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The Antibacterial Activity Of Bee Venom On Selected Pathogenic Bacteria

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ABSTRACT

Honey bee venom of *Apis mellifera carnica* with LD_{50} equal to 290µg/mouse, was analyzed by HPLC (high performance liquid chromatography) to perform its activity and found that it contains the most important active compounds which are Melittin (52.1%), Phospholipase A2(11.9%), and Apamin (2.3%) then tested against selected pathogenic bacteria *invitro*. The selected bacteria were Methicillin resistant *Staphylococcus aureus* (MRSA), *Proteus sp., E.coli ,Salmonella* sp., The results showed that bee venom has high significant effect on Gram positive bacteria (MRSA), and from moderate to low significance on gram negative bacteria (*Proteus* sp., *E.coli , Salmonella* sp.) therefore Bee venom could be a promising alternative antibiotic.

INTRODUCTION

Bee venom (BV) therapy (BVT) is the therapeutic application of honeybee venom (HBV) to the treatment of various diseases. BVT has been used as a traditional medicine to treat a variety of Conditions, such as arthritis, rheumatism, back pain, cancerous tumors, and skin diseases (Hider, 1988), BV contains a variety of peptides including melittin, apamin, adolapin, and the MCD peptide. It also contains enzymes (e.g., PLA2), biologically active amines (e.g., histamine and epinephrine) and nonpeptide components (including lipids, carbohydrates and free amino acids ;(Lariviere & Melzack, 1996). These substances were claimed to directly or indirectly express its potency and medical efficacy. Bee venom has been suggested as an effective healing agent for alleviating persistent pain and treating several ailments including different rheumatic disorders involving inflammation and degeneration of connective tissue (different types of arthritis) (kwon *et al.* 2001).

Bee venom has long been known to have a natural antimicrobial effect (Bechinger 1997). The antibacterial properties of BV are due to the potential action of melittin (Asthana 2004), which has very low cell selectivity and acts strongly on the cell membrane lipid through pores forming channels.

MATERIALS AND METHODS

Materials

- Bee venom samples (*Apis mellifera carnica*) were kindly donated from the Beekeeping Research Department, Plant Protection Research Institute, Agriculture Research Centre at Dokki, Giza governorate, Egypt.
- Four bacterial strains Methicillin resistant *Staphylococcus aureus (MRSA), Proteus sp., E.coli, Salmonella sp*., collected from microbiology lab of Al-Azhar hospital, were used.
- 25 Swiss albino mice (16-18gm)

Methods

Qualitative HPLC (Division, 2004; Ionete & Dinca, 2013)

Sample preparation:

One mg of powdered honeybee venom was transferred into a screw capped tube and dissolved in 10 mL of pure water by mixing with a vortex mixer for 3 min. then it is ready for HPLC analysis.

Standard preparation:

One mg of standard bee venom containing (mellitin, phospholipase A2, apamin) (Sigma co.) Was dissolved in 1mL of pure water, then it was ready for HPLC analysis.

Chromatographic conditions:

The high performance liquid chromatographic analysis was performed using model yl9100 (Korean), consisting of two pumps, the column compartment, and the PDA detector. Samples were separated on a C18 column at a flow rate of 1 mL/min. The mobile phase was acetonitrile (eluent A) and 0.4% phosphoric acid in water (eluent B) at 25°C with isocratic elution 50%: 50%, The volume of all injections was 20 µL. The Detection was made at 230nm. **(Chmielewska & Szczêsna 2004; Ionete & Dinca, 2013)**.

Determination of LD_{50} of bee venom.

The LD_{50} of venoms were determined according to the method of British Pharmacopeia, 2000, using male albino Swiss mice 16-18 gm B.W. Ascending concentrations of 5 dose levels of the freshly prepared venom solutions in normal saline were arranged in a geometric progression starting by a dose which kills approximately 0-20% of the animals and ending by a dose which kills approximately 80-100 % of the injected animals. Each dose level was tested in 4 mice, and all injections were given intravenously, and deaths and survivals of injected animals were recorded after 24 hrs from the time of injection. The uncorrected % lethality at each dose level was calculated from the numbers of survivals and deaths directly obtained at that dose level. However, in order to avoid the interference of accidental survivals or deaths resulting from abnormal resistance or susceptibility of tested animals, i.e. by the data were recalculated after correction of the numbers of survivals at each dose level by adding consideration of the numbers of survivals at higher dose level, and correction of numbers of deaths by adding the number of lower deaths at deaths at each dose level. It is assumed that mice surviving at a given dose level would have survived at a lower dose level, and conversely, mice which died at a given dose level would have died at any higher dose level. Therefore at each dose level, the number of survivals at higher dose levels was added to the uncorrected number of survivals obtained at that dose level; and the number of deaths at lower dose levels was added to the uncorrected number of deaths obtained at that dose level. The accumulated corrected % lethality at each dose was determined from the accumulated corrected deaths and accumulated corrected survivals at that dose level (Bradford, M. M 1976). Determination of LD_{50} represents lethal activity of bee venom which depends on its main components, melittin and phospholipase A2, that combination of the two at their natural 3:1 mixture in bee venom revealed that the lethal activity of the lethal activity of the mixture was about the same as crude bee venom (Schmidt, 1995).

Calculation of LD_{50}: When the differences in accumulated % lethality at the 5 dose were statistically significant, then the 50% point dose (LD_{50}) was calculated according to Reed and Muench (1938)from the following equation:

Log LD_{50} = log dose next below50 % + (log increasing factor × proportionate distance).

Proportionate distance = (% mortality next above -% mortality next below) / (% mortality next above +% mortality next below)

Antibacterial activity of bee venom

The Antibacterial activity was performed using the Agar-well diffusion method. Different concentrations of bee venom solutions (5%, 2.5%, and 1.25%) were obtained to show which conc. would affect on the bacterial strains.

A small touch taken by a sterilized steak of each strain of bacterial culture was added on 20 ml molten agar media then poured in a petridish (9cm), allowed to cool in room temp. 100µ of bee venom solution (different concentrations) were added in each well, allowed to diffuse in one hr. in room temperature. The diameter of the inhibition zone (mm) was measured after overnight (24 hrs) incubation. (Katircio & Mercan, 2006).

Statistical analysis: the obtained results are processed statistically according to Bolton 1997; comparison between groups was significance was done using T-Test represented by mean ± standard deviation (SD).

RESULTS AND DISCUSSION

In the present study, the antibacterial activity and chemical composition of bee venom were determined. Chemical properties are important parameters for Bee venom characterization and to correlate between antibacterial activity and chemical composition.

Table 1 and Figure 1 describe HPLC separation profile of the identified compounds of honeybee (*Apis mellifera carnica*) venom, and show that the percent amount of Mellitin equal 52.1%± 0.75, phospholipase A2 equal 15.91%±0.25, and Apamin equal 2.3% ±0.13. these results agree with Choi *et al.*, 2015, and Chmielewska & Szczêsna 2004 who found that Melittin content varied from 61.15 to 70.15 and averaged 64.40%, Phospholipase A2 content came within a range of 11.24 to 15.05, and averaged 13.00%, and Apamin content was between 2.09 to 4.18, averaging 3.10%. Kim, 1997 reported that the principal components of the venom are phospholipase A2 (PLA2; 10–12%), the melittin peptide (40–50%), and Apamin (2-3%), while Haghi *et al*. 2013 reported that the amount of melittin in honey- bee venom samples detected by HPLC ranged from 21.9 to 66.4 %.

Table 1: HPLC study of BV sample test

Retention time (Min)	Amount % (mean ± SD)	Compound name
2.073	52.1± 0.75	MELLITIN
3.057	15.91±025	PHOSPHOLIPASE
8.340	2.3±0.13	APAMINE

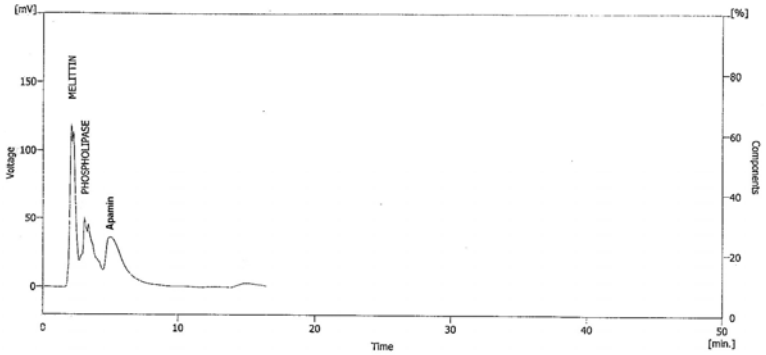

Fig. 1: Hplc study of BV sample test

Evaluation of bee venom potency was represented by determination of LD_{50} as shown in Table 2.

By calculation of these previous results in Table 2 according to Reed and Muench (1938), bee venom LD50 was equal to 290µg/mouse, this value converted to 16.11 µg/gm (or 0.016mg/gm) by calculation. This result represented that the Egyptian bee venom was less toxic than other BV which previously evaluated by Gary *et al.,* 1988 who found that $LD_{50}$of the crude bee venom was about 76 µg and added that the toxicity of bee venom resulted from Melittin and Phospholipase A2 that acts synergistically. While Kim, 1992 found that LD_{50} of BV was 2.97±0.32mg/kg when injected intravenously in mice. The difference in LD_{50} values may be due to the difference in *Apis mellifera* species according to geographical distribution (Haberomann, 1972).

Table 2: Bee venom potency in mice

Dose µg/mouse	**Directly observe**		**Corrected**		**Lethality %**
	Death	**survival**	**Death**	**Survival**	
190	0	5	0	15	00.0
228	1	4	1	10	09.0
273	2	3	3	6	33.0
328	3	2	6	3	66.6
393	4	1	10	1	90.0

As shown in Table 3, and images from group A (A1:A4), group B (B1:B4) and group C (C1:C4), Antibacterial activity was determined in our study and found that The bee venom of Genus *Apis mellifera* exhibited a noticeable inhibition zone against all the selected clinical isolates; one Gram positive (MRSA) methicillin resistant *Staph.aureus*,& three Gram negative bacteria *Salmonella sp., E.coli., Proteus sp*. Different conc. of bee venom (5%, 2.5%, 1.25%) were done to study its effect, and inhibition zones were noticed in these conc. of bee venom on the selected strains which were used in the present study.

Table3: Effect of bee venom on different pathogens:

Bacterial strains	Inhibition zones(mm)		
	5%BV	2.5% BV	1.25% BV
E.coli	1	0.6	0.6
MRSA	0.7	0.7	0.7
Proteus SP.	1	1	0.9
Salmonella SP.	0.9	0.7	0.6

At 5% bee venom concentration; inhibition zones were (1mm, 0.7mm, 1mm, and 0.9mm) for *E.coli*, MRSA, *Proteus sp., Salmonella sp*. Respectively, as shown in (Fig. 2) group A (A1 to A4) . At 2.5% bee venom concentration; inhibition zones were (0.6mm, 0.7mm,1mm, and 0.7mm) for *E.coli*, MRSA, *Proteus sp., Salmonella sp*. Respectively, as shown in (Fig.3) group B (B1 to B4). At 1.25% bee venom concentration; inhibition zones were (0.6mm, 0.7mm, 0.9mm, and 0.6mm) for *E.coli*, MRSA, *Proteus sp.,and Salmonella sp*. Respectively, as shown in (Fig.4) group C (C1 to C4) .from the above data we found that Gram negative bacteria (*E.coli*, *Proteus sp., and Salmonella sp)* exhibited a wider inhibition zone in the higher conc., then become smaller as the conc. decreased. This result agree with (Han *et al*., 2007) who proved that Korean bee venom has indeed an antibacterial effects against both gram-negative (*Enterococcus faecium, and E.coli*) and gram-positive bacteria (Methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus, Streptococcus*

intermedius, Streptococcus oralis and Streptococcus uberis) , and also (Hegazi *et al., 2015)* who noticed that the gram negative (*Klebsiella pneumoniae, Escherichia coli and Pseudomonas aeruginosa)* seemed to be the least sensitive bacteria to bee venom while gram positive *(Staphylococcus aureus, Streptococcus pyogenes),* were more affected by tested venoms of *Apis mellifera* Carniolan. These results may be due to Melittin activity as said by Hanulová *et al*. 2009, through pore formation, which causes membrane permeation as said by Matsuzaki *et al.* 1997 or by cell lyses in both prokaryotic and eukaryotic cells in a non-selective manner as said by Papo and Shai 2003. While Kondo and Kanai 1986 found that bee Venom fraction (melittin), affected mycobacteria and staphylococci but not *E. coli. Ad hoc.* Choi *et al.*, 2015 recorded that the viabilities of the MRSA strains treated with purified melittin decreased to levels comparable to those observed when the whole bee venom used, these results thought to be due to melittin and PLA2 synergism.

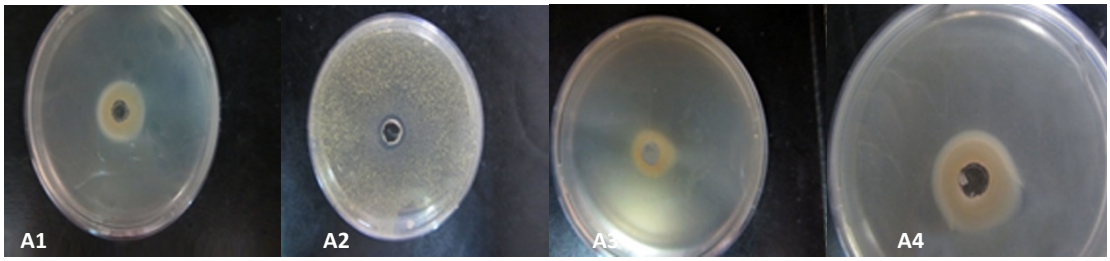

Fig. 2 : Group A (A1:A4) represents the inhibition zones of the 4 bacterial strains (5% bee venom conc.)A1=E.Coli, A2=MRSA, A3=proteus, A4=salmonella.

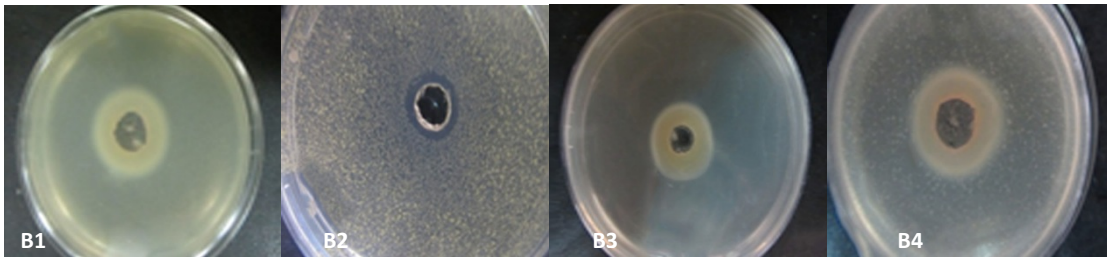

Fig. 3: Group B (B1:B4) represents the inhibition zones of the 4 bacterial strains (2.5% bee venom conc.)B1= E. Coli, B2=MRSA, B3=proteus, B4=salmonella

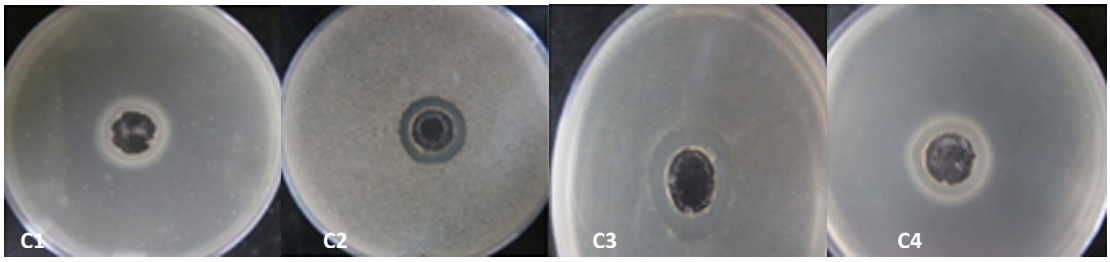

Fig. 4 : Group C (C1:C4) represents the inhibition zones of the 4 bacterial strains (1.25% bee venom conc.) c1=E.coli, c2=MRSA, c3=proteus, c4= salmonella

CONCLUSION

From the previous result and discussion, we can conclude that antibacterial activity of honey bee venom (*Apis mellifera carnica*) maybe due to the synergism between its major components and their amount percent in the given sample. Since honey bee venom inhibited the growth of the selected clinical isolates ,therefore it could be a potential alternative natural antibiotic.

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