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Original Article

Investigating the Antibacterial Potency of Scorpion (Scorpiops yagmuri) Crude Venom in Tehsil Babozai, Swat, Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Background: Scorpion venom, a complex mixture of proteins, peptides, and other substances, is widely recognized. Scorpion venom is a complex mixture of proteins, enzymes, bioactive peptides, and numerous small molecules, such as nucleotides, inorganic ions, and neurotransmitters. A diverse range of species produce these bioactive chemicals, and some of these organic molecules have been demonstrated to have antibacterial properties.

Objective: To examine the antibacterial properties of scorpion venom against selected bacteria.

Methods: This laboratory-based experimental study used various techniques, such as UV light, rubber bands, and flushing water into their nests, to collect scorpions. Electrical stimulation of the telson was used to collect venom. The disk diffusion method was used to assess the antibacterial activity of crude scorpion (Scorpiops yagmuri) venom against a variety of bacteria, including Proteus vulgaris, Proteus mirabilis, Providencia stuartii, and Morganella morganii.

Results: The findings showed that scorpion (Scorpiops yagmuri) crude venom demonstrated antibacterial activity against various bacteria, with zone of inhibition diameters as follows, after applying 25 µL of venom to each bacterial culture: Proteus vulgaris (1.6 cm), Proteus mirabilis (5.6 cm), Providencia stuartii (5.5 cm), and Morganella morganii (5.4 cm).

Conclusion: The findings suggest that, to varying degrees, crude venom from the scorpion (Scorpiops yagmuri) may exhibit antibacterial activity against the studied bacteria.

INTRODUCTION

Scorpions, belonging to the Arachnida class, are famous for their venom, a complex mixture of proteins, peptides, and other substances. The type of scorpion venom and its biochemical composition can vary substantially between species and even populations of the same species. A complex and perfect composition of proteins, enzymes, bioactive peptides, and a variety of small molecules, such as nucleotides, inorganic salts, and neurotransmitters, make up scorpion venoms. When defending themselves or attacking, scorpions use their venom [1, 2]. Because of their importance and pharmacological traits, scorpion venom toxins are the most studied and relevant. Although scorpion venom is known to have harmful effects on living things (tissues and cells), it has also shown a lot of potential for use in medicinal research [3, 4]. In many respects, scorpions are the only arachnids. The scorpions first appeared and developed into extremely complex animals some 430 million years ago. With about 32,016 genes encoding proteins, 800 well-characterized active polypeptides, and stable structures, the scorpion's genome is the largest to be sequenced among the Arthropoda. Two groups have been established for these arthropods based on their geographic range. Since the venom gland of scorpions is located on the tip of the stinger before the tail, their distinct distribution of the venom system provides evidence for the independent development of venomous species [5]. There are 2,231 species of scorpions worldwide, divided into 20 families and 208 genera. 1500 species are venomous, and about 50 are very dangerous to people. Hemiscorpius, Androctonus, Tityus, Leiurus, Buthus, and Mesobuthus are a few of these 50 species [6]. The primary chemical function of animal venoms, which are complex secretions made up predominantly of bioactive proteins and peptides, is to defend and immobilize prey. Venom is found in all major animal lineages and has evolved independently across the animal kingdom [7]. A potent and varied mixture of bioactive peptides, proteins, enzymes, and numerous tiny molecules (such as nucleotides, neurotransmitters, and inorganic salts) make up scorpion venoms. For both aggressive and defensive purposes, scorpions use their venom. Most toxins in scorpion venoms target voltage-gated potassium, sodium, chloride, and calcium ion channels in smooth, cardiac, and skeletal muscles, as well as neuronal cells [8]. Scorpion venoms, which fall into two categories, α-toxins and β-toxins, are the most researched and significant because of their significant effect on humans. A-toxins cause the prolonged inactivation of voltage-gated Na^+ channels, but the opening of similar channels with a higher negative potential is caused by β-toxins. Low doses of α-toxins decrease excitability and depolarize the cell membrane, whereas higher doses increase the likelihood of an excitable cell stroke, resulting in paralysis and cardiac arrhythmia. B-toxins trigger paralytic muscle spastic responses. Although scorpion venom toxins are known to have harmful effects on living things (tissues and cells), they have also demonstrated a great deal of promise for pharmaceutical and medication development [5]. Numerous chemicals found in scorpion venom block or alter different ion channels in excitable cells. Many short-chain peptides (30–40 residues) are strong and specific K^+ channel blockers; for example, charybdotoxin, isolated from scorpion venom, is a selective blocker of the two potassium (K^+) channel isoforms (KCa3.1 and Kv1.3), which are targets of immunosuppression. Similarly, sodium (Na) channels, which are the targets of pain therapy, may be blocked by long-chain peptides (60–75 residues). These peptides are used to develop many medications [5]. It was determined that Opisthorchin 1 and Parabutoporin are effective against Gram-negative bacteria, such as E. coli ATCC, E. coli DH5α, P. aeruginosa, H. influenzae, and K. pneumoniae, after melittin and mastoparan from scorpions (Parabuthus schlechteri, Opisthophtalmus carinatus) were tested against a variety of Gram-positive and Gram-negative bacteria. Gram-positive bacteria such as B. subtilis ATCC, B. subtilis IP, S. aureus, S. pneumoniae, N. asteroides, and E. faecalis are susceptible to the effects of melittin and mastoparan [9].

MATERIALS AND METHODS

Study Design

This laboratory-based experimental study was conducted to evaluate the antibacterial activity of crude venom obtained from Scorpiops yagmuri against selected Gram-negative bacterial species using an agar disc diffusion assay. The antibacterial efficacy of the venom was compared with that of a standard antibiotic by measuring zones of inhibition.

Study Setting

The study was conducted at the Center for Animal Sciences and Fisheries and the Department of Microbiology, University of Swat, Khyber Pakhtunkhwa, Pakistan, from January 2025 to December 2025. Scorpion collection was performed in Tehsil Babozai, District Swat, while venom extraction and antibacterial testing were conducted under controlled laboratory conditions.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize the inhibition zones produced by crude venom from Scorpiops yagmuri and the standard antibiotic against the tested bacterial species. Results are presented as inhibition zone diameters (cm). Because this was an exploratory laboratory-based experimental study, only descriptive statistics were performed, and no inferential statistical comparisons were undertaken.

Figure 1. Laboratory maintenance of Scorpiops yagmuri specimens before venom extraction

Figure 1. Findings of laboratory maintenance of Scorpiops yagmuri specimens.

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Figure 2: Collection of crude venom by electrical stimulation of the telson.

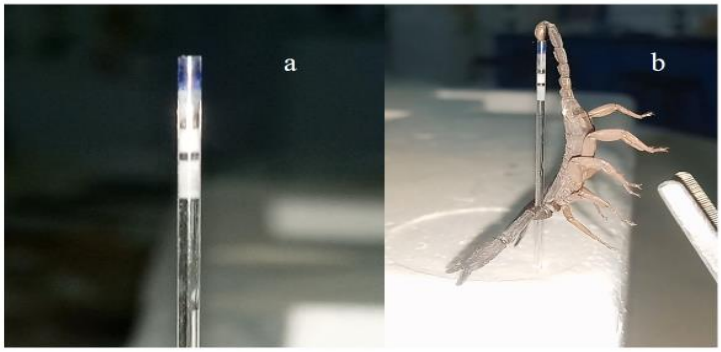


Figure 2. Collection of crude Scorpiops yagmuri venom by electrical stimulation of the telson. (A) Venom collected in a capillary tube. (B) Electrical stimulation of the telson during venom extraction.

RESULTS

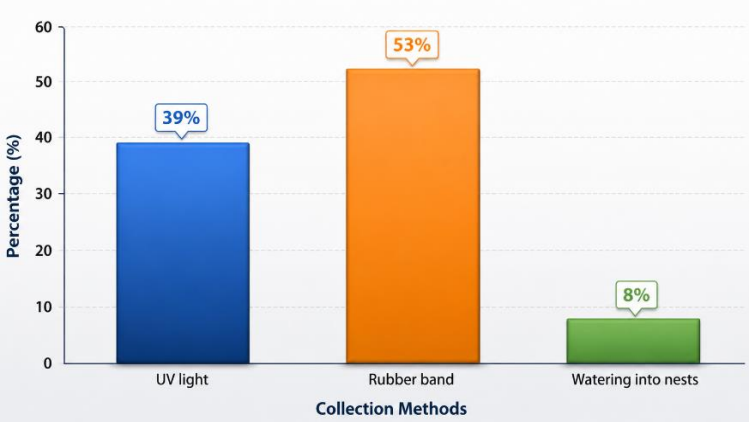
A total of 100 adult *Scorpiops yagmuri* specimens were successfully collected from Tehsil Babozai, District Swat, using three collection techniques. The rubber-band method was the most effective, accounting for 53 (53%) specimens, followed by ultraviolet (UV) light collection with 39 (39%) specimens. Flushing water into burrows yielded the fewest specimens, accounting for only 8 (8%) of the total collection (Figure 3). After collection, all scorpions were maintained under controlled laboratory conditions for venom extraction. Crude venom was successfully obtained from all specimens and processed for antibacterial evaluation. The antibacterial activity of crude *Scorpiops yagmuri* venom was evaluated against four clinically important Gram-negative bacterial species using the Kirby–Bauer agar disc diffusion method. Antibacterial activity was observed against all tested bacterial isolates, although the magnitude of inhibition varied among species. The greatest inhibition zone was recorded against *Proteus mirabilis* (5.6 cm), followed by *Providencia stuartii* (5.5 cm) and *Morganella morganii* (5.4 cm). The smallest inhibition zone was observed against *Proteus vulgaris* (1.6 cm), indicating comparatively lower susceptibility to the crude venom. When compared descriptively with the standard antibiotic, crude *Scorpiops yagmuri* venom produced larger inhibition zones against *Proteus mirabilis*, *Providencia stuartii*, and *Morganella morganii*. In contrast, the standard antibiotic produced a larger inhibition zone against *Proteus vulgaris*. The comparative inhibition zone diameters are presented in Table 1**,** representative inhibition zones are shown in Figure 4, and the comparative graphical analysis is presented in Figure 5**.**

Table 1. Comparison of Inhibition Zone Diameters Produced by Crude Scorpiops yagmuri Venom and the Standard Antibiotic Against Selected Gram-Negative Bacterial Species.

Bacterial Species	Venom (cm)	Standard Antibiotic (cm)
Proteus vulgaris	1.6	2.3
Proteus mirabilis	5.6	2.7
Providencia stuartii	5.5	1.7
Morganella morganii	5.4	1.7

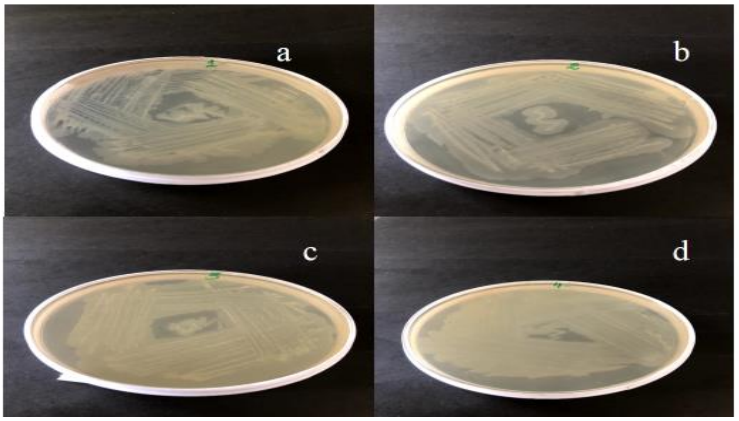
Values represent Inhibition zone diameters (cm) measured using the Kirby–Bauer agar disc diffusion method. Crude Scorpiops yagmuri venom demonstrated greater antibacterial activity than the standard antibiotic against Proteus mirabilis, Providencia stuartii, and Morganella morganii. In contrast, the standard antibiotic produced a larger inhibition zone against Proteus vulgaris.

Figure 3: Percentage distribution of Scorpiops yagmuri specimens collected using ultraviolet (UV) light, the rubber-band method, and flushing water into burrows.



Percentage distribution of adult Scorpiops yagmuri specimens collected from Tehsil Babozai, District Swat, using the rubber-band method, ultraviolet (UV) light, and flushing water into burrows. The rubber-band method yielded the highest proportion of specimens (53%), followed by UV light (39%) and flushing water into burrows (8%).

Figure 4: Inhibition zones observed against (a) Proteus vulgaris, (b) Proteus mirabilis, (c) Providencia stuartii, and (d) Morganella morganii



Representative Mueller–Hinton agar plates showing inhibition zones produced by crude Scorpiops yagmuri venom against (a) Proteus vulgaris, (b) Proteus mirabilis, (c) Providencia stuartii, and (d) Morganella morganii following incubation using the Kirby–Bauer agar disc diffusion method.

DISCUSSION

Scorpion venoms are increasingly recognized as a valuable source of biologically active molecules with significant antimicrobial potential. The present study showed that the crude venom of Scorpiops yagmuri has antibacterial activity against all four Gram-negative bacterial species. Proteus mirabilis (5.6 cm), Providencia stuartii (5.5 cm), and Morganella morganii (5.4 cm) showed the maximum inhibition zone, and Proteus vulgaris showed comparatively less. The results indicated the presence of bioactive compounds in crude S. yagmuri venom that can inhibit the growth of clinically significant Gram-negative bacteria and support the therapeutic potential of scorpion venom-derived antimicrobial peptides (AMPs) [11,12].Scorpion collection efficiency is an important factor that can affect venom yield and further experimental studies. The rubber-band collection method yielded the highest number of scorpion specimens (53%), with ultraviolet (UV) light catching the next-largest number (39%) and flushing water into burrows the lowest number

(8%). Other studies have indicated this, such as those by Tahir et al., who showed that manual collection at night, coupled with species-specific behavior, helps optimize catch while minimizing animal injury [12]. Collection efficiency is important, as some venom is produced and of good quality; other venom may be produced and of poor quality due to environmental conditions, physiological conditions, and handling.The antibacterial activity in this study was similar to that reported in other studies, which have shown scorpion venoms to have broad-spectrum antimicrobial activity. The Kirby–Bauer disc diffusion method is a reliable method for assessing antibacterial activity, and it was used in the present study to compare the venom activity with the standard antibiotic [11]. The larger inhibition zones of crude venom of S. yagmuri against P. mirabilis, P. stuartii and M. morganii indicate that the venom contains peptides that can disrupt the growth of bacteria. The results corroborate previous studies which showed that scorpion venom peptides directly interact with bacterial membranes, leading to destabilization, pore formation and eventually to bacterial cell death [13,14].Previously, several antimicrobial peptides isolated from scorpion venom exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria. Hadrurin, isolated from Hadrurus aztecus, a scorpion native to Mexico, was among the first scorpion peptides shown to have broad-spectrum antimicrobial activity by disrupting bacterial cell membranes [13]. Similarly, the antimicrobial peptides parabutoporin and opistoporin were shown to be active against Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Haemophilus influenzae, indicating that the antimicrobial peptides are conserved among various scorpion species [14–16]. Despite the lack of isolation of peptides from S. yagmuri venom in the present study, the observed antibacterial effect indicates the presence of peptides with similar membrane-active properties.In recent studies, scorpion venom peptides have also been recognized as being of pharmaceutical significance. Recent improvements in proteomic and transcriptomic techniques have led to the discovery of many new antimicrobial peptides that are more stable, more selective, and less toxic to mammalian cells [17–20]. Structure–activity relationship studies have revealed that modest changes in sequence, charge distribution, and amphipathic features of peptides can result in a significant increase in antibacterial activity with decreased cytotoxic effects. These advances have renewed interest in scorpion venom as a potential source of future antimicrobial agents, especially in the present age of increasingly prevalent antimicrobial resistance [18–21].Multidrug-resistant Gram-negative pathogens are among the biggest threats to modern medicine. Proteus, Providencia and Morganella spp. are among the organisms that are becoming resistant to many of the antibiotics commonly used to treat them, and are therefore difficult to treat [22,23]. This has led to growing interest in the study of natural antimicrobial peptides that operate outside the "normal" antimicrobial resistance pathways. In contrast to many conventional antibiotics, which inhibit certain metabolic pathways, scorpion venom peptides mainly act by disrupting the integrity of bacterial membranes, and the development of bacterial resistance may be less likely [19,22]. This mechanism may explain the promising inhibitory activity observed in the present study. These combinations might achieve greater bacterial killing while using lower doses of antibiotics to which the bacteria are susceptible, thereby minimizing toxicity and the development of antimicrobial resistance [20,24,25]. Synergistic effects were not examined in the present study but will be investigated in the future with clinically relevant antibiotics against MDR bacterial isolates using purified S. yagmuri venom peptides.While these are promising results, there are several limitations to note. The results were limited to other clinically important pathogens, as only four Gram-negative bacterial species were included. In addition, crude venom, rather than purified venom fractions, was tested; thus, the specific antimicrobial peptides responsible for the observed activity are unknown. Only disc diffusion was used to assess the antibacterial activity; minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), time-kill curves, and antibiofilm activity were not determined. Furthermore, cytotoxicity to mammalian cells was not evaluated; therefore, the therapeutic safety profile was not assessed. Further studies should use peptide sequencing, mass spectrometry, transcriptomics, and chromatographic purification to identify the active components of the venom. In vivo efficacy and toxicity studies, along with further evaluation against multidrug-resistant clinical isolates, will be crucial before possible clinical use is considered [21,26-29]. The present study contributes to the growing body of evidence indicating that scorpion venoms are a natural source of antimicrobial compounds. The antibacterial activity found in crude Scorpiops yagmuri venom against clinically significant Gram-negative bacteria further suggests that the venom's bioactive peptides are worth further investigation for their potential as new antimicrobial agents that can combat antibiotic resistance globally [24,28].

CONCLUSION

The present study highlights the antibacterial potential of crude venom from Scorpiops yagmuri collected in Tehsil Babozai, Swat, KP, Pakistan. The venom exhibited promising inhibitory effects against selected bacterial strains, suggesting the presence of bioactive molecules with therapeutic potential. These findings suggest that scorpion venom could serve as a valuable source for the development of new antibacterial agents, particularly at a time when antibiotic resistance is an increasing global concern. While encouraging, the results also emphasize the need for further detailed studies to isolate, characterize, and evaluate the safety of the active compounds before any clinical application can be considered.

Author Contributions (ICMJE)

Zaid Nabi: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing – Original Draft Preparation.

Rohi Naz: Conceptualization, Supervision, Methodology, Validation, Writing – Review & Editing.

Shabnam: Investigation, Laboratory Analysis, Data Collection, Data Curation, Writing – Review & Editing.

SM Naeem: Formal Analysis, Methodology, Validation, Visualization, Writing – Review & Editing.

SA Nawaz: Resources, Project Administration, Supervision, Critical Review of the Manuscript, Writing – Review & Editing.

All authors contributed substantially to the conception and design of the study, acquisition, analysis, or interpretation of data; participated in drafting or critically revising the manuscript; approved the final version of the manuscript; and agree to be accountable for all aspects of the work in accordance with the International Committee of Medical Journal Editors **(ICMJE)** authorship criteria.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Consent for Publication

Not applicable.

Conflict of Interest

The authors declare that they have no competing interests.

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Ethical Approval

All procedures involving live scorpions were conducted in accordance with institutional guidelines for the ethical handling of research animals and were approved by the Center for Animal Sciences and Fisheries, University of Swat (Reference No. **D606/CAS&F/UoS/2025).**

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