İ. Çorak Öcal et al. / Hacettepe J. Biol. & Chem., 2024, 52 (4), 237-242

Research Article

Hacettepe Journal of Biology and Chemistry

journal homepage: www.hjbc.hacettepe.edu.tr

Antibacterial Effects of Crude Venom from *Aegaeobuthus gibbosus* (Brulle, 1832)

Aegaeobuthus Gibbosus (Brulle, 1832) Akrep Ham Zehrinin Antibakteriyel Etkisi

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ABSTRACT

Scorpion venom, is a neurotoxic secretion produced by the venom glands in telson of scorpions, consisting of many proteins, peptides, and biologically active compounds, and it is commonly used in catching and digesting prey. The peptides within scorpion venoms hold significant pharmacological importance, particularly in the fields of cancer treatment, analgesics, and anesthesia. In recent years, bacteria developing resistance to antibiotics drew a significant level of interest. The present study investigates the antibacterial activity of the venom obtained from *Aegaeobuthus gibbosus* (Brulle, 1832). The antibacterial effects were observed by applying crude venom by using the dripping method on *Escherichia coli*, *Proteus vulgaris*, and *Klebsiella pneumoniae* bacteria in suitable environments. As a result, it was determined that the venom of *Aegaeobuthus gibbosus* is effective against *Escherichia coli, Proteus vulgaris,* and *Klebsiella pneumoniae* bacteria.

Key Words

Aegaeobuthus gibbosus, Venom, Antibacterial activity, *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris.*

ÖZ

Akrep zehri, akreplerin telsonlarında bulunan zehir bezleri tarafından üretilen, birçok protein, peptid ve biyolojik yönden etkin bileşiklerden oluşan nörotoksik etkili bir salgı olup, genellikle avını yakalamada ve sindirimde kullanılır. Akrep zehirlerinin içerisindeki peptidler farmakolojik olarak oldukça önemlidir. Özellikle kanser, ağrı kesiciler grubu ve anestezide yararlanılmaktadır. Son yıllarda antibiyotiklere karşı direnç kazanan bakteriler dikkat çekmektedir. Bu çalışmada A. gibbosus (Brulle, 1832) türüne ait zehrin antibakteriyel aktivitesi araştırılmıştır. Araştırmada *Escherichia coli, Proteus vulgaris* ve *Klebsiella pneumoniae* bakterilerine uygun ortamda damlatma yöntemi ile ham zehir uygulanarak antibakteriyel etkileri gözlemlenmiştir. Sonuç olarak *A. gibbosus* zehrinin *Escherichia coli*, *Proteus vulgaris* ve *Klebsiella pneumoniae* bakterilerine karşı etkili olduğu tespit edilmiştir.

Anahtar Kelimeler

Aegaeobuthus gibbosus, Venom, Antibakteriyel aktivite, *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris.*

Article History: Feb 19, 2024; Revised: May 13, 2024; Accepted: May 15, 2024; Available Online: Oct 2, 2024.

DOI: https://doi.org/10.15671/hjbc.1439515

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INTRODUCTION

The human body constitutes one of the most suitable environments for numerous microorganisms that pose a vital threat, such as bacteria, fungi, parasites, and viruses.

Humans live closely with microorganisms and are exposed to pathogenic microorganisms. Some pathogens, which have global medical significance, include *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* [1]. These pathogens are thought to be responsible for food poisoning, sepsis, and neonatal infections. There are various reports indicating the drug resistance of clinical isolates of these pathogens [2]. Therefore, there is an urgent need for new and innovative treatments to combat antibiotic-resistant pathogens.

Venoms obtained from animals comprise a complex pharmacological molecule cocktail that can assist when existing antibacterial agents prove insufficient. Various species have been producing venom for millions of years, either to capture prey or as a defense mechanism. Based on this principle that what makes a poison or drug toxic is the dose, the antibacterial effects of venoms were examined and studied in many fields. Venoms contain pharmacologically active molecules that affect human physiology. Venoms obtained from various organisms such as scorpions, bees, spiders, ants, caterpillars, and sea snails are used in developing new drugs for treating various diseases [3].

Despite the intimidating nature of their venomous effects, scorpion venoms became a popular research topic in studies analyzing the venom structure. Scorpion venom is a mixture of approximately 70-600 different compounds, including polypeptides, nucleotides, lipids, biogenic amines, heterocyclic compounds, and inorganic salts. Various scorpion venom peptides were shown to be valuable sources for drug discovery due to their activities in blocking ion channels, as well as anticancer and antibacterial activities [3-4].

There are multiple bioactive components in scorpion venoms. The effects of these venoms range from mild symptoms to those causing sudden respiratory failure through allergic reactions. Despite some species often resulting in fatal outcomes, the pharmacological significance of venom is considered highly valuable due to its complex structure and rich content [5].

Nowadays, the extensive use of high doses of numerous antibiotics led to the development of antibiotic resistance in various bacteria. Particularly, a substantial resistance developed in hospital-acquired infections. Therefore, the number of actively usable antibiotics in patient treatment is limited [6].

From a global public health perspective, antibiotic resistance in bacterial infections became a serious problem. Antimicrobial peptides from scorpion venom are considered to be promising alternative treatment options to traditional antibiotics. The peptide-based components of scorpion venom exert their effects through various pharmacological targets such as ion channels and receptors.

There are various resistance mechanisms in Gram-negative bacteria. The primary cause of increasing resistance is generally attributed to mobile resistance genes on plasmids that can easily spread among bacterial populations and chemical differences in the bacterial cell wall. The S-layer is attached to the outer membrane in Gram-negative bacteria, whereas it adheres to the peptidoglycan layer in Gram-positive ones. This explains why Gram-negative bacteria develop resistance more rapidly. Resistance genes undergo mutation in the cell wall, altering the peptide structure and hindering the entry of antibiotics into the cell by following a Key and Lock model.

This study aims to determine the antibacterial effects of scorpion venom using three bacterial species: *Escherichia coli*, *Proteus vulgaris*, and *Klebsiella pneumoniae*.

MATERIAL and METHOD

Venom Extraction

The crude venom of *Aegaeobuthus gibbosus* (Brulle, 1832) scorpions was used in the present study. Scorpions collected from the Ballıca region in Çankırı province were brought to the laboratory for venom extraction. The electrical stimulation method was used for obtaining crude venom. Scorpions' sensitivity to electricity in the telson region was enhanced by dripping 0.9% physiological saline onto the articulations. The scorpion's stinger was fixed to the microcapillary tube by using forceps and then subjected to electrical current by using the electrostimulation method with two electrode

tips at voltages ranging between 6V and 12V. The crude venom extracted was transferred to microcapillary tubes, sealed at both ends with Parafilm to prevent air contact, and then stored at -20°C until further use.

Bacteria Studies

Gram-negative *P. vulgaris*, *K. pneumoniae*, and *E. coli*, bacteria belonging to the Enterobacteriaceae family and the Eosin Methylene Blue Agar, which is suitable for isolation the most, were used. The medium consists of a mixture of Peptone, Lactose, Sucrose, Dipotassium hydrogen phosphate, Eosin Y, Methylene Blue, Agar, and Distilled water. Thirty-six grams of the mixture was taken and dissolved in 1L distilled water. Magnetic stir bars were added to the agar mixture, and the stirring process continued in a heated magnetic stirrer until a homogeneous solution was achieved and the optimal temperature was achieved. It was then sterilized at 121 °C for 15 minutes in an autoclave. The agar medium was cooled to 60 °C, shaken, and poured into sterilized Petri dishes [7].

Microorganisms were inoculated onto EMB agar and incubated at 37 °C for 24 hours. Microorganisms that showed reproduction were dissolved in ID Broth, and a 0.5 McFarland turbidity standard was prepared. *P. vulgaris*, *K. pneumoniae*, and *E. coli* Gram-negative bacteria were obtained from the samples taken from the hospital. The samples were inoculated onto EMB agar by using the single colony method. In the end, 50 µl of the prepared inoculum was drawn with micropipettes and spread onto EMB agar by using inoculation loops.

Gram staining was performed after 48 hours of incubation at 37 °C. The bacteria obtained from the Gram staining results were re-inoculated into the agar for obtaining pure cultures. Gram-negative bacteria with known incubation results were identified based on the morphological appearance on EMB agar.

Antibacterial Activity Test

The agar drop method was employed by using crude scorpion venom to assess the antibacterial activity. Bacterial strains, standardized to 0.5 microliters according to the McFarland standard, were swabbed onto EMB agar plates. Each petri dish received a 50 µl inoculum, and the plates were incubated at 37°C for 24 hours. Two marked control sections on each plate were designated for the application of crude venom, distilled water (as a negative control), and alcohol (as a positive control), with 5 µl from each substance. After a brief incubation for agar diffusion, the plates were left to incubate at 37°C for an additional 24 hours. The experiment was conducted in triplicate.

RESULTS

The drip method was employed with 5 microliters of crude venom directly applied to bacteria on petri dishes. After a 24-hour incubation period, it was observed that bacterial growth did not occur in the section of the Petri dishes where the venom was applied. Zones formed in the venom-applied areas (Figure 2), and the zone diameters were measured by using a ruler (Figure 3). The zone diameters are presented in Table 1.

Figure 1. A. *Aegaeobuthus gibbosus* (Brulle, 1832) Habitus, B. Scorpions reared in the laboratory environment.

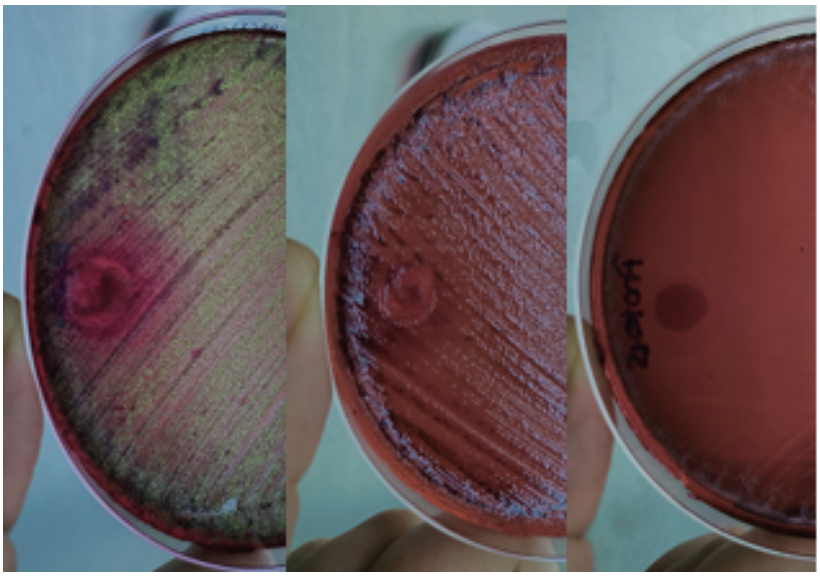

Figure 2. Inhibition zone diameters for *E.coli-Klebsiella-Proteus* bacteria, respectively.

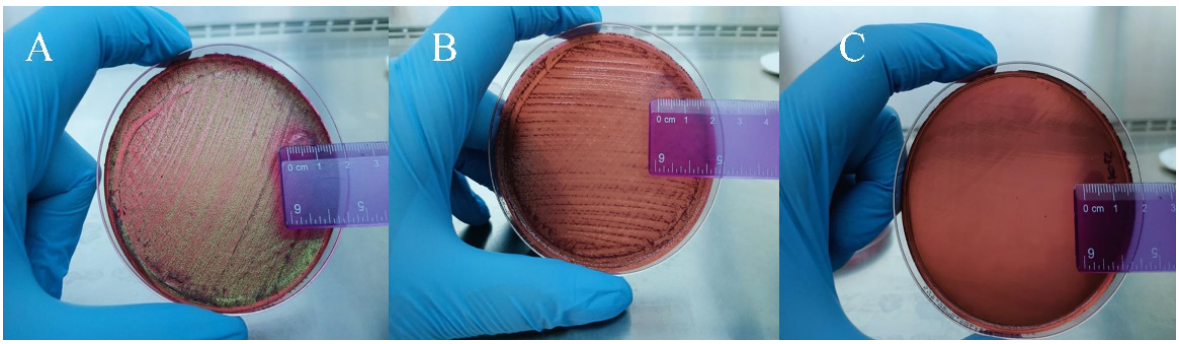

Figure 3. A. Zone formed with *E. coli*, B. Zone formed with *Klebsiella*, C. Zone formed with Proteus.

Table 1. Antibacterial activity inhibition zone diameters.

Bacteria	Zone Diameter
Escherichia coli	10.0±0.3 mm
Proteus vulgaris	7.0±0.2 mm
Klebsiella pneumoniae	8.0±0.2 mm

DISCUSSION

In the study carried out by Conder et al. [8] on the peptides of *Pandinus imperator* scorpion venom, the researchers used the disk diffusion method to observe the antibacterial effects of peptides against *Klebsiella pneumoniae* and *Bacillus subtilis* bacteria. In the present study, *Aegaeobuthus gibbosus* venom was also noted to be effective against *Klebsiella pneumoniae* bacteria.

In a study carried out by Doğan [3] with the venom of *Protoiurus kraepelini* scorpion, the liquid nutrient dilution method was utilized, and it was revealed that it has antibacterial effects on *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) bacteria. This study also demonstrated the effectiveness of *Aegaeobuthus gibbosus* venom against *Escherichia coli* (Gram-negative) bacteria.

Kamaoğlu [9] applied the crude venom of *Calchas nordmanni* scorpion to various Gram-positive and Gram-negative bacteria, and they observed inhibition zones in some bacterial species but no inhibition zones in others.

Ekmekcioğlu [10] investigated the antibacterial activities of the crude venom of *Androctonus crassicauda* scorpion against *E. coli* and *A. baumannii* bacteria and confirmed its effectiveness against these bacteria.

In their study, Corzo et al. [11] argued that the selective effect of crude venom is more pronounced in Gram-negative species when compared to Gram-positive species. Their study investigated the antibacterial effects of the obtained crude venom on three different bacteria known for their resistance to a broad antibiotic spectrum. Utilizing Gram-negative *E. coli, K. pneumoniae*, and *P. vulgaris* incubated with crude venom, the present study yielded results confirming its antibacterial effects.

Despite the undeniable effect of scorpion venom on Gram-negatives, the study carried out by Koç et al. [12] on *Aegaeobuthus gibbosus* (Formerly known as *Mesobuthus gibbosus*) scorpion species and Gram-negative *E. coli* and Gram-positive *S. aureus* bacteria reported similar effects. However, no antibacterial effect was observed on other Gram-positive bacteria, *E. faecalis* and *M. luteus*. The differences in results from various studies could be attributed to the distinct bacterial strains used and the methodological variations employed in the research.

In previous studies carried out on scorpion venoms, various methods were used to investigate the antibacterial effects of peptides against bacteria. These studies revealed the rich peptide composition of scorpion venom. In the present study using the disk diffusion method, it was demonstrated that the crude venom had no effect on bacteria. However, various inhibition zones were observed when the crude venom was applied using the drop-plate method. Even though the methods and bacteria used were not consistent across different studies, the antibacterial effect of scorpion venom is evident on bacteria.

The present study was designed to examine the therapeutic potential of utilizing the rich peptide composition of scorpion venom by investigating the dosage-dependent therapeutic effects of isolated toxins. Despite variations in methods and bacteria, the antibacterial effect of scorpion venom on bacteria highlights its presence. The investigation aims to harness the potential of these toxins, once separated, to exhibit therapeutic effects in the opposite direction. It is believed that some isolated toxins, when used in appropriate doses, may be effective in treating infections, pain, and cardiac dysfunction, ultimately leading to the development of a beneficial medication.

The strong immune system evolved by scorpions over the course of evolutionary processes is an important factor preventing their susceptibility to many bacteria and microorganisms. The key components in this immune system include peptides having antibacterial properties discovered in the venom. Through molecular-level examination and isolation of crude venom, it can be hypothesized that scorpion venom, which is rich in proteins, might offer broad-spectrum protection against bacterial infections [11-13].

The molecular-level exploration of scorpion venom structure holds significant importance in the advanced generation of raw materials through experiments that will elucidate the peptide diversity and effects. The diversification of studies focusing on venom content illumination can lead to the creation of an advanced resource containing a multitude of bioactive components. This, in turn, could contribute to the establishment of a novel pharmacology domain, where phyto-based drugs are combined with animal-derived bioactive substances, resulting in more robust mechanisms of action.

Acknowledgments

This study was produced from the master's thesis with the same title, completed by Tuba Amucaoğlu from Çankırı Karatekin University, Institute of Science and Technology, Department of Biology, under the supervision of Asst. Prof. İlkay Çorak Öcal.

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