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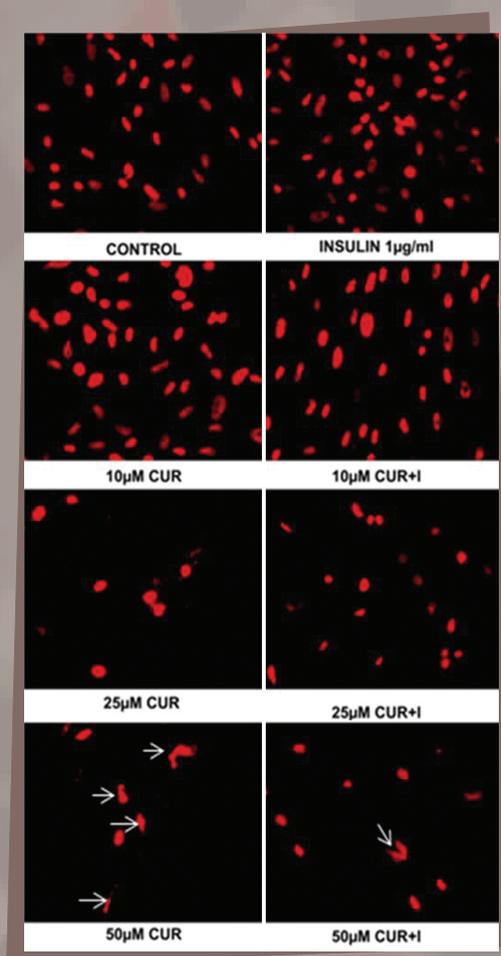


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Antibacterial activity of the venom of *Heterometrus xanthopus*

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ABSTRACT

Heterometrus xanthopus (Scorpion) is one of the most venomous and ancient arthropods. Its venom contains anti-microbial peptides like hadrurin, scorpine, Pandinin 1, and Pandinin 2 that are able to effectively kill multidrug-resistant pathogens. The present study was conducted to evaluate the anti-bacterial activity of *H. xanthopus* venom. Six Gram-positive and Gram-negative bacterial strains were tested against 1/100, 1/10, and 1/1 fractions of distilled water diluted and crude venom. 1/100 and 1/10 dilutions were not successful in any of the six bacterial strains studied while the 1/1 dilution was effective on *Bacillus subtilis* ATCC 6633, *Salmonella typhimurium* ATCC 14028, and *Pseudomonas aeruginosa* ATCC 27853 with highest zone of inhibition were obtained on *B. subtilis*. Crude venom was effective against *Enterococcus faecalis* ATCC 14506, *B. subtilis*, *S. typhimurium*, and *P. aeruginosa*. The most effective results were observed on *B. subtilis*.

KEY WORDS: Antibiotics, antimicrobial activity, extracts, *Heterometrus xanthopus* venom

Introduction

Scorpions are venomous arthropod animals belonging to the class Arachnida.^[1] There are thirteen families and about 1,400 described species and subspecies of scorpions.^[2] All scorpions are venomous but approximately 50 of them have enough poison to kill a person.^[3,4] The oldest known scorpions lived around 430 million years ago in the Silurian period, on the bottom of shallow tropical seas, hence regarded as the oldest terrestrial arthropods.^[5]

Venoms from scorpions are complex mixtures of compounds (neurotoxins, enzyme inhibitors, salts, etc.). Scorpions use venoms for immobilization of prey and protection against predators. Scorpion venoms consist of a complex of several toxins that exhibit a wide range of biological properties and actions, as well as chemical compositions, toxicity, pharmacokinetic, and pharmacodynamic characteristics.^[6] Small basic proteins present in scorpion venoms are responsible for the neurotoxic activities of the venoms. Keeping in view of all these facts the present study was conducted to evaluate the antibacterial activity of *Heterometrus xanthopus* by utilizing crude and diluted venom.

Materials and Methods

Materials

All agars, sulphuric acid, barium chloride, sodium chloride, ethyl alcohol, nutrient broth, and other chemicals used in this study were of procured from Oxoid (UK), Fluka Chemika, and Difco Laboratories, USA.

Scorpion Procurement and Extraction Procedure

Scorpion (*H. xanthopus*) was collected by excavation from burrows in Mehra Alikhan, dry location in Haripur District, Khyber Pakhtunkhwa, Pakistan. The scorpions were fed on spiders, centipedes, grasshoppers, and cockroaches in glass cages (38W×58L×38H cm) with open tops for easy monitoring. Cage floor was covered up till four inches height with soil in order to provide natural habitat. These cages were kept at room temperature.^[7]

Electricity control (12 V) Huawei AC/DC Adaptor (Model: UE4112005006) was used for venom extraction [Figure 1]. Venom was obtained from mature *H. xanthopus* from an opening near the tip of the bulb-shape venomous gland stinger by applying 12 V electrical stimulus. Electrical stimulus was applied by touching electrodes in the joints of last two segments of tail. One drop of sodium chloride solution was applied on each of these two joints to maximize flow of electricity. The venom was collected in 1.5 ml eppendorf tube.

Preparation of Venom Dilutions

Crude venom as well as 1/1, 1/10, and 1/100 dilutions were prepared in sterile distilled water. 1/1 diluted venom was prepared by mixing equal volume of venom and distilled

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water. 1/10 diluted venom was prepared by mixing 1 part of venom with 9 parts of distilled water (v/v) while 1/100 diluted venom was prepared by mixing 1 part of venom with 99 parts of distilled water with the help of micropipette.

Gram-positive and Gram-negative bacterial strains

Reference bacterial strains, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 49452, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, and *Bacillus subtilis* ATCC 19659 were obtained from American Type Culture Collection (ATCC) and were maintained on Nutrient agar slants (Oxoid, UK) at 4°C

Purity testing of each organism

Each organism is inoculated from working culture of nutrient broth (Merck) on their respective selective media for control as well as for purity testing, i.e., *P. aeruginosa* on (PCA) Pseudomonas Cetrimide Agar (Oxoid, CM0579), *S. typhimurium* on (XLD) Xylose Lysine Deoxycholate Agar (Oxoid, CM0469), *S. aureus* on (MSA) Mannitol Salt Agar (Oxoid, CM0085), *E. faecalis* on (S and B) Slanetz and Bartley (Oxoid, CM0377), *E. coli* on (EMB) Eosin Methylene Agar (Oxoid, CM0069), *B. subtilis* on Mannitol Egg Yolk Polymyxin Agar (MYP; Oxoid, CM0929) and incubated at 37°C for 24 h.

Evaluation of antimicrobial activity

After incubation, one colony of each bacterium from their respective selective agar medium was inoculated into 5 ml

Figure 1: Milking venom from *Heterometrus xanthopus* scorpion by 12V electric shock



nutrient broth and incubated for 4-6 h at 37°C. The inocula were standardized by matching turbidity with a McFarland standard (No. 0.5). The test culture was spread evenly on the surface of pre-sterilized plastic petri dish containing solidified Mueller Hinton Agar (MHA; Oxoid CM 0337) with a sterile cotton swab. A total of 15 µl venom from eppendorf tube was dropped on the surface of each swabbed plate with the help of micropipette. These plates were then incubated at 37°C for 24 h (Culture box, DH-2500). Resulting zones of inhibition were measured in centimeters for all bacterial plates after incubation. Same procedure was repeated for 1/1, 1/10, and 1/100 dilution.

Results

Antimicrobial activity of crude venom

Antimicrobial activity of crude venom was presented in Table 1. *H. xanthopus* crude venom was active against four bacterial strains. The clear zone of inhibition (30 mm) was seen against *B. subtilis* followed by *S. typhimurium* with 20 mm zone of inhibition. *E. faecalis* and *P. aeruginosa* showed (12 mm) each zone of inhibition on crude extract. However, the crude venom appeared to lack activity against the *E. coli* and *S. aureus*.

Inhibitory zone with 1/1, 1/10, and 1/100 diluted venom

1/1 diluted venom formed 30 mm zone of inhibition against *B. subtilis* showing no change of inhibition in comparison with crude venom [Figure 2]. *P. aeruginosa* showed zone of inhibition of 20 mm that was much higher than crude venom (12 mm). The possible reason for higher antibacterial activity is due to more activation of antibacterial peptides (AMP) in water in comparison with crude venom as previously described through RNA and DNA sequence by Gao *et al.*^[8] 1/1 diluted venom showed 11 mm zone of inhibition against *S. typhimurium*. *S. typhimurium* showed reduced activity on 1/1 diluted venom in comparison with crude venom (20 mm). 1/1 diluted venom was found inactive against *E. coli*, *E. faecalis*, and *S. aureus*. Similar results of inactiveness of *E. coli* and *S. aureus* were seen on crude venom but in comparison a higher zone of inhibition of 12 mm against *E. faecalis* was seen on crude venom.

All bacterial strains showed resistance against 1/10 and 1/100 times diluted venom, with no zone of inhibition on Mueller Hinton Agar.

Discussion

Scorpion species often use to spray venom on their own bodies to disinfect them from possible saprophytic organisms

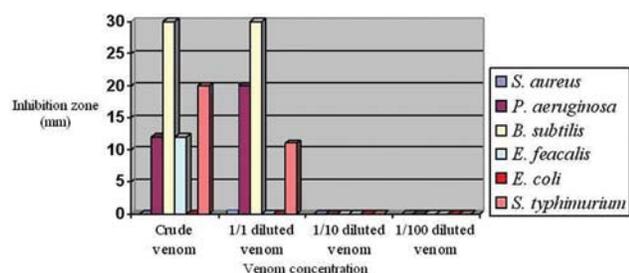
Table 1:

Venom's activity against bacteria with different concentrations

Bacterial Strain	Crude venom inhibition zone	1/1 Dilution Inhibition zone	1/10 Dilution Inhibition zone	1/100 Dilution Inhibition zone
<i>B. subtilis</i>	30 ± 0.56 mm	30 ± 0.67 mm	Nil	Nil
<i>E. coli</i>	Nil	Nil	Nil	Nil
<i>E. faecalis</i>	12 ± 0.54 mm	Nil	Nil	Nil
<i>P. aeruginosa</i>	12 ± 0.60 mm	20 ± 0.71 mm	Nil	Nil
<i>S. aureus</i>	Nil	Nil	Nil	Nil
<i>S. typhimurium</i>	20 ± 0.45 mm	11 ± 0.60 mm	Nil	Nil

B. subtilis=*Bacillus subtilis*, *E.coli*=*Escherichia coli*, *E. faecalis*=*Enterococcus faecalis*, *P. aeruginosa*=*Pseudomonas aeruginosa*, *S. aureus*=*Staphylococcus aureus*, *S. typhimurium*=*Salmonella typhimurium*

Figure 2: Venom's activity against bacterial cultures with different concentrations



including bacteria and fungi, showing that venom of these scorpions could contain some sort of antibiotic potential.^[9] Scorpion venom contains peptides which exhibit anti-microbial properties.^[10,11] Kievit *et al.*^[12] reported that an ingredient in the venom of the “deathstalker” scorpion could help in gene therapy for effective treatment for brain cancer. The scientists describe a new approach that could solve these problems. Key ingredients of their gene delivery system are chlorotoxin, the substance in deathstalker scorpion venom that can slow the spread of brain cancer, and nanoparticles of iron oxide.^[12,13]

In this study, crude venom was effective against *B. subtilis* showed zone of inhibition 30 mm. These results are similar with that of spider venom activity reported by Benli and Yigit.^[14] *E. coli* showed resistance against venom with no zone of inhibition is in relation with least effect of scorpion venom from southern Africa.^[15] The results of *E. faecalis* showed 12 mm inhibition zone, Benli and Yigit^[14] reported that *E. faecalis* was resistant against spider venom, whereas our finding indicates effectiveness of scorpion venom against *E. faecalis*. The results of *P. aeruginosa* are in comparison with Benli and Yigit.^[14] *S. aureus* showed no zone of inhibition in our finding, whereas *S. aureus* showed zone of inhibition on spider venom.^[14,16]

H. xanthopus venom with 1/1 dilution showed different results. The zone of inhibition of *B. subtilis* is identical to the results obtained from antibacterial study of two different spider species venom.^[14,16] *E. coli* and *S. aureus* showed resistance against 1/1 diluted venom. These results are in comparison with results of San *et al.*^[17] They obtained similar results with antimicrobial activity of venoms from snakes. 1/1 diluted venom made inhibition zone of 11 mm against *S. typhimurium*, while 9 mm inhibition zone was reported against *S. typhimurium* when bee venom was tested against it.^[18] All tested bacteria were found resistant to 1/10 and 1/100 diluted *H. xanthopus* venom. The possible reason behind this might be because at some point when venom gets too much diluted the antibacterial activity subsides.^[14,18]

Conclusion

The data presented in the present research provides a strong base that venom peptide/protein is involved in antibacterial responses. This work paves the way for further characterizing immune related components involved in *H. xanthopus* venom, antibacterial response at the molecular level, which will undoubtedly expand our understandings in innate immunity. Polypeptide component's involvement in antibacterial response

of venom, offers clues for the search of new antibacterial template from scorpion resources for drug design and for high potential for clinical use.

Acknowledgments

“This manuscript is about antibacterial activity of *Heterometrus Xanthopus* (Scorpion) in comparison with antibiotics. This research is unique and novel because there was not so much available literature available on this topic and recent research mainly focus on plant extracts and other relevant things but none of them put emphasis on scorpion powerful venom medicinal value. We have conducted for the first time the complete antimicrobial profile of this venom and also compared it with standard antibiotics. This research provides a path for further comprehensive research on scorpion medicinal value.”

References

- Gouge DH, Olson C, Smith KA, Baker P. Scorpions. University of Arizona Cooperative Extension AZ1223; 2001.
- Dunlop JA, Penney D, Tettie OE, Anderson LI. How many species of fossil arachnid are there? *Arachnol* 2008;36:267-72.
- Osnaya-Romero N, de Jesus Medina-Hernandez T, Flores-Hernandez SS, Leon-Rojas G. Clinical symptoms observed in children envenomed by scorpion stings, at the children's hospital from the state of Morelos, Mexico. *Toxicon* 2001;39:781-5.
- Isbister GK, Graudins A, White J, Warrel D. Antivenom treatment in Arachnidism. *Clin Toxicol* 2003;41:291-300.
- Ali SA, Zaidi ZH, Abbasi A. Oxygen transport proteins: I. Structure and organization of hemocyanin from scorpion (*Buthus indicus*). *Comp Biochem Physiol* 1995;112:225-32.
- Petricevich VL. Scorpion venom and the inflammatory response. *Mediators Inflamm* 2010;90:1-16.
- Candido DM, Lucas S. Maintenance of scorpions of the genus *tityus koch* (scorpiones, buthidae) for venom obtention at instituto butantan, são paulo, brazil. *J Venom Anim Toxin Incl Trop Dis* 2004;10:86-97.
- Gao B, Caihuan T, Shunyi Z. Inducible antibacterial response of scorpion venom glands. *Peptides* 2007;28:2299-305.
- Torres-Larios A, Gurrola GB, Zamudio FZ, Possani LD. Hadrurin, a new antimicrobial peptide from the venom of the scorpion *Hadrurus aztecus*. *Eur J Biochem* 2002;267:5023-31.
- Gordon YJ, Romanowski EG, McDermott AM. A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. *Curr Eye Res* 2005;30:505-15.
- Brown KL, Hancock RE. Cationic host defense (antimicrobial) peptides. *Curr Opin Immunol* 2006;18:24-30.
- Kievit FM, Veisoh O, Fang C, Bhattarai N, Lee D, Ellenbogen RG, *et al.* Chlorotoxin labeled magnetic nanovectors for targeted gene delivery to glioma. *ACS Nano* 2010;4:4587-94.
- In: Wulff H, Miller MJ, Hänsel W, Grissmer S, Cahalan MD, Chandy KG, editors. Design of a potent and selective inhibitor of the intermediate-conductance Ca²⁺-activated K⁺ channel, IKCa1: A potential immunosuppressant. USA: Proc Nat Acad Sci; 2000.
- Benli M, Yigit N. Antibacterial activity of venom from funnel web spider *Agelena labyrinthica* (Araneae: agelenidae). *J Venom Anim Toxin Incl Trop Dis* 2008;14:641-50.
- Moerman L, Bosteels S, Noppe W, Willems J, Clynen E, Schoofs L, *et al.* Antibacterial and antifungal properties of alpha-helical, cationic peptides in the venom of scorpions from southern Africa. *Eur J Biochem* 2002;269:4799-810.
- Haerberli S, Kuhn-Nentwig L, Schaller J, Nentwig W. Characterisation of antibacterial activity of peptides isolated from the venom of the spider *Cupiennius aslei* (Araneae: Ctenidae). *Toxicon* 2000;38:373-80.
- San TM, Vejjayan J, Shanmugan K, Ibrahim H. Screening antimicrobial activity of venoms from snakes commonly found in Malaysia. *Applied Sci* 2010;10:2328-32.
- Tatu E. Contributions to the antimicrobial action of bee venom. *Apiacta* 1989;24:13-7.

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