

Evaluation of the Antibacterial Activity of Bee Venom from Different Sources

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Abstract: The objective of this investigation was to evaluate the antibacterial activity of bee venom from different sources against selected Gram-positive and Gram-negative bacterial strains of medical importance. Three different samples of bee venom (BV) collected from the honeybee *Apis mellifera* as well as whole bee extract were assessed for their potential use as antibacterial agents against five pathogenic bacterial strains including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*. Both bee venom and whole bee extract exhibited antibacterial activity against all five bacterial strains with different levels according to the type. The minimum inhibitory concentration (MIC) of BV and whole bee extract were determined with values ranging from 1000-3800 µg/ml. The results of the current study indicate that BV inhibits the growth and survival of bacterial strains and that BV can be used as complementary antimicrobial agent against pathogenic bacteria.

Key words: Antimicrobial Activity • Bee Venom • *Apis mellifera*

INTRODUCTION

The composition of bee venom produced by the glands of *Apis mellifera* has been well documented [1-3]. Bee venom comprises a very complex mixture of active peptides, enzymes and amines [4, 5].

The therapeutic application of bee venom has been used in traditional medicine to treat different diseases [6,7]. Bee venom exhibits a variety of biological activities including its cytotoxic activity against malignant cells *in vitro* [8]. It also inhibited the proliferation of mammary cell carcinoma [9]. Bee venom was found to be effective in arthritis [10], rheumatism, pain, tumors and skin diseases [11] rheumatoid arthritis and osteoarthritis [12].

Moreover, the antimicrobial activity of BV was documented on both Gram-negative and Gram-positive bacteria including *Escherichia coli* and *Salmonella* spp. [13,14], *Enterobacter cloacae*, *E. coli* and *Citrobacter freundii* [15] *Staphylococcus aureus*, Coagulase-negative *Staphylococcus* and *E. coli* [16]. The aim of the present study was to evaluate the antibacterial activity of bee venom from different sources against selected Gram-positive and Gram-negative bacterial strains of medical importance.

MATERIALS AND METHODS

Preparation of Bee Venom Sac in Ethanol: One hundred life bees were collected from bee hive. The stingers were grasped with venom sac by forceps and placed in a jar containing ethanol. The solution was mixed very well using vortex as described by Hegazi *et al.* [17]. The solution was left to dry at 37°C. The dried venom was dissolved in sterile normal saline to reach a concentration of 20%.

Preparation of Homogenized Whole Bees: One hundred life bees were collected from bee hive and killed by freezing. These bees were washed with 70 % ethyl alcohol then by sterile distilled water. The bees were homogenized in 10 ml sterile normal saline and then centrifuged at 1500 rpm for 15 minutes. The supernatant was collected after centrifugation and sterile normal saline was added to reach a total volume of 10 ml.

Deride Bee Venom: In this investigation, two samples of dried bee venom from two different places were used. The first sample was whole bee venom (Apitox) obtained from (Apitronic Services, Richmond, B.C.,

Canada). The second sample was whole bee venom obtained from Egyptian Vaccine and Serum Organization (Vacsera).

Antibacterial Assay: Five bacterial strains from human origin were used: *S. aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *E. coli* and *Pseudomonas aeruginosa*. The bacterial suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard (1.5×10^8 organisms/ml) tubes. It was further diluted to obtain a final of 5×10^6 organisms/ml. *S. aureus* was enriched on polymyxin agar [18] as a selective media, while *K. pneumoniae*, *E. coli* and *P. aeruginosa* were enriched on MacConkey broth. All bacteria were subcultured on nutrient broth for further bacterial propagation [19]. The broth was inoculated by the 0.20 µl/10 ml broth with *S. aureus*, *S. pyogenes*, *K. pneumoniae*, *E. coli* and *P. aeruginosa*, then 40 µl of 20 % bee venom or whole bee extract was added. Tetracycline was used as standard antimicrobial agent. The tubes were incubated at 37°C for 24 h. The growth of control bacterial strains as well as inhibition of the bacterial growth due to bee venom was measured by spectrophotometric assay as turbidity at 420 nm wave length. The mean values of inhibition were calculated from triple reading in each test.

Determination of Minimum Inhibitory Concentrations (MIC): The MIC were determined by broth dilution method [17,20-22]. The bacterial strains were grown in broth media to a mid-logarithmic phase at 1.0×10^6 to 3.0×10^8 CFU/ml. Two hundred microliter of a mid-logarithmic phase culture of bacteria was added to 10 µl of the BV (range of final concentration; 1-200 µg) in 96 well plate.

One well containing 200 µl of bacterial inoculates served as a bacterial control, while another well containing 200 µl of bacteria free broth media and 10 µl of sterile distilled water were used as a negative control. Cultured plates were incubated at 37°C for 24 h. The inhibition of bacterial growth was determined by measuring the absorbance at 560 nm using ELISA reader. Results were expressed as MIC, the lowest concentration of the BV that reduces growth by more than 90% of the strains.

Statistical Analysis: Means and standard deviations of the data collected for each experiment were calculated using Microsoft Excel and statistical significance determined by t-test and one-way ANOVA. Differences in survival were considered significant at $P < 0.05$ [23].

RESULTS

The effect of bee venom and whole bee extract on the growth of tested bacteria is shown in Table 1. Bee venom (Apitox) was the most effective against *S. aureus*, *S. pyogenes* and *E. coli* with a turbidity of (0.167 ± 0.003), (0.106 ± 0.001) and (0.114 ± 0.029) respectively. Tetracycline was the most effective against both *K. pneumoniae* and *P. aeruginosa* with turbidity of (0.095 ± 0.001) and (0.057 ± 0.002) respectively, followed by bee venom (Apitox) with turbidity of (0.155 ± 0.003) and (0.125 ± 0.007) respectively. Bee venom from Vacsera followed bee venom (Apitox) in its activity against *S. aureus*, *S. pyogenes* and *K. pneumoniae* with a turbidity of (0.267 ± 0.001), (0.116 ± 0.001) and (0.305 ± 0.007) respectively. Meanwhile, bee venom from ethanolic sac extract followed (Apitox) in its activity against *P. aeruginosa* with turbidity of (0.317 ± 0.006).

Table 1: Influence of bee venom on growth inhibition of different bacterial strains

Treatment	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>
Bacterial Normal growth	1.701 ± 0.015*	0.901 ± 0.15	1.559 ± 0.005	1.400 ± 0.001	1.450 ± 0.005
Apitox	0.167 ± 0.003	0.106 ± 0.001	0.155 ± 0.003	0.125 ± 0.007	0.114 ± 0.029
Vacsera	0.267 ± 0.001	0.116 ± 0.001	0.305 ± 0.007	0.325 ± 0.004	0.664 ± 0.009
Ethanolic sac extract	0.351 ± 0.001	0.501 ± 0.001	0.552 ± 0.002	0.317 ± 0.006	0.910 ± 0.012
Whole Bee extract	0.401 ± 0.009	0.306 ± 0.001	0.415 ± 0.005	0.457 ± 0.005	0.681 ± 0.004
Tetracycline (50ug)	0.369 ± 0.004	0.349 ± 0.001	0.095 ± 0.001	0.057 ± 0.002	1.049 ± 0.003

* Growth Inhibition = Inhibition of the growth measured by turbidity at 420 nm analyzed by spectrophotometer.

Table 2: The minimal inhibitory concentration (MIC) of bee venom on different bacteria

Treatment	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>
Bacterial Normal growth	----	----	----	----	----
Apitox	1600 *	1000	1200	2400	1800
Vacsera	2800	1000	1000	2600	2100
Ethanolic sac extract	3600	2400	2400	3600	3800
Whole Bee extract	2600	1000	1700	2400	2400
Tetracycline (50ug)	1000	1800	1800	2100	4400

* MIC: Minimal inhibitory concentration (µg/ml)

The results of minimum inhibitory concentration of BV were determined (Table 2). The results showed high variation in MIC of different samples against the tested bacteria. For *S. aureus*, the lowest MIC was shown by Tetracycline followed by bee venom sample (Apitox) with values of 1000 and 1600 µg/ml respectively. For *S. pyogenes*, the lowest MIC (1000 µg/ml) was shown by bee venom (Apitox), Vacsera as well as whole bee extract. Meanwhile, the lowest MIC (1000 µg/ml) for *K. pneumoniae*, was shown by bee venom from Vacsera. For *P. aeruginosa*, the lowest MIC was shown by Tetracycline followed by (Apitox) with values of 2100 and 2400 µg/ml respectively. For *E. coli*, the lowest MIC (1800 µg/ml) was shown by bee venom (Apitox).

DISCUSSION

Bee venom extraction is usually done by the electro-shock method as a standard procedure. The protein content of bee venom extracted by surgical removal of sacs was found to be different from that collected using electro-shock method [24]. In the current study, we used bee venom extracted by surgical removal of sacs in comparison with two bee venom samples, Apitox and Vacsera, extracted by milking the bees using electric current. We also used an extract of homogenized whole bees due to its different chemical composition with more peptides and flavonoids as reviewed by Hegazi [7]. Bee venom (Apitox) and bee venom from Vacsera were found to be the most effective antibacterial agents against the tested pathogens. The antimicrobial activity of bee venom was documented in earlier studies. Park *et al.* [16] clearly demonstrated that honey bee venom inhibited the growth of seventeen Gram-positive and partially two Gram-negative out of 44 bacterial strains isolated from bovine mastitis in Korea.

The antimicrobial activity of honeybee venom may be due to the presence of several peptides like melittin, apamin, adolapin, mast cell degranulating peptide, enzymes, biologically active amines and non-peptide component [25,26]. Fennel *et al.* [27] reported that the venom contains mellitin that is active against Gram-positive more than Gram-negative bacteria. These results were in agreement with Kondo and Kanai [28] who found that mycobacteria and staphylococci were affected by bee venom fraction (melittin), but not *E. coli*. Ortel and Markwrtdt [29] quantitatively determined the zones of inhibition. They found that Gram positive organisms were

sensitive at lower concentrations of bee venom than Gram negative. In contrast, a stronger activity on *E. coli* had been reported previously for bee venom [30,31]. Although, in earlier study Hegazi *et al.* [17] showed that bee products were less effective against *E. coli*, the current study provides evidence that bee venom has antibacterial activity against both Gram-positive and Gram-negative bacteria with no significant differences between both groups.

In a separate study to evaluate the combined effect of bee venom and antibiotics on *S. aureus*, it was reported that bee venom (8µg/ml) and kanamycin (10µg/ml) exhibited synergistic activity against a kanamycin resistant strain of *S. aureus*; 4-10; mean 6.6 µg/ml [32,33] Han *et al.* [34] studied, bee venom collected from honey bees *Apis mellifera* and assessed its potential use as an antimicrobial agent against fish pathogenic bacteria. They found that bee venom exhibited antibacterial activity against *Edwardsiella tarda*, *Vibrio ichthyenteri* and *Streptococcus iniae*. Han *et al.* [35] found that Korean Bee Venom (KBV) has a potential antibacterial effect against mastitis pathogens. The highest inhibitory zone of 21.4 mm was observed against *S. aureus*. followed by MRSA at 21.2 mm against the standard of 10.8.. It was evident from the study that the KBV has indeed antibacterial effects against both Gram-negative and Gram positive bacteria. Investigators have reported that the level of antibacterial activity against Gram-negative and Gram-positive bacteria is different among different antibacterial agents [36]. The experimental data of the current study confirms the previous work which suggested that bee venom can inhibit growth and survival of some bacterial strains. Further studies are needed to standardize the potential use of BV as complementary antimicrobial agent against pathogenic bacteria.

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