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Effect of some Honey Bee and Wasp Products on some Pathogenic Bacteria and Fungi: *In vitro* Study

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ABSTRACT

The present study was conducted to evaluate the antimicrobial activity of some honey bee and wasp products against different pathogenic strains of Gram (+ve), Gram (-ve) and also against microscopic fungi. All the compounds were tested *in vitro* for their antibacterial activity against *Streptococcus pneumonia, Bacillus substilis, Staphylococcus aureus* and Methicillin- Resistant *Staphylococcus aureus* (Gram - positive bacteria); *Pseudomonas aeruginosa* , *Escherichia coli* and *Salmonella typhimurium* (Gram - negative bacteria) using nutrient agar medium. Antifungal activity was carried out against *Aspergillus fumigatus*, *Syncephalastrum racemosum, Geotricum candidum* and *Candida albicans* using Sabouraud Dextrose Agar medium. Ampicillin, Vancomycine, Ciprofloxacin and Amphotricine B were used as standard drugs for Gram positive, MRSA, Gram negative and fungi respectively. DMSO (dimethylsulfoxide) was used as solvent control. The compounds were tested at a concentration of 5 mg/ml against both bacterial and fungal strains. Propolis exhibited prominent antibacterial and antifungal activities against Gram (-ve), Gram (+ve) and fungi following by bee venom and wasp venom, respectively in this investigation. The result suggests that propolis could be developed as a natural antibacterial and antifungal drug.

Key words: Antimicrobial activity, bee products, propolis, bee venom, wasp venom, pathogenic bacteria, microscopic fungi

Introduction

The wide spread use of antibiotics and chemicals against harmful microorganisms has increased and lead to development of the microbial resistance for many of them. On the other hand, chemical food preservatives used for centuries to prevent bacterial and fungal spoilage of foods represent health risks and economic cost. Food poisoning refers to illness arising from eating contaminated food by bacteria, viruses, environmental toxins, or toxins present with in the food itself. The application of natural compounds with antimicrobial properties into food products might provide an alternative to the chemical preservatives currently employed. (Paulo *et al*., 2002). Since ancient times Greeks, Romans, Chinese and Egyptians have speculated about honey and bee product's curative properties (Zumla and Lulat, 1989).

In recent years attention has been focused on the use propolis, a resinous substance collected by bees, as a health supplement suited to consumers. Propolis has different biological activities (Popova *et al*., 2005, Silici and Kutluca, 2005 and Uzel *et al*., 2005). The antimicrobial activity of propolis is reflected in its constituent, which may differ from area to area and from season to season depending on its chemical composition. Flavonoid and esters of phenolic acids are generally regarded to be responsible for the antimicrobial activity of propolis (Hegazi *et al*., 2000; Sforcin *et al*., 2000).

In recent years, venoms and venom components from animals have shown potential antimicrobial activity. These include venom of wasps, common honeybees, spiders, snakes and scorpions (Dani *et al*., 2003; Torres-Larios *et al*., 2002). Their antimicrobial activities is mostly revealed to the presence of peptides (Choi and Kange, 2001; Dani *et al.,* 2003; Perumal Samy *et al.,* 2007; Sforça *et al.,* 2004).

Venom components from predator wasps including hornets (genera *Vespa* and *Dolichovespula*), yellow jackets (genus *Vespula*) and paper wasps (genus *Polistes*) have been extensively studied. Their toxins are complex mixtures of amines, small peptides and high molecular weight proteins such as enzymes, allergens and toxins (Biggs *et al.,* 2007). Venoms from these stinging wasps are important weapons both in the defense of the colony and capture of prey.

This study aimed to compare potent antimicrobial activities of some honey bee products namely; bee venom, propolis of *Apis mellifera* and wasp venom of *Vespa orientalis* reared under the same environmental conditions.

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Materials and Methods

Honey bee colonies

The tested honey bee colonies of *Apis mellifera* were situated in the apiary of the Honey bee Research Dept., Plant Prot. Institute, Ministry of Agriculture, Giza, Egypt. (Spring of 2015).

Sampling

Propolis

Propolis was obtained using glass slides placed on to the top bars of combs in tested colonies according to the method of Mohanny (2005).

Adhered propolis was weekly collected by scratching with a sharp and clean blade, packed and kept at – 5°C till use.

Bee Venom

Venom of honey bee was obtained by the electric shock device unit of Mohanny (2005). After drying on glass plate, the whole bee venom was scratched with a sharp knife and quickly packed in opaque glass vials and kept at – 5°C till use.

Wasp venom

Vespa orientalis specimens were collected from honey bee research department. Wasps were paralyzed at 4°C; their venom glands were dissected and immersed in liquid nitrogen. The glands were then crushed in a clean mortar using pestle and liquid nitrogen. Twenty milliliters of 0.1 M buffer phosphate (pH = 7.4) were added to the powdered sample immediately after the evaporation of liquid nitrogen. The suspension was then transferred into a clean tube and further homogenized. Each tube containing the sample was centrifuged at 8,000 × *g* for 15 minutes at 4°C. The supernatant was transferred into another tube, lyophilized and kept at - 20°C until further assay.

Antimicrobial assay: -

The antimicrobial activity of the tested products was examined in Regional Center for Mycology and Biotechnology Antimicrobial unit test organisms (RCMB) as follow:

Test organisms

The tested Gram (+ve) bacteria were, *Streptococcus pneumonia, Bacillis subtilis*, *Staphylococcus aureus and* Methicillin-Resistant *Staphylococcus aureus*. On the other hand the tested Gram (- ve) bacteria were, *Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhimurium*. The chosen fungi were, *Aspergillus fumigatus, Syncephalastrum racemosum*, *Geotricum candidum* and *Candida albicans*.

Method of testing:

The sterilized molten agar media was poured onto the sterilized Petri dishes (20 ml, each petri dish (9cm)) and allowed to solidify in room temperature. Wells of 6 mm diameter were made in the solidified media with the help of sterile borer. A sterile swab was used to evenly distribute microbial suspension over the surface of solidified media and solutions of the tested samples were added to each well with the help of micropipette. The plates were incubated at 37°C for 24 hrs. in case of antibacterial activity and 48 hrs. at 25°C for antifungal activity. This experiment was carried out in triplicate and zones of inhibition were measured in mm. scale.

Minimum inhibitory concentration (MIC):

The MIC was determined by the broth micro dilution method using 96-well micro-plates (Saini *et al*., 2005). Each sample (1.0 mg) was dissolved in DMSO (1 mL) to obtain 1000 µg/mL stock solution, then the concentrations of 500, 250, 125, 62.5, 31.3, 15.6 and 7.81, 3.9, 1.95, 0.98 and 0.49 µg/mL from the samples were prepared and applied against the microscopic organisms. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC).

Results and Discussion

The obtained data (Table 1 & 2) indicated that all the tested honey bee and wasp products showed antimicrobial activities against the tested bacteria and fungi, but varied in their potencies. Propolis was the most effective followed by bee venom then wasp venom.

Propolis

The effect of propolis on the growth of tested bacteria and fungi was shown in Table 1. Propolis was the most effective against *Streptococcus pneumoniae*, *Bacillis subtilis*, *Staphylococcus aureus* and MRSA (Gram–positive bacteria) with inhibition zone (22.4 ± 1.2), (22.4 ± 0.25), (20.6 ± 1.2) and (18.3± 0.25) respectively. The results of minimum inhibitory concentration of propolis were determined (Table 2). The results showed high variation in MIC of propolis against *Streptococcus pneumoniae*, *Bacillis subtilis*, *Staphylococcus aureus* and MRSA (Gram- positive bacteria) with values 1.95, 0.98, 1.95 and 7.81 µg/ml respectively.

The effect of propolis against *Pseudomonas aeruginosa* , *Escherichia coli* and *Salmonella typhimurium* (Gram-negative bacteria) was shown in Table1 with inhibition zone (20.4± 0.25), (23.6± 0.63) and (22.6 ± 0.25) respectively , with the lowest MIC values, since *Escherichia coli*, *Salmonella typhimurium* seemed to be the most sensitive 0.98 µg/ml, followed by *Pseudomonas aeruginosa* 3.9 µg/ml .

According to the obtained data, the tested microscopic fungi, *Aspergillus fumigatus* (20.1 ± 0.63) mm was the most sensitive and the sensitivity of the microscopic fungi decreased: *Geotricum candidum* > *Syncephalastrum racemosum* and *Candida albicans* (18.2± 1.2), (17.3±0.58) and (17.3±0.58) respectively. The MIC values were varied, the lowest MIC (3.9 µg/ml) was shown for *Aspergillus fumigatus* then *Geotricum candidum* , *Syncephalastrum racemosum* and *Candida albicans* with MIC values (7.81, 15.63, 15.63 respectively.

A number of studies have presented evidence that propolis has strong antimicrobial properties (Ozcan, 2000; Banskota *et al*., 2001; Sforcin, 2007; Viuda- Martos, *et al*., 2008). Bankova *et al*., (1995) examined the activity of different fractions of Brazilian propolis towards *Staphylococcus aureus*, and observed that the antibacterial activity is mainly due to polar phenolic compounds. Kujumgiev *et al*., (1999) reported that all the propolis samples used in these experiments were active against the Gram-positive bacteria.

Castaldo and Capasso (2002) reported that propolis samples showed *in vitro* antimicrobial activity mainly against Gram-positive (*Staphylococcus* spp. and *Streptococcus* spp.) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa*). The variation in the antibacterial activities of tested extracts may be due to the different substance compounds and their phenolic constituents (Castaldo and Capasso, 2002; Nagai *et al*., 2003; Basim *et al*., 2006). Melliou *et al*., (2007) reported that the volatiles of Greek propolis inhibited four different species of Gram-negative bacteria (*E. coli, E. cloacae, K. pneumoniae, P. aeruginosa*). However, Brazilian and Korean propolis extracts inhibited the Gram-negative bacterium *S. typhimurium* but failed to inhibit the Gram-negative *Pseudomonas aeruginosa* (Choia *et al*., 2006). The recent review of Simone-Finstrom and Spivak (2010) also summarizes the importance of the antimicrobial action of propolis in regards of bee health.

Bee venom

From the obtained results, bee venom exhibit antibacterial and antifungal effects, with a significant MICs value, since *Streptococcus pneumonia* and *Bacillis subtilis* seemed to be the most sensitive (21.9±0.58), (21.2±0.67) mm respectively and the sensitivity of the Gram (+ve) bacteria decreased as follows: *Staphylococcus aureus*, then MRSA (20.1± 2.1), (16.3± 0.67) mm respectively. The MICs value of the tested sample was recorded as follow: (1.95, 1.95, 3.9 and 31.25 µg /ml), against *Streptococcus pneumonia*, *Bacillis subtilis*, *Staphylococcus aureus* and MRSA respectively, while the bee venom show an inhibitory effect on Gram (-ve) bacteria as follow: (20.4±1.2), (18.1±0.46), (18.3±0.67) for *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* respectively. The results of minimum inhibitory concentration of BV were determined in Table 2. The results showed significant variation against the tested bacteria. The lowest MIC value (3.9 µg /ml) was recorded for *E.coli* then *Pseudomonas aeruginosa* and *Salmonella typhimurium* i.e. Gram- positive were more affected by tested venoms compared to Gram-negative bacteria.

According to the obtained results, the tested microscopic fungi, *Aspergillus fumigatus* (19.2±0.63) mm was the most sensitive, and the sensitivity of the microscopic fungi decreased: *Geotricum candidum* , *Candida albicans* (16.9±0.58), (16.4±1.2) respectively, while BV showing no effect on *Syncephalastrum racemosum*.

Table 1: Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (5mg/ml) concentration of tested samples. Results are depicted in the following table:

Sample / Tested microorganisms	Wasp V	Prop	Bee v	St.
FUNGI	Mean zone of inhibition mm			Amphotericin B
Aspergillus fumigatus (RCMB 02568)	15.4± 0.58	20.1 ± 0.63	19.2± 0.63	23.7± 1.2
Syncephalastrum racemosum (RCMB 05922)	NA	17.3 ± 0.58	NA	19.7± 0.58
Geotricum candidum (RCMB 05097)	14.2± 0.25	18.2± 1.2	16.9± 0.58	28.7± 0.72
Candida albicans (RCMB 05036)	13.6± 1.2	17.3± 0.58	16.4± 1.2	25.4± 1.2
Gram Positive Bacteria:				*Ampicillin*
Streptococcus pneumoniae (RCMB 010010)	17.3± 0.63	22.4 ± 1.2	21.9±0.58	23.8± 0.58
Bacillis subtilis (RCMB 010067)	20.3± 0.63	22.4 ± 0.25	21.2±0.67	32.4± 1.2
Staphylococcus aureus (RCMB 000106)	13.6± 0.44	20.6 ± 1.2	20.1 ± 2.1	26.2± 0.72
Methicillin-Resistant Staphylococcus aureus				*Vancomycine*
MRSA 2658 RCMB	NA	18.3± 0.25	16.3±0.67	20.3± 1.2
Gram negativeBacteria:				*Ciprofloxacin*
Pseudomonas aeruginosa (RCMB 010043)	11.2± 0.58	20.4± 0.25	18.1±0.46	20.6± 1.2
Escherichia coli (RCMB 010052)	13.4± 0.58	23.6± 0.63	20.4±1.2	23.4± 0.63
Salmonella typhimurium (RCMB 010072)	16.2± 1.2	22.6 ± 0.25	18.3±0.67	25.4± 1.5

*The test was done using the diffusion agar technique, Well diameter: 6.0 mm …… …… (100 µl was tested), RCMB: Regional Center for Mycology and Biotechnology Antimicrobial unit test organisms *NA: No activity, data are expressed in the form of mean ± SD*

In this respect, Kondo and Kanai (1986) found that mycobacteria and staphylococci were affected by bee venom fraction (melittin), but not *E. coli*. Also, Hegazi *et al.*, (2002) showed that bee products were less effective against *E. coli*. Benton and Mulfinger (1989) and Rybak *et al.*, (1994) reported that bee venom (8µg/ml) + kanamycin (10µg/ml) exhibited synergistic activity against a kanamycin- resistant strain of *S. aureus*.

Wasp venom

Vespa orientalis crude venom displayed a significant effect against different Gram-positive, Gram-negative bacterial strains and fungus emplyed in this study. The corresponding inhibition zones and MICs are listed in Table 1 & 2. Wasp venom caused a marked inhibition in bacterial growth with inhibition zones of (17.3±0.63), (20.3±0.63) and (13.6±0.44) mm for *Streptococcus pneumonia, Bacillis subtilis*, *Staphylococcus aureus* respectively, while there is no inhibitory effect on MRSA. The corresponding MICs of the crude venom were respectively found to be 15.63, 3.9, 62.5 µg/mL.

On the other hand, wasp venom caused a relatively low inhibition in bacterial growth of Gram (-ve) bacteria with inhibition zones of (11.2±0.58), (13.4±0.58), (16.2±1.2) mm for *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhimurium* respectively. The MICs value were respectively found to be 125, 62.5, 31.25 µg/mL.

According to the given data, *Aspergillus fumigatus* (15.4± 0.58) mm was the most sensitive followed by *Geotricum candidum* then *Candida albicans*, (14.2± 0.25) and (13.6± 1.2) mm respectively. Wasp venom did not show an inhibitory effect on *Syncephalastrum racemosum*. The MICs value were found to be 31.25, 62.5 and 62.5 µg/mL for *Aspergillus fumigatus, Geotricum candidum* and *Candida albicans* respectively.

In addition, the present findings indicate that the crude wasp venom is more effective against Gram-positive than Gram-negative bacteria which might be related to the difference in cell envelope structure. 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 (Schwarz and Reiter , 2001)

Table 2: Antimicrobial Activity as MICS (µg / ml) of tested samples against tested microorganisms

Tested microorganisms \ Sample	Wasp V	Prop	Bee v	St.
	Minimum inhibitory concentration (µg/ml)			
FUNGI				Amphotericin B
Aspergillus fumigatus (RCMB 02568)	31.25	3.9	3.9	23.7± 1.2
Syncephalastrum racemosum (RCMB 05922)	NA	15.63	NA	19.7± 0.58
Geotricum candidum (RCMB 05097)	62.5	7.81	15.63	28.7± 0.72
Candida albicans (RCMB 05036)	62.5	15.63	31.25	25.4± 1.2
Gram Positive Bacteria:				*Ampicillin*
Streptococcus pneumoniae (RCMB 010010)	15.63	1.95	1.95	23.8± 0.58
Bacillis subtilis (RCMB 010067)	3.9	0.98	1.95	32.4± 1.2
Staphylococcus aureus (RCMB 000106)	62.5	1.95	3.9	26.2± 0.72
Methicillin-Resistant Staphylococcus aureus				*Vancomycine*
MRSA 2658 RCMB	NA	7.81	31.25	20.3± 1.2
Gram negative Bacteria:				*Ciprofloxacin*
Pseudomonas aeruginosa (RCMB 010043)	125	3.9	7.81	20.6± 1.2
Escherichia coli (RCMB 010052)	62.5	0.98	3.9	23.4± 0.63
Salmonella typhimurium (RCMB 010072)	31.25	0.98	7.81	25.4± 1.5

**NA : No activity*

Conclusion

The present findings augment the role of honey bee and wasp products as inhibitors for pathogenic bacteria and fungi. The results suggested that propolis could be developed as a natural antifungal and antibacterial drug.

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