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Antibacterial activity of *Vespa orientalis* L. venom against some antibiotic resistant bacteria

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ABSTRACT

Vespa orientalis (wasp) venom was found to have antibacterial activity against medically important bacterial strains as Gram-positive bacteria: Methicillin–Resistant *Staphylococcus aureus, Bacillus subtilis* and *Streptococcus mutans* and Gram-negative bacteria as: *Escherichia coli, Klebsiella pneumonia* and *Salmonella typhimurium*. All bacterial strains were compared for their sensitivity to the wasp venom and tetracycline antibiotic by determining the inhibition zone and the minimum inhibitory concentration (MIC). In the present study MIC of venom against the above mentioned bacteria were 2×10^{-8}, 2×10^{-7}, 2×10^{-3}, 2×10^{-4}, 2×10^{-4} and 2×10^{-2}, respectively.

Key words: *Vespa orientalis*, venom, antibacterial activity, gram positive bacteria, gram negative bacteria.

INTRODUCTION:

The widespread and/or inappropriate use of antibiotics and chemicals against harmful microorganisms has led to microbial resistance[(1)]. Despite tremendous advances in biological sciences, the difficulty in identifying new mechanisms to kill bacterial pathogens is discouraging. Thus, finding alternative sources of new drugs or prototypes is of major interest to complementary medicine. In the hope of finding novel antimicrobial agents to control antibiotic-resistant bacteria, natural products are an important source of medicinal compounds. A wide variety of organisms produces such bioactive compounds and some of these natural substances have been shown to be able to kill bacteria[(2, 3)]. Venoms of a vast number of animal species represent complex mixtures of compounds (ions, biogenic amines, polyamines, polypeptide neurotoxins, cytolytic peptides, enzymes etc.) responsible for various medical effects[(4, 5, 6, 7)]. Venoms can also be useful and valuable as pharmacological tools in drug research, as potential drug design templates and as therapeutic agents[(8, 9)]. In recent years, venoms and venom components from animals have shown potential antibacterial activity. These include venom of wasps, common honeybees, spiders, snakes and scorpions[(10, 11, 12, 13, 14, 15, 16)]. Therefore, the present study was conducted to evaluate the antibacterial activity of *Vespa orientalis* venom against different strains of gram-positive and gram-negative bacteria with resistance to antibiotics.

MATERIALS AND METHODS

1.Collection of hornets wasps (*Vespa orientalis* L.):

Hornets wasps were collected during summer season (July-September) from hornet traps which settled between the honey bee nests at the Department of honey bee researches, Institute of plant protection, Ministry of Agriculture, Dokki, Giza, Egypt. The hornet traps were placed to trap hornets wasps as they feed on honey and workers.

2 . Venom extraction:

Samples of venom were collected from the oriental hornets (*Vespa orientalis* L.) by the electric shock method: A venom-collecting apparatus was a 42 × 50 × 58 cm cage-like box, with inner walls that were equipped with electric wires, which would be sequentially charged and discharged. The hornet that would come in contact with two adjacent wires would receive an electric shock of 21 volts for 3 seconds. After a lapse of 7 seconds, the wire is recharged and ready for the next electric shock. This 10-second cycle continues for duration of 5 minutes, during which hornet workers are made to sting on the plastic covering of a glass plate. Venom deposited on the glass plates for drying and then the whole wasp venom was scratched with a sharp knife and quickly packed in opaque glass vials and kept at – 5°C till used[17].

a) Bacterial strains

A panel of standard strains of Gram- positive bacteria was collected from the culture collection unit at the Regional Center for Mycology and Biotechnology (RCMB), Al- Azhar University: Gram- positive bacteria: *Bacillus subtilis* (RCMB 015), *Streptococcus mutans* (RCMB017) and Methicillin –Resistant *Staphylococcus aureus* (clinical isolate) and Gram-negative bacteria: *Klebsiella pneumonia* (RCMB 003), *Salmonella typhimurium* (RCMB 006) and *Escherichia coli* (RCMB 010052)were used to test the activity of the wasp venom.

b) Agar disc diffusion method for detecting antibacterial activity of wasp venom:

Twenty four hours old culture of each of the tested bacterial strains was used. Five ml of sterile distilled water was added to the culture tube and mixed by vortex mixture. Five drops of the suspension were added to 100 ml of nutrient agar medium at 45^0C. This was dispensed in petri dishes; 10 ml from each 2mg of the venom extract wwas dissolved in 10 ml dist. water. Analytical paper discs about 6mm in diameter were loaded with venom extracts and aseptically put on the surface of the selected plates with the different test organisms. The plates were left for 2 hours in a refrigerator for diffusion, and then the plates were incubated at 37^0C for 24 hours[18].

c) Determination of growth- inhibition zone:

Each experiment was repeated in 3 replicates, and the diameter of growth inhibition zones were measured[19]**.** Twenty four hours old culture of each of the tested microbial strains was used. Inoculums of the microorganism were uniformly spread on the sterile nutrient agar media, 0.2 mg of the extracted venom was dissolved in 1 ml dist. Water. Analytical paper discs about 6mm in diameter were loaded with venom extracts and aseptically put on the surface of the selected plates with the different test organisms and the plates were left for 2 hours in a refrigerator for diffusion. Then the plates were incubated at 37^0C for 24 hours.

d) Determination of Minimum inhibitory concentration (MIC)

MIC was determined by micro titer broth dilution method[20].Serial dilutions of the wasp venom started with 2mg of wasp venom was dissolved in 10ml of sterile dist. water. One ml of each venom concentration was added to a 3ml of growth medium in separate test tubes with 1ml of each bacterial strain. Then the tubes were allowed to incubate for 24 hours at 37°C. Broth tubes that appear turbid were indicative of bacterial growth, while tubes that

remain clear indicated no growth. Optical densities were measured after incubation using a spectrophotometer at wave length 600 nm.

RESULTS

1.Antibacterial activity of wasp venom:

Vespa orientalis L. crude venom showed a significant activity against different gram-positive and gram-negative bacterial strains used in this study. The corresponding inhibition zones are listed in Table (1) and Figures (1 , 2). During the growth of all tested bacteria, the diameter of inhibition zones was measured to be 18.9,18.4,10.5.18.5,16.4 and9.5 mm for *S. aureus* (MRSA), *B. subtilis*, *S. mutans*, *E. coli* , *K. pneumonia* and *S. typhimurium*, respectively, compared with low values of tetracycline antibiotic 9.4, 12.8, 15.4, 15.2, 12.8 and17.5 mm, respectively.

Table (1). Inhibitory effect of *Vespa orientalis* crude venom and tetracycline on different strains of bacteria.

Bacteria	**Growth inhibition zone in mm.**	
Gram +Ve	**Venom**	**Tetracycline**
S. aureus (MRSA)	18.9	9.4
B. subtilis	18.4	12.8
S. mutans	10.5	15.4
Gram –Ve		
E.coli	18.5	15.2
K. pneumonia	16.4	12.8
S. typhimurium	9.5	17.5

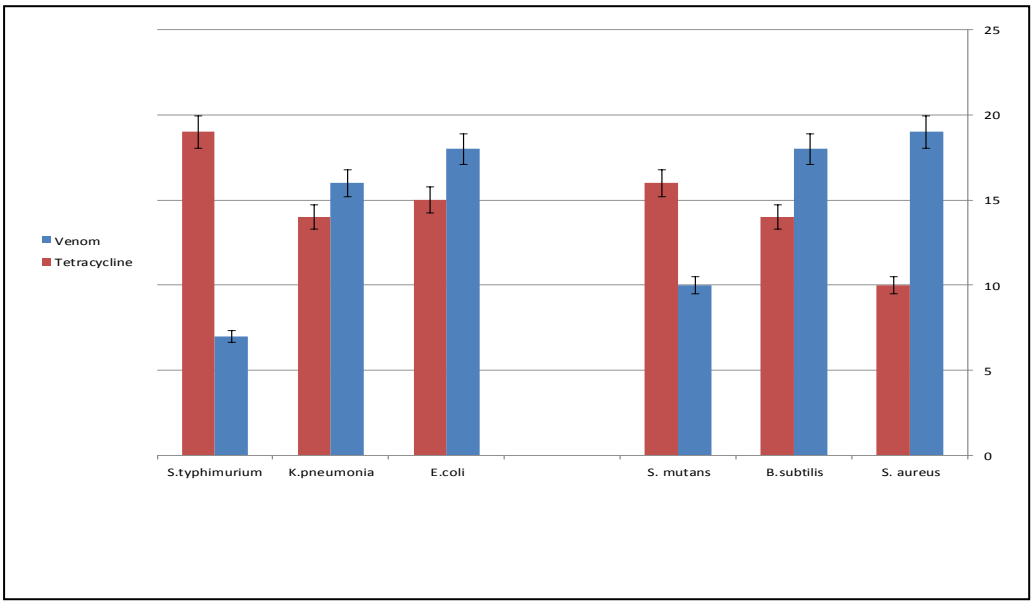

Fig. (1). Comparison of the inhibitory effect of both wasp venom and tetracycline against different bacterial strains.

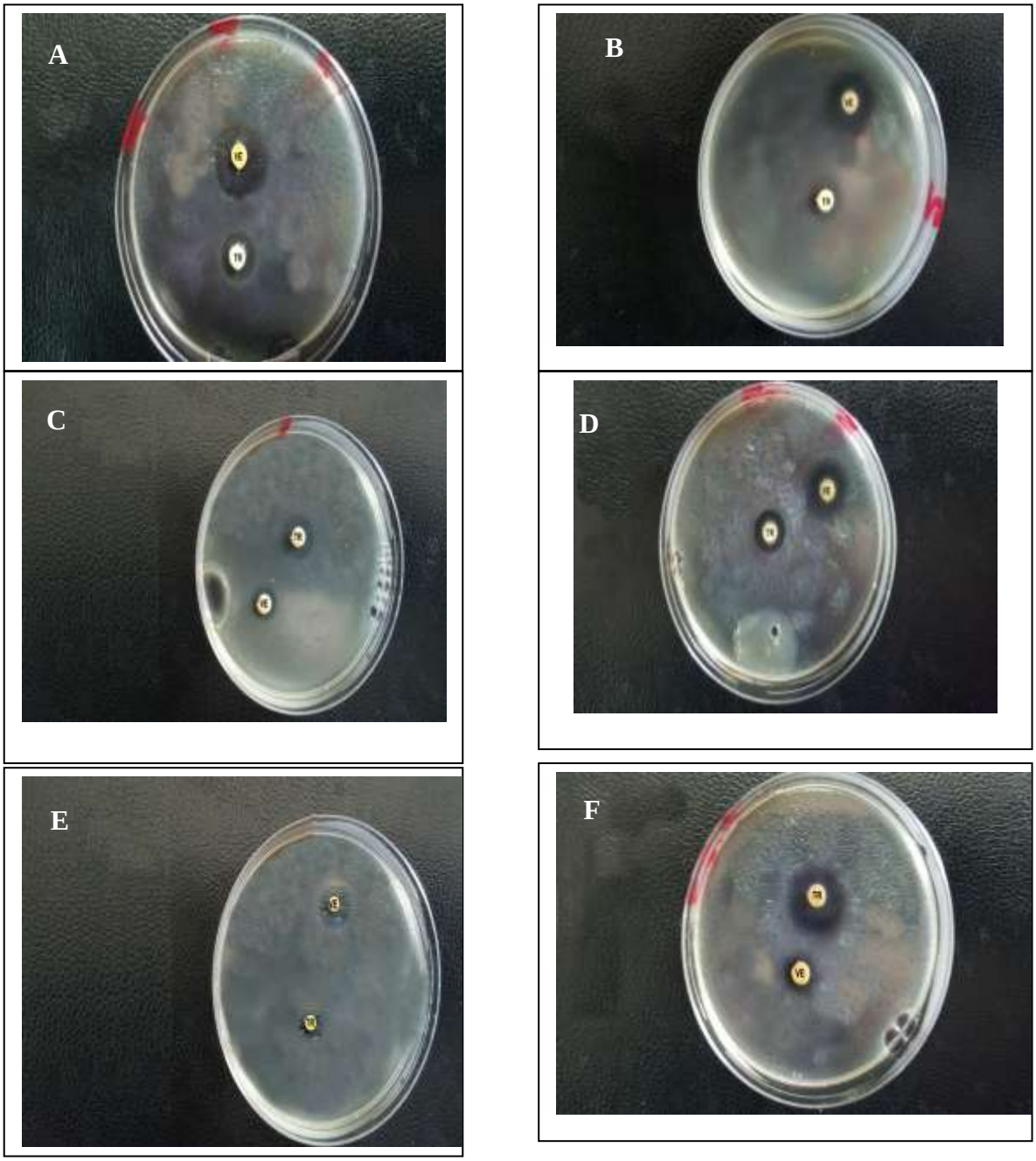

Fig. (2). The inhibitory effect of *Vespa orientalis* venom (VE:0.2 mg/disc) on the different bacterial growth; A: *Staphylococcus aureus*; B: *Bacillus subtilis*; C: *Streptococcus mutans*; D: *Escherichia coli*; E: *Klebsiella pneumonia*; F: *Salmonella typhimurium* compared with tetracycline antibiotic (Tr:0 .2 mg/disc).

2-Determination of minimum inhibitory concentration of wasp venom and tetracycline antibiotic against the tested bacterial strains:

Minimum inhibitory concentrations (MICs) were obtained by serial dilution method. As measuring the MICs were ranged between 2×10^{-8} and 2×10^{-2} mg/ml, after treated with the wasp venom and MICs were ranged between 2×10^{-3} and 2×10^{-7} mg/ml. after treated with tetracycline antibiotic (Table 2). All tested bacterial strains were found to be susceptible to the venom and among them, *S. aureus* (MRSA) was the most sensitive.

Table (2). Minimum inhibitory concentrations (MICs) obtained using serial dilutions of *Vespa orientalis* crude venom and tetracycline on different strains of bacteria.

Bacteria	**MIC (mg/ml)**	
Gram +Ve	**Venom**	**Tetracycline**
S. aureus (MRSA)	2×10^{-8}	2×10^{-3}
B.subtilis	2×10^{-7}	2×10^{-5}
S. mutans	2×10^{-3}	2×10^{-7}
Gram –Ve		
E.coli	2×10^{-4}	2×10^{-3}
K.pneumonia	2×10^{-4}	2×10^{-3}
S.typhimurium	2×10^{-2}	2×10^{-6}

DISCUSSION:

The present study describes the assessment of antimicrobial effects of *Vespa orientalis* crude venom against many pathogenic bacterial strains. The crude venom exhibited activity against both gram-positive and gram negative bacteria. However, the actual function of antimicrobial agents in these venoms is not clear yet. The toxins are complex mixtures of amines, small peptides and high molecular weight proteins such as enzymes, allergens and toxins[21]. Venoms from stinging wasps are important weapons both in the defense of the colony and capture of prey. To the best of our knowledge, only few of its components have been purified and characterized from parasitic Hymenoptera, such as metalloproteinase, serpin, calreticulin-like protein, aspartyl glucosaminidase-like protein and insecticidal toxins[22]. The antimicrobial property of wasp venoms is mostly due to their peptides. Amphipathic secondary structures with net positive charges are essential to the biological activities of peptides that interact with ionic components of bacterial membranes in different ways, sometimes resulting in irreversible damage to the cell[23]. One of the major targets for antimicrobial agents is the bacterial cell envelope, which is a complex, multiple macromolecular structures that undergoes highly ordered cycles of synthesis and hydrolysis, facilitating cell division while maintaining a protective barrier against environmental stress. There are several different classes of antibiotics that target specific cell envelope structures or enzymatic steps of cell wall synthesis[24]. The present results showed that the wasp venom was more active against Gram+ve bacteria than Gram-ve bacteria. The main difference between these two types of bacteria is the structure of bacterial cell wall. The biological membrane is a highly dynamic, complicated system, which is composed of weakly interacting protein molecules and lipids[25]. Cell wall of bacteria comprises a complex structure that is fundamentally different between gram positive and gram-negative bacteria. It consists of a polymer of disaccharides cross-linked by short chain peptides, forming a type of peptidoglycan. Cell wall in gram-positive bacteria is thick (15–80 nm), consisting of several layers of peptidoglycans and molecules of teichoic acids. In contrast, cell wall of gram-negative bacteria is relatively thin (10 nm) and is composed of a single layer of peptidoglycan surrounded by a membranous structure (the outer membrane) which may invariably contain lipopolysaccharides. Thus, the outer membrane is more hydrophobic in gram-negative than in gram-positive bacteria and constitutes a target for being attacked by hydrophobic agents and

other antibiotic agents[26, 27].The present results were in agreement with Jalaei *et al.*[28] in that venom markedly inhibited gram-positive bacteria than gram-negative ones.

Conclusions:

The crude venom of *Vespa orientalis* (wasp) efficiently has antibacterial activity against the growth of some gram-positive and gram-negative bacterial strains, even at a very low concentration when compared to that of tetracycline. The crude venom showed to be more efficient against gram-positive bacteria; Methicillin –Resistant *Staphylococcus aureus* (MRSA). Further investigation is required to determine the potential components that could be used as antimicrobial drugs, especially for treating antibiotic-resistant pathogens.

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النشاط المضاد لسم الدبور الشرقي فيسبا أورينتاليس لانواع من البكتريا المقاومة لبعض المضدات الحيوية

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المستخلص

أتضح من نتائج الدراسة أن السم المستخلص من الدبور الشرقي فيسبا أورينتاليس ذو نشاط مضاد لانواع من البكتريا ذات أهمية طبية المكور العنقودي المضاد للميثيسيللين(مرسا) ، العصوية الرقيقة والعقدية ميوتانز(كمثال للبكتريا موجبة الجرام) والبكتريا القولونية ، بكتريا كلبسيلا المسببة للالتهاب الرئوى ، وبكتريا السالمونيلا تيفيموريوم (كمثال للبكتريا سالبة الجرام). تم مقارنة جميع السلالات الجرثومية لحساسيتها لكلا من سم الدبور والتتراسيكلين مضاد حيوي من خلال تحديد منطقة تثبيط والحد الأدنى من التركيزات المثبطة المثبطة للسم ضد البكتيريا المذكورة أعلاه 2×10^{-8}، 2×10^{-7}، 2×10^{-3} ، 2×10^{-4} ، 2×10^{-4} ، 2×10^{-2} على التوالي.