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Phenolic Content, Antioxidant and Antimicrobial Activities of Egyptian and Chinese Propolis

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Abstract: Propolis is a resinous mixture that honeybees collect from tree buds, sap flows, or other botanical sources. It is used as a sealant for unwanted open spaces in the hive. Propolis is sticky at and above room temperature, 20°C (68°F). At lower temperatures, it becomes hard and very brittle. The aim of this study is to explore the phenolic contents and identify of the Egyptian and Chinese propolis and their biological activity potentiality, especially antioxidant and antimicrobial activity. Egyptian and Chinese propolis contained considerable amounts of phenolic compounds. The Egyptian propolis contains phenolic content a little bit greater than Chinese propolis. The Egyptian propolis showed an antioxidant activity higher than Chinese. IC_{50} of Egyptian propolis was (73.49 µg/ml) and (81.67 µg/ml) for Chinese propolis, whereas the IC_{50} for L-Ascorbic acid as positive control was (39.62 µg/ml). The HPLC analysis of Egyptian and Chinese propolis approved reasonable and different concentrations of phenolic compounds in both Egyptian and Chinese Propolis. The Egyptian propolis contains high concentration levels of tannic acid (10.64 µg/g), catechol (8.12 µg/g) and caffeic acid (7.435 µg/g). The Egyptian propolis showed a highest toxicity against *Bacillus subtilis DB 100 host and Streptococcus sp.* (IZD= 18 and 20 mm) respectively. On the other hand, Chinese propolis showed a highest antimicrobial activity and toxicity against *Candida albicans* and *Bacillus subtilis* (IZD=20mm) for both strains.

Key words: Honey Bee · Propolis · Antioxidant · Phenolic Content · Antimicrobial

INTRODUCTION

Propolis (bee glue) is the generic name for the resinous product of complex composition collected by *Apis mellifera* bees from bud and exudates of various plants mixed with bee secretions and beeswax. Physically it's a sticky material in room temperature but becomes hard and brittle at low temperature [1-3]. The color may be cream, yellow, green, light or dark brown. Some samples have a friable, hard texture, while other samples may be elastic and gummy [4]. Honeybees utilize propolis to diverse purposes, among them to seal openings in the hive. In addition to avoiding the entrance of intruders, this contributes to maintaining the hive inner temperature at around 35°C. Also, bees use it to seal cracks in hives, encapsulate invader carcasses, repairing combs and strengthening the thin borders [1, 2, 5]. This is obviously important to protect the hive from a widespread microorganisms specially bacteria and fungal infection. Propolis was very well known in ancient Egypt, to the priests who had monopolized medicine, chemistry and art on mummifying corpses [6]. The fact that propolis was also known to the old Greeks is demonstrated by the very Greek name of it Makashvili [7]. Abu Ali bin Sina (Avicenna) distinguishes two kinds of wax in his well-known work, the clean and the black wax [6].

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The clean wax is that which composes the comb wells where the bees rear the brood and store the honey and the black is the filth the hive. It is clear enough that the black wax is propolis that after Avicenna's testimony [8]. The chemical variability of propolis is, of course, due to its plant origin, collecting geographic locations the source plants might vary with respect to the local flora at the site of collection and seasons [4, 9- 11]. It is now generally accepted that bees collect resinous plant materials, produced by a variety of botanical processes, in different parts of plants. These are substances actively secreted by plants, as well as substances exuded from wounds in plants; they include lipophilic materials on leaves and leaf buds, mucilage, gums, resins and latices [12, 13]. The specificity of local flora is responsible for the chemical composition of propolis [14]. Propolis is typically composed of resin and vegetable balsams (50-70%), essential and aromatic oils and beeswax (30-50%), pollen (5-10%) and other constituents which are amino acids, minerals, vitamins A, B complex, E and the highly active bio-chemical substance known as bioflavenoid (Vitamin P), phenols and aromatic compounds [15-17]. The chemical compositions and biological activities of propolis are attributed to plant sources, geographical area and collecting season [9, 18]. More than 300 components have been identified in propolis samples. Flavonoids, aromatic acids, diterpenoid acids, triterpenoids and phenolic compounds are the major components of propolis [19-23]. In Mediterranean, propolis from Algeria, Croatia, Cyprus and Greece has a poplar-type chemical profile, while samples from Crete and South Greece are rich in diterpenes [24]. However, [25] mentioned that the major compounds of Ethiopian propolis were triterpenoids. Aliphatic acids, aromatic acids, alcohols, phenols, esters and other compounds were found in the Egyptian propolis and commercial one [26]. They identified fifty-seven compounds in Egyptian propolis, while a total of forty-four compounds have been tentatively identified in commercial propolis. Propolis has a wide range of biological activity and pharmacological effects as antibacterial and antifungal activity; therefore it is the defense of bees against infections [21]. It has potential to uncover new biologically active compounds with important pharmacological effects, especially antibacterial, antiviral, anti-inflammatory, antitumor, antioxidant, anticancer substances and new bioactive molecules [2, 10, 27-29]. The aim of this study is to explore and identify the phenolic contents of the Egyptian and Chinese propolis and their biological activity potentiality, especially antioxidant and antimicrobial activity.

MATERIALS AND METHODS

Samples Collection: Egyptian propolis samples collected from middle delta region, Egypt and the Chinese propolis samples collected from Anhui, China.

Sample Preparation: The propolis sample (20 g) was extracted with 90% ethanol (200 mL) by mixing for 24 h at room temperature in dark place. The crude extract was recovered by centrifugation (3000 g, 10 min) and dried under vacuum using a rotary evaporator.

Total Phenolic Content (TPC): The total phenolic compounds assay was carried out using the Folin-Ciocalteu reagent, following the method of [30] and based on the reduction of a phosphowolframate-phosphomolebdate complex by phenolics to blue reaction products. 1mg propolis extract was dissolved in 1ml methanol and 500 µl of dissolved sample was taken and added to 0.5 ml of distilled water and 0.125 ml of Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 minutes before addition of 1.25 ml of 7% Na_2CO_3. The solution was adjusted with distilled water to a final volume of 3 ml and mixed thoroughly. After incubation in the dark for 30 min, the absorbance at 650 nm was read versus the prepared blank. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000 µg/mL). Total phenolic content was estimated as µg Gallic acid equivalents (GAE)/g of dry weight sample.

Determination of Antioxidant Activities

DPPH Radical-Scavenging Activity: DPPH radical - scavenging activity was measured by direct hydrogen donation to the DPPH radical, as previously reported, with minor modifications [30]. For each sample, different concentrations ranging from 5 to 200 µg/mL were prepared with methanol. The reaction mixtures in the 96-well plates consisted of sample (100 µl) and DPPH radical (100 µl, 0.2 mM) dissolved in methanol. The mixture was stirred and left to stand for 15 min in dark. Then the absorbance was measured at 517 nm against a blank. All determinations were performed in triplicates. The percentage scavenging effect was calculated as:

$$\% \ Inhibition = [1 - (A_1 - A_2) / A_0] \times 100\%$$

where: A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample, A_2 is the absorbance of sample without DPPH radical. The scavenging ability of the samples was

expressed as IC_{50} value, which is the effective concentration at which 50% of DPPH radicals were scavenged. The IC_{50} values were calculated from the relationship curve of scavenging activities (%) versus concentrations of respective sample [31, 32].

HPLC Analysis of Phenolic Compounds: The phenolic compounds of the propolis samples were analyzed using high-performance liquid chromatography (HPLC) according [33]. Fifty (50) milligrams of propolis were extracted using 200 ml of ethanol at room temperature for 30 minutes. The extract was filtered through a paper filter and using methanol, the volume was adjusted to 10 ml. One milliliter of this sample was mixed with 0.5 ml Milli Q water and centrifuged for 3 minutes at 13000 rpm and the supernatant was used directly for HPLC analysis. Each propolis sample was extracted and analyzed in triplicate. Phenolic compounds were analyzed using HPLC (Agilent, Series 1100, Germany), an instrument containing a binary pump (G1316A), The column used was Zorbax, SB-C18, 4.6 x 75 mm with 3.5 µm particle size. The elution solvents were aq. 1.5% tetrahydrofuran + 0.25% orthophosphoric acid (A) and 100% methanol (B). The samples were eluted according to the following gradient: 0-5 min 100% A; 5-10 min 85% A, 15% B; 10-20 min 70% A, 30% B; 20-40 min 50% A, 50% B; 40-75 min 50% A, 50% B; 75-80 min100% B. The flow rate was 2 ml/min and the autoinjection volume was 20 µl. The temperature of the column and injector was +30°C and +20°C, respectively. The HPLC runs were monitored at 220 and 320 nm. Analyzed secondary metabolites were quantified against commercial standards. The identification of the compounds was based on the HPLC-MS-identification or on comparison of retention times and spectral characteristics as described in [34]. The quantification of the phenolic compounds is based on the commercial standards: chlorogenic acid; ferulic acid cinnamic acid, p -OH-cinnamic acid, caffeic acid, benzoic acid, vanillic acid, apigenin, Pinocembrin, Chlorogenic acid, Acacetin, Gallic acid, Itaconic acid, Protocatechoic acid, Catechin, Esculetin, Catechol, Tannic acid, Ferulic acid and Pyrogallol.

Determination of Antimicrobial Activity

Bacterial Strains: The tested bacterial strains in this study were *Candida Albicans, Bacillus Subtilis DB 100 host, Salmonella senftenberg and Streptococcus sp.* (Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain-Shams University, Cairo, Egypt). Nutrient broth was used to obtain the viable growth of microbes from their freeze-dried form. After 48 h, turbidity in test tube confirmed the growth of microbes that was compared and adjusted to McFarland 0.5 turbidity standard (108 colony-forming units per milliliter) [35, 36].

Preparation of Propolis Extract for Antimicrobial Test: Ten grams of propolis powder was added to 100 ml of DMSO (an inert solvent) and kept at a cool and dark place in an amber colored bottle [37]. Agar well diffusion assay was carried out to evaluate the antimicrobial potential of propolis [38]. Petri dishes containing 100 ml of brain heart infusion broth supplemented with 5 ml of 5% sheep blood were inoculated with approximately 100 µl of the respective microbial strain using swab technique. Wells of 8 mm diameter were cut into solidified agar media using a sterilized device. One hundred microliters of the propolis extract was poured in the wells and the plates were incubated at 37°C for 48 h. To ensure the consistency of all findings, the experiment was performed and repeated under strict aseptic conditions. The antibacterial activity of propolis extract was expressed in terms of the mean of diameter of inhibitory zone (in millimeters) produced by the extract at the end of incubation period [30].

Determination of Minimum Inhibitory Concentration: Minimum inhibitory concentration (MIC) is defined, as the lowest concentration of extract at which there will be no visible growth of the test organism. In the present study, MIC was determined using "serial tube dilution technique." The MIC of propolis for Egyptian and Chinese propolis was conventionally determined in triplicate for each strain by the macrodilution broth method as described by the National Committee for Clinical Laboratory Standards (NCCLS) [39, 40]. Serial two fold dilutions of propolis extract were prepared in macrodilution tubes and inoculated with constant amount of test bacteria and then all the test tubes were incubated at 37°C for 18–24 h. Each tube was mixed and examined for growth, comparing each tube to the control. For each test, DMSO was used as the control solvent.

Statistical Analysis: Triplicate determinations, mean and standard deviation were calculated. Calibration curve of standard was obtained for concentration vs. absorbance. All data were subjected for analysis using independent variable t-test.

RESULTS AND DISCUSSION

Total Phenolic Content: Total phenolic content in the propolis extract was carried out using the Folin-Ciocalteu reagent and the obtained results confirmed that, the Egyptian and Chinese propolis contains considerable amounts of phenolic compounds. Total phenolic content in propolis extracts were 137.52±0.003 and 123.08±0.005 µg GAE/g propolis extract for Egyptian and Chinese propolis respectively (Table 1). The Egyptian propolis contains phenolic compounds a little bit more than Chinese propolis. Actually there is no significant difference between the Egyptian and Chinese propolis in the total phenolic content. The obtained results agree with some previously published works, which studied the phenolic content of propolis [29, 41, 42]. The total amount of the phenolic compounds in Finnish propolis ranged from 79.8 to 156.3 µg/g, the average being 119.5 µg/g [41]. There are many limiting factors affecting on the concentration of phenolic compounds, type of solvents, extract temperature, stirring and the origin and source of the propolis [41-43]. This considerable content of phenolic compounds in either Egyptian or Chinese propolis makes it very important to human health and confirms a wide spectrum of its health benefits.

Antioxidant Activity: From the received data (Table 2), both Egyptian and Chinese propolis showed a high antioxidant activity potentiality. The Egyptian propolis showed an antioxidant activity a little bit higher than Chinese but there is no significant differences between them. IC_{50} of Egyptian propolis was 73.49 µg/ml and 81.67 µg/ml for Chinese propolis, whereas the IC_{50} for L-Ascorbic acid as positive control was 39.62 µg/ml. DPPH-stable free radical scavenging activity (% inhibition) of both Egyptian and Chinese propolis extracts and L-ascorbic acid increased as the concentration of propolis extract and L-ascorbic acid were increased (Table 2). L-ascorbic acid more effective than propolis at lower concentrations (5-60 ug/ml), nevertheless, the propolis showed antioxidant activity near L-ascorbic acid at higher concentrations (80-200ug/ml). These obtained results agreed with some published work about Egyptian and Chinese propolis. Ethyl acetate fraction of Chinese propolis showed significant antioxidant and free radical-scavenging capacities, phenolics contributed to the antioxidant activity of propolis collected in Anhui, China. Therefore, Chinese propolis and its phenolics might be used as a natural antioxidant [44]. All investigated propolis samples collect form different Egyptian provinces (Fayoum; Assiut; Souhag; Dakahlia; Sharkia and Ismailia) possess a good anti-oxidative potential [45-47]. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start

Table 1: Total phenolic content of Egyptian and Chinese propolis (expressed as mean of triplicates ±SD) (P>0.05)

Propolls extract	TPC Conc. µgGAE/g sample
EG	137.52±0.003
CH	123.08±0.005

Table 2: Antioxidant activity of Egyptian and Chinese Propolis (The values mentioned are the means of triplicates ±SD) (P>0.05)

	% Inhibition		
Sample Conc. (µg/ml)	Egyptian	Chinese	Ascorbic acid
5	9.34±0.03	8.98±0.09	36.28±0.16
10	12.87±0.04	12.17±0.16	49.68±0.15
20	25.76±0.02	25.53±0.02	57.41±0.08
40	36.71±0.12	34.63±0.31	64.76±0.13
60	46.23±0.23	38.24±0.22	69.43±0.25
80	55.61±0.36	49.63±0.18	75.21±0.32
100	63.49±0.28	56.38±0.07	86.34±0.05
120	80.15±0.31	78.31±0.19	91.34±0.36
140	89.7±0.06	89.45±0.37	98.87±0.29
160	95.56±0.34	93.76±0.51	99.32±0.41
180	98.36±0.04	97.86±0.42	99.89±0.32
200	99.20±0.28	99.13±0.06	99.96±0.36
IC_{50}	73.49±0.39	81.67±0.28	39.62±0.34

Table 3: HPLC analysis of phenolic compounds of propolis (P<0.05)

	Conc. µg/g	
Phenolic Compound	Egyptian	Chinese
Cinnamic acid	0.092±0.32	0.167±0.25
Vanillic acid	3.05±0.53	0.63±0.51
Chlorogenic acid	0.28±0.51	0.034±0.56
p-OH-cinnamic acid	0.089±0.02	0.125±0.63
Benzoic acid	2.31±0.34	0.91±0.24
Pinocembrin	1.120±0.26	0.632±0.25
Caffeic acid	7.435±0.36	1.287±0.63
Apigenin	0.32±0.34	1.54±0.18
Chlorogenic acid	4.22±0.28	0.81±0.29
Acacetin	Nil	0.21±0.76
Gallic acid	6.35±0.18	4.36±0.71
Itaconic acid	2.51±0.42	6.12±0.48
Protocatechoic acid	0.19±0.71	2.34±0.61
Catechin	4.22±0.19	3.66±0.51
Esculetin	6.39±0.36	4.69±0.38
Catechol	8.12±0.25	2.50±0.21
Tannic acid	10.64±0.81	4.13±0.24
Ferulic acid	5.14±0.54	0.19±0.53
Pyrogallol	1.96±0.26	6.32±0.23

Table 4: Antibacterial activities of Propolis, ampicillin and DMSO against various indicator bacteria. (+)= Inhibition zone detected, (-) = No inhibition zone detected. The values mentioned are the means of triplicates ±SD

Substances	Conc. (mg/ml)	*Candida Albicans*	*Bacillus Subtilis DB 100 host*	*Salmonella Senftenberg*	*Streptococcus sp.*
Propolis Egypt	300	+	+	+	+
	150	+	+	+	-
	75	+	+	+	-
	37.5	+	-	+	-
	18.75	+	+	-	-
	9.3	+	+	-	-
Propolis China	300 (mg/ml)	+	+	+	+
	150 (mg/ml)	+	+	-	-
	75 (mg/ml)	+	+	-	-
	37.5(mg/ml)	+	+	-	-
	18.75(mg/ml)	+	+	-	-
	9.3 (mg/ml)	+	+	-	-
Ampicillin	300 (mg/ml)	+	+	+	+
	150 (mg/ml)	-	-	+	+
	75 (mg/ml)	-	-	+	-
	37.5(mg/ml)	-	-	+	-
	18.75(mg/ml)	-	-	+	-
	9.3 (mg/ml)	-	-	+	-
DMSO	300 (mg/ml)	-	-	-	-
	150 (mg/ml)	-	-	-	-
	75 (mg/ml)	-	-	-	-
	37.5(mg/ml)	-	-	-	-
	18.75(mg/ml)	-	-	-	-
	9.3 (mg/ml)	-	-	-	-

chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols. The antioxidant activity of propolis may due to the ability of phenolic compounds to donate hydrogen ions that can attack the free radicals to prevent the oxidation reactions in the cell and preventing the oxidation and deterioration of food substances during storage as well. The high antioxidant activity of propolis makes it a good natural antioxidant that can use as a natural preservative and/or food additives to help guard against food deterioration.

HPLC Analysis of Phenolic Compounds: The HPLC analysis of Egyptian and Chinese propolis approved reasonable and different concentrations of phenolic compounds in both Egyptian and Chinese Propolis. Concentrations of some phenolic compounds (Vanillic acid, Chlorogenic acid, Benzoic acid, Ferulic acid, Pinocembrin, Caffeic acid, Chlorogenic acid, Gallic acid, Catechin, Esculetin, Catechol, Tannic acid and Ferulic acid) in the Egyptian propolis higher than that in Chinese one. In contrary, the concentration of (Cinnamic acid, p-OH-cinnamic acid, Apigenin, Acacetin, Itaconic acid and Pyrogallol) in Chinese propolis higher than that in Egyptian one. These differences in the concentration of phenolic compounds may cause the differences in antioxidant activities between Egyptian and Chinese propolis. The Egyptian propolis contained high concentrations of Tannic acid (10.64 µg/g), Catechol (8.12 µg/g) and Caffeic acid (7.435 µg/g). The Egyptian propolis was analyzed by GC-MS and 25 compounds were identified, seven compounds were identified in Egyptian propolis for the first time [45]. The constituents were phenolic acid esters (72.7 %); phenolic acids (1.1 %); aliphatic acids (2.4 %); dihydrochalcones (6.5 %); Chalcones (1.7 %); flavanones (1.9 %); flavones (4.6 %) and tetrahydrofuran derivatives (0.7 %) [44, 45].

Antimicrobial Activity: The antimicrobial activity was measured in terms of diameter of the inhibitory zones in a soft agar layer. From the obtained results in Table (4), both Egyptian and Chinese propolis showed a reasonable antimicrobial activity against tested strains (*Candida albicans, Bacillus subtilis DB 100 host, Salmonella Senftenberg, Streptococcus sp.)*. The Egyptian propolis showed a highest toxicity against *Bacillus subtilis DB 100 host and Streptococcus sp. (IZD= 18 and 20 mm) respectively*. On the other hand Chinese propolis extract showed a highest antimicrobial activity and toxicity against *Candida albicans* and *Bacillus subtilis* (IZD=20mm) for both strains. In contrary, both Egyptian and Chinese propolis showed the lowest antimicrobial activity against *Salmonella senftenberg* (IZD=10 and 8 mm) respectively. The moderate toxicity recorded against *Candida albicans* for Egyptian peoples (IZD= 16mm) and *Streptococcus sp.* for *C*hinese propolis (IZD= 12mm). Furthermore, both Egyptian and Chinese propolis showed toxicity against all tested microbial strains higher than ampicillin as standard antibiotic (Table 6), the minimum inhibitor, concentrations (MIC) was calculated for both Egyptian and Chinese propolis and the results recorded in (Table 5). Several researchers have studied the antimicrobial activity of propolis. The antibacterial activity of six propolis solutions from different geographic locations was active against various bacterial strains [48]. Another *in vitro* investigation also demonstrated the antimicrobial activity of Brazilian propolis against various periodontopathogens including Pg and Aa [49]. The Inhibitory activity of Brazilian propolis was investigated on Aa, *Fusobacterium nucleatum*, Pg and

Table 5: Minimum inhibitory Concentration (MIC) of propolis extract

	Antibacterial activity against indicator strain expressed in MIC (mg/ml)			
Substances	*Candida albicans*	*Bacillus subtilis*	*Salmonella senftenberg*	*Streptococcus sp.*
Egyptian propolis	9.3±1.03	9.3±0.97	37.5±1.03	300±1.02
Chinese propolis	9.3±1.21	9.3±1.05	300±1.02	300±1.03
Ampicillin	300±0.86	300±1.03	9.3±1.04	150±1.03
DMSO	-	-	-	-

Table 6: Antimicrobial activity of propolis extract. (-)= No inhibition zone detected. The values mentioned are the means of triplicates ±SD

	Inhibition zone diameter (mm)			
Strain	Egyptian	Chinese	Ampicillin	DEMSO
Candida albicans	16±0.16	20±0.43	8±0.39	-
Bacillus subtilis D 100 host	18±0.13	20±0.35	10±0.34	-
Salmonella senftenberg	10±0.16	8±0.27	8±0.18	-
Streptococcus sp.	20±0.51	12±0.13	10±0.37	-

Prevotella intermedia and found that all of the assayed bacterial species were susceptible to propolis extract [50]. The antimicrobial activity of Chinese propolis was investigated and approved that the Chinese propolis exhibited the maximum inhibitory zone of 25 mm for Pg and 14 mm for Aa at a concentration of 0.1 µg/ml and observed that propolis extract had a wide spectrum antimicrobial activity against *Candida albicans and Bacillus subtilis* [51]. All Egyptian propolis samples showed an inhibition in the growth of all examined bacteria but the inhibition varied according to the propolis origin [52, 42]. Evaluation of Egyptian propolis as immunostimulant, antiviral, antibacterial and antifungal agents were done and showed that the Egyptian propolis has such activities [46]. The differences in the level of the effectiveness of propolis extract as antimicrobial agent may refer to the differences of the concentration, types and carrier of phenolic compounds. The anti-bacterial activity of propolis extract might be due the ability of phenolic compounds to bind to bacterial cell walls and prevent cell division and growth [53, 30]. The mechanism of antifungal activity of phenolic compounds may refer to disruption of Ca_2+ and H+ homeostasis, up- and down-regulation of gene transcription similar to Ca_2+-stress and nutrient starvation [54], disruption of membrane integrity and impairment of ergosterol biosynthesis in Candida strains [55]. It is thus clear that both types of propolis Egyptian and Chinese have a good effectiveness as an anti-microbial, which nominated as a natural material that can be used in food preservation. More extended research work needed to prepare an antimicrobial product from propolis.

CONCLUSION

Propolis is a resinous mixture that honeybees collect from tree buds, sap flows, or other botanical sources. The composition of propolis varies from hive to hive, from district to district and from season to season. Propolis has a wide range of biological activity and pharmacological effects as antibacterial and antifungal activity; therefore it is the defense of bees against infections. It has potential to uncover new biologically active compounds with important pharmacological effects, especially antibacterial, antiviral, anti-inflammatory, antitumor, antioxidant, anticancer substances and new bioactive molecules. The aim of this study is to explore the phenolic compounds in the Egyptian and Chinese propolis and their antimicrobial potentiality. Egyptian and Chinese propolis contained a considerable amount of phenolic compounds. The Egyptian propolis contains phenolic content a little bit greater than Chinese propolis. Actually there is no significant difference between the Egyptian and Chinese propolis in the total phenolic content. The Egyptian propolis showed an antioxidant activity a little bit higher than Chinese but there is no significant differences between them. IC_{50} of Egyptian propolis was 73.49 µg/ml and 81.67 µg/ml for Chinese propolis, whereas the IC_{50} for L-Ascorbic acid as positive control was 39.62 µg/ml. The HPLC analysis of Egyptian and Chinese propolis approved reasonable and different concentrations of phenolic compounds in both Egyptian and Chinese Propolis. The Egyptian propolis contained high concentrations of Tannic acid (10.64 µg/g), Catechol (8.12 µg/g) and Caffeic acid (7.435 µg/g).

The Egyptian propolis showed a highest toxicity against *Bacillus subtilis DB 100 host and Streptococcus sp.* (IZD= 18 and 20 mm) respectively. On the other hand, Chinese propolis extract showed a highest antimicrobial activity and toxicity against *Candida albicans* and *Bacillus subtilis* (IZD=20mm) for both strains. Finally we can conclude that the Egyptian and Chinese propolis contains considerable concentrations of phenolic compounds that lead to their biological activity as antimicrobial agent.

REFERENCES

1. Ghisalberti, E.L., 1979. Propolis: a review. Bee World, 60: 59-84.
2. Banksota, A.H., Y. Tezuka and S.H. Kadota, 2001. Recent progress in pharmacological research of propolis. Phytotherapy Research, 15: 561-571.
3. Parolia, A., M. Tomas, M. Kundabala and M. Mohan, 2010. Propolis and its potential uses in oral health. Int. J. Med Medical Sci., 2(7): 210-215.
4. Salatino, A., E.W. Teixeira, G. Negri and D. Message, 2005. Origin and chemical variation of Brazilian propolis. Evidence-Based Complementary and Alternative Medicine, 2(1): 33-38.
5. Simone-Finstrom, M. and M. Spivak, 2010. Propolis and bee health: the natural history and significance of resin use by honey bees. Apidologie, 41: 295-311.
6. Hegazi, A.G., Z.N. Moharm, F. Abd Allah, E.A.B. El Sheikh, N.A. Ellithy and A.M. Khair, 1998. Influence of ethanolic extract of Egyptian propolis in mice bearing Ehrlich carcinoma on immune status. J. Union of Arab Biologists, 10(A): 387-396.
7. Makashvili, Z.A., 1978. From the history of propolis. In Remarkable hive product: Propolis. Scientific data and suggestions concerning its composition, properties and possible use in therapeutics. APIMONDIA standing commission on beekeeping technology and equipment, Bucharest.
8. Lotfy, M., 2006. Biological Activity of Bee Propolis in Health and Disease. Asian Pac. J. Cancer Prev., 7: 22-31.
9. Popova, M., V. Bankova, S. Bogdanov, I. Tsvetkova, C. Naydenski, G.L. Marcazzan and A.G. Sabatini, 2007. Chemical characteristics of poplar type propolis of different geographic origin. Apidologie, 38: 306-311.
10. Bankova, V., 2009. Chemical diversity of propolis makes it a valuable source of new biologically active compounds. J. ApiProduct and ApiMedical Sci., 1(2): 23-28.
11. Guo, S., S. Fu, Z. Shen, Z. Zhang and Q. Xu, 2011. Chemical composition, biological activity and application in animal science of propolis- A review. International Conference on Agricultural and Biosystems Engineering Advances in Biomedical Engineering, 1-2: 98-101.
12. Crane, E., 1988. Beekeeping: Science, Practice and World Resources. Heinemann press, London, pp: 367-372.
13. Silva, B.B., P.L. Rosalen, J.A. Cury, M. Ikegaki, V.C. Souza, A. Esteves and S.M. Alencar, 2008. Chemical composition and botanical origin of red propolis, a new type of Brazilian Propolis. Evidence-Based Complementary and Alternative Medicine, 5(3): 313-316.
14. Righi, A.A., G. Negri and A. Salatino, 2013. Comparative chemistry of propolis from eight Brazilian localities. Evidence-Based Complementary and Alternative Medicine, http:// dx.doi.org/ 10.1155/ 2013/267878.
15. Cirasino, L., A. Pisati and F. Fasani, 1987. Contact dermatitis from propolis. Contact Dermatitis, 16: 110-111.
16. Almas, K., A. Dahlan and A. Mahmoud, 2001. Propolis as a natural remedy: An update. Saudi Dental J., 13: 45-49.
17. Park, Y.K., S.M. Alencar and C.L. Aguiar, 2002. Botanical origin and chemical composition of Brazilian propolis. J. Agric. Food Chem., 50: 2502-2506.
18. Sforcin, J.M. and V. Bankova, 2011. Propolis: Is there a potential for the development of drugs? J. Ethnopharmacol, 133: 253-260.
19. Marcucci, M.C., 1995. Propolis: chemical composition, biological properties and the therapeutic activity. Apidologie, 26: 83-99.
20. Markham, K.R., K.A. Mitchell, A.L. Wilkins, J.A. Daldy and Y. Lu, 1996. HPLC and GC–MS identification of the major organic constituents in New Zealand propolis. Phytochemistry, 42: 205-211.
21. Bankova, V., S. De Castro and M. Marcucci, 2000. Propolis: Recent advances in chemistry and plant origin. Apidologie, 31: 3-15.
22. Chen, Y. W., S.W. Wu, K.K. Ho, S.B. Lin, C.Y. Huang and C.N. Chen, 2008. Characterization of Taiwanese propolis collected from different locations and seasons. J. Sci. Food Agric., 88: 412-419
23. Popova, M., C.N. Chen, P.Y. Chen, C.Y. Huang and V. Bankova, 2010a. A validated spectrophotometric method for quantification of prenylated flavanones in Pacific propolis from Taiwan. Phytochem. Anal., 21: 186-191.

24. Popova, M.P., K. Graikou, I. Chinou and V.S. Bankova, 2010b. GCMS profiling of diterpene compounds in Mediterranean propolis from Greece. Journal of Agricultural and Food Chemistry, 58(5): 3167-3176.
25. Rushdi, A.I., N. Adgaba, N.I.M. Bayaqoob, A. Al-Khazim, B.R.T. Simoneit, A.H. El-Mubarak and K.F. Al-Mutlaq, 2014. Characteristics and chemical compositions of propolis from Ethiopia. SpringerPlus 2014, 3: 253.
26. Abouzid, M.M. and A.A. Abd El-Kaway, 2013. Chemical composition of Egyptian and commercial propolis and its effects on liver function and lipid profiles in albino rats. J. Biological Chem. Env. Sci., 8(2): 323-340.
27. Hegazi, A.G., K. Faten and F.K. Abd El Hady, 1997. Chemical and biological studies of Egyptian propolis. International Symposium on Apitherapy, Cairo 8-9th March.
28. Kimoto, T., S. Arai, M. Kohguchi, M. Aga, Y. Nomura, M.J. Micallef, M. Kurimoto and K. Mito, 1998. Apoptosis and suppression of tumor growth by artepillin C extracted from Brazilian propolis. Cancer Detection and Prevention, 22: 506-15.
29. Hegazi, A.G. and F.K. Abd El Hady, 2002. Egyptian propolis: 3. Antioxidant, antimicrobial activities and chemical composition of propolis from reclaimed lands, Z. Naturforsch., 57c: 395-402.
30. El Sohaimy, S.A., 2014. Chemical Composition, Antioxidant and Antimicrobial Potential of Artichoke. The Open Nutraceuticals Journal, 7: 15-20.
31. Wang, H., X.D. Gao, G.C. Zhou, L. Cai and W.B. Yao, 2008. In vitro and in vivo antioxidant activity of aqueous extract from Choerospondias axillaris fruit. Food Chem., 106: 888-895.
32. Barbary, O.M., S.A. El-Sohaimy, M.A. El-Saadani and A.M.A. Zeitoun, 2010. Antioxidant, antimicrobial and anti-HCV activities of lignan extracted from flaxseed. Research Journal of Agriculture and Biological Sciences, 6(3): 247-256.
33. Croci, A.N., B. Cioroui, D. Lazar, A. Corciova, B. Ivanoscu and M. I. Lazar, 2009. HPLC evaluation of phenolic and polyphenolic acids from propolis. Farmacia, Vol. LVII(1): 52-57.
34. Julkunen-Tiitto, R. and S. Sorsa, 2001. Testing the drying methods for willow flavonoids, tannins and salicylates. J. Chem. Ecol., 27(4): 779-789.
35. Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. The American Journal of Clinical Pathology, 45: 493-496.
36. Nanasombat, S. and P. Lohasupthawee, 2005. Antibacterial activity of crude ethanolic extracts and essential oils of spices against salmonellae and other enterobacteria. KMITL Sci. Tech. J., 5: 527-38.
37. Norajit, K., N. Laohakunjit and O. Kerdchoechuen, 2007. Antibacterial effect of five Zingiberaceae essential oils. Molecules, 12: 2047-60.
38. Ozan, F., H. Ozer, H. Ozdemir, K. Eren and H. Yeler, 2008. A morphometric and histopathologic evaluation of the effects of propolis on alveolar bone loss in experimental periodontitis in rats. J. Periodontol., 79: 1089-94.
39. Allen, K.L., P.C. Molan and G.M. Reid. 1991. A survey of the antibacterial activity of some New Zealand honeys. J. Pharm. Pharmacol., 43: 817-22.
40. National Committee for Clinical Laboratory Standards, 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow anaerobically. Approved Standard. (4th ed.), 20(2) M7-A5.
41. Salonen, A., S. Saarnio and R. Julkunen- Tiitto, 2012. Phenolic compounds of propolis from the boreal coniferous zone. Journal of Apicultural Science, 56(1): 13-22.
42. Hegazi, A.G., A.M. Abdou and F. Abd Allah, 2014. Egyptian Propolis 11: Its antimicrobial activity with comparison with different localities. Int. J. Curr. Microbiol. App.Sci., 3(9): 530-538.
43. Ramanauskien, K., A.M. Inknien, V. Petrikait and V. Briedis, 2013. Total phenolic content and antimicrobial activity of different Lithuanian propolis solutions. Hindawi Publishing Corporation, Evidence-Based Complementary and Alternative Medicine, volume 2013: 2-5.
44. Yang, H., Y. Dong, H. Du, H. Shi, Y. Peng and X. Li, 2011. Antioxidant compounds from propolis collected in Anhui, China. Molecules, 16: 3444-3455.
45. Abd El-Hady, F.K. and A.G. Hegazi, 1994, Gas chromatography-mass spectrometry (GC/MS). Study of the Egyptian propolis 1- aliphatic, phenolic acids and their esters. Egypt. J. Appl.Sci., 9: 749-760.
46. Abd El-Hadya, F.K., A.G. Hegazi and E. Wollenweber, 2007. Effect of Egyptian propolis on the susceptibility of LDL to oxidative modification and its antiviral activity with special emphasis on chemical composition. Z. Naturforsch., 62c: 645-655.

47. Hegazi, A.G., A.A. Farghaly and F.K. Abd El Hady, 2001. Antiviral activity and chemical composition of European and Egyptian propolis. Proc. 37th Int. Apic. Congr. 28 Oct - 1 Nov 2001, Durban, South Africa.
48. Sonmez, S., L. Kirilmaz, M. Yucesoy, B. Yücel and B. Yilmaz, 2005. The effect of bee propolis on oral pathogens and human gingival fibroblasts. J. Ethnopharmacol., 102: 371-6.
49. Gebara, E.C, L.A. Lima and M.P. Mayer, 2002. Propolis antimicrobial activity against periodontopathic bacteria. Braz. J. Microbiol., 33: 365-9.
50. Santos, F.A., E.M. Bastos, M. Uzeda, M.A. Carvalho, L.M. Farias, E.S. Moreira and F.C. Braga, 2002. Antibacterial activity of Brazilian propolis and fractions against oral anaerobic bacteria. J. Ethnopharmacol., 80: 1-7.
51. Agarwal, G., G.G. Vemanaradhya and S.M. Dhoom, 2012. Evaluation of chemical composition and efficacy of Chinese propolis extract on Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans: An in vitro study. Contemp Clin Dent., 3(3): 256-261.
52. Abd El Hady, F.K. and A.G. Hegazi, 2001. Egyptian propolis: 2- chemical composition, antiviral and antimicrobial activities of East Nile delta propolis. Proc. 37th Int. Apic. Congr. 28 Oct - 1 Nov 2001, Durban, South Africa.
53. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-82.
54. Rao, A., Y. Zhang, S. Muend and R. Rao, 2010. Mechanism of antifungal activity of terpenoid phenols resembles calcium stress and inhibition of the TOR pathway. Antimicrob. Agents Chemother, 54(12): 5062-9.
55. Ahmad, A., A. Khan, F. Akhtar, S. Yousuf, I. Xess, L.A. Khan, N. Manzoor, 2011. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. Eur. J. Clin. Microbiol. Infect. Dis., 30(1): 41-50.