Antioxidant Activity and other Physicochemical Characteristics of Different Propolis Extracts

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Abstract: This study was to investigate the antioxidant activity of three different extracts of propolis (Ethanolic 50%, methanolic 100% and aqueous extracts) with some of their physicochemical aspects. Ethanolic extract was analyzed by LC-MS, while the methanolic extract was analyzed by GC-MS in order to screen polyphenols and volatile compounds in the sample, the identity of these components were achieved by means of comparing their mass spectra with previous published studies. Forrier transformation infrared spectroscopy (FTIR) was conducted on the methanolic extract and crude propolis samples to gain an overview of some physical characteristics. The antioxidant activities of the three extracts were measured by DPPH scavenging ability and FRAP assay. LC-MS and GC-MS allowed the identification of 19 phenolic and 17 volatile compounds. The most abundant phenolics found in our sample correspond with *p*-Coumaric benzyl ester, Pinobanksin-3-*O*-acetate, Caffeic acid benzyl ester and pinocembrin, as they showed the highest peaks in the total ion chromatogram (TIC). In addition, the most abundant volatile compounds showed a high level of agreement with δ-Cadinene and γ- Terpinen. Furthermore, Ethanol 50% extract showed the strongest antioxidant activity, which has a significant correlation with its high polyphenolic content.

Keywords: Propolis, antioxidant, Liquid Chromatography-Mass Spectrometry, Gas Chromatography-Mass Spectrometry, Folin-Ciocalteu.

1. INTRODUCTION

Propolis is a resinous, sticky, brownish hive product collected by honeybees (*Apis mellifera*), from various tree barks and buds, mainly from the poplar (Populus) genus, beech, horsechestnut, birch and conifer trees. Afterwards, the resin is mixed with salivary enzymes and beeswax [1].

Propolis is a substance used in the defense of the hive. Honeybees use propolis to seal cracks in their combs, in order to protect themselves from cold weather and humidity, and it is considered a strong biocide and protection tool against intruders [2, 3].

This product has a wide range of biological activities such as anti-inflammatory, antimicrobial, antioxidant, antitumor, antiulcer and anti-HIV; therefor, propolis was extensively used in folk medicine to treat colds, wounds, ulcers, rheumatism, sprains, heart disease and diabetes [4-6]. Its healing effects are due to its complex composition and the synergism among its constituents, mainly phenolic acids and esters, flavonoids (flavones, flavanones, flavonols, dihydroflavonols) [7, 8]. In addition to phenolics, propolis contains volatile compounds in low amount, which add an important biological value and give information about the origin of samples [9].

Flavonoids and biopolyphenols in propolis have an important free radical scavenging activity by depressing the propagation reaction of free radicals, which have a significant role in tissue injuries, inflammation and aging [10, 11]. This antioxidant effect of propolis is due to enzyme inhibition and chelating transition metals [10].

Raw propolis is composed of 50% resin, 30% wax, 10% essential oils, 5% pollen and 5% various organic compounds. Inert materials must be removed from propolis as it cannot be used as a raw material [1]. Extraction of propolis by solvents is a method to purify propolis from its impurities and to preserve the active polyphenolic fraction. Many studies were conducted to analyze the constituents and determine the antioxidant activities of propolis extracts by different extraction solvents. However, inconsistent results were obtained when comparing the scavenging capacity of ethanolic and aqueous extracts because of their different compositions [12].

Liquid chromatography coupled with mass spectrometry (LC-MS) is one of the most important technique used to separate complex mixtures qualitatively and quantitatively [1]. Whereas, headspace coupled with gas chromatography-mass spectrometry (HS-GC/MS) is used to analyze volatile

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ISSN: 2223-3806 / E-ISSN: 1927-5951/18

compounds, in different medicinal and aromatic plants, with a great separation capacity, sensitivity and selectivity [9, 13].

This study used LC-MS and HS-GC/MS techniques to predict the different constituents of the ethanolic propolis extract because ethanol is a good solvent to dewax propolis [1]. This was achieved by analyzing the constituents' mass spectra and comparing our results with previous published work. Furthermore, some physical aspects of propolis were studied using FTIR technique.

The antioxidant activity of different propolis extracts was determined by measuring their diphenylpicrylhydrazil (DPPH) scavenging capacity and ferric reducing antioxidant power.

2. MATERIALS AND METHODS

2.1. Propolis Samples

Green Propolis was harvested by beekeepers from Tartus (Syrian Arab Republic) in November 2016; it was stored at 2°-8°C.

2.2. Preparation of Propolis Extracts

2.2.1. Ethanolic 50% Extracts of Propolis (EEP)

EEP was prepared by macerating 4 g of propolis in 160 ml ethanol 50%. The mixture was subjected to ultrasound for 20 minutes and then stirred overnight at room temperature. The mixture was centrifuged at 6000 x g for 30 minutes and the supernatant was dried by rotavapor at 50°C to obtain the dry ethanol extract of green propolis (EEP). The dry extarcts were stored in freezer at -18°C until further use.

2.2.2. Methanol Extracts of Propolis (MEP)

MEP was prepared by macerating 25g of propolis in 77.5ml of methanol. The mixture was subjected to ultrasound for 15 minutes and then stirred overnight at room temperature, then it was centrifuged at 6000 x g for 30 minutes, the supernatant was dried by rotavapor at 30°C to obtain the dry extract (MEP), which was stored at -18°C.

2.2.3. Aqueous Extracts of Propolis (AEP)

AEP was prepared by soaking 25g of propolis in 70ml of distilled water; the mixture was ultrasounded for 30 minutes and then stirred overnight at room temperature. The sample was filtered and the filtrates were dried by rotavapor at 60°C to obtain the dry (AEP), then they were stored in a freezer.

2.3. LC-MS Analysis

The chromatographic system was Shimadzu® LCMS-2020 equipped with SPD-20A UV/VIS detector, and a single quadrupole mass spectrometer with ESI source, and ESI/APCI combination source called DUIS (Tokyo, Japan). A 5µm C_{18} symmetry column (250mm x 4.6 mm, Shimadzu) was used for the separation, at a flow rate of 1.2 mL/min. The column was maintained at 30°C, and the flow rate split 5:1 before ESI source. The dry ethanolic extract (EEP) was dissolved in ethanol 50% v/v, 10 µl injected in the LC system. The separation was performed by means of a linear gradient elution (eluent A, 0.1% formic acid; eluent B, acetonitrile). The gradient was as follows: 20% B for 60 min, 20-30%B for 4min, 30-40% B for 30min, 40-60% B for 20 min, 60-90% B for 20 min and 90%B for 10min. Chromatographic data were acquired at 290 nm. Mass spectrometer operated in negative and positive full-scan mode, in the range 50-1000 Da. The capillary voltage was set to 3.0 kV, the cone voltage was 20 V, the source temperature was 130°C, and the desolvating temperature was 350°C. Data were acquired by Labsolution software (Shimadzu). Pure nitrogen more than 99% purity was used; the gas pressure was 520 kPa (75 psi) and the flow rate was 1.5 L/min.

2.4. HS-GC/MS Analysis

The headspace extraction was carried out at 100 ◦C for 15 min, using a 0.6 g of triturated methanolic extract of propolis in a 10.0 mL glass flask. One milliliter of the vapor phase from headspace was injected in the gas chromatograph Agilent technology, 7890A, coupled with mass spectrometer Agilent technology, 5975C, provided with an electrical syringe (GC sampler 80) featured by Agilent PAL headspace (Agilent, Swiss). The injection, in splitless mode, used helium as carrier gas at a flow rate of 1.0 $mL.min^{-1}$. The injector's temperature set at 220°C. A fused-silica capillary column (5% phenyl–95% polydimethylsiloxane, 30 m × 0.25 mm, 0.25 µm) was used in the separation of the compounds. The oven temperature was programmed, from 40°C (2 min) to 200°C at a rate of 4°C min^{-1} and then to 250°C at a rate of 25°C min^{-1}. The mass spectrometer was used with electrospray ionization mode (70 eV), and mass scan range was from 40 to 600 Da. The temperatures of the ion source and the GC-MS interface were 150 and 230 ◦C, respectively. The obtained mass spectra of the compounds were compared with the GC/MS spectral library (Agilent MSD Productivity Chemstation E.02.01.1177).

2.5. Attenuated Total Reflection Forrier Transformation Infrared Spectroscopy (ATR-FTIR)

An infrared absorption spectrum was obtained for the crude propolis sample by using ALPHA FT-IR Spectrum BRUKER (Billerica, MA · USA).

2.6. Determination of Total Phenolic Content

The total phenolic contents were determined using Folin–Ciocalteu method according to the literature [14]. Briefly, Folin–Ciocalteu reagent (1 mL) was added to 1:10 diluted sample (1 mL) in a volumetric flask (25 mL). After 5 min, 1 mL sodium carbonate solution (6%) was added .After 40 min of incubation in a dark place at room temperature, the absorbance of the reaction mixture was measured at 760 nm. Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalent (mg GAE)/g of dry extract. All the experiments were performed in triplicate, and the results were expressed as mean ± SD. The correlation between the antioxidant capacities and total phenolic contents was analyzed using the simple linear regression, and the correlation coefficients (R^2) were calculated.

2.7. Free Radical Scavenging Activity on DPPH

The reaction mixture contained 0.1 mL of the extract (ethanolic, methanolic or aqueous) and 3.9 mL of the methanolic solution of the stable synthetic free radical, diphenylpicrylhydrazil (DPPH). The absorbance was recorded at 514 nm after incubation for 90 min in a dark place at room temperature [15]. Results were expressed as a percentage decrease with respect to control values. Vitamin C was used as a positive control (1mM & 10 mM).

2.8. Ferric-Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out according to the literature [16]. Briefly, the FRAP reagent was prepared from aqueous sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ (tripyridyltriazine) solution (40 mM HCl as solvent), and 20 mM iron (III) chloride solution in a volume ratio of 10:1:1, respectively. The FRAP reagent was prepared freshly and warmed to 37◦C in a water bath before use. 50 µL of the diluted sample was added to 1.5 mL of the FRAP reagent. The absorbance of the reaction mixture was then measured at 596 nm after incubation for 4 min in a dark place at room temperature. The standard curve was constructed using FeSO4solution. The FRAP values were obtained by comparing the absorbance change of the blue coloured ferrous- tripyridyltriazine complex at 596 nm in diluted extracts of propolis samples, with those containing ferrous ions in known concentrations.

3. RESULTS AND DISCUSSION:

3.1. LC-MS Analysis

The analysis was carried out in the positive and negative ion mode, but better results were obtained by negative one, because of its high sensitivity in the detection of phenolic compounds [1]. The chromatographic profile at 290 nm was illustrated in Figure **1** for the ethanolic 50% extract of propolis. It shows 19 peaks, suggesting a high diversity of the compounds present in the sample. Whereas the total ion chromatogram indiactes that the main peaks are (9, 10, 12 and 13) corresponding to *p*-Coumaric benzyl ester, Pinocembrin, Caffeic acid benzyl ester, Pinobanksin-3-*O*-acetate, respectively, which could be considered some of the most abundant phenolic acids and flavonoids in a temperate zone propolis. Due to this complexity in structures, the identification of the compounds in each LC fraction was made by mass spectrometry analysis of the $[M-H]^-$. Table **1** summarizes the results estimated by the analysis, whereas Figures **3** and **4** shows the chemical structures of these compounds. 19 compounds and their identity and structure were predicted by the search, the interpretation of MS fragmentations and comparison with previous studies [7, 17].

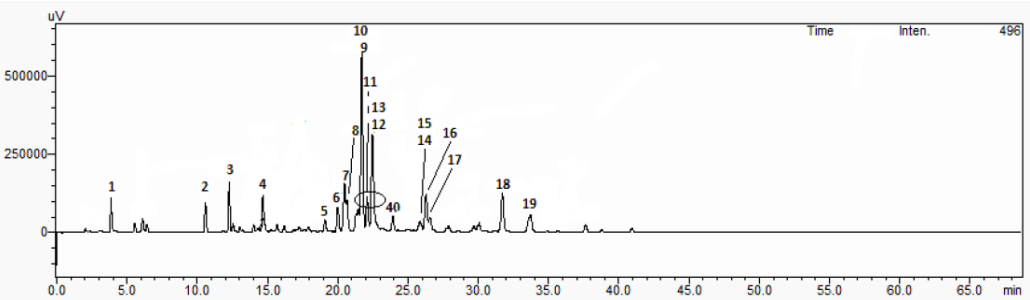


Figure 1: Total chromatographic profile at 290 nm of ethanolic 50% propolis extract

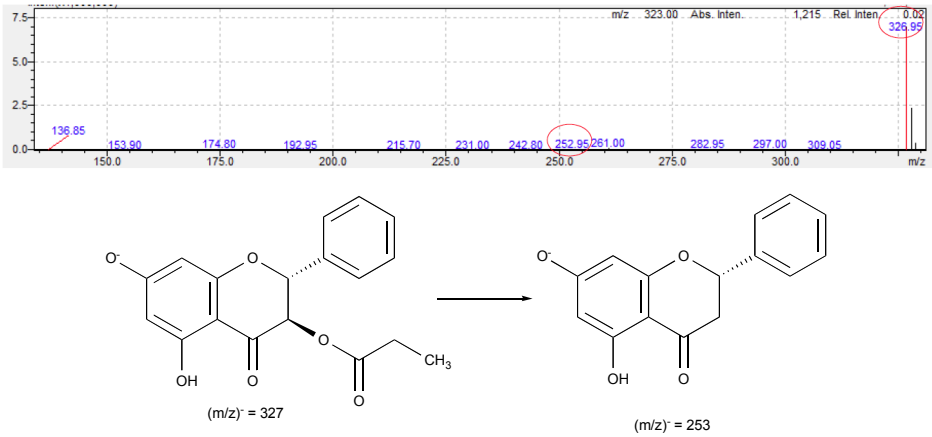
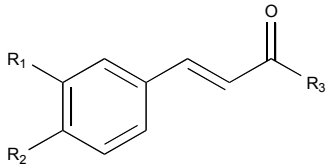


Figure 2: Pinobanksin-3-*O*-propionate (M_w 328) fragmentation pattern.



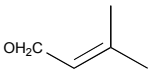
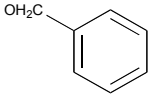
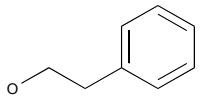
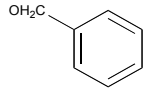
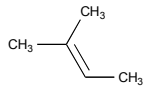
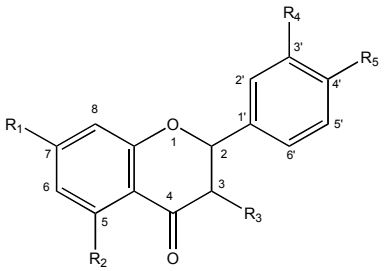
Phenolic acids	R_1	R_2	R_3
Caffeic acid	OH	OH	OH
Caffeic acid isoprenyl ester	OH	OH	
p-Coumaric benzyl ester	H	OH	
caffeic acid phenethyl ester	OH	OH	
Caffeic acid benzyl ester	OH	OH	
p-Coumaric-methyl-butenyl ester	H	OH	

Figure 3: Structures of several phenolic acids detected in the ethanolic 50% propolis extract.



Flavanones and dihydroflavonols	R1	R2	R3	R4	R5
Pinobanksin-5-methyl-ether (3)	OH	OCH_3	OH	H	H
Pinobanksin (4)	OH	OH	OH	H	H
Pinocembrin-5-methyl-ether (7)	OH	OCH_3	H	H	H
Pinocembrin (10)	OH	OH	H	H	H
Pinobanksin-3-*O*-acetate (13)	OH	OH	$OCOCH_3$	H	H
Pinobanksin-3-*O*-propionate (16)	OH	OH	$OCOC_2H_5$	H	H
Pinobanksin-3-*O*-hexanoate (17)	OH	OH	$OCOC_5H_{11}$	H	H
Pinobanksin-3-*O*-(pentanoate or 2-methyl-butyrate) (19)	OH	OH	$OCOC_4H_7$	H	H

Figure 4: Structures of several flavonoids detected in the ethanolic 50% propolis extract.

Table 1: Compounds Predicted in the Ethanolic 50% Propolis Extract and their RT and MS Characteristics

T_R (min)	Exact mass	Major ions *m/z* for $[M-H]^-$	Compound
3.9	180.2	178.9	Caffeic acid
11.8	302.2	300.9	Quercetin
12.2	286.1	284.9	Pinobanksin-5-methyl-ether
14.7	272.3	270.9	Pinobanksin
17.9	316.3	314.9	Quercetin-7-methyl-ether = Rhamnetin
19.2	330.2	328.9	Quercetin-7-methyl-X-methyl-ether
20.1	248.3	246.9	Caffeic acid isoprenyl ester
20.4	270.3	268.9	Pinocembrin-5-methyl-ether
21.7	254.2	252.9	*p*-Coumaric benzyl ester
21.7	256.3	254.9	Pinocembrin
22.0	284.0	282.9	CAPE
22.4	270.2	268.9	Caffeic acid benzyl ester
22.4	314.3	312.9	Pinobanksin-3-*O*-acetate
25.3	232.2	230.9	*p*-Coumaric-methyl-butenyl ester
25.6	232.2	230.9	*p*-Coumaric-methyl-butenyl ester
26.4	328.3	326.9	Pinobanksin-3-*O*-propionate
26.7	370.3	369.0	Pinobanksin-3-*O*-hexanoate
31.7	354.3	353.0	Chlorogenic acid
33.8	356.3	354.9	Pinobanksin-3-*O*-(pentanoate or 2-methyl-butyrate)

The ethanolic extract showed the typical temperate region composition, suggesting the existence of one or more plant sources of propolis resin like *Populus* species as the main one which is in agreement with previous published work [18, 19]. However, it is difficult to determine all the botanical sources of polyphenols predicted in the sample due to the high complexity of the phenolic fraction.

The main predicted compounds in our propolis sample were a mixture of phenolic acids and flavonoids and their derivatives as shown in Table **1** and Figures **3** and **4**.

3.1.1. Phenolic Acids

The main predicted phenolic acid was caffeic acid which had the retention time 3.9 minutes and m/z value of 179, that corresponds with the loss of CO_2 (– 44 Da). Other predicted phenolic acids were esterified and/or methylated derivatives of caffeic and coumaric acids which have higher retention times, due to the reversed phase chromatographic conditions. These results are in accordance with the previous literature [20]. It is important to note that both *p*-Coumaric benzyl ester and Caffeic acid isoprenyl ester were abundantly present in the sample as was found in other phenolic extracts of propolis taken from other temperate zones [20]. In addition, chlorogenic acid was identified which is the ester of caffeic acid and quinic acid (m/z 353, RT 31.7 min). Figure **2** shows the typical predicted fragmentation pattern of pinobanksin-3-O-propionate.

3.1.2. Flavonoids

Flavonoids predicted in our propolis sample can be classified in two major groups: Flavanones and dihydroflavonols that were present either in their free forms or as esterified or methylated derivatives. These two classes differ in their chemical structures and therefor their fragmentation patterns. 9 flavonoids were predicted and identified by finding their $[M-H]^-$ molecular ions and comparing their MS fragmentation with literature data. The main aglycones detected were: Quercetin, Pinobanksin, Pinocembrin, Table **1**, these compounds show two important fragments at m/z 165 and 151 resulting from the retro Diels-Alder mechanism [21]. It is worthy to note that the main flavonoid aglycons without B-ring substituent, pinobanksin and pnocembrin, are the typical constituents of temperate poplar propolis [5, 12] and they are present in high amounts by comparison with other flavonoids in the

studied sample. The common fragmentation pattern of the flavonoids present in this sample, includes the loss of CO_2 group (– 44 Da) which comes in accordance with previous literature [7, 20]. The esterified derivatives shown in Table **1**: Pinobanksin-3-*O*-acetate, Pinobanksin-3-*O*-propionate, Pinobanksin-3-*O*-hexanoate and Pinobanksin-3-*O*-pentenoate, and the methylated derivatives: Pinobanksin-5-methyl-ether, Quercetin-7-methyl-ether and Pinocembrin-5-methyl-ether.

3.2. HS-GC/MS Analysis

Volatile compounds play a major role in defining the quality of propolis, even though they represent only 10% of the active compounds in propolis. In addition, these compounds have many important biological activities adding a great value to propolis [5]. The main volatile compounds identified in our sample and shown in Table **2** are: i) Sesquiterpene hydrocarbons such as β-Cubebene, δ-Cadinene (15.18%) and α- Muurolene which could be considered the most abundant components in propolis as stated in previous studies [5]. It is important to state that δ-Cadinene which showed the highest peak in our sample is an important indicator of superior quality in autumn harvested propolis [22]. ii) Other important classes present in the sample were monoterpene hydrocarbons represented by α-Pinene, α-Thujene, α-Terpinene and γ- Terpinen. iii) Oxygenated monoterpenes such as p-Menth-1-en-4-ol, Thymol. iv) Bicyclic monoterpene such as Sabinene and Camphene. It is important to note that methyl salicylate was detected in this sample in a small abundance. As well as, cymene an alkylbenzene related to monoterpenes and carvacrol a monoterpenoid phenol.

3.3. Infrared Spectroscopy (ATR-FTIR)

The FT-IR absorption peaks of the crude propolis sample were (v, cm^{-1}): 2916, 2848, 1736, 1463, 1168 and 719 which are attributed to different chemical functions present in propolis.

3.4. Quantification of Total Phenolic Content in Different Propolis Extracts

The total phenolic contents of various extracts are presented in Table **3**. The amount of total phenolics ranged from 85.7 to 263.5 mg in equivalence of gallic acid per gram of extract, the highest one was of the ethanol 50% extract followed by the methanolic extract (212.5 mg *gallic acid equiv* /g *extract*), and the lowest was that of the aqueous extract.

All samples were obtained from the same region and have a similar botanical origin, different polyphenolic concentrations were observed.

Table 2: Volatile Compounds Predicted in the Methanolic Propolis Extract and their RT and Area Percentage

Peak	RT (min)	Area%	Compound
1	8.88	5.30	α-Thujene
2	9.09	5.74	α-Pinene
3	10.54	1.55	Sabinene
4	12.12	5.37	α-Terpinene
5	12.42	1.98	Cymene
6	12.57	1.91	Sabinene
7	13.71	7.38	γ- Terpinen
8	15.81	1.91	Phenylethyl Alcohol
9	18.10	4.57	p-Menth-1-en-4-ol
10	18.60	1.53	1-Dodecanamine (Armeen)
11	18.70	1.55	Methyl salicylate (Betula)
12	21.05	2.24	Thymol
13	21.41	2.58	carvacrol
14	24.86	5.85	Camphene
15	28.77	6.45	α- Muurolene
16	29.46	15.18	δ-Cadinene
17	32.88	3.05	β-Cubebene

Table 3: Total Phenolic Content of Different Propolis Extracts

Extract	Total phenolics (mg gallic acid equv./g of extract)
Ethanolic 50% Ext.	263.5 ∓ 3.4
Methanolic Ext.	212.5 ∓ 0.9
Aqueous Ext.	85.8 ∓ 1.1

The data shown in Table **3**, would show that the polyphenols of the ethanolic 50% extract have a higher capacity to reduce the Folin reagent, than that present in methanolic and aqueous extracts. Such capacity could be associated with the concentration and the number of hydroxyl groups of the phenolic compounds found in each extract. In fact, it is known for pure compounds and also complex mixtures, that Folin's index correlates with the number of phenolic hydroxyl groups present in the chemical structure of the molecules [23].

In this context, LC-MS analysis of ethanolic 50% extract indicated the presence of the flavonols: quercetin and kaempferol that are considered good antioxidants due to the o-dihydroxy groups, and the presence of the o- hydroxyketo group that can chelate cupric ion. Moreover, phenolic acids detected in this extract such as caffeic and chlorogenic acids have a very good antioxidant capacity due to their structures rich in hydroxyl groups [24].

Another important and powerful antioxidant found in ethanolic propolis extract, by LC-MS analysis, is CAPE, a flavonoid like compound [8], contributes in the explanation of the higher capacity of this extract to reduce FC reagent by comparison with methanolic and aqueous extracts.

3.5. DPPH Scavenging Activity

The experiments were conducted on three different extract types (ethanolic 50%, methanolic and aqueous), and on three different concentrations of these extracts (200, 500, and 700) µg/mL for each, as shown in Table **4**, the highest DPPH scavenging activity was displayed by the ethanolic 50% extract at all concentrations (25.0∓ 2.6, 56.3 ∓ 1.1and 66.6 ∓ 0.7, respectively) as they contain the highest phenolic fraction as shown before. It is important to note that the ethanolic 50% extract (700µg/mL) showed even higher scavenging activity than that of ascorbic acid (10 mM). The aqueous extract showed the lowest scavenging activity (9.9 ∓ 0.5, 20.2 ∓ 2.8 and 29.2 ∓ 0.4, respectively) which also correlates with its low phenolic fraction.

The antioxidant activity of phenolic compounds is related to their ability to donate hydrogen atoms or electron(s), which requires the presence of one or more hydroxyl group in their structures, as mentioned previously [25].Therefore, the total antioxidant activity estimated from the evaluation of DPPH radical scavenging activity, of different extracts, depends on the number of hydroxyl groups of phenolic compounds found in each one.

The highest DPPH scavenging activity of the ethanolic 50% extract comes in correlation with our previous findings as it has the most powerful FC reducing capacity, therefore the highest content of polyphenols rich in hydroxyl groups as confirmed by LC-MS analysis.

3.6. Ferric-Reducing Antioxidant Power (FRAP) Assay

In this method, the antioxidants found in the sample reduces the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to a ferrous (Fe^{+2}) blue colour complex at a low pH [26]. The latter complex has a maximum absorbance at 593 nm, which is measured for all

Table 4: DPPH Radical Scavenging Activities of Different Propolis Extracts

Sample conc. (µg/mL)	Relative scavenging activity (%)		
	Ethanol 50% extract	Methanol extract	Aqueous extract
200	25.0 ∓ 2.6	19.9 ∓ 2.6	9.9 ∓ 0.5
500	56.3 ∓ 1.1	34.2 ∓ 1.5	20.2 ∓ 2.8
700	66.6 ∓ 0.7	40.8 ∓ 5.2	29.2 ∓ 0.4

The scavenging activity of ascorbic acid (1mM & 10mM) was (6.9 ∓ 0.7 & 59.8 ∓ 1.2) respectively.

Table 5: FRAP Assay Results of Differnet Propolis Extracts

Sample conc. (µg/mL)	FRAP µmol equiv. Fe(II)	
	Ethanol 50% extract	Methanol extract
200	236.3 ∓ 5.1	336.7 ∓ 5.9
500	656.0 ∓ 15.1	924.7 ∓ 13.7
700	862.0 ∓ 19.3	1579.3 ∓ 15.1

samples and compared with those containing ferrous ions at known concentrations ranging from 100 to 2000 µmol.

The FRAP assay was used for assessing ‘‘antioxidant power’’ of different propolis samples (ethanolic 50% and methanolic) prepared at three different concentrations (200, 500, and 700) µg/L for each. The FRAP values of propolis ethanolic crude extracts ranged from 236.3 ∓ 5.1 to 862 ∓ 19.3 µmol *equiv. Fe(II)*, whereas the FRAP values of propolis methanolic crude extracts ranged from 336.7 ∓ 5.9 to 1579.3 ∓ 15.1µmol *equiv. Fe(II)* as shown in Table **5**. The previous results indicate higher reducing capacities of the methanolic extracts compared with the ethanolic 50% extracts at all concentrations.

3.7. Correlation between Antioxidant Capacities and Total Phenolic Content

A simple linear regression analysis was used to analyze the correlation between the antioxidant capacities and the total phenolic content of ethanolic 50% and methanolic propolis extracts. Concerning the DPPH scavenging activity, there was a positive relationship between the total phenolic content (TPC) and the DPPH scavenging activity, and the correlation coefficient R^2 was (0.8425), which indicates that the scavenging power of these extracts could be due to their phenolic content. These results were in agreement with (Silva, *et al* 2011) [27], but the correlation coefficient R^2 was small (0.5016) between FRAP values and total phenolic content (TPC) of propolis extracts. Thus, the high correlation obtained between the DPPH scavenging activity and TPC, whereas the weak correlation observed between the FRAP and TPC indicates that phenolic compounds in these extracts might contribute in scavenging free radical, but not responsible for any reducing oxidant abilities.

4. CONCLUSION

Both LC-MS and HS-GC/MS represent effective techniques to screen the main constituents of propolis, giving a detailed overview about its composition, quality and origin. This study clearly demonstrates the powerful antioxidant activity of ethanolic 50% extract as a high correlation was found between the polyphenol content and the reducing power of the analyzed extracts. This structural overview supported by other assays that measure the antioxidant activity emphasizes on the importance of propolis as a promising substitute to many inefficient chemical medicines.

ACKNOWLEDGEMENT

The manuscript is based on my thesis.

DATA AVAILABILITY

The data used to support the findings of this study are included within the article.

FUNDING STATEMENT

This work was supported by Damascus University.

REFERNCES

[1] Volpi N, Bergonzini G. Analysis of flavonoids from propolis by on-line HPLC-electrospray mass spectrometry. J Pharm Biomed Anal 2006; 354: 361-3. https://doi.org/10.1016/j.jpba.2006.04.017

[2] Miguel MG, Antunes MD. Is propolis safe as an alternative medicine? J Pharm Bioall Sci 2011; 479:495-4. https://doi.org/10.4103/0975-7406.90101

[3] Toreti VC, *et al*. Recent progress of propolis for its biological and chemical compositions and its botanical origin. Evid Based Complement Alternat Med 2013; 1: 13-2013. https://doi.org/10.1155/2013/697390

[4] Gulcin I, *et al*. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food Chem Toxicol 2010; 2227: 2238-8.

[5] Huang S, *et al*. Recent advances in the chemical composition of propolis. Molecules 2014; 19610: 19632-12. https://doi.org/10.3390/molecules191219610

[6] Silva JC, *et al*. Antimicrobial activity, phenolic profile and role in the inflammation of propolis. Food Chem Toxicol 2012; 1790: 1795-5. https://doi.org/10.1016/j.fct.2012.02.097

[7] Gardana C, *et al*. Analysis of the polyphenolic fraction of propolis from different sources by liquid chromatography-tandem mass spectrometry. J Pharm Biomed Anal 2007; 390: 399-3. https://doi.org/10.1016/j.jpba.2007.06.022

[8] Ramos AFN, Miranda JL. Propolis: a review of its anti-inflammatory and healing actions. J Venom Anim Toxin Incl Trop Dis 2007; 697: 710-4. https://doi.org/10.1590/S1678-91992007000400002

[9] Pellati F, Prencipe FP, Benvenuti S. Headspace solid-phase microextraction-gas chromatography-mass spectrometry characterization of propolis volatile compounds. J Pharm Biomed Anal 2013; 103: 111-84. https://doi.org/10.1016/j.jpba.2013.05.045

[10] Russo A, Longo R, Vanella A. Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. Fitoterapia 2002; S21: 29-73. https://doi.org/10.1016/S0367-326X(02)00187-9

[11] Nakajima Y, *et al*. Water extract of propolis and its main constituents, caffeoylquinic acid derivatives, exert neuroprotective effects via antioxidant actions. Life Sci 2007; 370: 377-4. https://doi.org/10.1016/j.lfs.2006.09.017

[12] Sun C, *et al*. Effect of Ethanol/Water Solvents on Phenolic Profiles and Antioxidant Properties of Beijing Propolis Extracts. Evid Based Complement Alternat Med 2015; 1: 9-2015. https://doi.org/10.1155/2015/595393

[13] Penton ZE. Headspace gas chromatography. In: J. Pawliszyn, editors. Comprehensive Analytical Chemistry. Elsevier Science 2002; pp. 279-296.

[14] Ötles S. Analysis of Polyphenols in Foods. In: Ötles S, editors. Methods of analysis of food components and additives. 2nd ed. Boca Raton: CRC Press, 2012; pp. 199-260.

[15] Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem 2005; 1841: 1856-6. https://doi.org/10.1021/jf030723c

[16] Li S, *et al*. Antioxidant capacities and total phenolic contents of infusions from 223 medicinal plants. Ind Crops Prod 2013; 289: 298-51. https://doi.org/10.1016/j.indcrop.2013.09.017

[17] Falcao SI, *et al*. Phenolic profiling of Portuguese propolis by LC-MS spectrometry: uncommon propolis rich in flavonoid glycosides. Phytochem Anal 2013; 309: 318-4. https://doi.org/10.1002/pca.2412

[18] Banskota AH, Tezuka Y, Kadota S. Recent progress in pharmacological research of propolis. Phytother Res 2001; 561: 571-7. https://doi.org/10.1002/ptr.1029

[19] Dias LG, Pereira AP, Estevinho LM. Comparative study of different Portuguese samples of propolis: pollinic, sensorial, physicochemical, microbiological characterization and antibacterial activity. Food Chem Toxicol 2012; 4246: 4253-12. https://doi.org/10.1016/j.fct.2012.08.056

[20] Falcao SI, *et al*. Phenolic characterization of Northeast Portuguese propolis: usual and unusual compounds. Anal Bioanal Chem 2010; 887:897-2. https://doi.org/10.1007/s00216-009-3232-8

[21] Pellati F, *et al*. HPLC-DAD and HPLC-ESI-MS/MS methods for metabolite profiling of propolis extracts. J Pharm Biomed Anal 2011; 934: 948-5. https://doi.org/10.1016/j.jpba.2011.03.024

[22] Nunes CA, Guerreiro MC. Characterization of Brazilian green propolis throughout the seasons by headspace GC/MS and ESI-MS. J Sci Food Agric 2012; 433: 438-2. https://doi.org/10.1002/jsfa.4596

[23] Bridi R, *et al*. International Regulations of Propolis Quality: Required Assays do not Necessarily Reflect their Polyphenolic-Related *In Vitro* Activities. J Food Sci 2015; C1188: 1195-6.

[24] Vinson JA, *et al*. Plant Flavonoids, Especially Tea Flavonols, Are Powerful Antioxidants Using an *in Vitro* Oxidation Model for Heart Disease. J Agric Food Chem 1995; 2800: 2802-11. https://doi.org/10.1021/jf00059a005

[25] Debbache N, Atmani D, Atmani D. Chemical analysis and biological activities of Populus nigra, flower buds extracts as source of propolis in Algeria. Ind Crops Prod 2014; 85: 92-53. https://doi.org/10.1016/j.indcrop.2013.12.018

[26] Mohammad Zadeh S, *et al*. Antioxidant power of Iranian propolis extract. Food Chemistry 2007; 729: 733-3.

[27] Silva V, *et al*. Antioxidant activity of uruguayan propolis. In vitro and cellular assays. J Agric Food Chem 2011; 6430: 6437-12. https://doi.org/10.1021/jf201032y

Received on 11-04-2018 Accepted on 20-04-2018 Published on 27-04-2018

DOI: https://doi.org/10.6000/1927-5951.2018.08.02.5