Antimicrobial effects of wasp (*Vespa orientalis*) venom

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Background and objective
The discovery of novel naturally occurring antimicrobial agents is one of the most promising approaches for overcoming the growing threat of antibiotic-resistant pathogens. Venomous animals from different ecological niches and taxonomic groups have recently gained attention in the search for new antimicrobials to treat infectious diseases. Therefore, the main aim of the present study was to investigate the antimicrobial activity of Orient hornet venom.
Materials and methods
Different concentrations of wasp venom were tested for their antimicrobial effect against two gram-negative bacteria (*Salmonella typhimurium*, *Escherichia coli*), two gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*), and one yeast like fungi (*Candida albicans*). The antimicrobial activity was analyzed using the well diffusion method, where zones of inhibition were used as indicators of antimicrobial activity.
Results and conclusion
The venom exhibited notable antimicrobial activity against all tested pathogens. Gram-positive bacterial strains were found to be more sensitive than both gram-negative bacterial strains and fungal strain. The highest inhibition zones were determined to be 24.3±1.9, 29.3±1.5, 17.3±1.8, 14.0±1.7, and 15.7±1.5 mm for *S. aureus*, *B. cereus*, *S. typhimurium*, *E. coli*, and *C. albicans*, respectively. The corresponding minimum inhibitory concentration values were determined to be 0.32, 0.16, 0.625, 1.25, and 0.625 mg/ml, respectively. These results offer insights into the antimicrobial potency of wasp venom and provide a basis for further pharmacological research.

Keywords:
agar well diffusion, antimicrobial activity, minimum inhibitory concentration, venom, *Vespa orientalis*

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Introduction

Antimicrobial resistance is one of the greatest challenges in today's world. It threatens the efficient protection against infections caused by viruses, bacteria, fungi, and parasites. This problem is progressively becoming more and more intense in terms of frequency and severity especially regarding antibiotic resistance in bacteria. Because of the overuse and/or misuse of antibiotics through the past decades, many pathogens have evolved resistance to them via natural selection [1]. Nowadays, resistance is seen to nearly all antibiotics that have been developed [2]. The antibiotic resistance crisis – in which common infections and minor injuries can kill – is a very real possibility in the 21st century rather being a faraway fantasy.

Although there are some potential alternatives to antibiotic treatment such as phage therapy [3] and passive immunization [4], the mainstream approach relies on developing new antimicrobial medicines to replace those that are becoming less effective. The bioactive natural products represent an important source of new antimicrobial agents with novel mechanisms of action that are broadly effective and less likely to induce antimicrobial resistance. These bioactive natural substances have shown reduced instances of adverse effects and good therapeutic potential. It is anticipated that the search for antimicrobial leads from natural sources will yield better results than from combinatorial chemistry and other synthetic procedures. Although a wide variety of organisms produces such bioactive compounds, the research to obtain these natural substances has been focused mainly on medicinal plants, algae, and fungi.

In recent years, venoms of a large number of animal species such as snakes, scorpions, spiders, wasps, and honeybees have shown activity against viruses, fungi, and most importantly antibiotic-resistant bacteria [5,6]. Wasp venom, in particular, is a rich source of bioactive compounds that has been evolved to capture prey and make a defense against predators and/or

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microorganisms. It is composed of a complex mixture of active amines, small peptides, and high-molecular-weight proteins such as enzymes, allergens, and toxins [7].

In view of the increasing demand for natural products and the growing threat of multidrug-resistant microorganisms, exploration of biologically active substances from different ecological niches and taxonomic groups is of utmost importance. Considering the recent reports on the antimicrobial activity of hymenopteran insects, the main aim of the present study was to investigate the antimicrobial potential of the venom of Oriental hornet (*Vespa orientalis*) against different strains of gram-positive bacteria, gram-negative bacteria, and yeast.

Materials and methods

Microorganisms and culture media

The antimicrobial activity of wasp venom was determined against a panel of pathogens. Two gram-negative bacteria (*Salmonella typhimurium* NCTC 12023 ATCC 14028; *Escherichia coli* ATCC 25922), two gram-positive bacteria (*Bacillus cereus* ATCC 33018; *Staphylococcus aureus* ATCC 25923) and one yeast like fungi (*Candida albicans* CAIM-22) were provided by the Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. Bacterial strains were maintained on nutrient agar whereas fungal strain was cultivated on potato dextrose agar. All cultures were stored at 4°C.

Antimicrobial assay

Antimicrobial activity tests were conducted by using the agar well diffusion method [8]. Overall, 15 ml of the appropriate agar medium (nutrient agar for bacterial strains and potato dextrose agar for fungal strain) was added into petri dishes. The melted and tempered (40°C) agar was previously inoculated with 200 μl of the target microorganism cell suspension. The freshly grown suspensions were prepared by diluting microbial cultures of the target strain to achieve a microbial concentration of 10^8 CFU/ml. The agar plates were solidified for 1 h and then, using a sterile cylinder, wells of 8-mm diameter were made and filled up with 100 μl of the diluted stock solutions of wasp venom (5, 2.5, 1.25, 0.625, 0.32, 0.16, and 0.08 mg/ml). Wells containing standard antimicrobials (tetracycline for bacteria and nystatin for fungi) served as a positive control (50 μg/ml). The plates were incubated for 24 h at 37°C and 48 h at 28°C for bacteria and fungi, respectively. The antimicrobial activities of the wasp venom were evaluated by measuring the inhibition zones around the wells. The inhibition zones were measured with a ruler and were determined by a clear zone of more than or equal to 2 mm around the well. Minimum inhibitory concentration (MIC) was determined by the lowest concentration of venom that inhibits the visible growth of the microorganism being investigated around the well.

Statistical analysis

Statistical analysis was performed using IBM SPSS (IBM Corp., Armonk, N.Y., USA) statistics subscription. Factorial analysis of variance (two-way analysis of variance) was employed to elucidate the effect of two independent factors on the antimicrobial activity response. The variables used were venom concentrations (with six values) and microorganism types (with five values). The interaction between

Table 1 Antimicrobial activity (mm) of wasp venom against selected pathogens

Venom concentration (mg/ml)	Zone of inhibition (mm)					Mean/ concentrations
	Gram-positive bacteria		Gram-negative bacteria		Yeast	
	Staphylococcus aureus	*Bacillus cereus*	*Salmonella typhimurium*	*Escherichia coli*	*Candida albicans*	
0.16	ND	2.3±0.3	ND	ND	ND	0.47^{A}
0.32	2.7±0.3	4.3±0.9	ND	ND	ND	1.40^{A}
0.625	5.3±1.3	9.0±1.5	3.3±0.7	ND	2.7±0.3	4.07^{B}
1.25	9.7±1.9	17.0±1.7	6.3±1.5	2.7±0.7	5.0±1.2	8.13^{C}
2.5	16.7±1.8	24.3±1.8	10.7±1.9	6.7±1.2	9.0±1.7	13.47^{D}
5	24.3±1.9	29.3±1.5	17.3±1.8	14.0±1.7	15.7±1.5	20.13^{E}
Mean/Microorganisms	9.78^{c}	14.39^{d}	6.28^{b}	3.89^{a}	5.39^{b}	
Tetracycline	26.3±0.7	27.3±1.2	22.0±1.2	21.3±1.5		
Nystatin					24.3±0.9	

Values represent the mean±SE of three repeated experiments with five replicates each. Values with majuscule letters (uppercase) represent the means of venom concentrations while values with minuscule letters (lowercase) represent the means of microorganisms. Values that are not represented by the same letters are significantly different ($P<0.05$). Nystatin (50 μg/ml) and tetracycline (50 μg/ml) served as positive controls for yeast and bacteria, respectively. ND, not detected.

both independent factors (tested microbe×venom concentration) was also included in the analysis. Five samples were used for each treatment and the experiment was repeated three times. Means and SE were obtained from analysis for each treatment. Data were presented either as bar graphs or as means±SE and were compared with Duncan's multiple range test at a 5% probability level.

Results

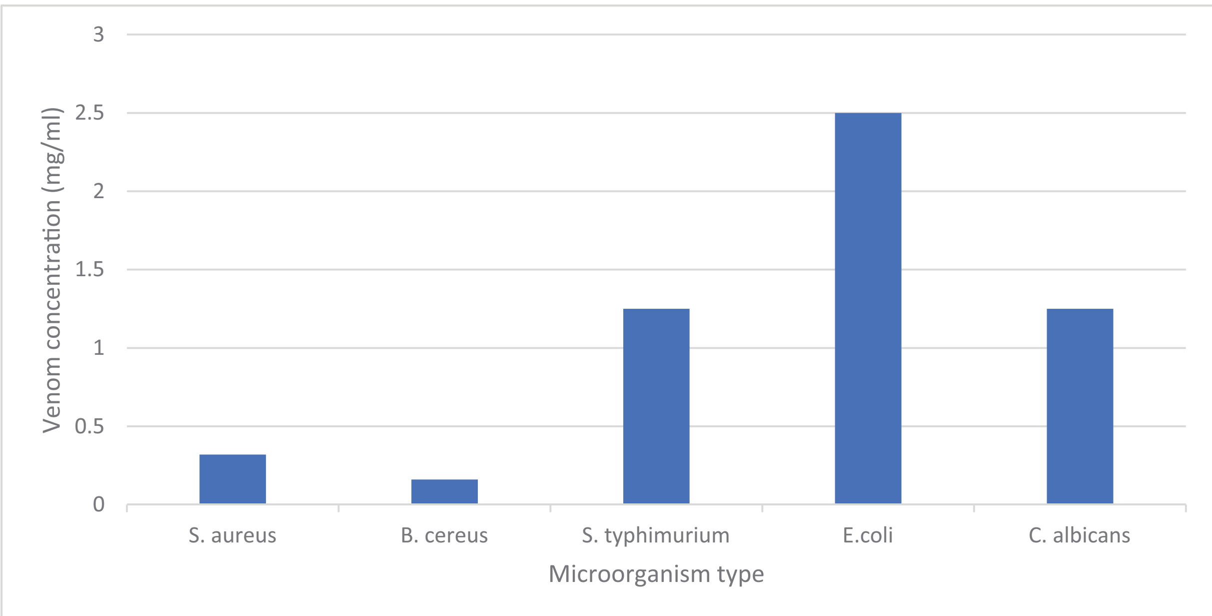
The present study demonstrates a comprehensive evaluation of the antimicrobial activity of different concentrations of wasp venom against a broad spectrum of pathogens. The results reveal that wasp venom was potentially effective against all tested microorganisms with variable potency (Table 1). Factorial analysis of variance indicates a significant effect of the microorganism type, venom concentration, and the interaction between them on the antimicrobial activity. The highest inhibition zones were recorded against *B. cereus* (29.3±1.5 mm) followed by *S. aureus* (24.3±1.9 mm) whereas *S. typhimurium*, *E. coli* and *C. albicans* exhibited the lowest inhibition zones (17.3±1.8, 14.0±1.7, and 15.7±1.5 mm, respectively) at the same tested concentration of wasp venom (5 mg/ml). It was also observed that as the concentrations of wasp venoms increased, the diameter of inhibition zones also increased. The wasp venom at a low concentration (0.32 mg/ml) inhibited the growth of only *S. aureus* and *B. cereus*, with inhibition zones of 2.7±0.3 and 4.3±0.9 mm, respectively. A moderate concentration (0.625 mg/ml) extended this inhibition to include *S. typhimurium* and *C. albicans* with inhibition zones of 3.3±0.7 and 2.7±0.3 mm, respectively. A higher concentration (1.25 mg/ml) inhibited the growth of the most resistant strain in our study, *E. coli*, with an inhibition zone of 2.7±0.7 mm. In this research, tetracycline (for bacteria) and nystatin (for yeast), which were used as positive controls, showed strong antimicrobial activities against the tested gram-positive bacteria, gram-negative bacteria, and yeast with inhibition zones ranging from 21.3±1.5 to 27.3 ±1.2 mm.

The MIC values of the wasp venom against *B. cereus*, *S. aureus*, *S. typhimurium*, *E. coli*, and *C. albicans* indicate its strong antimicrobial potency (Fig. 1). These values are 0.16, 0.32, 0.625, 1.25, and 0.625 mg/ml in that order. The present findings denote that wasp venom is more effective against gram-positive bacteria than gram-negative bacteria and fungi as indicated by the relatively lower MIC values of gram-positive bacteria (ranging from 0.16 to 0.32 mg/l).

Discussion

To address the rapid emergence of pathogens resistance to the classical antibiotics, naturally occurring antimicrobial agents are promising candidates in the search for novel therapeutic agents. Wasp venom has been reported recently to have strong antimicrobial properties. The antimicrobial activity of wasp venom

Figure 1



Minimum inhibitory concentrations of wasp venom towards certain strains of gram-positive and gram-negative bacteria and yeast.

is mostly owing to its peptides such as mastoparans and protonectins [9]. Although the exact mechanism of action of the antimicrobial peptides inside the bacterial cytoplasm is not clear yet, the most commonly accepted mechanism implies the binding of the peptides to the cell membrane surface which creates a mechanical disruption followed by the total destruction of the cell membranes [10]. The typical features for antimicrobial peptides are the formation of a well-defined secondary structure, α-helical conformation, and the presence of both hydrophobic and hydrophilic regions creating an amphiphilic nature necessary for the proposed mode of action of these antimicrobial peptides [11].

Results on the sensitivity of the tested microorganisms to wasp venom revealed that all tested types of microorganisms were susceptible with different magnitudes. This suggests that the mechanism of action of the active principle(s) of wasp venom is applicable on a broad spectrum of microorganisms. Moreover, gram-positive bacterial strains were found to be more susceptible than both gram-negative bacterial strains and fungal strain. This response might be consistent with the cell wall structure of these microorganisms. Although the bacterial cell wall is composed primarily of peptidoglycan [12], the fungal cell wall is composed largely of chitin and other polysaccharides [13]. In addition, gram-negative bacteria have an extra hydrophilic outer membrane consisting fundamentally of lipopolysaccharides, which provides a formidable barrier and excludes certain drugs and antibiotics from penetrating the cell [14]. This renders the gram-negative bacteria generally more resistant to venom and some other antibiotics than the gram-positive bacteria. Our results are in accordance with several recent studies that have reported on the antimicrobial potentiality of wasp venom against gram-positive bacteria, gram-negative bacteria, yeast, and filamentous fungi [15,16].

In the present investigation, the increase of venom concentration was accompanied by a parallel increase in the diameter of the inhibition zone. A similar trend has been observed in snake venom [17] as well as in many other bioactive natural products such as extracts from medicinal plants [18]. However, the degree of increment differed from one microorganism to another. Hence, the sensitivity to various concentrations of the wasp venom depends on the microorganism type.*S. aureus* is a human pathogen that causes a wide spectrum of diseases, ranging from minor skin infections to fatal necrotizing pneumonia. Although *S. aureus* infections were historically treatable with common antibiotics, the emergence of methicillin-resistant *S. aureus* (MRSA) is now a major clinical problem [19]. MRSA infections are difficult to treat because of their multidrug-resistance properties. Infections caused by *S. aureus*, above all other antibiotic-resistant strains, have reached epidemic proportions globally [20]. Importantly, some recent studies have reported on the effectiveness of some antimicrobial peptides derived from the venoms of honeybee, scorpion, and snake against MRSA [21–23]. In this investigation, *S. aureus* was found to be the second most susceptible microorganism to the wasp venom. Therefore, the current finding might be timely and significant, as the wasp venom could be screened and further developed into an alternative treatment for MRSA.

Conclusion

The present study demonstrates a comprehensive evaluation of the antimicrobial activity of different concentrations of wasp venom against a broad spectrum of pathogens. The results indicated the antimicrobial activity of wasp venom. All tested pathogens were susceptible to wasp venom; gram-positive bacterial strains were found to be more sensitive than both gram-negative bacterial strains and fungal strain. The results of this research provide scientific insights into the antimicrobial potency of wasp venom and form a basis for further pharmacological research in this field.

Data availability

All data generated or analyzed during this study are included within the article.

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Conflicts of interest

There are no conflicts of interest.

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