International Journal of Endorsing
HEALTH SCIENCE RESEARCH

IJEHSR

Original Article

Evaluation of the antimicrobial potential of partially purified proteins/peptides of Yellow Scorpion Buthus sindicus against Carbapenem-Resistant Acinetobactor baumannii & Pseudomonas aeruginosa.

Sehrish Urooj[1], **Rafat Amin**[2] **& Zeba Haque**[3]

[1]Department of Biochemistry, Institute of Basic Sciences, DUHS, Karachi-Pakistan.
[2]Department of Biochemistry, Institute of Basic Biomedical Sciences, DUHS, Karachi-Pakistan.
[3]Department of Pathology, Dow College of Pharmacy, DUHS, Karachi-Pakistan.

Doi: 10.29052/IJEHSR.v9.i2.2021.238-247
Corresponding Author Email:
sehrishurooj2015@gmail.com
Received 04/01/2021
Accepted 25/05/2020
First Published 01/06/2021

OPEN ACCESS

Abstract

Background: Increasing antimicrobial resistance of microbes is one of the leading challenges faced by the medical community and human health. This study focused on a local scorpion species (Buthus sindicus) of Pakistan known for its potent antibiotic properties against Carbapenem-resistant Acinetobacter baumannii and Pseudomonas aeruginosa.

Methodology: Venom extracted from Buthus sindicus tested against Carbapenem-resistant A. baumannii and P. aeruginosa in both crude and partially purified forms. The antibacterial Screening was performed by the Agar-disc diffusion method, using different concentrations of venom while using commercially available antibiotics as positive controls.

Results: Among both two species P. aeruginosa and A. baumannii, were tested, there is no zone of inhibition found in any form of Buthus sindicus in the Disc Diffusion Method. Scorpion venom was processed with deionized water, PBS, and Tris-HCl and observed no difference in antibacterial activity.

Conclusion: Our study concluded that no antimicrobial activity was found against any selected drug-resistant pathogens in the venom of Buthus sindicus, probably because of the lack of disulphide bonds.

Keywords

Antimicrobial Resistance, Buthus Sindicus, Multidrug-Resistant Pathogens, Scorpion Venom, Antimicrobial Activity.

Check for updates

Introduction

An increased incidence of Multidrug-resistant bacterial infections has reported globally. The World Economic Forum Global Risk (WEFGR) reported that multidrug resistance (MDR) is one of the ultimate threats to human health. A progressive compromise in the efficacy of modern antimicrobial medicines has been observed due to increased antimicrobial resistance (AMR), creating a massive obstacle in treating various infectious diseases[1-4]. Approximately 50% of the antibiotics used worldwide contain beta-lactam rings; resistance to these antibiotics is a grave problem concerning the medical world today[5,6]. According to World Health Organization (WHO) report listing the pathogens that are a priority, twelve organisms are considered prime concern[7].

Acinetobacter baumannii and Pseudomonas aeruginosa are two pathogens selected from the list for this study. These bacteria have developed resistance against multiple antibiotics, and WHO emphasizes an urgent need to create new antibiotics/antibacterial agents against them to counter the diseases caused by them[8, 9].

As the members of ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.) pathogens, A. baumannii and P. aeruginosa are known to cause lethal nosocomial infections frequently associated with admission to ICU (Intensive Care Unit)[10]. A. baumannii is mainly involved in wound infections, bacteremia, ventilator-associated pneumonia, urinary tract infections, and meningitis[11,12]. While P. aeruginosa is an opportunistic organism associated with bloodstream infections[13,14]. Infections of P. aeruginosa are associated with increase sputum production, pulmonary hemorrhage and vascular leakage in the lungs. Pseudomonas also causes other diseases such as Pneumonia, Endocarditis, Otitis, Conjunctivitis, Dermatitis, Urinary tract infections and infections involving the central nervous system[15, 16]. Carbapenem used to treat A. baumannii and P. aeruginosa infections. Still, the organisms have acquired resistance to this antibiotic by establishing an increased production of Carbapenemases (Carbapenem degrading enzyme), structural changes in Penicillin Binding Protein (PBP), efflux pump overexpression, and loss of genetic expression of porin proteins[17-19].

Discovering new biologically active compounds with higher efficacy and lesser side effects from natural resources like plants and animals is the hot topic of research to battle AMR[20]. Scorpion venom is known to contain antimicrobial properties against certain microbes, increasingly recognized as a rich source of proteins/peptides with antimicrobial functions. Antimicrobial activities of scorpion venom and the combination of protein complexes present in its structure provide stencils for drug designing. These biomolecules have been considered as potential alternatives for today's antibiotics[21, 22].

This study aimed to explore the antimicrobial potential of the venom of scorpion Buthus sindicus, found in Pakistan, which has not been reported in the literature until now. This study was performed to explore the evidence that scorpion Buthus sindicus contains bacteriostatic and/or bactericidal activity against multidrug resistant Pseudomonas aeruginosa and Acinetobactor baumannii.

Methodology

Scorpion venom Collection

A total of 300 scorpions belonging species Buthus sindicus got purchased from rural areas of Sindh via professional snake charmers who were well-versed in collecting and identifying these arthropods. Out of 300, venom was extracted from 250 scorpions, as rest lacked the venom for unknown reasons. Venom was collected in fine glass sterile micropipette by electrical stimulation (15 volts) of telson (last segment in the abdomen) Venom of 250 scorpions was dissolved in deionized water and centrifuged at 15000 rpm for 10 minutes. Supernatant was stored at -80℃ until further use[23-25].

Preparation of Scorpion venom

Venom processed with Deionized water

15µl of venom taken from 5 scorpions was collected in each of the 20 eppendorf tubes to be treated with deionized water. 50µl of sterile deionized water was added in each eppendorf containing venom and centrifuged at 15000 rpm for 10mins. The supernatant was pooled separately and stored at -80ºC till further processing[23, 25].

Venom processed with Tris-HCl.

The amount of scorpion venom was 15 µl taken from 5 scorpions was collected in each of the ten eppendorf tubes to be treated with Tris-HCl. 50µl of 1M Tris-HCl was added in each eppendorf tube containing venom and centrifuged at 15000 rpm for 10mins. The supernatant was pooled separately and stored at -80ºC till further processing[23, 24].

Venom processed with PBS

15µl of venom taken from 5 scorpions was collected in each of the ten eppendorf tubes to be treated with Phosphate Buffer Solution (PBS). 50 µl of 1M PBS was added in each eppendorf tube containing venom and centrifuged at 15000 rpm for 10mins. The supernatant was pooled separately and stored at -80ºC for further use[23, 25].

To separate any possible microorganisms present in the collected supernatants of scorpion venom prepared with deionized water, Tris-HCl, and Phosphate buffer was separately passed via Nylon syringe filters with 0.22 µm pore size. The filtrate was collected and stored at -80ºC[26].

Partial Purification of Scorpion Venom

Partial purification of protein/peptide was performed by Amicon ultracentrifugation filter tubes of 10kDa, used to separate scorpion venom in low (>10kDa) and high (<10kDa) molecular weight components[27].

Bacterial Strains Collection

Carbapenem-resistant A. baumannii and P. aeruginosa collected from Dow Diagnostic Research and Reference Laboratory (DDRRL), DUHS.

Identification of Microorganism

The identification and validation of both selected strains were made in Microbial Bio-resources and Biotechnology Laboratory (MBBL), DRIBBS. Colonial Morphology of P. aeruginosa and A. baumannii were observed on Muller Hinton Agar (MHA) by incubating the plates for 24 hours at 37ºC. Both pathogens are gram-negative bacteria and microscopically produce pink short rods (Gram Staining)[28]. Biochemical testing was done by Oxidase test[29], Methyl red test, and Voges-Proskauer test[30] for P. aeruginosa. While A. baumannii was biochemically identified by Methyl red, Voges-Proskauer[30], Coagulase[31], and Catalase tests[32].

Experimental Protocol Protein Estimation

NanoDrop spectrophotometer was used to estimate protein concentration in crude and partially purified venom. 3 µl Venom (sample) volume was used for the purpose. The wavelength was set at 280 nm. The deionized water, Tris-HCl, and PBS were used as control separately.

Inoculations of test plates

Bacterial Plates of MH agar were prepared for susceptibility test in two different ways: Muller Hinton agar test plate was prepared by spreading 10 µl of inoculum of each strain (1:100 diluted of 0.5 McFarland maintained) evenly on the plate surface through sterile glass spreader and in another way 1% of soft agar was used for the preparation of bacterial lawn[33].

Agar disc-diffusion

Antimicrobial Screening of collected venom was carried out through a disc diffusion susceptibility test following Clinical Laboratory Standard Institute Protocols. 100 µl of bacterial inoculums (of a 0.1 A600) were spread onto sterile Muller-Hinton Agar plates until the surface of the plate became dry. Sterile paper discs were placed onto the MH agar surface, and different concentrations of scorpion venom sample (20 µl) were added per disc in replicates. The disc containing 20 µl of

distilled water/buffer served as normal control, and discs containing antibiotics were used as drug controls. The plates were incubated at 37°C for 24 h, following which the diameter of inhibition zones was measured[33].

Determination of Antimicrobial activity of Diluted Scorpion venom by Agar-disc diffusion method

The pure culture of each strain was separately inoculated in 5 ml of Tryptic soy broth (TSB) and incubated for 24 hours at 37ºC. O.D was maintained up to 0.1A (0.5 McFarland) by adding freshly prepared TSB. Dilution (1:100) of each culture was mixed in soft agar (1%) and evenly distributed onto Muller Hinton agar plates and dried under the hood. Six plain sterile discs were placed on MH agar plates. 20 µl of diluted venom (1, 10, 50, 100, 250, and 500) was separately added with two positive control (respective antibiotic for the test organism) and negative control (Deionized water). Test plates were incubated for 24 hours at 37ºC[33].

Determination of Antimicrobial activity of Crude venom by Agar-disc diffusion method

The pure culture of each strain was inoculated in 5 ml of Tryptic soy broth (TSB) and incubated for 24 hours at 37ºC. O.D was maintained up to 0.08-0.1A (0.5 McFarland), and mixed 1 ml of each culture (1:100) in agar (1%), and evenly distributed onto Muller Hinton agar plates and air-dried. Two plain sterile discs and two antibiotic discs were placed on MH agar plates. 20µl of crude venom and 20 µl of deionized water were added on plain discs separately. Test plates were incubated for 24 hours at 37ºC[33].

Experimental Protocol of Antimicrobial activity of scorpion venom with PBS

Each strain with maintained O.D up to 0.08-0.1A (0.5 McFarland) was incubated in Tryptic soy broth for 24 hours at 37ºC. Diluted culture (1:100) was mixed in agar (0.6%), and evenly distributed onto Muller Hinton agar plates, and dried. Two plain sterile discs and 2 antibiotic discs were placed on MH agar plates. 20 µl of each scorpion venom and PBS were added on separate plain discs and incubated for the plates for 24 hours at 37˚C[33].

Experimental protocol for antimicrobial activity of scorpion venom mixed with Tris-HCl

The pure culture of each strain was inoculated in 5ml of Tryptic soy broth (TSB) and incubated for 24 hours at 37ºC. O.D of overnight culture was maintained up to 0.08 (0.5 McFarland). Diluted culture (1:100) of each strain was mixed in soft agar (0.6%) and evenly distributed onto Muller Hinton agar plates. Two plain sterile discs and two antibiotic discs were placed on MH agar plates. 20 µl scorpion venom and Tris-HCL were added on separate plain discs and dried. Test plates were incubated for 24 hours at 37ºC[33].

Antimicrobial activity of filtrate (<10KDa) and retentate (>10KDa) of Scorpion Venom

Each bacterial strain was inoculated in 5 ml of sterile TSB (Tryptic Soy Broth) incubated for 24 hours at 37˚C. 100µl of each diluted bacterial strain (1:100) were separately poured onto MHA plates, spread evenly till dry. Placed three plane sterile discs and two antibiotic discs on MH agar plates. 20 µl of each crude scorpion venom, filtrate (<10kDa), retentate (>10kDa), and deionized water were added on separate plain discs and dried. Test plates were incubated for 24 hours at 37ºC[33].

Results

Protein Estimation of crude and partially purified venom

The protein concentration of crude venom with deionized water was 10.89 mg/ml, Crude venom with PBS (Phosphate Buffered Saline) was 12.24 mg/ml, and crude venom with Tris-HCl was 10.27 mg/ml. In partially purified venom concentration of protein infiltrate was 10.34 mg/ml, and retentate was 2.8 mg/ml estimated by NanoDrop spectrophotometer.

Confirmatory test for Pseudomonas aeruginosa and Acinetobactor baumannii

In macroscopic morphology, P. aeruginosa and Acinetobactor baumannii produced irregular

cream-coloured and punctiform beige-coloured colonies on Muller Hinton agar, respectively. Under the microscope, both pathogens appeared pink-coloured small rods in gram staining. P. aeruginosa was oxidase-positive; it confirmed the presence of the cytochrome oxidase enzyme. P. aeruginosa was negative for both Methyl red/Voges-Proskauer Tests. Acinetobactor baumannii was coagulase-negative, and catalase enzyme positive observed release of oxygen bubbles.

Antimicrobial Activity by Agar-disc diffusion Method

In the present study, the sensitivity of two multidrug resistance bacterial pathogens P. aeruginosa and A. baumannii, were analyzed by the Agar disc diffusion method against crude form (Figure 2, 5), diluted form (Figure 1, 4), and partially purified (Figure 3, 6) form of scorpion venom of Buthus sindicus. In this study, we used Muller Hinton Agar as a growth medium (Figure 1-6), Phosphate buffer solution, Deionized water (Figure 1-6), and Tris-HCl were used as a negative control, and commercially prepared Antibiotic discs were used as a positive control. Both 2 Bacteria were susceptible to both Polymyxin and Colistin antibiotics (Figure 1-6). Among both two species P. aeruginosa and A. baumannii, were tested, there is no zone of inhibition found in any form of scorpion venom of Buthus sindicus (Figure 1-6). Scorpion venom was processed with Deionized water, PBS, and Tris-HCl; there is no difference in antibacterial activity observed by using Deionized water (Figure 1-6), Phosphate buffer saline and Tris-HCl.

Table 1: List of Bacteria for which Antibiotics are urgently required published by WHO

Organism	Disease	Antibiotic Resistant
Acinetobactor baumannii	Hospital-derived Nosocomial infections	Carbapenem-resistant
Pseudomonas aeruginosa	Hospital-derived Nosocomial infections	Carbapenem-resistant
Enterobacteriaceae (Salmonella, E. coli, Shigella, Klebsiella)	Hospital-derived Nosocomial infections	Carbapenem-resistant, ESBL-producing
Enterococcus facium	Blood stream infections in hospitalized patients	Vancomycin-resistant
Staphylococcus aureus	Skin, Respiratory tract, Food poisoning Infections	Methicilin-resistant, Vancomycin-intermediate and resistant
Helicobactor Pylori	Chronic Gastritis, Peptic Ulcer, Gastric Cancer	Clarithromycin-resistant
Campylobactor spp.	Food-Borne Diseases	Fluoroquinolone-resistant
Salmonella	Typhoid fever, food poisoning	Fluoroquinolone-resistant
Neisseria gonorrhoeae		
Streptococcus pneumoniae	Meningitis, Pneumonia	Penicillin-non-susceptible
Haemophilus influenza	bacteremia, pneumonia, epiglottis and acute bacterial meningitis	Ampicillin-resistant
Shigella spp.	Intestinal Diseases	Fluoroquinolone-resistant

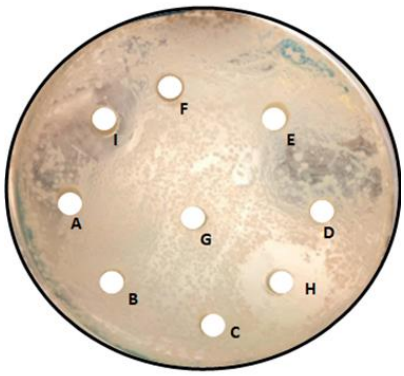

Figure 1: Pseudomonas aeruginosa; Antimicrobial activity of Diluted Scorpion venom by Agar-disc diffusion method; A: 1 µg/ml, B: 10 µg/ml, C: 50 µg/ml, D: 100 µg/ml, E: 250 µg/ml,F: 500 µg/ml, G: Deionized Water, H: Colistin, I: Polymyxin

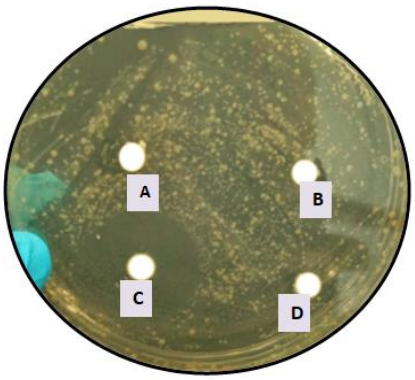

Figure 2: Pseudomonas aeruginosa; Determination of Antimicrobial activity of Crude venom by Agar-disc diffusion method; A: Crude Venom, B: Deionized Water, C:

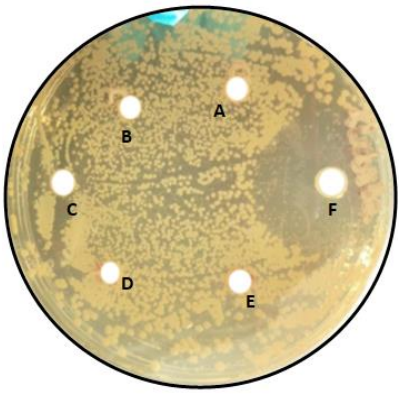

Figure 3: Pseudomonas aeruginosa; Antimicrobial activity of filtrate (<10KDa) and retentate (>10KDa) of scorpion venom; A: Crude Venom, B: Deionized Water, C: Filtrate, D: Retentate, E: Polymyxin, F: Colistin

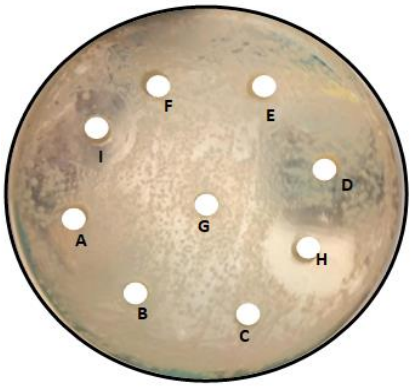

Figure 4: Acinetobactor baumannii; Antimicrobial activity of Diluted Scorpion venom by Agar-disc diffusion method; A: 1 µg/ml, B: 10 µg/ml, C: 50 µg/ml, D: 100 µg/ml, E: 250 µg/ml,F: 500 µg/ml, G: Deionized Water, H: Colistin, I: Polymyxin

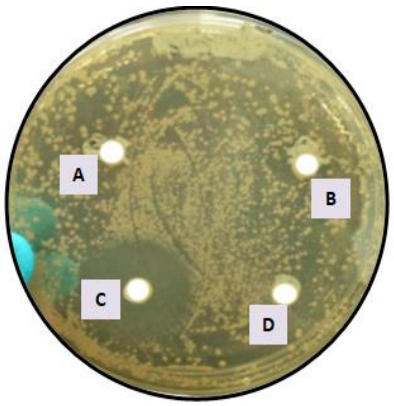

Figure 5: Acinetobactor baumannii; Antimicrobial activity of Crude venom by Agar-disc diffusion method; A: Crude Venom, B: Deionized Water, C: Colistin, D: Polymyxin

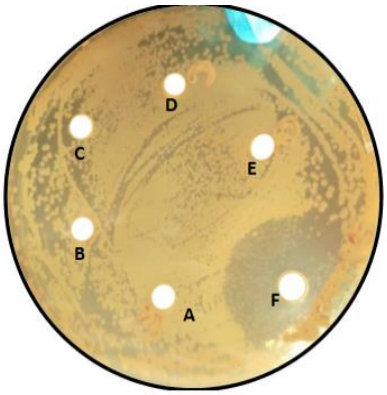

Figure 6: Acinetobactor baumannii; Antimicrobial activity of filtrate (<10KDa) and retentate (>10KDa) of scorpion venom; A: Crude Venom, B: Deionized Water, C: Filtrate, D: Retentate, E: Polymyxin, F: Colistin

pathogen P. aeruginosa and A. baumannii to seek its antibiotic potential against these organisms, as scorpion venom is known for its anti-cancer, antiviral, antimalarial, and antibacterial activities[34]. It also contains various biomolecules that modulate cell membrane ion channels and their receptors[35]. Antimicrobial peptides in venom may have different mechanisms to kill organisms such as bacteria, viruses, fungi, and parasites that might threaten the scorpion's integrity. According to the literature, scorpion venom of several species are active against both Gram-positive and Gram-negative bacteria either in a crude or purified form. Some examples of antimicrobial peptides (AMPs) are BmKn1, BmKbpp, IsCT, IsCT2, Hadrurin, and Parabutoporin, isolated from the venom of different scorpion species, are predicted to accumulate easily in the bacterial cell membrane to form pores and destroy it[36-38].

To evaluate the antimicrobial activity of the venom of scorpion Buthus sindicus was diluted with three different chemicals, including Phosphate buffer solution, deionized water, and Tris-HCl separately. Venom was tested against two of the multidrug-resistant pathogens enlisted in the critical category by WHO in 2017, namely Pseudomonas aeruginosa and Acinetobactor baumannii.

The Susceptibility test of the scorpion Buthus sindicus was studied by the Agar disc-diffusion method. No zone of inhibition was found when the venom of Buthus sindicus was tested against both pathogens. In 2017, Abdul Rahman et al. tested 10 MDR bacterial and a fungal species against the crude venom of scorpion Androctonus bicolor and Androctonus crassicauda and found both species lacking the antimicrobial activity against Pseudomonas aeruginosa, E. coli, Klebsiella pneumonia, Staphylococcus aureus, Enterococcus faecalis, and Acinetobactor baumannii. Abdul Rahman et al. concluded that the selected scorpions might not contain any effective proteins or enzymes responsible for bactericidal activity[39, 40]. However, Hassan et al. (1984) stated that A. crassicauda is the most poisonous scorpion in the world. The Lethal Dose50 value of A. crassicauda is 0.32± 0.02 mg/kg in mice which makes the scorpion among the most toxic species worldwide[41]. Furthermore, Androctonus bicolor (black fat-tailed scorpion) is also considered to be the most lethal scorpion in the world[42].

Buthus sindicus may not contain proteins/peptides or enzymes responsible for antimicrobial activity against Acinetobactor baumannii and Pseudomonas aeruginosa. We further authenticated our results by using two different buffers PBS and Tris-HCl, along with deionized water for processing of venom in agar well diffusion, and the results were identified as above. In this study, B. sindicus was not active against A. baumannii and P. aeruginosa, but it may exhibit antimicrobial activity against other bacterial species such as venom of Buthus martensii and Buthotus hottenota contain antimicrobial activity against E. aerogens and S. aureus, but lacks any antimicrobial activity against Proteus mirabilis, E. coli, Pseudomonas aeruginosa and Proteus vulgaris[43].

Based on the Chemical properties of venom peptides are divided into two major categories Non-Disulphide-bridged peptides (NDBPs) and Disulphide-bridged peptides (DBPs). DBPs mainly affect membrane-bound ion (K, Na, Cl, and Ca) channels and altered their normal cellular physiology[44, 45]. It is experimentally proven in the literature that A. bicolor envenomation caused severe Hypercalciumia, Hypernaterimia, Hypomagnesaemia, and Hyperkalemia in rats[36]. Reported peptides of B. sindicus by S. Ali et al. (1998) contain Disulphide Bridge in its structure. Out of 5 low molecular weight peptides, four contain disulphide bonds, namely Bs5, Bs6, Bs8, and Bs14, while only one (Bs10) is basic non-cysteinic and thermolabile in nature[23]. Crude venom fraction of Buthus sindicus studied via HPLC i.e. Bs36, Bs38, Bs40, and Bs41 designated as Buthus sindicus depressant insect toxins (Bs-dprIT1 to Bs-dprIT4) are found to be toxic against insect Larvae, Cockroaches and Blowflies, but it does not have any effect on mice[46].

According to a study, 34 out of 40 venoms tested for Anti-cancer, Antiparasitic, Anti-malarial,

Bradykinin-potentiating, and Anti-bacterial activities displayed NDBPs instead of DBPs[45, 48]. It might be concluded that as venom taken from Buthus sindicus lacks NDBPs, as reported by Ali et al. [23], it may be the cause of it lacking the antimicrobial activity. A similar situation was seen in the case of an antimalarial peptide named meucin-24, which shares structural similarity with two different antimicrobial peptides named magainin-1 and magainin-2 isolated from frog[47]. Meucin-24 showed no toxicity to microorganism) and mammals, it's only inhibited the development of Plasmodium falciparum and Plasmodium berghei (malarial parasite). The property of this selective inhibition of meucin-24 is due to the lack of hydrophilic/hydrophobic balance (amphipathic architecture)[49], a distinctive structural arrangement is significant for antimicrobial activity. Furthermore a structural similarity was found between the peptides isolated from the venom of Buthus sindicus[23] and a peptide named buthinin from the blood of scorpion Androctomus autralis; while buthinin was found active against E. coli and Micrococcus luteus, peptides extracted from the venom of B. sindicus were inactive against the tested MDR pathogens[49].

This study was designed to explore the antimicrobial potentials of scorpion Buthus sindicus, which has not been testified in the literature up till now. The major health problem of MDR bacteria can be overcome by considering different natural sources such as scorpion venom for the development of novel drugs. The present study provided evidence that venom of scorpion Buthus sindicus may not contain antimicrobial activity against carbapenem resistance Pseudomonas aeruginosa and Acinetobacter baumannii. Due to lack of resources, scorpion venom proteins/peptides of Buthus sindicus could not further be purified in smaller peptides for detailed study. In the present study, no antimicrobial activity was found in either crude or partially purified form of venom taken from specie Buthus sindicus. It is highly recommended that venom proteins and peptides of scorpion Buthus sindicus should be further processed and isolated for sequencing to understand its structure and cellular processes. It is possible that Venom of Buthus sindicus contains antimicrobial activity against other pathogens, which are not tested in this study. Therefore it is recommended that this venom should be further tested against other pathogens to gain insight into its antibiotic potential.

Conclusion

Our study concluded that no antimicrobial activity was found against any selected drug-resistant pathogens in the venom of scorpion Buthus sindicus, probably because of the lack of Disulfide bonds, as discussed. Further studies are required on non-resistant bacterial strains to evaluate if the venom contains any effect against these microbes. Besides this, venom may also be investigated for biological properties other than an antibiotic activity which may prove beneficial in the medical field.

Conflicts of Interest

The authors have declared that no competing interests exist.

Acknowledgement

We are very thankful to Ms. Samina in Dow Diagnostic Research and Reference Laboratory (DDRRL), DUHS for providing Bacterial Strains.

Funding

The author(s) received no specific funding for this work.

References

1. Clímaco EC, de Oliveira ML, Pitondo-Silva A, Oliveira MG, Medeiros M, Lincopan N, da Costa Darini AL. Clonal complexes 104, 109 and 113 playing a major role in the dissemination of OXA-carbapenemase-producing Acinetobacter baumannii in Southeast Brazil. Infec, Genet and Evol. 2013;19:127-133.
2. O'Neill J. Review on antimicrobial resistance: tackling drug-resistant infections globally: final report and recommendations. Rev Antimicr Resist: Tack Drug-Resist Inf Glob: Final Rep and Recom. 2016;2016:80

3. MacLean RC, San Millan A. The evolution of antibiotic resistance. Science. 2019;365(6458):1082-1083.
4. Mobarki N, Almerabi B, Hattan A. Antibiotic resistance crisis. Int J Med Dev Ctries. 2019;40(4):561-564.
5. Bonomo RA. β-Lactamases: a focus on current challenges. Cold Spring Harb Perspect Med. 2017;7(1):a025239.
6. Pandey N, Cascella M. Beta lactam antibiotics. StatPearls [Internet]. 2020.
7. World Health Organization. WHO publishes list of bacteria for which new antibiotics are urgently needed. 2017.
8. Ambrosi C, Scribano D, Aleandri M, Zagaglia C, Di Francesco L, Putignani L, Palamara AT. Acinetobacter baumannii virulence traits: a comparative study of a novel sequence type with other Italian endemic international clones. Front in microbiol. 2017;8:1977.
9. de Carvalho LP, Kreidenweiss A, Held J. Drug Repurposing: A Review of Old and New Antibiotics for the Treatment of Malaria: Identifying Antibiotics with a Fast Onset of Antiplasmodial Action. Molecules. 2021;26(8):2304.
10. Cerqueira GM, Peleg AY. Insights into Acinetobacter baumannii pathogenicity. IUBMB life. 2011;63(12):1055-1060.
11. Thaden JT, Park LP, Maskarinec SA, Ruffin F, Fowler VG, van Duin D. Results from a 13-year prospective cohort study show increased mortality associated with bloodstream infections caused by Pseudomonas aeruginosa compared to other bacteria. Antimicrob Agents and Chemother. 2017;61(6): e02671-16.
12. Garnacho-Montero J, Timsit JF. Managing Acinetobacter baumannii infections. Curr Opin Infect Dis. 2019;32(1):69-76.
13. Turner KH, Wessel AK, Palmer GC, Murray JL, Whiteley M. Essential genome of Pseudomonas aeruginosa in cystic fibrosis sputum. PNAS. 2015;112(13):4110-4115.,
14. Le HN, Tran VG, Vu TT, Gras E, Le VT, Pinheiro MG, Aguiar-Alves F, Schneider-Smith E, Carter HC, Sellman BR, Stover CK. Treatment efficacy of MEDI3902 in Pseudomonas aeruginosa bloodstream infection and acute pneumonia rabbit models. Antimicro Agents Chemother. 2019;63(8): e00710-19.
15. Rosales-Reyes R, Gayosso-Vázquez C, Fernández-Vázquez JL, Jarillo-Quijada MD, Rivera-Benítez C, Santos-Preciado JI, Alcántar-Curiel MD. Virulence profiles and innate immune responses against highly lethal, multidrug-resistant nosocomial isolates of Acinetobacter baumannii from a tertiary care hospital in Mexico. PloS one. 2017;12(8):e0182899.
16. Tümmler B. Emerging therapies against infections with Pseudomonas aeruginosa. F1000Research. 2019;8.
17. Vandendriessche T, Olamendi-Portugal T, Zamudio FZ, Possani LD, Tytgat J. Isolation and characterization of two novel scorpion toxins: The α-toxin-like CeII8, specific for Nav1. 7 channels and the classical anti-mammalian CeII9, specific for Nav1. 4 channels. Toxicon. 2010;56(4):613-623.
18. McCracken MG, Adam HJ, Blondeau JM, Walkty AJ, Karlowsky JA, Hoban DJ, Zhanel GG, Mulvey MR. Characterization of carbapenem-resistant and XDR Pseudomonas aeruginosa in Canada: results of the CANWARD 2007–16 study. J Antimicro Chemother. 2019;74(Supplement_4):iv32-iv38.
19. Isler B, Doi Y, Bonomo RA, Paterson DL. New treatment options against carbapenem-resistant Acinetobacter baumannii infections. Antimicro Agents Chemother. 2019 Jan 1;63(1): e01110-18.
20. Liu W, Ling N, Guo J, Ruan Y, Wang M, Shen Q, Guo S. Dynamics of the antibiotic resistome in agricultural soils amended with different sources of animal manures over three consecutive years. J Hazardous Materials. 2021;401:123399.
21. das Neves RC, Mortari MR, Schwartz EF, Kipnis A, Junqueira-Kipnis AP. Antimicrobial and antibiofilm effects of peptides from venom of social Wasp and scorpion on multidrug-resistant Acinetobacter baumannii. Toxins. 2019;11(4):216-224.
22. Amorim-Carmo B, Daniele-Silva A, Parente A, Furtado AA, Carvalho E, Oliveira JW, Santos EC, Silva MS, Silva SR, Silva-Júnior AA, Monteiro NK. Potent and broad-spectrum antimicrobial activity of analogs from the scorpion peptide stigmurin. Int J Mol Sci. 2019;20(3):623.
23. Ali SA, Stoeva S, Schütz J, Kayed R, Abassi A, Zaidi ZH, et al. Purification and primary structure of low molecular mass peptides from scorpion (Buthus sindicus) venom. Comp Biochem Physiol A Mol Integr Physiol. 1998;121(4):323-32.
24. Liu G, Yang F, Li F, Li Z, Lang Y, Shen B, Wu Y, Li W, Harrison PL, Strong PN, Xie Y. Therapeutic potential of a scorpion venom-derived antimicrobial peptide and its homologs against antibiotic-resistant gram-positive bacteria. Front in Microbio. 2018;9:1159.
25. Alajmi R, Al-ghamdi S, Barakat I, Mahmoud A, Abdon N, Al-Ahidib M, Abdel-Gaber R. Antimicrobial Activity of Two Novel Venoms from Saudi Arabian Scorpions (Leiurus quinquestriatus and Androctonus crassicauda). Int J Peptide Res Therape. 2020;26(1):67-74.

26. Shao Y, Xia M, Liu J, Liu X, Li Z. Composition and profiles of volatile organic compounds during waste decomposition by the anaerobic bacteria purified from landfill. Waste Management. 2021;126:466-75.
27. Merck. Amicon® Pro Purification System with 10kDa Amicon® Ultra-0.5 Device. Amicon Pro Purification System - A Centrifugal Tool collapsing protein purifcation with concentraion & buffer exchange. 2020. Available from: https://www.merckmillipore.com/INTL/en/product/Amicon-Pro-Purification-System-with-10kDa-Amicon-Ultra-0.5-Device,MM_NF-ACS501024#documentation.
28. Bartholomew JW, Mittwer T. The gram stain. Bacteriological Rev. 1952;16(1):1-29.
29. Shields P, Cathcart L. Oxidase test protocol. Am Soc Microbio. 2010.
30. McDevitt S. Methyl Red and Voges-Proskauer Test Protocols. Am Soc for Microbio. 2009;8:1-9.
31. Katz DS. Coagulase Test Protocol: Am Soc for Microbio. 2010.
32. Reiner K. Catalase test protocol. Am Soc for Microbio. 2010.
33. Wayne P. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. INFORM SUPPL. 2011;31(1):100-121.
34. Ghosh A, Roy R, Nandi M, Mukhopadhyay A. Scorpion venom–toxins that aid in drug development: a review. Int J peptide Res Therape. 2019;25(1):27-37.
35. Zeng XC, Corzo G, Hahin R. Scorpion venom peptides without disulfide bridges. IUBMB life. 2005;57(1):13-21.
36. Al-Asmari A, Khan H, Manthiri R. Effect of Androctonus bicolor scorpion venom on serum electrolytes in rats: A 24-h time–course study. Human & Exper Toxico. 2016;35(3):293-296.
37. Seyfi R, Kahaki FA, Ebrahimi T, Montazersaheb S, Eyvazi S, Babaeipour V, Tarhriz V. Antimicrobial peptides (AMPs): roles, functions and mechanism of action. Int J Peptide Res Therape. 2020;26(3):1451-1463.
38. Li Z, Yuan Y, Li S, Deng B, Wang Y. Antibacterial activity of a scorpion-derived peptide and its derivatives in vitro and in vivo. Toxicon. 2020;186:35-41.
39. Al-Asmari AK, Alamri MA, Almasoudi AS, Abbasmanthiri R, Mahfoud M. Evaluation of the in vitro antimicrobial activity of selected Saudi scorpion venoms tested against multidrug-resistant microorganisms. J Global Antimicro Resist. 2017;10:14-18.
40. Alajmi R, Al-ghamdi S, Barakat I, Mahmoud A, Abdon N, Al-Ahidib M, Abdel-Gaber R. Antimicrobial Activity of Two Novel Venoms from Saudi Arabian Scorpions (Leiurus quinquestriatus and Androctonus crassicauda). Int J Peptide Res Therape. 2020;26(1):67-74.
41. Ismail M, Abd-Elsalam M, Al-Ahaidib M. Androctonus crassicauda (Olivier), a dangerous and unduly neglected scorpion—I. Pharmacological and clinical studies. Toxicon. 1994;32(12):1599-1618.
42. Nevo Y, Spirer Z. Myocardial and central nervous system involvement in scorpion envenomation by Androctonus bicolor bicolor. Harefuah. 1991;120(8):453-455.
43. Li Z, Hu P, Wu W, Wang Y. Peptides with therapeutic potential in the venom of the scorpion Buthus martensii Karsch. Peptides. 2019;115:43-50.
44. Perumal Samy R, Gopalakrishnakone P, Thwin M, Chow T, Bow H, Yap E, et al. Antibacterial activity of snake, scorpion and bee venoms: a comparison with purified venom phospholipase A2 enzymes. J Applied Microbio. 2007;102(3):650-659.
45. Almaaytah A, Albalas Q. Scorpion venom peptides with no disulfide bridges: a review. Peptides. 2014;51:35-45.
46. Ali SA, Stoeva S, Grossmann JG, Abbasi A, Voelter W. Purification, characterization, and primary structure of four depressant insect-selective neurotoxin analogs from scorpion (Buthus sindicus) venom. Arc Biochem Biophy. 2001;391(2):197-206.
47. Cruciani RA, Barker JL, Durell SR, Raghunathan G, Guy HR, Zasloff M, Stanley EF. Magainin 2, a natural antibiotic from frog skin, forms ion channels in lipid bilayer membranes. Eur J Pharmacol: Mol Pharmacol. 1992;226(4):287-296.
48. Jiménez-Vargas JM, Ramírez-Carreto S, Corzo G, Possani LD, Becerril B, Ortiz E. Structural and functional characterization of NDBP-4 family antimicrobial peptides from the scorpion Mesomexovis variegatus. Peptides. 2021;141:170553.
49. Ehret-Sabatier L, Loew D, Goyffon M, Fehlbaum P, Hoffmann JA, van Dorsselaer A, et al. Characterization of novel cysteine-rich antimicrobial peptides from scorpion blood. J Bio Chem. 1996;271(47):29537-29544.