IJPSR (2023), Volume 14, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 05 September 2022; received in revised form, 18 October 2022; accepted 17 November 2022; published 01 May 2023

ISOLATION OF ANTIBACTERIAL PEPTIDE FROM *VIGNA ACONITIFOLIA* L.

D. Swathi * and N. Mallikarjun

Department of Studies and Research in Microbiology, Sahyadri Science College, Kuvempu University, Shivamogga - 577203, Karnataka, India.

Keywords:

Antibacterial, Chromatography, Drought-resistance, Peptides, Pulverized

Correspondence to Author:
D. Swathi

Research Scholar,
Department of Studies and Research in Microbiology, Sahyadri Science College, Kuvempu University, Shivamogga - 577203, Karnataka, India.

E-mail: swathidshet@gmail.com

ABSTRACT: *Vigna aconitifolia L*. represents a genus in the Fabaceae family and is one of the most drought-resistant legumes, commonly grown in arid and semi-arid regions of India. Therapeutic effects of *Vigna aconitifolia L.* have been reported in several publications, but there are only a few reports on the biological activities of the *Vigna aconitifolia L.* proteins. In the present study, antibacterial activities of the *Vigna aconitifolia L.*, peptides have been investigated. Homogenized seed flour was pulverized, and its protein content was extracted in a phosphate buffer. After centrifugation, by adjusting pH, peptides from the supernatant were isolated. Gel filtration (G-25) and Ion-exchange chromatography on the seed extract gave fairly pure peptide fractions of which inhibitory activities were done by agar well diffusion method against *Shigella flexineri, Bacillus cereus, Salmonella typhimurium, Staphylococcus aureus, Enterococcus faecalis, Vibrio cholerae* bacterial pathogens. The results indicate that a 30kDa of the peptide was identified and exhibited good antibacterial activity against bacterial pathogens. Among all strains, *Shigella flexineri* and *Staphylococcus aureus* exhibit good results with a clear zone of inhibition.

INTRODUCTION: Antibacterial peptides (ABPs) are part of antimicrobial peptides with small glycine, and cysteine-rich amino acid residues of polypeptides known as "host defense peptides" and a part of the innate immune response found among all classes of life [1-9]. Antibacterial peptides have a molecular weight of 4-50kDa, which plays a role in defense of the plant immune system and inhibits various bacterial species [2, 5]. In the past two decades, a considerable increase in resistance in pathogenic bacteria against common antibiotics has emerged.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(5).2477-82

This article can be accessed online on www.ijpsr.com

DOI link: http://doi.org/10.13040/IJPSR.0975-8232.14(5).2477-82

Those bacteria, such as *Escherichia coli, Salmonella sp, and Klebsiella sp,* although they were susceptible to the usual antibiotic therapy some time ago, have now made the main cause of challenging efforts in treating nosocomial infections [3]. Rising bacterial resistance faster against synthetic antibiotics has turned attempts to search for antibiotics from natural sources, again [11]. Among different categories of natural substances, proteinaceous materials isolated from plant sources have given promise in finding new natural compounds with good antimicrobial activities.

In this way, many small proteins with some activity against microbial pathogens have been identified in plant seeds [14]. *Vigna aconitifolia L*. represents a genus in the Fabaceae family (sub-family Papilionaceae). *Vigna aconitifolia L*. in generalis known as mat bean, moth bean, matki, Turkish

gram, or dew bean. It is one of the most drought-resistant legumes, commonly grown in arid and semi-arid regions of India. Itsfamine-resistant merits made it fight soil erosion. With its high protein content, the *Vigna aconitifolia L*. has been identified as possibly a more significant food source in the future. Therapeutic effects of *Vigna aconitifolia* L. have been reported in several publications, but there are only a few reports on the biological activities of the *Vigna aconitifolia L*. proteins [4]. The present study used chromatographic approaches to extract and purify antibacterial peptides from Vigna aconitifolia L. seeds.

MATERIALS AND METHODS:

Sample Collection: The two varieties [V1 and V2] *Vigna aconitifolia L*. seeds were obtained from the local market in Dharwad, Karnataka. The seeds collected were identified and authenticated in the Department of Studies and Research in Botany, Sahyadri Science College, Shivamogga, Karnataka. India.

Bacterial Strains: All the bacterial strains used in this study were collected from the National Collection of Industrial Microorganisms (NCIM), Pune, India, and Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial strains include *Staphylococcus aureus* (NCIM 2073), *Enterobacter faecalis* (NCIM 5253), *Salmonella typhimurium* (NCIM 2501), *Bacillus cereus* (NCIM 2217), *Vibrio cholerae* (MTCC 2906), *Shigella flexineri* (NCIM 5265). The cultures were preserved at 4^{o}C and subcultured at regular intervals.

Extraction and Purification of the Moth Bean Seeds: Fresh seeds of moth bean were collected and rinsed with 0.1% of sodium hypochlorite solution for 10 min and then washed with distilled water for the removal of dirt and debris. Seeds were dried under sterile conditions and pulverized into fine powder; 30 gm of seed sample was homogenized with 100 ml of 0.1 M Phosphate buffer (pH 7). The homogenate was then centrifuged at 6000 rpm for 15 minutes at 4 ℃. The supernatant thus obtained was used as the crude peptide extract. The obtained crude extract was saturated to 40% and 80% relative ammonium sulfate precipitation by adding solid ammonium sulfate (12.6 g) with constant stirring at 4 ℃. This solution was incubated overnight at 4 ℃. The solution was centrifuged at 8500 rpm for 15 minutes at 4℃. The obtained pellets were re-dissolved in 10 ml of 10mM Tris buffer and stored at 4 ℃. The resulting suspension was extensively dialyzed against distilled water. The dialyzed solutions were recovered and subjectedto further purification by chromatographic methods [13].

Gel Filtration Chromatography: The column filled with Sephadex G-25 pre-equilibrated with 0.1 M phosphate buffer (pH 7) was initially used to separate proteins from the F/3-10 fraction. The salt-precipitated sample (80 %) was loaded in the column and 40 ml of 0.1 M phosphate buffer (pH 7) was added to it. The fractions of 1 ml volume were collected at the flow rate of 1 ml/5mins. All the collected fractions were assessed for protein content by 280nm methods [14].

Ion Exchange Chromatography: The fractions showing the highest protein content (fractions 3-10) were pooled and used for Ion exchange chromatography [6], DEAE- Sephadex column pre-equilibrated with 0.025M Tris- NaCl buffer. The fractions were prepared at different ionic strengths, these fractions were collected at the flow rate of 1 ml/5min, and a stepwise increase in ionic strength eluted the protein. The amount of protein in these fractions was estimated by Lowry's method using Bovine serum albumin (BSA) as the standard [7].

SDS-PAGE: The purification process was monitored throughout by SDS-PAGE, performed according to the method described by Laemmle [8]. The sample buffered contained (Tris-HCl 6g, Glycine 14.4g, SDS 1g, distilled water- 1000ml, pH-8.2). After the run, the gel was removed from plates and put in staining solution (40ml methanol, 5ml acetic acid, 55ml distilled water, 0.01g Coomassie brilliant blue dye) overnight and later destained with destaining solution (40ml methanol, 5mL acetic acid, 55mL distilled water) until the bands were visible.

Antibacterial Assay: Antibacterial activity of the crude and purified peptide concentrates from seed sample was determined using Agar well diffusion method [13] against bacterial pathogens such

as *Staphylococcus aureus, Enterobacter faecalis, Salmonella typhimurium, Bacillus cereus, Vibrio cholerae, Shigella flexineri.* The test organism (100ul) was inoculated on the surface of the Nutrient Agar medium. Isolated peptides of different concentrations (100ul, 150ul, 200ul) were loaded onto each well and incubated at 37°C for 24 h. The zone of inhibition was measured, and the assays were performed in triplicates.

RESULTS: The present study was carried out to extract, purify, and isolate antibacterial peptides from *Vigna aconitifolia* L. seeds.

Spectrometric Determination of Protein: The concentration of total protein content present in the seeds is shown in **Table 1**. The protein concentration of moth bean in crude extract and purified elutes were represented in mg/100gm of seeds. Both varieties of moth bean show the highest protein content thus, it is further selected for antibacterial activity.

Antibacterial Activity of Crude Peptide Extract: The antibacterial activity of the crude peptide concentrates from seeds of moth beans was determined by Agar well diffusion assay. Both varieties (V-1 and V-2) exhibit good inhibitory activity against six pathogenic bacteria **Table 2**.

Data represents that among all strains, *Shigella flexineri* and *Staphylococcus aureus* exhibit good results with a zone of inhibition of 20±1.24 and 21.6±1.44 **Fig. 1,** respectively followed by Enterobacter *faecalis* 15±1.05, *Salmonella typhimurium* 12±1.05, *Bacillus cereus* 10±1 *Vibrio cholera* 10±0.5 even at low concentration (100µl/well).

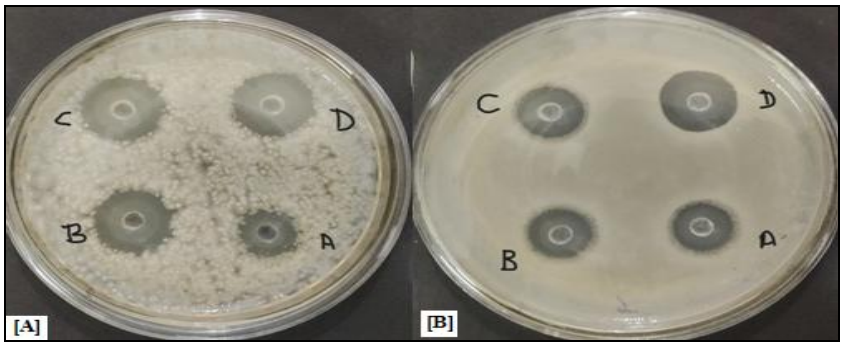

FIG. 1: ANTIBACTERIAL ACTIVITY OF CRUDE PEPTIDE AGAINST BACTERIAL STRAINS [A] *Shigella flexineri* and [B] *Staphylococcus aureus.* In the figure*:* A=100 µl,B=150 µl,C=200 µl,D=Standard antibiotic.

TABLE 1: PURIFICATION STAGES OF PEPTIDES FROM VIGNA ACONITIFOLIA L (V-1 AND V-2)

Peptide purification stage	Amount of protein, mg/100 g of seeds	Protein yield, %
Protein extract	22900	100
Precipitate 30% $(NH4)_2SO4$	1200	52.4
50% $(NH4)_2SO4$	1083	47.2
70% $(NH4)_2SO4$	900	39.3
After Gel filtration	450	19.65

TABLE 2: ANTIBACTERIAL ACTIVITY OF CRUDE PEPTIDE ISOLATED FROM THE SEED OF VIGNA ACONITIFOLIA L.(V-1 AND V-2) AT DIFFERENT CONCENTRATIONS

Bacteria	Diameter of zone of inhibition(mm)						Antibiotic
	Variety-1			Variety-2			Streptomycin
	100	150	200	100	150	200	
Staphylococcus aureus	17.6±1.44	19.6±1.44	21.6±1.44	17±1.05	19±1.05	21±1.05	27±0.5
Enterobacter faecalis	9.3±1.05	11.3±0.5	15±1.05	11±1.05	12±1.05	14±1.05	28±0.2
Salmonella typhimurium	8±1.05	10±1.05	12±1.05	8±0.5	10±0.5	12±0.5	27±0.8
Bacillus cereus	-	9±1	10±1	-	10±1.05	11±1.05	27±0.4
Vibriocholerae	-	9±0.5	10±0.5	-	10±0.5	11±0.5	27±0.4
Shigella flexineri	16±1.24	19±1.24	20±1.24	16±0.5	18±0.5	21±0.5	26±0.2

Where "-" shows, inhibition not detected, all values were expressed in ± Standard Error Mean

Sodium Dodecyl Sulfate-polyacrylamide Gel Electrophoresis: Peptide fractions obtained were subjected to SDS-PAGE. The migration of molecular mass standards was indicated in kDa on

the left-hand side. Based on Lowry's method [7] of protein estimation results, the total amount of peptide was determined, and 33µg of peptide sample was loaded on wells. The molecular profiling of the sample was observed and indicated the presence of a peptide with a molecular weight ranging from 16 kDa to 175kDa. According to the previously reported studies, the peptide bands with molecular weight 30kDa **Fig. 2** distinguished in this study were used for further activity.

TABLE 3: ANTIBACTERIAL ACTIVITY OF ELUTED PEPTIDE ISOLATED FROM THE SEED OF VIGNA ACONITIFOLIA L(V-1 AND V-2) AT DIFFERENT CONCENTRATIONS

Bacteria	Diameter of zone of inhibition(mm)						Antibiotic
	Variety-1			Variety-2			Streptomycin
	100	150	200	100	150	200	
Staphylococcus aureus	12±1.44	14±0.5	18±1.05	11±1.44	14±0.5	16±1.05	27±0.5
Enterobacter faecalis	-	10±0.5	11±0.5	-	10±0.5	11±0.5	28±0.2
Salmonella typhimurium	-	10±0.5	10±0.5	-	10±0.5	10±0.5	27±0.8
Bacillus cereus	-	10±0.5	10±0.5	-	10±0.5	10±0.5	27±0.4
Vibriocholerae	-	10±0.5	10±0.5	-	10±0.5	10±0.5	27±0.4
Shigella flexineri	11±1.44	15±1.05	18±1.5	10±0.5	12±1.05	15±1.5	26±0.2

Where "-" shows, inhibition not detected, all values were expressed in ± Standard Error Mean

Antibacterial Activity of Eluted Peptide Sample: To check the antibacterial activity of eluted peptide band, the peptide was eluted from an elution buffer, further inhibitory activity was done against six bacterial pathogens. As shown in **Fig. 2.** A shows a clear zone of inhibition of 19±1.24 at 150 µl of eluted peptide content and 20±1.24 at 200 µl of eluted peptide content against bacterial pathogen *Shigella flexineri*. [B] shows a zone of inhibition of 14±0.5 mm with 150 µl of eluted peptide content and 18±1.05 at 200 µl of the peptide against *Staphylococcus aureus*, the negative control is Tris-HCl (pH=8.8).

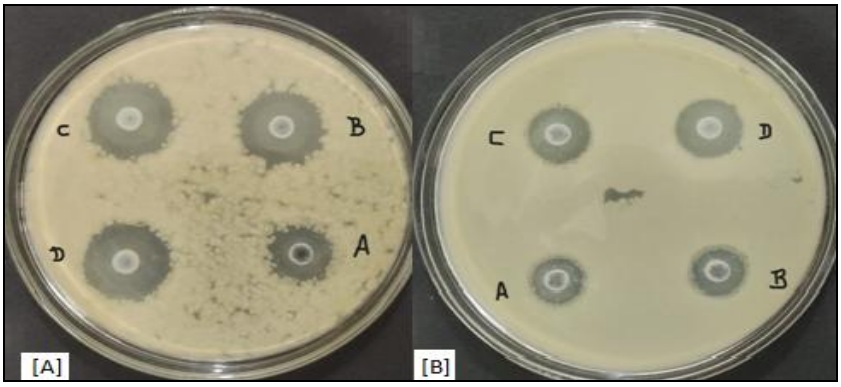

FIG. 2: ANTIBACTERIAL ACTIVITY OF ELUTED PEPTIDE AGAINST BACTERIAL STRAINS [A]*Shigella flexineri* and [B]*Staphylococcus aureus.* In the figure: A=100 µl,B=150 µl,C=200 µl,D=Standard antibiotic.

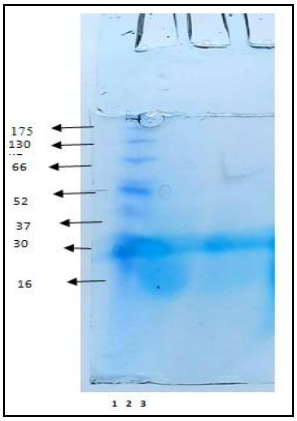

FIG. 3: SDS-PAGE ANALYSIS OF PEPTIDE EXTRACT: 1. SHOWS THE MOLECULAR MARKER, 2. CRUDE EXTRACT 3. PURIFIED PEPTIDE

Statistical Analysis: All the experiments were carried out in triplicates (n=3). The statistical analysis of the data was carried out by analysis of the variance (ANOVA). Results were considered significant when $p<0.05$.

DISCUSSION: This study described the isolation of antibacterial peptides from *Vigna aconitifolia L*; we found that the isolated peptide exhibited strong antibacterial activity against gram-positive and gram-negative bacteria that as *Staphylococcus aureus* and *Shigella flexineri* with a goodinhibition zone. In the previous study, antimicrobial peptides with gram-positive and gram-negative bacteria

growth-inhibiting abilities have been reported [9]. Numerous molecules with antibacterial activity have been isolated from plants [10, 12]. According to the World Health Organization (WHO), medicinal plants would be a very effective source of obtaining a variety of active compounds and drugs. Therefore, such plants should be investigated to understand their properties, efficiency, and safety [16]. Hence an attempt is made to explore the possible antimicrobial peptides generated through the medicinal plants [17]. In previous studies, the antibacterial peptide was isolated from different plant sources. Similar to our study, we found potential antibacterial peptides isolated from *capsicum* [15]. Several antimicrobial peptides have been reported [17], purified and characterized by using different techniques like ion exchange chromatography and RP-HPLC, which are quite expensive and time-consuming [18]. Here, we have isolated the antibacterial proteins/peptides from *Vigna aconitifolia L* by cost-effective and time-saving methods for plant protection and therapeutic potential.

CONCLUSION: It is concluded that traditional plants may represent new sources of antimicrobials with the use of AMPs as single therapeutic antibiotic agents. The current study identified a novel antibacterial peptide from *Vigna aconitifolia L*. The results showed that 30kDa **Fig. 3** of the peptide was identified and exhibited broad-spectrum antibacterial activity, which can be further used to develop novel therapeutic agents and biologically active components. Further Proteomic analysis is in progress to ensure the authenticity of the results regarding the peptide activity tested in our study.

ACKNOWLEDGEMENT: We are grateful to the Department of Microbiology, Sahyadri Science College, Shimoga, Karnataka, for supporting us in this work. We take the privilege to acknowledge Dr. N. Mallikarjun, Department of Microbiology, Sahyadri Science College, Shimoga, Karnataka, for his contribution to the project and manuscript preparation. We are thankful to the Department of OBC for a financial assistantship.

CONFLICTS OF INTEREST: The authors declare that they do not have any conflict of interest.

REFERENCES:

1. Barashkova AS and Rogozhin EA: ZEQ Isolation of antimicrobial peptides from different plant sources: Does a general extraction method exist. Plant Methods 2020; 16: 143.
2. Andras Fodor, Birhan Addisie Abate, Peter Deak and Laszlo Fodor: Multidrug Resistance (MDR) and collateral sensitivity in Bacteria, with special attention to Genetic and Evolutionary aspects and to the perspectives of AMP-A review. Pathogens 2020; 9: 522.
3. Shelenkov A, Slavokhotova A, Odintsova T. Predicting antimicrobial and other cysteine-rich peptides in 1267 plant transcriptomes. Antibiotics 2020; 9: 60.
4. Sushmita Singh and Imtiyaz Ansari: A Pharmacognostic and pharmacological review on *Vigna aconitifolia* (Moth bean). The Pharma Innovation J 2018; 7(10): 491-495.
5. Pires ÁS, Rigueiras PO, Dohms SM, Porto WF and Franco OL: Structure-guided identification of antimicrobial peptides in the spathe transcriptome of the non-model plant, arum lily (*Zantedeschia aethiopica*). Chem Biol Drug Des 2019; 93: 1265–75.
6. Kozaki D, Sago Y, Fujiwara T, Mori M, Kubono C, Koga T, Mitsui Y and Tachibana T: Ion-Exclusion/Cation-Exchange Chromatography Using Dual-Ion-Exchange Groups for Simultaneous Determination of Inorganic Ionic Nutrients in Fertilizer Solution Samples for the Management of Hydroponic Culture. Agronomy 2021; 11(9): 1847
7. Arianna Yglesias-Rivera, Ana Zenaida Viera-Morales, Yania Suarez-Perez, Jose Raul Torres-Viltres and Rayner Ochoa-Cardentey: Validation of modified Lowry for the determination of total protein concentration of *Rhopalurus junceus* scorpion venom. Biomedical Journal of Scientific & Technical Research 2020; 28(3): 21627-21632.
8. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-5.
9. Shen Y, Xu L, Huang J, Serra A, Yang H, Tam JP: Potentides: new Cysteine-Rich Peptides with Unusual Disulfide Connectivity from *Potentilla anserina*. Chem Bio Chem 2019; 20: 1995–2004.
10. Finkina EI, Melnikova DN, Bogdanov IV and Ovchinnikova TV: Peptides of the innate immune system of plants. Part II. Biosynthesis, biological functions and possible practical applications. Russ J Bioorganic Chem 2019; 45: 55–65.
11. Santos-Silva CAD, Tricarico PM, Vilela LMB, Roldan-Filho RS, Amador VC,d'Adamo AP, Rego MdS, Benko-Iseppon AM and Crovella S: Plant Antimicrobial peptides as potential tool for Topic Treatment of Hidradenitis Suppurative Front Microbiol 2021; 12: 795217.
12. Torres MDT, Sothiselvam S, Lu TK and de la Fuentez-Nunez C: Peptide design principles for antimicrobial applications. J Mol Biol 2019; 431: 3547-3567.
13. Manisha Thapliyal, Anjali Bisht and Ajeet Singh: Isolation of antibacterial protein/peptide from *Ficus glomerata* leaf. Int J Curr Pharm Res 2016; 8(4): 24-27.
14. Petre B: Toward the discovery of host-defense peptides in plants. Front Immunol 2020; 11: 1825.
15. Maracahipes AC, Taveira GB, Sousa-Machado LY, Machado OLT and Rodrigues RCarvalho AO: Characterization and antifungal activity of plant peptide expressed in the interaction between *Capsicum annuum* fruits and the anthracnose fungus. Biosci Rep 2019; 39: BSR20192803.

16. Swee-Seong Tang, Zakaria H Prodhan, Sudhangshu K Biswas, Cheng-Foh Le and Shamala D Sekaran: Antimicrobial peptides from different plant sources: Isolation, characterization, and purification, Phytochemistry 2018; 07: 002.
17. Li J, Hu S, Jian W, Xie C and Yang X: Plant antimicrobial peptides: structures, functions and applications. Bot Stud 2021; 62: 5.
18. de la Torre BG and Albericio F: Peptide therapeutics. Molecules 2020; 25: 2293.

How to cite this article:
Swathi D and Mallikarjun N: Isolation of antibacterial peptide from *Vigna aconitifolia* L.. Int J Pharm Sci & Res 2023; 14(5): 2477-82. doi: 10.13040/IJPSR.0975-8232.14(5). 2477-82.