



ANALYSIS OF ACTIVE COMPONENTS AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) PROFILING OF EMPEROR SCORPION (*PANDINUS IMPERATOR*) AGAINST SELECTED BACTERIAL STRAINS

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ABSTRACT

This study was carried out by collecting fifty *Pandinus imperator* Scorpions aseptically extracting the venom and tested on selected bacteria. The extracted venom was in crude (non-lyophilized) state but lyophilized to compare their different efficacy and was passed through a membrane filter. The Bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, and *Acinetobacter baumannii*) were isolated from drinking water sources of the University of Abuja after which it was preserved in slant for further use. Appropriate differential and selective agar were used to culture and sub-culture the isolates and serotyped while Analytical Profile Index (API) kits were used to confirm the isolated bacteria. Column chromatography of the venom to determine the active ingredient was also done after which high-performance liquid chromatography was carried out. The result shows in mean lowest zone for crude venom against isolated bacteria was 3mm at 25% against *Acinetobacter baumannii* while the highest was 11.5mm at 100% against *Pseudomonas aeruginosa*, the lowest for crude venom against serotyped was 4mm at 25% against The lowest MIC was 25% and 20mg/ml while the highest is 50% and 30mg/ml, the lowest MBC was 50% and 30mg/ml while the lowest was 75% and 40mg/ml. Column chromatography and High-Performance Liquid Chromatography were used to determine Pi3 and Pi5 which were found to be the active components in the venom. ANOVA statistical analysis carried out showed no significant difference in the activities of both venom states.

Keywords: Antimicrobial Peptides; Antibacterial Activity; Emperor Scorpion; *Pandinus Imperator*; Scorpion Venom; Column Chromatography, High-Performance Liquid Column Chromatography

1 INTRODUCTION

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan, as well as killing viruses. The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world.

Resistance to antibiotics is a rising concern, healthcare professionals, with antimicrobial activities in plants, bacteria, and animals (1; 2). Antimicrobial peptides (AMPs) are key effectors of the innate immune response of animals and show antimicrobial therapy potency (3; 4; 5; 6; 7). Most AMPs that display hydrophobic and cationic properties have a molecular mass below 25 to 30 kDa (8; 9; 1). So far, more than 2000 natural AMPs have been isolated, sequenced, and submitted until now. Some natural antimicrobial peptides are being developed for use either as antibiotics for topical use in healthcare or as preservatives in the food industry. Apart from the extreme diversity in their primary and secondary structures, AMPs have *in vitro* effects on a wide spectrum of bacteria (Gram-negative and Gram-positive) and cells, including parasites, tumor cells, fungi, and viruses (3). AMPs have non-specific processes involved in the antimicrobial action. The unique property of an antimicrobial peptide is its ability to interact selectively with bacterial lipid membranes and cause disruption. This property could decrease the likelihood of microbes acquiring resistance to AMPs. This could give AMPs a major advantage over conventional antibiotics, and in response, these peptides have been extensively investigated as potential antimicrobial agents (10; 11; 12; 13).

Scorpions are particularly resistant to bacterial aggressions, and it is of interest to analyze the molecules responsible for this resistance (14). The scorpion venoms have three different groups of ingredients and peptides, based on their molecular mass with enzyme activity such as hyaluronidases, phospholipases, and sphingomyelinase (15). The second group contains mainly a peptide fraction with molecular masses around and lower than 10 kDa such as toxins and cytolytic compounds. The third group is composed of components such as ions, free amino acids, biogenic amines, neurotransmitters, acylpolyamines, heterocyclic compounds, and alkaloids (16; 17). Different antimicrobial peptides such as micropores have been identified in plants and animals (18; 19), BmKb1 and BmKn2 (20), Hadrurin (21) and Mucroporin (15).

Caerin is an antimicrobial peptide that was first found in amphibian, Australian green tree frogs (22). Later different variants were isolated from several Australian frogs of the *Litoria* genus (23; 24) and African frog (12). In recent years, caerin and caerin-like antimicrobial peptides were been found in scorpions such as *Mesobuthus lupus* and *Mesobuthus martensii* (25). Antibacterial activity of caerin on Gram-positive and negative bacteria has been shown with only small variation in their minimum inhibitory concentration (MIC) values (26; 27).

Objective

- Extract and Quantify the different components of *Pandinus imperator* venom by high-performance liquid chromatography (HPLC) and column chromatography

2 MATERIAL AND METHODS

Gwagwalada is one of the six Local Government Area Councils of the Federal Capital Territory of Nigeria, together with Abaji, Kuje, Bwari, Kwali; and the Abuja Municipal Area Council (AMAC). Gwagwalada is also the name of the main town in the Local Government Area, which has an area of 1,043 km² and a population of 157,770 at the 2006 census. It is located at an elevated of 210 meters above sea level. Its coordinates are 8°56'29" N and 7°5'31" E in DMS (Degree Minutes Seconds) or 8.94139 and 7.09194 (in decimal degrees). Its UTM position is KQ98 and it is Joint Operation Graphics reference is NC32-13 (28).

2.1 Media and preparation

All media used were prepared according to the manufacturer's standard of preparation.

2.2 Sample collection

2.2.1 Method of venom collection

Fifty (50) numbers of Emperor Scorpion (*Pandinus imperator*) which were identified in Zoology Department, Faculty of Science, University of Abuja, venoms were collected by the electrical stimulation method described by (29). A series of regular currents were applied to shock the scorpion until the venom was ejected. For that purpose, the body of the scorpion was immersed in a saline solution for better electrical conduction and gave a shock with the electrode by using a simple 12-volt battery. The venom droplet was recovered in a sterile test tube after which part of the venom in the test tube was placed in a container containing ice and used within 30 minutes while another part was kept frozen until

use. Venom was recovered using distilled water and centrifuged (10,000 g). The supernatant was lyophilized (freeze-dried) and then kept at 20 °C until use. All venom collected was passed through the membrane filter.

2.2.2 Collection of Test organisms

20 ml of drinking water samples were from sources of drinking water supply in the University of Abuja main site, mini campus, and staff quarters for the isolation of selected test bacteria. All the samples were taken to the Microbiology Laboratory University of Abuja for culturing, isolation, and identification of the isolates.

2.3 Preparation of samples

2.3.1 Preparation of The Emperor Scorpion (*Pandinus imperator*) venom

The venom of *Pandinus imperator* was collected and measured in percentage since venom is in liquid state. Raw venom represents 100% while 50%, 25%, and 12.5% were diluted with 50%, 75%, and 87.5% of appropriate neutral diluents (sterile water) respectively, and also 10, 20, 30, and 40 mg/ml for the lyophilized.

2.3.2 Preparation of test organism

The samples collected were taken to the laboratory in a sterile plastic container. Using the serial dilution method, they were cultured on an enrichment medium, incubated, and incubated on MacConkey agar at 37°C for 24 hours. The suspected colonies were further sub-cultured on selective medium (CHROM agar, Eosin methylene blue (EMB), and Cetrimide agar) on which the colonies were red, metallic green sheen, and blue-green/yellow-green color, then the single colony was picked, screened and identified based on Analytical Profile Index (API). The serotyped organism was sub-cultured on selective media and confirmed based on the normal standard biochemical techniques (30; 31).

3 QUANTITATIVE DETERMINATION OF DIFFERENT COMPONENTS OF THE EMPEROR SCORPION VENOM USING (HPLC) PURIFICATION

HPLC studies were carried out using the KNAUER (Dr.Ing. Herbert Knauer GmbH, Berlin, Germany) instrument made up of the following elements: HPLC PUMP K-1001, detector (UV K-2501), column oven, manual injector with 20 µm loop and EUROCHROM 2000 V 2.05 computer software. In the investigations for the development of the method for separation and identification of scorpion venom components the following parameters were checked using literature data (32; 33):

- (1)- Chromatographic columns with C18 packing, 25 cm x 4 mm (4.6 mm), 5 µm, of different pore size: 100 (Knauer Eurosfer-100 C18, Dr.Ing. Herbert Knauer GmbH, Berlin, Germany), 180 (DISCOVERY®C18, Supelco Park, Bellefonte PA, USA) and 300 (SUPELCOSIL™ LC-318, Supelco Park, Bellefonte PA, USA)
- (2)- Conditions for linear gradient elution: 0%B - 45%B for 60 min, 0%B 60%B, for 60 min, 5%B - 80%B for 40 min; - flow rate of mobile phase 1.5 ml/min, 2.0 ml/min and 2.5 ml/min;
- (3)-Separation temperature: 25°C, 30°C and 35°C; Chromatographic separation will be performed using the following eluents: eluent A - 0.1% TFA in water, B - 0.1% TFA in the solution: acetonitrile: water (80:20). Venom compounds were identified using a UV detector at 220 nm wavelength. The external standard method (one-point calibration) was used for quantization of Imcroporin BmKb1, BmKn2, Hadrurin, Mucroporin, Caerin like and any other peptides that were present. The whole procedure was validated by determining the detection limit, precision, accuracy, repeatability, and linearity of the method. Validation was performed for the assay on the column DISCOVERY®C18.

3.1 Statistical analysis

Descriptive statistics were used to analyze the data into simple percentages and standard deviation of the mean, Microsoft Excel and statistical significance determined by ANOVA were used to know if there was any statistically significant difference between the venoms and isolates. Differences in survival were considered significant when $P \leq 0.05$.

4 RESULTS

Table 1: Physical characteristics of Pandinus Imperator Venom

Color	odour	Form	PH	Room Temperature (28°C)
Colourless	Odourless	Transparent Liquid	4.5	Dried up easily

The result of the Physio-chemical characteristic of *Pandinum Imperator* Venom is shown in table i. the venom was observed to be colourless, odourless and transparent liquid which dries easily at room temperature.

Table 2: Analytical Profile Index (API-20E)

Bacteria Identification Using Analytical Profile Index (API)	
Preference	Identity
Permanent site	<i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i> <i>Acinetobacter baumannii</i>
Mini Campus	<i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i> <i>Acinetobacter baumannii</i>
Staff Quarters	<i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i> <i>Acinetobacter baumannii</i>

The result of the Analytical profile index of the bacterial identification of isolates shows the different bacteria isolated from the three water sources studied were *E. coli*, and *P. aeruginosa* and *A. baumannii* (Table 2)

Column Chromatography non-Lyophilized *Pandinus imperator* Venom

Table 3: Column Chromatography of non-Lyophilized *Pandinus Imperators* venom

Microorganisms	Pi1	Pi2	Pi3	Pi4	Pi5
<i>Escherichia coli</i>	+	+	-	+	-
<i>Pseudomonas aeruginosa</i>	+	+	-	+	-
<i>Acinetobacter baumannii</i>	+	+	-	+	-

P1 means *Pandinus imperator* toxins 1; Key: + = Bacterial growth; - = No bacterial growth

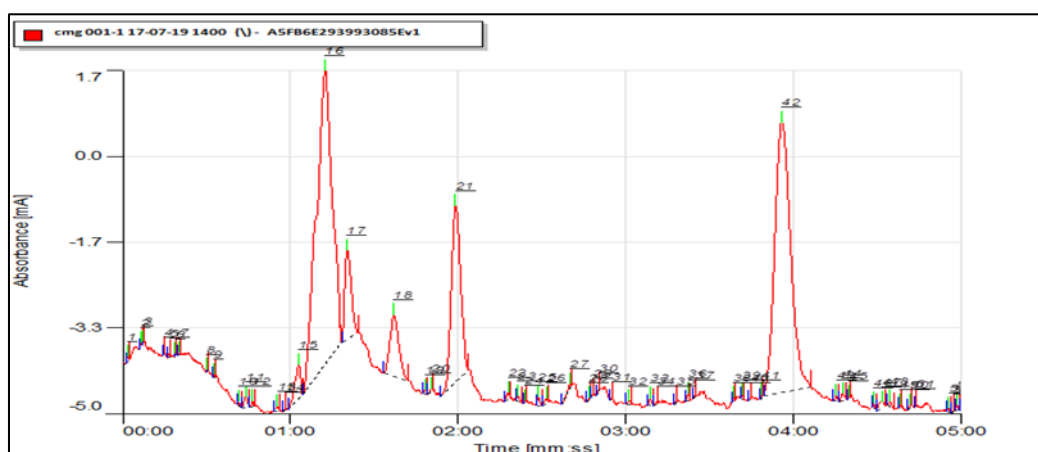


Figure 1: High Purance Liquid Chromatography

5 CONCLUSION

From this research work, it can be concluded or deduced that Venom of *Pandinus imperator* called Emperor Scorpion found in West Africa is an antibacterial agent that is non-toxic to animals, the scorpion venom either in the lyophilized or crude state has same potency and can both be safely used as a therapeutic agent.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

No conflict of interest to be disclosed.

REFERENCES

- [1] Meylears, K., Cerstiaens, A., Vierstraete, E., Baggerman, G., Michiels, C.W., DeLoof, A. and Schoofs, L. Antimicrobial Compounds of Low Molecular Mass is Constitutively Present in Insects: Characterisation of β Alanyl Tyrosine. *Current studies of Pharmaceutical Design* 2002; 8: 99-110.
- [2] Shaini, T., Shreyas, K., Ram, S.B., Jayaraman, V.K. and Susan, I.T. CAMP: a useful resource for research on antimicrobial peptides. *Nucleic-acids Research* 2010; 38: 774-780.
- [3] Bulet, P., Stöcklin, R. and Menin, L. Anti-microbial peptides: from invertebrates to vertebrates. *Immunology Reviewed* 2004; 198 (1): 169-184.
- [4] Vancompernelle, S.E., Taylor, R.J., Richter, K.O., Jiang, J., Youree, B.E., Bowie, J.H., Tyler, M.J., Conlon, J.M., Wade, D., Aiken, C., Dermody, T.S., Kewalramani, V.N., Rollins-Smith, L.A. and Unutmaz, D. Antimicrobial peptides from amphibian skin potentially inhibit human immunodeficiency virus infection and transfer of virus from dendritic cells to T cells. *Journal of Virology* 2005; 79(18): 11598-11606.
- [5] Dhople, V., Krukemeyer, A. and Ramamoorthy, A. The human beta Defensin 3, an antibacterial peptide with multiple biological functions. *Biochemistry and Biophysics Acta* 2006; 1758(9): 1499-1512.
- [6] Yibao, M., Ruiming, Z., Yawen, H., Songryong, L., Jun, L., Yingliang, W., Zhijian, C. and Wenxin, L. Transcriptome analysis of the venom gland of the scorpion *Scorpiops jendeki*: implication for the evolution of the scorpion venom arsenal. *Biomedical Genomics* 2009; 10 (290): 1-15.
- [7] Ramamoorthy, A. Beyond NMR spectra of antimicrobial peptides: Dynamical images at atomic resolution and functional insights. *Solid State Nucleic Magnesium Research* 2009; 35: 201-207.
- [8] Marsh, E.N., Buer, B.C. and Ramamoorthy, A. Fluorine-a new element in the design of membrane-active peptides. *Molecular Bio-system* 2009; 5(10): 1143-1147.
- [9] Reddy, K.V.R., Yedery, R.D. and Aranha, C. Antimicrobial peptides: premises and promises. *International Journal of Antimicrobial Agents* 2004; 24 (6): 536–547.
- [10] Harris, F., Dennison, S.R. and Phoenix, D.A. Anionic Antimicrobial Peptides from Eukaryotic Organisms. *Current studies of Protein Peptide Science* 2009; 10: 585-606.
- [11] Thennarasu, S., Huang, R., Lee, D.K., Yang, P., Maloy, L., Chen, Z. and Ramamoorthy, A. Limiting an antimicrobial peptide to the lipid water interface enhances its bacterial membrane selectivity: a case study of MSI-367. *Biochemistry* 2010; 49(50): 10595-10605.
- [12] Ramamoorthy, A., Lee, D.K., Narasimhaswamy, T. and Nanga, R.P. Cholesterol reduces pardaxin's dynamics-a barrel-stave mechanism of Membrane disruption investigated by solid-state NMR. *Biochemical and Biophysiological Acta* 2010; 1798(2): 223-237.
- [13] Thennarasu, S., Lee, D.K., Poon, A., Kawulka, K.E., Vederas, J.C. and Ramamoorthy, A. Membrane permeabilization, orientation, and antimicrobial mechanism of subtilisin A. *Chemistry of Physiological Lipids* 2005; 137(1-2): 38-51.
- [14] Sabatier, L.E., Loew, D., Goyffon, M., Fehlbaum, P., Hoffmann, J.A., Dorsselaer, A.V. and Bulet, P. Characterization of novel cysteine-rich antimicrobial peptides from scorpion blood. *Journal of Biological* 1996; 11(2):24-27.
- [15] Chao, D., Yibao, M., Zhenhuan, Z., Ruiming, Z., Qian, W., Yingliang, W., Zhijian, C. and Wenxin, L. Mucroporin, the first cationic host Defense peptide from the venom of *Lychas mucronatus*. *Antimicrobial Agents of chemotherapy* 2008; 52(11): 3967-3972.
- [16] Ma, Y., Zhao, Y., Zhao, R., Zhang, W., He, Y., Wu, Y., Cao, Z., Guo, L. and Li, W. Molecular diversity of toxic components from the scorpion *Heterometrus petersii* venom revealed by proteomic and transcriptome analysis. *Proteomics* 2010; 10(13): 2471-2485.
- [17] Kuhn-Nentwig, L. Antimicrobial and cytolytic peptides of venomous arthropods CMLS. *Cellular Molecular of Life Science* 2003; 60: 2651-2668.

- [18] He, Q.Y., He, Q.Z., Deng, X.C., Yao, L., Meng, E., Liu, Z.H. and Liang, S.P. ATDB: a uni-database platform for animal toxins. *Nucleic Acids Research* 2008; 36: 293-297.
- [19] Zhao, Z., Ma, Y., Dai, C., Zhao, R., Li, S., Wu, Y., Cao, Z. and Li, W. Imcroporin, a new cationic antimicrobial peptide from the venom of the scorpion *Isometrus maculatus*. *Antimicrobial Agents Chemotherapy* 2009; 53: 3472.
- [20] Zeng, X.C., Wang, S.X., Zhu, Y., Zhu, S.Y. and Li, W.X. Identification and functional characterization of novel scorpion venom peptides with no disulfide bridge from *Buthus martensii* Karsch. *Peptides* 2004; 25(2): 143-50.
- [21] Torres-Larios, A., Gurrola, G.B., Zamudio, F.Z. and Possani, L.D. Hadrurin, a new antimicrobial peptide from the venom of the scorpion *Hadrurus aztecus*. *European Journal of Biochemistry* 2000; 267: 5023-5031.
- [22] Wong, H., Bowie, J.H. and Carver, J.A. The solution structure and activity of caerin 1.1, an antimicrobial peptide from the Australian green tree frog, *Litoria splendida*. *European Journal of Biochemistry* 1997; 247: 545-557.
- [23] Brian, C.S.C., Carver, J.A., Lindner, R.A., Bowie, J.H., Wong, H. and Lie, W. Caerin 4.1, an Antibiotic Peptide from the Australian Tree Frog, *Litoria caerulea*. The NMR-Derived Solution Structure. *Australian Journal of Chemistry* 2000; 53(4): 257-265.
- [24] Steinborner, S.T., Currie, G.J., Bowie, J.H., Wallace, J.C. and Tyler, M.J. New antibiotic caerin 1 peptide from the skin secretion of the Australian tree frog *Litoria chloris*. Comparison of the activities of the caerin 1 peptides from the genus *Litoria*. *Journal of Peptide Research* 1998; 151 (2): 121-126.
- [25] Luo, F., Zeng, X.C., Hahin, R., Cao, Z.J., Liu, H. and Li, W.X. Genomic organization of four novel non-disulfide-bridged peptides from scorpion *Mesobuthus martensii* Karsch: gaining insight into the evolutionary mechanism. *Peptides* 2005; 26(12): 2427-2433.
- [26] Boland, M.P. and Separovic, F. Membrane interactions of antimicrobial peptides from Australian tree frogs. *Biochemistry and Biophysics Acta* 2006; 1758(9): 1178-1183.
- [27] Chia, C.S., Gong, Y., Bowie, J.H., Zuegg, J. and Cooper, M.A. Membrane binding and perturbation studies of the antimicrobial peptides caerin, citropin, and maculatin. *Biopolymers* 2011; 96(2): 147-157.
- [28] Wikipedia (2016)
- [29] Ozkan, O. and Filazi, A. The determination of acute lethal dose-50 (LD50) levels of venom in mice, obtained by different methods from Scorpions. *Androctonus crassicauda* (Olivier 1807). *Acta Parasitology Turcica* 2004; 28(1):50-53.
- [30] Chessbrough, M. Medical laboratory manual for Tropical Countries. *Microbiology II* 1998; 196-205.
- [31] Adegoke, O.S., Owoicho, I., Obekpa, Nnamso, J., Ibuotenang, D. and Shaibu, S.E. Phytochemical Synthesis of Copper Nanoparticles Using Hibiscus Rosa-Sinensis Flower Extract and Assessment of their Antibacterial and Antioxidant Potentials. *UMYU Journal of Microbiology Research*, 2025; 10 (2) 188 – 200
- [32] Szókán, G. Y., Horvath, J., Almas, M., Saftics, G. Y., and Palocz, A. Liquid chromatographic analysis and separation of polypeptide components from honey bee venoms. *Journal of liquid chromatography*, 1994; 17(16), 3333-3349.
- [33] Szókán, G., Hadfi, S., Krizsán, K., Liembeck, A., Krecz, I., Almás, M. and Somlai, C. HPLC determination of enantiomeric purity of protected amino acid derivatives used in peptide synthesis. *Journal of Liquid Chromatography & Related Technologies*, 1994; 17(13), 2759-2775.