterococcus hirae, Escherichia coli Pseudomonas aeruginosa*	69
6	Rs-AFP1 Rs-AFP2	*Raphnus sativus*	Antifungal	*Alternaria brassicola, Botrytis cinerea, Fusarium culmorum, Fusarium oxysporum, Pyricularia oryzae, Verticiliium dahlia,*	51, 70, 71
7	Flower specific gamma-thionin (Nad1)	*Nicotiana tabacum*	Antifungal	*Fusarium oxysporum Botrytis cinerea*	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 activity [74]. 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 respectively [77].

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 μM.

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-didulfide 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	*Viola hederacea*	Antiviral	HIV virus	79
2	Kalata- B8	*Oldenlandia affinis*	Antiviral	HIV virus	76
3	Circulin – F (CIRF) Circulin – E (CIRE) Circulin – D(CIRD) Circulin – C(CIRC)	*Chassalia parviflora*	Antiviral	HIV virus	82
4	Cycloviolin- A Cycloviolin- B Cycloviolin- C Cycloviolin- D	*Leonis cymosa*	Antiviral	HIV-1 virus	83
5	Kalata- B2	*Oldenlandia affinis*	Insecticidal	*Hellicoverpa armigera*	84, 85
6	Circulin-A Circulin-B	*Chassalia parviflora*	Antibacterial Antifungal	*Micrococcus luteus, Escherichia coli, Proteus vulgaris, Klebsiella oxytoca* *Candida albicans, Candida kefyr, Candida tropicalis*	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 venoms [90]. 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 family [91].

Peptide showed antifungal activity against several fungi. Snakin-1 (StSN1) with 63 amino acid residues was isolated from the potato tubers [90]. 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 bacteria [92]. cDNA of mature StSN2 peptide encoded for signal sequence which is preceded by a sequence of 15 acidic residues. St-PTHl 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 cells [93]. 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)	*Solanum tuberosum*	Antibacterial	*Clavibacter michiganensis, Ralsotonia solanacearum*	90, 92
	Snakin-2 (StSN2)		Antifungal	*Botrytis cinerea, Fusarium solani, Fusarium culmorum, Fusarium oxysporum f.* sp. *Lycopersici, Plectosphaerella cucumeria, Collectotrichum graminicola, Aspergillus flavus*	

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 chitin [95]. 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 strains [96]. 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 brasiliansis* 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 fungus [98]. 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 residues [99]. 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 peptides [101]. 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	*Amaranthus caudatus*	Antibacterial	Gram positive bacteria: *Bacillus megaterium, Sarcina lutea*	103
			Antifungal	*Alternaria brassicola, Ascophyta pisi, Botrytis cinera, Fusarium culmorum*	
2	Ee-CBP (Bark) Ee-CBP (leaves)	*Euonymus europaeus*	Antifungal	*Fusarium culmorum, Fusarium oxysporum, Mycosphaerella eumusae, Neurospora crassa*	104
3	Ar-AMP	*Amaranthus retroflexus*	Antifungal	*B. cinerrea, F. Culmorum, H. satium, A. consortiale*	105
4	EAFP2 EAFP1	*Eucommis ulmoides*	Antifungal	*Phytophthora infestans, Ascochyta lycopersici, Alternaria nicotianae, Fusarium moniliforme, Fusarium oxysporum*	99, 106, 107
5	PN-AMP-1 PN-AMP-2	*Ipomoea nil*	Antibacterial	*Bacillus subtilis*	108
			Antifungal	*Botrytis cinerea, Fusarium oxysporum, Phytophthora parasitica, Pythium sp.*	
			Anti yeast	*Saccharomyces cerevisae*	
6	Fa-AMP1 Fa-AMP2	*Fagopyrum esculentum*	Antibacterial	*Clavibacter michiganesis, Erwinia carotovora, Agrobacterium radiobacter, Agrobacterium rhizogenes*	109
			Antifungal	*Fusarium oxysporum, Geotrichum candidum*	

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 death [110].

Tassin *et al*., isolated antifungal *Ace*-AMP1 peptide from the seeds of onion [111]. 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 3_{10}-helix turn and a β turn.

LTPs of 9 kDa cysteine-rich cationic peptide was isolated from *Vigna unguiculata* seeds [112]. 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 pepper [114].

This peptide showed antifungal activity against *Fusarium oxysporum, Colletotrium lindemunthianum, Saccharomyces cerevisiae, Pichia membranifaciens, Candida tropicali* 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)	*Leonurus artemisia*	Antibacterial	*Bacillus subtilis* *Pseudomonas solanacearum* *Ralstonia solanacearum*	115
			Antifungal	*Alternaria alternate, Alternaria brassicae, Aspergillus niger, Bipolaris maydis, Botrytis cinerea, Cerospora personata, Colletotrichum gloeosporiodes*	
2	Hv- LTP Cw-18 (PKG2316) Hv-LTP-1 (LTP4.1) (CW21)	*Hordeum vulgare*	Antifungal	*Fusarium solani, Pseudomonas solanacearum, Clavibacter michiganensis*	116
3	IWF1 (Bv-LTP), JWF2 (Bv-LTP2)	*Beta vulgaris*	Antifungal	*Cercospora beticola*	117
4	Ace-AMP1	*Allium cepa*	Antibacterial	*Bacillus megaterium* *Sarcina lutea*	118
			Antifungal	*Alternaria brassicola, Ascockyta pisi Botrytis cinerea, Colletotrickum lindemutkianum, Fusarium culmorum, Pyricularia oryzae*	
5	Pa-LTP1	*Phaseolus aureus*	Antibacterial Antifungal	*Staphylococcus aureus* *Fusarium oxysporum, Pythium aphanidermatum, Sclerotium rolfsii*	119,120
6	Lc-LTP4, Lc-LTP8, Lc-LTP6, Lc-LTP5, Lc-LTP2	*Lens culinaris*	Antibacterial	*A.tumefaciens*	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 60^0C 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. Carboxy-amidation 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 ascolinicum, 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 ascolinicum* 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 property [128]. 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 amphipathic [129]. 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 form *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 database [130]. 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 Brassicacea*e*. 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 Medicine [131]. 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.

ACKNOWLEDGEMENT: We thank Dr. B. C. Josan, Principal of DAV College and DAV management committee for providing infrastructure facilities.

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Centers for Disease Control and Prevention, Office of Infectious Disease. Antibiotic resistance threats in the United States. 2013.13-19.
2. Ventola CL: The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and Therapeutics 2015; 40(4): 277.
3. Read AF and Woods RJ: Antibiotic resistance management. Evolution, Medicine and Public Health 2014; (1): 147.
4. Gross M: Antibiotics in crisis. Current Biology 2013; 23 (24):1063-1065.
5. Chakrabarti A: Drug resistance in fungi-an emerging problem. Regional Health Forum 2011; 15(1): 1-7.
6. Hassan M, Kjos M, Nes IF, Diep DB and Lotfipour F: Natural antimicrobial peptides from bacteria: characteristics and potential applications to fight against antibiotic resistance. Journal of Applied Microbiology 2012; 113(4): 723-736.
7. Lehrer RI and Ganz T: Antimicrobial peptides in mammalian and insect host defence. Current Opinion in Immunology 1999; 11(1): 23-27.
8. Zasloff M: Antimicrobial peptides of multicellular organisms. Nature 2002; 415(6870): 389-395.
9. Ganz T and Lehrer RI: Antimicrobial peptides of vertebrates. Current Opinion in Immunology 1998; 10(1): 41-44.
10. Singh AR, Bajaj VK, Sekhawat PS and Singh K: Phytochemical estimation and antimicrobial activity of aqueous and methanolic extract of *Ocimum Sanctum* L. Journal of Natural Product and Plant Resources 2013; 3(1): 51-58.
11. Mahady GB: Medicinal plants for the prevention and treatment of bacterial infections. Current Pharmaceutical Design 2005; 11(19): 2405-2427.
12. Nawrot R, Barylski J, Nowicki G, Broniarczyk J, Buchwald W and Goździcka-Józefiak A: Plant antimicrobial peptides. Folia Microbiologica 2014; 59(3): 181-196.
13. Stotz HU, Waller F and Wang K: Innate immunity in plants: the role of antimicrobial peptides. Antimicrobial Peptides and Innate Immunity. Springer Basel 2013; 29-51
14. Lay FT and Anderson MA: Defensins-components of the innate immune system in plants. Current Protein and Peptide Science 2005; 6(1): 85-101.
15. Pelegrini P, del Sarto RP, Silva ON, Franco OL and Grossi-de-Sa MF: Antibacterial peptides from plants: what they are and how they probably work. Biochemistry Research International 2011; 2011: 1-9.
16. Epand RM and Vogel HJ: Diversity of antimicrobial peptides and their mechanisms of action. Biochimica et Biophysica Acta (BBA)-Biomembranes 1999; 1462(1): 11-28.
17. Sitaram N and Nagaraj R: Interaction of antimicrobial peptides with biological and model membranes: structural and charge requirements for activity. Biochimica et Biophysica Acta (BBA)-Biomembranes 1999; 1462(1): 29-54.
18. Shai Y: Mode of action of membrane active antimicrobial peptides. Peptide Science 2002; 66(4): 236-248.
19. Yeaman MR and Yount NY: Mechanisms of antimicrobial peptide action and resistance. Pharmacological Reviews 2003; 55(1): 27-55.
20. Gazit E, Boman A, Boman HG and Shai Y: Interaction of the mammalian antibacterial peptide cecropin P1 with phospholipid vesicles. Biochemistry 1995; 34(36): 11479-11488.
21. Matsuzaki K, Murase O, Fujii N and Miyajima K: Translocation of a channel-forming antimicrobial peptide, magainin 2, across lipid bilayers by forming a pore. Biochemistry 1995; 34(19): 6521-6526.
22. Matsuzaki K, Murase O, Fujii N and Miyajima K: An antimicrobial peptide, magainin 2, induced rapid flip-flop of phospholipids coupled with pore formation and peptide translocation. Biochemistry 1996; 35(35):11361-11368.
23. Matsuzaki K: Magainins as paradigm for the mode of action of pore forming polypeptides. Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes 1998; 1376(3): 391-400.
24. Matsuzaki K: Why and how are peptide–lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. Biochimica et Biophysica Acta (BBA)-Biomembranes 1999; 1462(1): 1-10.
25. Matsuzaki K, Sugishita KI, Ishibe N, Ueha M, Nakata S, Miyajima K and Epand RM: Relationship of membrane curvature to the formation of pores by magainin 2. Biochemistry 1998; 37(34): 11856-11863.
26. Ludtke SJ, He K, Heller WT, Harroun TA, Yang L and Huang HW: Membrane pores induced by magainin. Biochemistry 1996; 35(43): 13723-13728.
27. Bahar AA and Ren D: Antimicrobial peptides. Pharmaceuticals 2013; 6(12): 1543-1575.
28. Silva PM, Gonçalves S and Santos NC: Defensins: antifungal lessons from eukaryotes. New edge of antibiotic development: antimicrobial peptides and corresponding resistance 2014; 75.
29. Majewski J and Stec B: X-ray scattering studies of model lipid membrane interacting with purothionin provide support for a previously proposed mechanism of membrane lysis. European Biophysics Journal 2010; 39(8): 1155-1165.
30. Fernandez De Caleya RF, Gonzalez-Pascual B, García-Olmedo F and Carbonero P: Susceptibility of phytopathogenic bacteria to wheat purothionins *in-vitro*. Applied Microbiology 1972; 23(5): 998-1000.
31. Ebrahim-Nesbat F, Behnke S, Kleinhofs A and Apel K: Cultivar-related differences in the distribution of cell-wall-bound thionins in compatible and incompatible interactions between barley and powdery mildew. Planta 1989; 179(2): 203-210.
32. Evans J, Wang YD, Shaw KP and Vernon LP: Cellular responses to Pyrularia thionin are mediated by Ca2+ influx and phospholipase A2 activation and are inhibited by thionin tyrosine iodination. Proceedings of the National Academy of Sciences 1989; 86(15): 5849-5853.

33. Kramer KJ, Klassen LW, Jones BL, Speirs RD and Kammer AE: Toxicity of purothionin and its homologues to the tobacco hornworm, *Manduca sexta* L. (Lepidoptera: Sphingidae). Toxicology and applied pharmacology 1979; 48(1): 179-183.
34. Tam JP, Wang S, Wong KH and Tan WL: Antimicrobial peptides from plants. Pharmaceuticals 2015; 8(4): 711-757.
35. Ponz F, Paz-Ares J, Hernández-Lucas C, Carbonero P and García-Olmedo F: Synthesis and processing of thionin precursors in developing endosperm from barley (*Hordeum vulgare* L.). The EMBO journal 1983; 2(7): 1035.
36. Epand RM and Vogel HJ: Diversity of antimicrobial peptides and their mechanisms of action. Biochimica et Biophysica Acta (BBA)-Biomembranes 1999; 1462(1): 11-28.
37. Castagnaro A, Maraña C, Carbonero P and Garcia-Olmedo F: Extreme divergence of a novel wheat thionin generated by a mutational burst specifically affecting the mature protein domain of the precursor. Journal of Molecular Biology 1992; 224(4): 1003-1009.
38. Rao U, Stec B and Teeter MM: Refinement of purothionins reveals solute particles important for lattice formation and toxicity. Part 1: α1-purothionin revisited. Acta Crystallographica Section D: Biological Crystallography 1995; 51(6): 904-913.
39. Stec B, Rao U and Teeter MM: Refinement of purothionins reveals solute particles important for lattice formation and toxicity. Part 2: Structure of β-purothionin at 1.7 Å resolution. Acta Crystallographica Section D: Biological Crystallography 1995; 51(6): 914-924.
40. Milbradt AG, Kerek F, Moroder L and Renner C: Structural characterization of hellethionins from *Helleborus purpurascens*. Biochemistry 2003; 42(8): 2404-2411.
41. Duvick JP, Rood T, Rao AG and Marshak DR: Purification and characterization of a novel antimicrobial peptide from maize (*Zea mays* L.) kernels. Journal of Biological Chemistry 1992; 267(26): 18814-18820.
42. Fujimura M, Ideguchi M, Minami Y, Watanabe K and Tadera K: Purification, characterization, and sequencing of novel antimicrobial peptides, Tu-AMP 1 and Tu-AMP 2, from bulbs of tulip (*Tulipa gesneriana* L.). Bioscience, Biotechnology, and Biochemistry 2004; 68(3): 571-577.
43. Fujimura M, Ideguchi M, Minami Y, Watanabe K and Tadera K: Amino acid sequence and antimicrobial activity of chitin-binding peptides, Pp-AMP 1 and Pp-AMP 2, from Japanese bamboo shoots (*Phyllostachys pubescens*). Bioscience, Biotechnology, and Biochemistry 2005; 69(3): 642-645.
44. Pal A, Debreczeni JE, Sevvana M, Gruene T, Kahle B, Zeeck A and Sheldrick GM: Structures of viscotoxins A1 and B2 from European mistletoe solved using native data alone. Acta Crystallographica Section D: Biological Crystallography 2008; 64(9): 985-992.
45. Bogomolovas J, Simon B, Sattler M and Stier G: Screening of fusion partners for high yield expression and purification of bioactive viscotoxins. Protein expression and purification 2009; 64(1):16-23.
46. Samuelsson G and Pettersson B: The disulfide bonds of viscotoxin A3 from the European mistletoe (*Viscum album* L., Loranthaceae). Acta Chem Scand 1971; 25(6): 2048-2054.
47. Schrader G and Apel K: Isolation and characterization of cDNAs encoding viscotoxins of mistletoe (*Viscum album*). European Journal of Biochemistry 1991; 198(3): 549-553.
48. Coulon A, Mosbah A, Lopez A, Sautereau AM, Schaller G, Urech K, Rougé P and Darbon H: Comparative membrane interaction study of viscotoxins A3, A2 and B from mistletoe (*Viscum album*) and connections with their structures. Biochemical Journal 2003; 374(1): 71-78.
49. Stotz HU, Thomson J and Wang Y: Plant defensins: defense, development and application. Plant Signaling and Behavior 2009; 4(11): 1010-1012.
50. Terras FR, Eggermont K, Kovaleva V, Raikhel NV, Osborn RW, Kester A, Rees SB, Torrekens S, Van Leuven F and Vanderleyden J: Small cysteine-rich antifungal proteins from radish: their role in host defense. The Plant Cell 1995; 7(5): 573-588.
51. Colilla FJ, Rocher A and Mendez E: γ-Purothionins: amino acid sequence of two polypeptides of a new family of thionins from wheat endosperm. FEBS letters 1990; 270(1-2): 191-194.
52. Méndez E, Rocher A, Calero M, Girbés T, Citores L and Soriano F: primary structure of ω-hordothionin, a member of a novel family of thionins from barley endosperm, and its inhibition of protein synthesis in eukaryotic and prokaryotic cell-free systems. The FEBS Journal 1996; 239(1): 67-73.
53. Moreno M, Segura A and García-olmedo F: Pseudothionin-St1, a potato peptide active against potato pathogens. European Journal of Biochemistry 1994; 223(1): 135-139.
54. Thevissen K, Ghazi A, De Samblanx GW, Brownlee C, Osborn RW and Broekaert WF: Fungal membrane responses induced by plant defensins and thionins. Journal of Biological Chemistry 1996; 271(25): 15018-15025.
55. Thevissen K, Osborn RW, Acland DP and Broekaert WF: Specific binding sites for an antifungal plant defensin from Dahlia (*Dahlia merckii*) on fungal cells are required for antifungal activity. Molecular Plant-Microbe Interactions 2000; 13(1): 54-61.
56. Thevissen K, Warnecke DC, François IE, Leipelt M, Heinz E, Ott C, Zähringer U, Thomma BP, Ferket KK and Cammue BP: Defensins from insects and plants interact with fungal glucosylceramides. Journal of Biological Chemistry 2004; 279(6): 3900-3905.
57. Janssen BJ, Schirra HJ, Lay FT, Anderson MA and Craik DJ: Structure of Petunia hybrida defensin 1, a novel plant defensin with five disulfide bonds. Biochemistry 2003; 42(27): 8214-8222.
58. Liu YJ, Cheng CS, Lai SM, Hsu MP, Chen CS and Lyu PC: Solution structure of the plant defensin VrD1 from mung bean and its possible role in insecticidal activity against bruchids. Proteins: Structure, Function, and Bioinformatics 2006; 63(4): 777-786.
59. Aerts AM, François IE, Meert EM, Li QT, Cammue BP and Thevissen K: The antifungal activity of RsAFP2, a plant defensin from *Raphanus sativus*, involves the induction of reactive oxygen species in *Candida albicans*. Journal of Molecular Microbiology and biotechnology 2007; 13(4): 243-247.
60. Aerts AM, Bammens L, Govaert G, Carmona-Gutierrez D, Madeo F, Cammue B and Thevissen K: The antifungal plant defensin HsAFP1 from *Heuchera sanguinea* induces apoptosis in *Candida albicans*. Frontiers in Microbiology 2011; 2:47.
61. Mello EO, Ribeiro SF, Carvalho AO, Santos IS, Da Cunha M, Santa-Catarina C and Gomes VM, Antifungal activity of PvD1 defensin involves plasma membrane permeabilization, inhibition of medium acidification, and induction of ROS in fungi cells. Current Microbiology 2011; 62(4):1209-1217.

62. Hayes BM, Bleackley MR, Wiltshire JL, Anderson MA, Traven A and van der Weerden NL: Identification and mechanism of action of the plant defensin NaD1 as a new member of the antifungal drug arsenal against *Candida albicans*. Antimicrobial agents and chemotherapy 2013; 57(8):3667-3675.
63. Padovan L, Segat L, Tossi A, Calsa J, Ederson AK, Brandao L, Guimarães RL, Pandolfi V, Pestana-Calsa MC, Belarmino LC and Benko-Iseppon AM: Characterization of a new defensin from cowpea (*Vigna unguiculata* L. Walp.). Protein and peptide letters 2010; 17(3): 297-304.
64. Lay FT, Brugliera F and Anderson MA: Isolation and properties of floral defensins from ornamental tobacco and petunia. Plant Physiology 2003; 131(3):1283-1293.
65. Chen JJ, Chen GH, Hsu HC, Li SS and Chen CS: Cloning and functional expression of a mungbean defensin VrD1 in Pichia pastoris. Journal of Agricultural and Food Chemistry 2004; 52(8): 2256-2261.
66. Meyer B, Houlne G, Pozueta-Romero J, Schantz ML and Schantz R: Fruit-specific expression of a defensin-type gene family in bell pepper (upregulation during ripening and upon wounding). Plant physiology 1996; 112(2): 615-622.
67. Houlne G, Meyer B and Schantz R: Alteration of the expression of a plant defensin gene by exon shuffling in bell pepper (*Capsicum annuum* L.). Molecular and General Genetics 1998; 259(5): 504-510.
68. Kragh KM, Nielsen JE, Nielsen KK, Dreboldt S and Mikkelsen JD: Characterization and localization of new antifungal cysteine-rich proteins from *Beta vulgaris*. Molecular Plant Microbe Interactions 1995; 8(3): 424-434.
69. Zhang Y and Lewis K: Fabatins: new antimicrobial plant peptides. FEMS Microbiology Letters 1997; 149(1): 59-64.
70. Terras FR, Schoofs HM, De Bolle MF, Van Leuven F, Rees S.B, Vanderleyden J, Cammue BP and Broekaert WF: Analysis of two novel classes of plant antifungal proteins from radish (*Raphanus sativus* L.) seeds. Journal of Biological Chemistry 1992; 267(22): 15301-15309.
71. Fant F, Vranken W, Broekaert W and Borremans F: Determination of the three-dimensional solution structure of *Raphanus sativus* antifungal protein 1 by 1 H NMR. Journal of Molecular Biology 1998; 279(1):257-270.
72. Gu Q, Kawata EE, Morse MJ, Wu HM and Cheung AY: A flower-specific cDNA encoding a novel thionin in tobacco. Molecular and General Genetics 1992; 234(1): 89-96.
73. Lay FT, Schirra HJ, Scanlon MJ, Anderson MA and Craik DJ: The three-dimensional solution structure of NaD1, a new floral defensin from *Nicotiana alata* and its application to a homology model of the crop defense protein alfAFP. Journal of Molecular Biology 2003; 325(1): 175-188.
74. Gould A, Ji Y, L Aboye T and Camarero AJ: Cyclotides, a novel ultrastable polypeptide scaffold for drug discovery. Current Pharmaceutical Design 2011; 17(38): 4294-4307.
75. Gran L: On the effect of a polypeptide isolated from "Kalata-Kalata" (*Oldenlandia affinis* DC) on the oestrogen dominated uterus. Basic & Clinical Pharmacology and Toxicology 1973; 33(5-6): 400-408.
76. Daly NL, Clark RJ, Plan MR and Craik DJ: Kalata B8, a novel antiviral circular protein, exhibits conformational flexibility in the cystine knot motif. Biochemical Journal 2006; 393(3): 619-626.
77. Cammue BP, De Bolle MF, Terras FR, Proost P, Van Damme J, Rees SB, Vanderleyden J and Broekaert WF: Isolation and characterization of a novel class of plant antimicrobial peptides form *Mirabilis jalapa* L. seeds. Journal of Biological Chemistry 1992; 267(4): 2228-2233.
78. Tam JP, Lu YA, Yang JL and Chiu KW: An unusual structural motif of antimicrobial peptides containing end-to-end macrocycle and cystine-knot disulfides. Proceedings of the National Academy of Sciences 1999; 96(16): 8913-8918.
79. Chen B, Colgrave ML, Daly NL, Rosengren KJ, Gustafson KR and Craik DJ: Isolation and characterization of novel cyclotides from *Viola hederaceae* solution structure and anti-hiv activity of vhl-1, a leaf-specific expressed cyclotide. Journal of Biological Chemistry 2005; 280(23): 22395-22405.
80. Ng YM, Yang Y, Sze KH, Zhang X, Zheng YT and Shaw PC: Structural characterization and anti-HIV-1 activities of arginine/glutamate-rich polypeptide Luffin P1 from the seeds of sponge gourd (*Luffa cylindrica*). Journal of Structural Biology 2011; 174(1): 164-172.
81. Oparin PB, Mineev KS, Dunaevsky YE, Arseniev AS, Belozersky MA, Grishin EV, Egorov TA and Vassilevski AA: Buckwheat trypsin inhibitor with helical hairpin structure belongs to a new family of plant defence peptides. Biochemical Journal 2012; 446(1): 69-77.
82. Gustafson KR, Walton LK, Sowder RC, Johnson DG, Pannell LK, Cardellina JH and Boyd MR: New Circulin Macrocyclic Polypeptides from Chassalia parvifolia 1. Journal of natural products 2002; 63(2):176-178.
83. Hallock YF, Sowder RC, Pannell LK, Hughes CB, Johnson DG, Gulakowski R, Cardellina JH and Boyd MR: Cycloviolins A-D, Anti-HIV Macrocyclic Peptides from *Leonia cymosa* 1. The Journal of Organic Chemistry 2000; 65(1): 124-128.
84. Craik DJ, Daly NL, Bond T and Waine C: Plant cyclotides: a unique family of cyclic and knotted proteins that defines the cyclic cystine knot structural motif. Journal of molecular biology 1999; 294(5):1327-1336.
85. Jennings C, West J, Waine C, Craik D and Anderson M: Biosynthesis and insecticidal properties of plant cyclotides: the cyclic knotted proteins from *Oldenlandia affinis*. Proceedings of the National Academy of Sciences 2001; 98(19): 10614-10619.
86. Hayashi K, Suketa Y and Suzuki T: Chemical structure of circulin B. Cellular and Molecular Life Sciences 1968; 24(7): 656-657.
87. Derua R, Gustafson KR and Pannell LK: Analysis of the disulfide linkage pattern in circulin A and B, HIV-inhibitory macrocyclic peptides. Biochemical and Biophysical Research Communications 1996; 228(2): 632-638.
88. Gustafson KR, Sowder RC, Henderson LE, Parsons IC, Kashman Y, Cardellina JH, McMahon JB, Buckheit Jr RW, Pannell LK and Boyd MR: Circulins A and B. Novel human immunodeficiency virus (HIV)-inhibitory macrocyclic peptides from the tropical tree *Chassalia parvifolia*. Journal of the American Chemical Society 1994; 116(20): 9337-9338.
89. Koltay A, Daly NL, Gustafson KR and Craik DJ: Structure of circulin B and implications for antimicrobial activity of the cyclotides. International Journal of Peptide Research and Therapeutics 2005; 11(1): 99-106.
90. Segura A, Moreno M, Madueño F, Molina A and García-Olmedo F: Snakin-1, a peptide from potato that is active against plant pathogens. Molecular Plant-Microbe Interactions 1999; 12(1): 16-23.
91. Terras FR, Goderis IJ, Van Leuven F, Vanderleyden J, Cammue BP and Broekaert WF: *In-vitro* antifungal activity of a radish *(Raphanus sativus* L.) seed protein

homologous to nonspecific lipid transfer proteins. Plant physiology 1992; 100(2):1055-1058.
92. Berrocal-Lobo M, Segura A, Moreno M, López G, Garcıa-Olmedo F and Molina A: Snakin-2, an antimicrobial peptide from potato whose gene is locally induced by wounding and responds to pathogen infection. Plant Physiology 2002; 128(3): 951-961.
93. Daneshmand F, Zare-Zardini H and Ebrahimi L: Investigation of the antimicrobial activities of Snakin-Z, a new cationic peptide derived from *Zizyphus jujuba* fruits. Natural Product Research 2013; 27(24): 2292-2296.
94. Archer BL: The proteins of *Hevea brasiliensis* latex. 4. Isolation and characterization of crystalline hevein. Biochemical Journal 1960; 75(2): 236.
95. Van Parijs J, Broekaert WF, Goldstein IJ and Peumans WJ: Hevein: an antifungal protein from rubber-tree (*Hevea brasiliensis*) latex. Planta 1991; 183(2): 258-264.
96. Li SS and Claeson P: Cys/Gly-rich proteins with a putative single chitin-binding domain from oat (*Avena sativa*) seeds. Phytochemistry 2003; 63(3): 249-255.
97. Kiba A, Saitoh H, Nishihara M, Omiya K and Yamamura S: C-terminal domain of a hevein-like protein from *Wasabia japonica* has potent antimicrobial activity. Plant and Cell Physiology 2003; 44(3): 296-303.
98. Lee OS, Lee B, Park N, Koo JC, Kim YH, Karigar C, Chun HJ, Jeong BR, Kim DH, Nam J and Yun JG: Pn-AMPs, the hevein-like proteins from Pharbitis nil confers disease resistance against phytopathogenic fungi in tomato, *Lycopersicum esculentum*. Phytochemistry 2003; 62(7): 1073-1079.
99. Huang RH, Xiang Y, Tu GZ, Zhang Y and Wang DC: Solution structure of Eucommia antifungal peptide: a novel structural model distinct with a five-disulfide motif. Biochemistry 2004; 43(20): 6005-6012.
100. Odintsova TI, Vassilevski AA, Slavokhotova AA, Musolyamov AK, Finkina EI, Khadeeva NV, Rogozhin EA, Korostyleva TV, Pukhalsky VA, Grishin EV and Egorov TA: A novel antifungal hevein-type peptide from *Triticum kiharae* seeds with a unique 10-cysteine motif. The FEBS Journal 2009; 276(15): 4266-4275.
101. Dubovskii PV, Vassilevski AA, Slavokhotova AA, Odintsova TI, Grishin EV, Egorov TA and Arseniev AS: Solution structure of a defense peptide from wheat with a 10-cysteine motif. Biochemical and Biophysical Research Communications 2011; 411(1): 14-18.
102. Kini SG, Nguyen PQ, Weissbach S, Mallagaray A, Shin J, Yoon HS and Tam JP: Studies on the chitin binding property of novel cysteine-rich peptides from *Alternanthera sessilis*. Biochemistry 2015; 54(43): 6639-6649.
103. Broekaert WF, Marien W, Terras FR, De Bolle MF, Proost P, Van Damme J, Dillen L, Claeys M, Rees SB and Vanderleyden J: Antimicrobial peptides from *Amaranthus caudatus* seeds with sequence homology to the cysteine/glycine-rich domain of chitin-binding proteins. Biochemistry 1992; 31(17): 4308-4314.
104. Van den Bergh KP, Rougé P, Proost P, Coosemans J, Krouglova T, Engelborghs Y, Peumans WJ and Van Damme EJ: Synergistic antifungal activity of two chitin-binding proteins from spindle tree (*Euonymus europaeus* L.). Planta 2004; 219(2): 221-232.
105. Lipkin A, Anisimova V, Nikonorova A, Babakov A, Krause E, Bienert M, Grishin E and Egorov T: An antimicrobial peptide Ar-AMP from amaranth (*Amaranthus retroflexus* L.) seeds. Phytochemistry 2005; 66(20): 2426-2431.
106. Huang RH, Xiang Y, Liu XZ, Zhang Y, Hu Z and Wang DC: Two novel antifungal peptides distinct with a five-disulfide motif from the bark of *Eucommia ulmoides* Oliv. FEBS Letters 2002; 521(1-3):87-90.
107. Xiang Y, Huang RH, Liu XZ, Zhang Y and Wang DC: Crystal structure of a novel antifungal protein distinct with five disulfide bridges from *Eucommia ulmoides* Oliver at an atomic resolution. Journal of structural biology 2004; 148(1): 86-97.
108. Koo JC, Lee SY, Chun HJ, Cheong YH, Choi JS, Kawabata SI, Miyagi M, Tsunasawa S, Ha KS, Bae DW and Han CD: Two hevein homologs isolated from the seed of *Pharbitis nil* L. exhibit potent antifungal activity. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology 1998; 1382(1): 80-90.
109. Fujimura M, Minami Y, Watanabe K and Tadera K: Purification, characterization, and sequencing of a novel type of antimicrobial peptides, Fa-AMP1 and Fa-AMP2, from seeds of buckwheat (*Fagopyrum esculentum* Moench.). Bioscience, Biotechnology, and Biochemistry 2003; 67(8): 1636-1642.
110. Selitrennikoff CP: Antifungal proteins. Applied and Environmental Microbiology 2001; 67(7): 2883-2894.
111. Tassin S, Broekaert WF, Marion D, Acland DP, Ptak M, Vovelle F and Sodano P: Solution structure of Ace-AMP1, a potent antimicrobial protein extracted from onion seeds. Structural analogies with plant nonspecific lipid transfer proteins. Biochemistry 1998; 37(11): 3623-3637.
112. Diz MS, Carvalho AO and Gomes VM: Purification and molecular mass determination of a lipid transfer protein exuded from *Vigna unguiculata* seeds. Brazilian Journal of Plant Physiology 2003; 15(3):171-175.
113. Lin P, Xia L and Ng TB: First isolation of an antifungal lipid transfer peptide from seeds of a Brassica species. Peptides 2007; 28(8):1514-1519.
114. Cruz LP, Ribeiro SF, Carvalho AO, Vasconcelos IM, Rodrigues R, Cunha MD and Gomes VM: Isolation and partial characterization of a novel lipid transfer protein (LTP) and antifungal activity of peptides from chilli pepper seeds. Protein and Peptide Letters 2010; 17(3): 311-318.
115. Yang X, Xiao Y, Pei Y and Zhen C: "LJAFP, a novel non-specific lipid transfer protein-like antimicrobial protein from motherwort (*Leonurus japonicus*) confers disease resistance against phytopathogenic fungi and bacterium in transgenic tobacco. Submitted (MAR-2005) to the EMBL/GenBank/DDBJ databases.
116. Molina A, Segura A and García-Olmedo F: Lipid transfer proteins (nsLTPs) from barley and maize leaves are potent inhibitors of bacterial and fungal plant pathogens. FEBS Letters 1993; 316(2): 119-122.
117. Nielsen KK, Nielsen JE, Madrid SM and Mikkelsen JD: New antifungal proteins from sugar beet (*Beta vulgaris* L.) showing homology to non-specific lipid transfer proteins. Plant molecular biology 1996; 31(3): 539-552.
118. Cammue BPA, Thevissen K, Hendriks M, Eggermont K, Goderis IJ, Proost P, Van Damme J, Osborn RW, Guerbette F, Kader JC and Broekaert WF: A potent antimicrobial protein from onion seeds showing sequence homology to plant lipid transfer proteins. Plant Physiology 1995; 109(2): 445-455.
119. Wang SY, Wu JH, Ng TB, Ye XY and Rao PF: A non-specific lipid transfer protein with antifungal and antibacterial activities from the mung bean. Peptides 2004; 25(8): 1235-1242.

120. Lin KF, Liu YN, Hsu STD, Samuel D, Cheng CS, Bonvin AM and Lyu PC: Characterization and structural analyses of nonspecific lipid transfer protein 1 from mung bean. Biochemistry 2005; 44(15):5703-5712.
121. Finkina EI, Balandin SV, Serebryakova MV, Potapenko NA, Tagaev AA and Ovchinnikova TV: Purification and primary structure of novel lipid transfer proteins from germinated lentil (*Lens culinaris*) seeds. Biochemistry (Moscow) 2007; 72(4): 430-438.
122. Pelegrini PB, Murad AM, Silva LP, dos Santos RC, Costa FT, Tagliari PD, Bloch Jr C, Noronha EF, Miller RN and Franco OL: Identification of a novel storage glycine-rich peptide from guava (*Psidium guajava*) seeds with activity against Gram-negative bacteria. Peptides 2008; 29(8): 1271-1279.
123. Yodyingyong S, Chaichana C, Nuchsuk C, Roytrakul S, T-Thienprasert NP and Ratanapo S: Partial purification of cytotoxic peptides against gastric cancer cells from protein hydrolysate of *Euphorbia hirta* Linn. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnology Engineering 2013; 7:74-77.
124. Remuzgo C, Oewel TS, Daffre S, Lopes TR, Dyszy FH, Schreier S, Machado-Santelli GM and Machini MT: Chemical synthesis, structure–activity relationship, and properties of shepherin I: a fungicidal peptide enriched in glycine-glycine-histidine motifs. Amino Acids 2014; 46(11): 2573-2586.
125. Ramos MV, Souza DP, Gomes MTR, Freitas CDT, Carvalho CPS, Júnior PAVR and Salas CE: A phytopathogenic cysteine peptidase from latex of wild rubber vine *Cryptostegia grandiflora*. The Protein Journal 2014; 33(2): 199-209.
126. Mandal SM, Dey S, Mandal M, Sarkar S, Maria-Neto S and Franco OL: Identification and structural insights of three novel antimicrobial peptides isolated from green coconut water. Peptides 2009; 30(4):633-637.
127. Al Akeel R, Al-Sheikh Y, Mateen A, Syed R, Janardhan K and Gupta VC: Evaluation of antibacterial activity of crude protein extracts from seeds of six different medical plants against standard bacterial strains. Saudi Journal of Biological Sciences 2014; 21(2):147-151.
128. Golla K, Vutukuru SS, Rani JU, Meghanath P and Pasha C: Screening of small peptides from various germinating seeds having antimicrobial activity. Journal of Pharmaceutical and Biomedical Analysis 2016; 11: 2278-3008.
129. Sharma S, Verma HN and Sharma NK: Cationic bioactive peptide from the seeds of *Benincasa hispida*. International Journal of Peptides 2014.
130. Hammami R, Hamida JB, Vergoten G and Fliss I: Phyt AMP: a database dedicated to antimicrobial plant peptides. Nucleic Acids Research 2009; 37: D963-D968.
131. http://www.bsienvis.nic.in/KidsCentre/BSI_3933.aspx

How to cite this article:
Samriti, Biswas R and Biswas K: Plant antimicrobial peptides: a novel approach against drug resistant microorganisms. Int J Pharm Sci Res 2018; 9(1): 1-15.doi: 10.13040/IJPSR.0975-8232.9(1).1-15.