

# In vitro evaluation of antimicrobial and antioxidant activities of honeybee venom and propolis collected from various regions in Egypt

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## Background and objective

Honeybee products are commonly used as food and medicine. Recently, pharmacological properties of bee venom and propolis have been reported. However, the geographic origin of bee venom and propolis influences their chemical composition and biological activities. The antimicrobial and antioxidant properties of bee venom and propolis collected from different regions in Egypt were evaluated.

## Materials and methods

Bee venom and propolis were collected from the regions of Kafr-Elsheikh, Fayoum, and Giza in Egypt. The antimicrobial and antioxidant effects of bee venom and propolis extracts obtained with various solvents were evaluated using the well-diffusion method and the 1,1-diphenyl-2-picrylhydrazyl free radical scavenging assay, respectively.

## Results and conclusion

The antimicrobial activities of bee venom extracts were greater than those of propolis extracts, and ethanol extracts were more efficient than chloroform and water extracts. Extracts obtained from the Kafr-Elsheikh region were the most active, whereas those from the Giza region were less effective. Gram-positive bacteria were more sensitive than gram-negative bacteria and fungi. Propolis extracts were more effective antioxidants than bee venom extracts. The activities of extracts from the Kafr-Elsheikh or the Fayoum regions were comparable and greater than those of the corresponding extracts from the Giza region. Ethanolic extraction provided the greatest antioxidant potential. The biological activity of Egyptian bee venom or propolis varies significantly depending on the extraction solvent and geographical area of collection. These results provide insights into the antimicrobial and antioxidant properties of Egyptian bee venom and propolis and constitute a basis for further phytochemical and pharmacological research.

## Keywords:

agar well diffusion, antimicrobial activity, antioxidant property, bee venom, 1, 1-diphenyl-2-picrylhydrazyl radical, propolis

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## Introduction

Antibiotic resistance constitutes a great pharmaceutical challenge. A growing number of infections are harder to treat as antibiotics become less effective [1]. This global crisis reflects the worldwide overuse and/or misuse of antibiotics and the lack of new antibiotic agents [2]. As resistance to nearly all antibiotics has been reported [3], the WHO has emphasized the urgency to design new antimicrobial molecules to avoid common infections and minor injuries from becoming lethal again [4].

Oxidative stress, which results from an imbalance between free radicals and antioxidants, contributes to the development and progression of various human

illnesses. Overwhelming free radical levels disrupt the body's antioxidant defense system, thereby damaging cellular membranes and macromolecules and leading to cell death [5]. Antioxidants neutralize free radicals via their free radical scavenging activity (RSA). Although many synthetic antioxidant agents have been developed, their high cost, lack of availability, and adverse effects remain major setbacks. Therefore, new antioxidants with lesser adverse effects need to be developed.

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Many natural bioactive compounds have promising antimicrobial and antioxidant properties [6,7]. Their safer and broader mechanisms of action are less likely to induce antimicrobial resistance [8]. Bioactive compounds are produced by a wide variety of organisms including honeybees (*Apis mellifera*).

Honeybees might be the oldest beneficial insects known to humankind. Their products, including honey, royal jelly, propolis, venom, and pollen, are used as food and medicine since time immemorial. Recently, the antimicrobial and antioxidant properties of bee venom and propolis have been demonstrated [9]. However, the high chemical variability of bee venom and propolis, which depends on honeybee species, geographical locations, and botanical sources, has hindered their chemical applications [10]. Moreover, genetic variability within the same species has also been reported not only in honeybees but also in other higher life forms [11–13].

Most studies used honeybees from Europe and Latin America. Only a few reports concern bee venom and propolis from Egypt, although Egypt is a source of bioactive compounds due to its diverse climatic conditions, terrains, and flora [14]. Here, we evaluated the antimicrobial and antioxidant properties of bee venom and propolis collected from different regions in Egypt.

## Materials and methods

### Bee venom and propolis samples

Bee venom and propolis samples were provided by the Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture and Land reclamation, Cairo, Egypt. These samples were collected from three different localities in Egypt (Kafr-Elsheikh, Fayoum, and Giza).

### Bacterial and fungal strains

A total of five strains from gram-positive bacteria, gram-negative bacteria, and yeast-like fungi (*Salmonella typhimurium* NCTC 12023/ATCC 14028, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 33018, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* CAIM-22) were provided by the Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The fungal strain was maintained on potato dextrose agar, whereas bacterial strains were cultivated on nutrient agar.

### Preparation of extracts

All of the six dried samples (three bee venom and three propolis samples) were crushed into a soft powder. The extracts were prepared using 80% ethanol, chloroform, or water as solvents. Overall, 5 g of each sample was macerated separately in 50 ml of each solvent and maintained in a shaker, at room temperature, for 3 days. Afterward, the liquid extracts were separated from the solid residues by filtration using Whatman No. 1 filter and were evaporated. The resulting dry extracts were dissolved in the extraction solvent at a final concentration of 40 mg/ml stock solutions. The 18 stock solutions were stored at  $-20^{\circ}\text{C}$  in well-sealed dark glass containers until testing.

### Antimicrobial assay

Antimicrobial activities were assessed with the agar well-diffusion method [15]. In brief, 1 ml of fresh bacterial or fungi culture was pipetted in the center of a sterile Petri dish. Then, 15 ml of nutrient agar medium for bacterial strains or potato dextrose agar medium for fungal strain was poured into the Petri dish containing the inoculum and mixed well. A sterile cylinder (8 mm in diameter) was used to make wells into the solidifying agar plates containing inoculums. After solidification, 100  $\mu\text{l}$  of the diluted stock solutions (extracts) was added to the wells. Wells containing solvents (100  $\mu\text{l}$ ) were used as negative controls, and wells containing reference antibiotic (tetracycline for bacteria and nystatin for fungi) served as positive controls (50  $\mu\text{g}/\text{ml}$ ). The incubation period was either 24 h for bacteria or 48 h for yeast. Antimicrobial activity was determined by measuring the zone of inhibition (including the well diameter) after the incubation period.

### Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was determined with the agar dilution method [16]. Six tubes containing 8 ml of sterile broth medium (nutrient broth for bacterial strains and potato dextrose broth for fungal strain) were used for each extract to perform serial two-fold dilutions. Then, 8 ml of each extract dilution (range, 8–0.25 mg/ml) was added to 8 ml of sterile liquid agar medium at  $45^{\circ}\text{C}$  (final concentration: 4–0.125 mg/ml). This mixture was homogenized, poured into plates, and allowed to solidify. Microbial suspensions of  $10^4$  colony-forming unit/spot were then inoculated to each plate. Spots were dried at room temperature, and then plates were incubated upside-down at  $37^{\circ}\text{C}$  for 24 h. Inoculated agar plates without extract served as positive controls, whereas negative controls included solvents.

### Antioxidant assay

Free RSA (antioxidant effect) was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to a previously described procedure [17]. This method is based on the discoloration of DPPH in the presence of antioxidants. In brief, 0.5 ml of extracts at different concentrations (0.25, 0.5, 1, and 2 mg/ml) was added to 2.5 ml of 0.004% DPPH in methanol. The mixture was incubated in dark at room temperature for 30 min. After incubation, the absorbance was measured at 517 nm using a spectrophotometer. DPPH in methanol was used as a control, and ascorbic acid was used as a reference. The antioxidant activity of the samples was expressed in percentage of DPPH radical reduction. RSA was calculated using the following formula:

$$\text{RSA}(\%) = \left\{ \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right\} \times 100,$$

where  $A_{\text{control}}$  is the absorbance of DPPH without extract and  $A_{\text{sample}}$  is the absorbance of DPPH with extract.

### Statistical analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences subscription software

(IBM, Armonk, New York, USA). All experiments were performed in triplicates, and data are presented as means  $\pm$  SEs. Significant differences were determined by one-way analysis of variance followed by Tukey's test. A  $P$  value of less than 0.05 was considered statistically significant.

### Results

Table 1 shows the microbial inhibitory potential of different solvent extracts from bee venom and propolis harvested from different regions. The inhibition zone diameters were different depending on the pathogens for a same treatment. In addition, different treatments of the same pathogen resulted in different inhibition zone diameters. In general, irrespective of the type of bee product (bee venom or propolis) or solvent (ethanol, chloroform, or water), extracts from the Kafr-Elshiekh region had stronger effects than the corresponding extracts from the Fayoum and Giza regions. All Kafr-Elshiekh extracts exhibited an antimicrobial activity against all tested microorganisms with inhibition zones ranging from 2.7  $\pm$  0.3 to 35.7  $\pm$  1.2 mm. They were more effective against gram-positive bacteria (*B. cereus* and *S. aureus*) than against gram-negative bacteria

**Table 1** Antimicrobial activity of various solvent extracts (4 mg/ml) of different bee venom and propolis sources collected from several regions in Egypt toward certain strains of gram-positive, gram-negative bacteria, and yeast

Region	Bee product	Solvent extract	Zone of inhibition (mm)				
			<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhimurium</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
Kafr-Elshiekh	Propolis	Ethanol	30.7 $\pm$ 0.9	29.3 $\pm$ 1.2	20.3 $\pm$ 0.9	9.0 $\pm$ 0.6	11.3 $\pm$ 0.7
		Chloroform	12.7 $\pm$ 0.3	13.0 $\pm$ 0.6	7.7 $\pm$ 0.3	2.3 $\pm$ 0.3	2.7 $\pm$ 0.3
		Water	13.3 $\pm$ 0.7	13.7 $\pm$ 0.3	8.0 $\pm$ 0.6	2.3 $\pm$ 0.3	3.0 $\pm$ 0.6
	Bee venom	Ethanol	35.7 $\pm$ 1.2	35.0 $\pm$ 1.0	26.3 $\pm$ 0.7	14.7 $\pm$ 0.7	16.3 $\pm$ 0.3
		Chloroform	19.0 $\pm$ 0.6	19.3 $\pm$ 0.3	13.3 $\pm$ 0.3	6.7 $\pm$ 0.3	8.0 $\pm$ 0.6
		Water	19.7 $\pm$ 0.3	19.3 $\pm$ 0.7	14.0 $\pm$ 0.6	7.3 $\pm$ 0.9	8.3 $\pm$ 0.9
Fayoum	Propolis	Ethanol	20.0 $\pm$ 0.6	19.7 $\pm$ 0.3	10.0 $\pm$ 0.6	2.7 $\pm$ 0.3	4.3 $\pm$ 0.3
		Chloroform	7.7 $\pm$ 0.3	8.0 $\pm$ 0.7	3.3 $\pm$ 0.3	ND	ND
		Water	8.3 $\pm$ 0.7	7.7 $\pm$ 0.7	3.0 $\pm$ 0.6	ND	ND
	Bee venom	Ethanol	25.3 $\pm$ 0.3	24.3 $\pm$ 0.3	16.3 $\pm$ 0.7	8.3 $\pm$ 0.9	8.0 $\pm$ 1.0
		Chloroform	14.0 $\pm$ 1.0	13.0 $\pm$ 1.0	8.3 $\pm$ 0.3	2.7 $\pm$ 0.7	3.3 $\pm$ 0.3
		Water	13.7 $\pm$ 0.9	13.3 $\pm$ 0.9	7.7 $\pm$ 0.3	2.3 $\pm$ 0.3	2.7 $\pm$ 0.3
Giza	Propolis	Ethanol	13.0 $\pm$ 0.6	13.7 $\pm$ 0.7	2.7 $\pm$ 0.7	ND	ND
		Chloroform	2.7 $\pm$ 0.7	2.7 $\pm$ 0.3	ND	ND	ND
		Water	3.0 $\pm$ 0.6	2.3 $\pm$ 0.3	ND	ND	ND
	Bee venom	Ethanol	18.7 $\pm$ 0.7	19.0 $\pm$ 0.6	7.7 $\pm$ 0.3	ND	ND
		Chloroform	8.0 $\pm$ 0.6	7.7 $\pm$ 0.3	ND	ND	ND
		Water	7.7 $\pm$ 0.3	7.3 $\pm$ 0.3	ND	ND	ND
Tetracycline			26.7 $\pm$ 0.3	26.3 $\pm$ 0.7	21.3 $\pm$ 0.3	20.0 $\pm$ 0.6	
Nystatin							24.3 $\pm$ 0.3

All results are expressed as mean  $\pm$  SE from three experiments ( $n=3$ ). Tetracycline and nystatin (50  $\mu$ g/ml) served as positive controls for bacteria and fungi, respectively. ND, not detected.

(*S. typhimurium* and *E. coli*) or fungi (*C. albicans*). Moreover, the antimicrobial activity of Kafr-Elshiekh bee venom extracts was greater than that of propolis extracts, and ethanol extracts had higher antimicrobial activities than chloroform and water extracts. Regarding the extracts from the Fayoum region, the same trend of a greater antimicrobial activity obtained with the bee venom and ethanol as solvent was observed. *B. cereus*, *S. aureus*, and *S. typhimurium* were more susceptible to the extracts from the Fayoum region. *E. coli* and *C. albicans* were sensitive to all bee venom extracts and the ethanolic propolis extract; however, propolis chloroform and water extracts failed to inhibit their growth. The largest inhibition zone was measured for *B. cereus* exposed to ethanolic bee venom extracts (25.3 ± 0.3 mm), whereas the lowest one was observed for *E. coli* treated with aqueous propolis extracts (2.3 ± 0.3 mm). The inhibitory activity of extracts from the Giza region was the greatest for ethanolic extracts of bee venom. However, unlike the Kafr-Elshiekh and Fayoum extracts, extracts obtained from the Giza region, regardless of the solvent, were inefficient against *E. coli* and *C. albicans*. Additionally, *S. typhimurium* growth was not affected by chloroform and water extracts of bee venom and propolis, whereas it was decreased by bee venom and propolis ethanol extracts, with inhibition zones of 7.7 ± 0.3 and 2.7 ± 0.7 mm, respectively. *B. cereus* and *S. aureus* were

sensitive to all Giza extracts with inhibition zones ranging from 2.3 ± 0.3 to 19.0 ± 0.6 mm. Solvents (100 µl of ethanol, chloroform, or water) had no effect on the pathogen growth as no clear inhibition zone (≥ 2 mm) was found around the wells of all negative controls. On the contrary, the standard drugs (tetracycline for bacteria and nystatin for fungi) used as positive controls exhibited strong antimicrobial effects toward gram-positive bacteria, gram-negative bacteria, and fungi, with inhibition zones ranging from 20.0 ± 0.6 to 26.7 ± 0.3 mm.

Table 2 shows the values of MIC obtained for the different solvent extracts of bee venom and propolis tested. All extracts were effective against *B. cereus* and *S. aureus*, but these effects depended on the region of origin of the bee product, the type of bee product, and the type of solvent. As expected, the lowest MIC values, ranging from 0.125 to 1 mg/ml, were obtained with the extracts from Kafr-Elshiekh. The MIC values were intermediary for the extracts from the Fayoum region (range, 0.25–2 mg/ml) and the highest (0.5–4 mg/ml) for Giza extracts. Additionally, MIC values obtained with chloroform and water extracts were comparable and were greater than those measured with ethanol extracts. Furthermore, bee venom extracts exhibited stronger antimicrobial effects than propolis extracts as shown by their smaller MIC values. All extracts from the Kafr-Elshiekh and Fayoum regions

**Table 2** Minimum inhibitory concentration of various solvent extracts of different bee venom and propolis sources collected from several regions in Egypt towards certain strains of gram-positive and gram-negative bacteria and yeast

Region	Bee product	Solvent extract	MIC (mg/ml)				
			<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhimurium</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
Kafr-Elshiekh	Propolis	Ethanol	0.25	0.25	1.0	2	2
		Chloroform	1	1	2	4	4
		Water	1	1	2	4	4
	Venom	Ethanol	0.125	0.125	0.5	1	1
		Chloroform	0.5	0.5	1	2	2
		Water	0.5	0.5	1	2	2
Fayoum	Propolis	Ethanol	0.5	0.5	2	4	4
		Chloroform	2	2	4	ND	ND
		Water	2	2	4	ND	ND
	Venom	Ethanol	0.25	0.25	1	2	2
		Chloroform	1	1	2	4	4
		Water	1	1	2	4	4
Giza	Propolis	Ethanol	1	1	4	ND	ND
		Chloroform	4	4	ND	ND	ND
		Water	4	4	ND	ND	ND
	Venom	Ethanol	0.5	0.5	2	ND	ND
		Chloroform	2	2	ND	ND	ND
		Water	2	2	ND	ND	ND

All results are expressed as mean ± SE from three experiments (n=3). Tetracycline and nystatin (50 µg/ml) served as positive controls for bacteria and fungi, respectively. ND, not detected.

were active against *S. typhimurium*, with MIC values ranging from 0.5 to 4 mg/ml. In contrast, only ethanolic bee venom and propolis extracts from the Giza region inhibited *S. typhimurium* growth, with MIC values of 2 and 4 mg/ml, respectively. *E. coli* and *C. albicans* were affected the most by Kafr-Elshiekh extracts, with MIC values ranging from 1 to 4 mg/ml. Regarding products from the Fayoum region, MIC values ranging from 2 to 4 mg/ml were obtained for all bee venom extracts and ethanol extract of propolis, whereas propolis chloroform and water extracts did not affect the growth of *E. coli* and *C. albicans*. None of the analyzed extracts from the Giza region were active against *E. coli* and *C. albicans*. Overall, *B. cereus* and *S. aureus* were the most susceptible microorganisms (with no difference between their MIC values), *S. typhimurium* was mildly affected and *E. coli* and *C. albicans* were the most resistant (with no difference between their MIC values).

The antioxidant properties of ethanol, chloroform, and water extracts of bee venom and propolis were evaluated using the DPPH method with ascorbic acid as control. As shown in Table 3, all extracts displayed different degrees of free RSA. In general, more DPPH radicals were scavenged by bee venom

extracts than by propolis extracts, regardless of the region of origin or the solvent used. In addition, ethanolic extraction was more effective to generate extracts with strong antioxidant properties. Furthermore, extracts originating from the Kafr-Elshiekh and Fayoum regions (no significant differences between both regions) were better free radical scavengers than those from the Giza region. On a whole, ethanolic extracts of propolis collected from the Kafr-Elshiekh or Fayoum region at a concentration of 0.2 mg/ml had the highest RSA ( $94.0 \pm 1.5$  and  $93.7 \pm 1.8\%$ , respectively, with no significant difference between both regions). In contrast, the antioxidant properties of identical concentrations of chloroform and water extracts from bee venom collected from the Giza region were comparable and were the lowest ( $44.0 \pm 1.5$  and  $43.0 \pm 1.2\%$ , respectively, with no significant difference between both types of extracts). All extracts inhibited the production of DPPH radicals in a dose-dependent manner, with the efficiency increasing with the concentration. At a high concentration (0.2 mg/ml), the antioxidant activities of ethanolic extracts from propolis collected from the Kafr-Elshiekh and Fayoum regions were statistically similar to that of the powerful antioxidant reference, ascorbic acid, used as control.

**Table 3** Antioxidant activity of various solvent extracts of different bee venom and propolis sources collected from several regions in Egypt

Region	Bee product	Solvent extract	1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (%)			
			25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
Kafr-Elshiekh	Propolis	Ethanol	$45.3 \pm 0.9^b$	$53.7 \pm 1.2^b$	$83.0 \pm 1.2^b$	$94.0 \pm 1.5^a$
		Chloroform	$36.3 \pm 1.3^c$	$38.0 \pm 1.0^{def}$	$70.3 \pm 0.9^c$	$82.3 \pm 1.5^b$
		Water	$36.7 \pm 1.2^c$	$37.7 \pm 0.7^{ef}$	$70.7 \pm 1.2^c$	$81.7 \pm 1.2^b$
	Venom	Ethanol	$37.7 \pm 0.9^b$	$45.0 \pm 1.5^c$	$57.3 \pm 1.5^d$	$71.7 \pm 1.3^c$
		Chloroform	$25.7 \pm 1.8^d$	$28.0 \pm 1.2^g$	$40.3 \pm 1.5^e$	$53.0 \pm 1.0^d$
		Water	$26.0 \pm 1.7^d$	$28.3 \pm 1.9^g$	$39.7 \pm 1.3^e$	$54.0 \pm 1.2^d$
Fayoum	Propolis	Ethanol	$44.3 \pm 1.5^b$	$53.0 \pm 1.2^b$	$81.3 \pm 1.5^b$	$93.7 \pm 1.8^a$
		Chloroform	$35.7 \pm 1.2^c$	$44.3 \pm 1.2^{cd}$	$70.0 \pm 1.2^c$	$82.3 \pm 1.2^b$
		Water	$35.0 \pm 1.2^c$	$43.7 \pm 0.9^{cde}$	$71.3 \pm 0.9^c$	$81.3 \pm 0.9^b$
	Venom	Ethanol	$36.7 \pm 1.8^c$	$42.3 \pm 1.3^{cde}$	$55.0 \pm 1.2^d$	$70.0 \pm 1.7^c$
		Chloroform	$23.3 \pm 1.3^d$	$26.0 \pm 1.5^{gh}$	$38.0 \pm 1.7^{ef}$	$55.3 \pm 1.5^d$
		Water	$24.7 \pm 1.2^d$	$27.0 \pm 1.2^g$	$40.3 \pm 1.7^e$	$54.3 \pm 1.5^d$
Giza	Propolis	Ethanol	$33.7 \pm 1.5^c$	$43.3 \pm 0.9^{cde}$	$70.0 \pm 1.2^c$	$82.7 \pm 1.2^b$
		Chloroform	$20.3 \pm 1.3^d$	$31.7 \pm 1.2^{fg}$	$60.3 \pm 0.7^d$	$70.3 \pm 1.9^c$
		Water	$21.7 \pm 1.5^d$	$32.0 \pm 1.0^{fg}$	$61.3 \pm 1.3^d$	$70.0 \pm 1.5^c$
	Venom	Ethanol	$21.0 \pm 1.5^d$	$30.0 \pm 1.2^g$	$41.3 \pm 1.5^e$	$55.3 \pm 1.3^d$
		Chloroform	$10.3 \pm 1.8^e$	$20.3 \pm 1.3^{hi}$	$31.0 \pm 1.0^g$	$44.0 \pm 1.5^e$
		Water	$8.0 \pm 1.2^e$	$19.3 \pm 1.5^i$	$31.7 \pm 1.3^{fg}$	$43.0 \pm 1.2^e$
Ascorbic acid			$74.3 \pm 0.9^a$	$85.0 \pm 0.6^a$	$94.7 \pm 1.2^a$	$96.0 \pm 1.0^a$

All results are expressed as mean  $\pm$  SE from three experiments ( $n=3$ ). Values with different letters within the columns are significantly different ( $P < 0.05$ ) according to Tukey's test.

## Discussion

In recent decades, natural products of plant, animal, or microbial origins have been intensively investigated for their health benefits. They might constitute not only the food but also the medicine of the future. The popularity of bee venom and propolis has increased because of their pharmacological activities, including antimicrobial, anticancer, anti-inflammatory, and anticoagulation effects [18,19]. These biological properties have been attributed to different bioactive compounds. Indeed, propolis contains mainly phenolics and flavonoids [18,20], and melittin is the major component of bee venom [19]. This distinct composition of bee venom and propolis might explain the higher antioxidant and lower antimicrobial actions of propolis extracts than those of bee venom extracts from the same geographical and botanical origins. Phenolic compounds are hydrogen donors capable of directly scavenging free radicals and reducing oxidative damage that makes them potent antioxidants [5]. Additionally, several studies have shown a strong positive relationship between phenolic compound contents and antioxidant potential [21,22]. In contrast, the antioxidant activity of melittin, a 26-amino acid peptide, is very poor [19]. Therefore, the antioxidant effect of bee venom might result from other bee venom extract components, such as antioxidant enzymes, including superoxide dismutase 1 present in honeybee venom gland tissue [23]. Melittin has very strong antimicrobial properties. It has the ability to kill a broad range of microorganisms including viruses, fungi, protozoa, and gram-positive and gram-negative bacteria, including drug-resistant ones such as methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococci* [24–27]. This small amphipathic peptide belongs to an emerging class of antimicrobials called antimicrobial peptides (AMPs). AMPs, also known as host defense peptides, are small proteins present naturally in various life forms and provide defense against microbial infections. Most AMPs kill microbial pathogens directly by permeabilizing microbial membranes, whereas some act indirectly by modulating the host defense systems [28,29]. Because the development by microbes of a resistance against AMPs is slower or delayed compared with that against conventional antibiotics, AMPs constitute prospective alternative therapeutics [30].

All examined pathogens were sensitive to bee venom and propolis extracts. This suggested that the mode of action of bioactive constituents of venom and propolis extracts is common to a broad range of microorganisms. Furthermore, the sensitivity of gram-positive bacteria was greater than that of

gram-negative bacteria and fungus, possibly because of their distinct cell wall and membrane structures. Indeed, bacterial cell walls are composed primarily of peptidoglycans, and fungal cell walls contain mostly chitin and other polysaccharides. Additionally, an extra hydrophilic outer membrane consisting mainly of lipopolysaccharides is found in gram-negative bacteria and functions as a barrier to protect the membrane [14]. In agreement with our observations, several studies have demonstrated a greater sensitivity of gram-positive bacteria than that of gram-negative bacteria [20,31,32].

The present work revealed significant differences in the effects of extracts obtained with the same solvent depending on the region of origin. These distinct biological effects of extracts from different regions might reflect their different compositions in bioactive ingredients. The regions might differ in regard of their botanical sources, climate conditions, and honeybee species or ecotypes, which affect the physicochemical properties, ingredient contents, and consequently the biological activities of propolis and bee venom. These findings are in accordance with a previous work [18,33,34] showing the influence of the geographic region on the quality and properties of propolis and bee venom. In addition, the present data demonstrated that extracts collected from the Kafr-Elsheikh governorate exhibited the highest antimicrobial and antioxidant activities, whereas those from the Giza governorate displayed the lowest biological activities. Interestingly, Kafr-Elsheikh Governorate is located on the countryside and harbors one of Egypt's richest floras. It is also, far less polluted, noisy and crowded and consequently constitutes a resource-rich environment for honeybees. In contrast, the Giza governorate is a main part of Greater Cairo. It contains the biggest city in Egypt, which is an industrial city and one of the most polluted and crowded cities in the world. Therefore, Giza governorate provides poor botanical resources for honeybees.

The solvent might affect the physical properties of the extracts, especially the solubility of phytoconstituents. The extraction capacities of solvents differ depending on their polarity and on the solute's chemical structure. Different solvent extracts have different soluble phytoconstituents in different amounts and hence have distinct biological activities [14]. Here, ethanol extracts had better antimicrobial and antioxidant effects than chloroform and water extracts. These results suggest that most of the bioactive constituents are soluble in ethanol. These findings are consistent with previous observations that the

best antimicrobial and antioxidant properties were obtained with ethanolic extraction and not with other solvents or even with other extraction methods such as supercritical fluids [32,35].

## Conclusion

The present study is a comprehensive evaluation of the antimicrobial and antioxidant properties of various solvent extracts from bee venom and propolis originating from different regions in Egypt. The results revealed the superiority of ethanol extracts over chloroform and water extracts. Extracts collected from the Kafr-Elsheikh region were the most active, whereas those from the Giza region were less effective. Although the antimicrobial activity of bee venom was higher than that of propolis, its antioxidant performances were lower. All tested microbes were susceptible to bee venom and propolis extracts, although gram-positive bacteria were more sensitive than gram-negative bacteria and fungi. Overall, these results provide insights into the antimicrobial and antioxidant potential of Egyptian bee venom and propolis and constitute a basis for further phytochemical and pharmacological research.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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