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Chemical Composition and Biological Evaluation of Algerian Propolis from Six Different Regions

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ABSTRACT

Propolis is considered a natural resin produced by the bee and is still used in folk medicine. Six propolis samples from *Apis mellifera* (P1-P6) collected from different regions in Algeria were investigated for their contents and biological activities. The obtained results revealed that propolis P1 exhibited the highest total phenolics (210.93 mg GAE/g propolis), total flavonoids (34.33 mg QE/g propolis), and tannins (23.36 mg CE/g propolis). For antioxidant activities, P1 showed strong free radical scavenging activity with EC_{50} values of 0.055, 0.0306, 0.109 and 0.071 mg/mL, respectively for DPPH, ABTS, **FRAP**, and phosphomolybdenum assays. On the other hand, all propolis demonstrated antibacterial activities against G+ve bacteria (*S. aureus*) with slightly higher activities that were associated with P1 and P5 (9.83 and 10.92mm, respectively). P5 exhibited the lowest MIC and MBC against *S. aureus* with values of 62.5 and 125 µg/ml, respectively. Furthermore, all propolis had moderate to low antimicrobial activities against *C. albicans* (yeast) with moderate activities for P1 and P6 (13.33 and 8.50 mm, respectively). Chemical profiling of the most bioactive propolis samples (P1, P4, and P5) using HPLC−fingerprint analysis mainly led to detecting phenolic acids and flavonoids in variable percentages.

Keywords: Propolis, antioxidants, antimicrobial, polyphenols, Algeria.

INTRODUCTION

Herbal medications are always adopted in therapeutic applications for their availability, simplicity, effectiveness, and fewer side effects relative to synthetic drugs. Propolis, also known as bee glue, is a natural substance with resinous properties and variable colors that is mainly produced by *Apis mellifera* via collecting from the exudates of multiple plant parts and their own salivary secretions [1–3].

It is basically produced for construction and the protection of bee's hive. In this sense, the Greeks came up with the propolis name that means the defense of the hive [4,5]. Historically humans applied propolis as an adhesive and embalming substance, in perfumery, and mostly in medicine and therapeutic fields [1,5] because of its antibacterial, antitumor, immunomodulatory, anti-inflammatory, antioxidant, antifungal, hepatoprotective, antidiabetic, anticancer, antiprotozoal, and antiviral activities [3,4,6–9].

About 300 compounds have been identified in propolis [3] including the phenolic compounds, which represent a wide class of organic compounds such as flavonoids, tannins and phenolic acids. Interestingly, the biological activity of propolis has been attributed to its phenolic

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ingredients [10]. Propolis has been reported to have potent antiradical and antimicrobial activities; in fact it is probably the strongest among the different bee products [11]. Propolis has been studied widely in different geographical locations since there are plenty of factors that affect its composition, such as the climate, the botanical floral and also the extraction process [12,13].

Overproduction and accumulation of reactive species within the human body lead to a phenomenon recognized as oxidative stress that initiates several health disorders like cancer, cardiovascular diseases, and inflammation. The destructive effects of such species can be diminished via utilizing naturally occurring antioxidant agents as free radical scavengers [14–17].

Additionally, the emergence of resistant pathogenic strains that fail to respond to existing drugs poses a huge challenge for health care providers and current research has been redirected to discover new antibiotics. Natural sources like medicinal plants, microbial extracts, and marine organisms [18,19] were extensively studied for the discovery of new safe and effective antibiotics to counteract the resistance problem. Moreover, several naturally occurring bioactive compounds have been reported for their antimicrobial effects against different microbial infections [20,21]. Therefore, this study aims to investigate the Algerian raw propolis samples collected from different areas for their chemical profiles as well as their antioxidant and *in vitro* antimicrobial activities.

MATERIALS AND METHODS

Propolis samples

Six samples of raw propolis were harvested from the wild from six different regions in Algeria namely: Tipaza (P1; Latitude: 36.59°N; Longitude: 2.44°E), Blida (P2; Latitude: 36.47°N; Longitude: 2.83°E), Bouira (P3; Latitude: 36.37°N; Longitude: 3.90°E) which locate in the north, Batna (P4; Latitude: 35.56°N; Longitude: 6.19°E) in the east, **Sidi-Bel-Abbes** (P5; Latitude: 35.21°N; Longitude: 0.63°W) in the west, and **Ghardaïa (P6;** Latitude**:** 32.49°N; Longitude**:** 3.64°E**) in the** Northern desert. Samples were collected during spring and winter of 2019. The samples were kept at 4°C until extraction, biological and chemical investigations were performed.

Chemicals and reagents

All solvents, standards and reagents were of highly analytical grade. Ethanol, Folin-Ciocalteu's reagent, Na_2CO_3, gallic acid, $AlCl_3$, quercetin, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) **and ascorbic acid** were obtained from Sigma-Aldrich (Steinheim, Germany). $ABTS^+$(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), potassium persulphate, BHT, Trolox, **$K_3[Fe(CN)_6]$,** trichloroacetic acid, $FeCl_3$, sulfuric acid, sodium phosphate and ammonium molybdate were obtained from Fluka Chemicals. Nutrient agar and Nutrient Broth media were purchased from HiMedia Laboratories Pvt. Ltd (Mumbai, India).

Extract preparation

The propolis was grated first, and then each sample of 1 g was dissolved in 30 mL of ethanol (70%) in a 50 mL flask and left for 96 hours at room temperature. Afterward, the mixture was filtered and the extraction was repeated. The two extracts were combined and diluted to 100 mL with 70% ethanol in a volumetric flask. Next the hydro-alcoholic extracts were analyzed to determine the total phenolics and flavonoids [22].

Total phenolic contents

Total phenolic contents in each tested extract were determined by the Folin-Ciocalteu's [23] method with minor modifications. Hydro-alcoholic extracts (0.1 mL) were mixed with 0.5 mL of Folin-Ciocalteu's reagent (10%) and 0.4 mL of (7.5%) Na_2CO_3, and the absorbance was measured at 765 nm after 30 min of incubation at room temperature. The total polyphenol content was calculated based on a standard curve prepared using gallic acid and expressed as milligrams of gallic acid equivalent (GAE) per gram of sample.

Total flavonoids contents

Total flavonoid contents in each tested extract were determined according to the reported procedures [24] with minor modification. An amount of 0.5 mL of $AlCl_3$ (2%) was added to 0.5 mL of extract, after 1 h the absorbance was measured at 420 nm. Total flavonoid contents were calculated as quercetin equivalent (mg QE/g) using a calibration curve.

Total tannins contents

Total tannins content were determined as previously described by [25]. Briefly, 50µL of the extract was added to 1500µL of vanillin-methanol solution (4%) and 750µL of concentrated hydrochloric acid, 20 min later the mixture was measured at 510 nm. The catechin solution was used as standard and treated the same manner.

2,2-diphenyl-1-picrylhydrazyl radical (DPPH) free radical

Various concentrations of each sample (100 µL) were added to DPPH-ethanol solution (3900 µL, 60 µM) as previously described [26] with minor alterations. After an hour of incubation, the absorbance was measured at 517 nm. **Ascorbic acid was selected** as an antioxidant reference and treated in the same manner, and the calculation was carried out via finding the inhibition percentage (I%), I%= $[(A_0–A_i/A_0)*100]$; A_0: Absorbance of DPPH free radical, A_i: Absorbance of the free radical with the antioxidant, and the EC_{50} (Half maximal effective concentration was estimated.

$ABTS^+$ free radical-scavenging activity

$ABTS^+$(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging evaluation was based on a previously published report [27]. Accompanying with ascorbic acid, BHT, and Trolox were used as antioxidant references. Initially the $ABTS^+$ radical with the absorbance Abs_{734nm}: 0.7 was prepared by reacting ABTS-aqueous solution (7mM) with the persulfate-ethanol solution (2.45 mM) during 16 hours in the dark, then 50 µL at different concentrations of the samples was added to 950 µL $ABTS^+$, and measured at 734 nm. Both I% and EC_{50} were adopted for the calculations.

Ferric reducing antioxidant assay

According to previous reports [28], 50µL of each sample with various concentrations were added to 500 µL of **Phosphate buffer solution (200mM, pH=6) and 500 µL of $K_3[Fe(CN)_6]$(1%) with 30s of shaking and incubation at 50°C in a water bath for 20 min,** Trichloroacetic acid (**500 µL,** 10%) was added to the previous mixture, **then** 500 µL of the supernatant of the last solution was mixed with water (500 µL) and $FeCl_3$ (100 µL, 0.1%). The absorbance was measured at 700 nm against a blank consisting of the same reagents with only ethanol 70% instead of samples, using ascorbic acid, BHT and Trolox as antioxidant references and the same calculation parameters.

Phosphomolybdenum total antioxidant capacity

The phosphomolybdenum scavenging activity was based on phosphomolybdenum reagent and each of **ascorbic acid,** BHT, and Trolox as antioxidant references. 0.1 mL of each sample was mixed with 1 mL of Phosphomolybdenum reagent [100 mL of sulfuric acid (0.5 mM), 100ml of sodium phosphate (28 mM) and 100 mL of ammonium molybdate (4mM)]. The reaction was carried out in the dark for 90 min under 95°C in a water bath, the absorbance was measured at 695 nm [29]. The same parameters of EC_{50} were used for the calculation.

Antimicrobial activity

The antimicrobial activity of the samples was investigated by the agar disc diffusion method. Four different test microbes namely: *Staphylococcus aureus* (G+ve bacteria), *Escherichia coli* (G-ve bacteria), *Candida albicans* (yeast), and *Aspergillus niger* (fungus) were used. Nutrient agar plates were heavily seeded uniformly with 0.1 mL of 10^5-10^6 cells/mL in case of bacteria and yeast. A Czapek-Dox agar plate seeded by 0.1 mL the fungal inoculum was used to evaluate the antifungal activities. The plates were kept at low temperature (4°C) for 2-4 hours to allow maximum diffusion. The plates were then incubated at 37°C for 24 hours for bacteria and at 30ºC for 48 hours. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter (mm). The experiment was carried out more than once and mean of readings was recorded [18].

MICs and MBCs evaluation

Staphylococcus aureus ATCC 6538 (G+ve bacteria) and *Escherichia coli* ATCC 25922 (G-ve bacteria) were used to evaluate the MIC values of the potent active fractions/compounds. The test strains were cultivated in 100 ml bottle with each test at 35°C for 24 hours on Mueller Hinton medium. Bacterial cells were collected by centrifugation at 5000rpm under aseptic conditions at 4°C and the cells were washed using sterile saline till the supernatant becomes clear. Cell suspension has been performed to achieve optical density of 0.5 to 1 (at 550 nm) giving actual colony forming units of $5x10^6$ cfu/ml. Resazurin solution was prepared by dissolving 270 mg tablet in 40 ml of sterile distilled water. 96-well sterile-microplates were prepared. 50 µl of test material in methanol was pipetted into the first row of the plate. 10 µl of Resazurin indicator solution was added followed by 10 µl of bacterial suspension. The plates were prepared in duplicate and placed in an incubator set at 37°C for 18–24 hours. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value. MBC has been done by streaking of the two concentrations higher than MIC and the plates exhibiting no growth were considered as MBC [30].

HPLC conditions

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Eclipse C18 column (4.6 mm x 250 mm i.d., 5 μm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid was added to acetonitrile (B) which does not affect the separation column at a flow rate 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A) ; 15-16 min (82% A) and 16-20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl for each of the sample solutions. The column temperature was maintained at 40 °C [31,32].

RESULTS

Total polyphenolic, flavonoid and tannins content in propolis extracts

According to Table 1, the phenolic contents in Algerian propolis ranged from 45.37 ± 11.01 to 210.93 ± 36.02 (mg GAE/g propolis) in P6 to P1 samples orderly, and the total flavonoid contents varied from 07.32 ± 0.11 to 34.33 ± 0.44 (mg QE/g propolis) relating to P4 and P1. In general, the propolis in northern areas of Algeria P1, P2, and P3 have higher content of both phenolics and flavonoids, especially the sample from Tipaza (P1). For the tannins, the content varied between 3.77 to 23.36 (mg CE/g propolis) in samples P6 and P1.

The antiradical activities of propolis extracts

Concerning the antioxidant activities, the EC_{50} parameter was used for all antioxidant activities assays. Table 1 shows that the EC_{50} of antiradical activities oscillated between 0.055-0.59 mg/mL (DPPH), 0.0033-0.354 mg/mL (ABTS), 0.109-0.377 mg/mL (FRAP), and from 0.055 to 0.47 mg/mL (phosphomolybdenum), these results indicate that samples P1 and P3 are the strongest antioxidants relative to the other samples. Sample P3 from Bouira region had a good capacity against the ABTS free radical which was estimated with 0.0033 mg/mL and it seems to be a very powerful antioxidant. As shown in Table 1 the value 0.109 mg/mL in both P1 and P3 had the highest values. For the phosphomolybdenum activity in table 1 all the five samples presented an intense capacity except the sample P6 in south region.

Table 1: Total polyphenolic, flavonoid and tannins contents, and antiradical activities of Algerian propolis extracts

Test/ Bio-assay	Tested propolis samples/ Standards								
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**Ascorbic acid**	**Trolox**	**BHT**
Total phenolic (mg GAE/g propolis)[1,2]	210.93 ± 36.02	107.56 ± 22.78	183.15 ± 15.18	56.65 ± 10.32	57.04 ± 9.37	45.37 ± 11.01	-	-	-
Total flavonoid (mg QE/g propolis)[3]	34.33 ± 0.44	29.16 ± 0.27	18.64 ± 0.63	07.32 ± 0.11	19.04 ± 0.31	09.52 ± 0.13	-	-	-
Total tannins (mg CE/g propolis)[4]	23.36 ± 1.91	6.53 ± 0.58	13.74 ± 0.82	23.17 ± 3.97	6.72 ± 0.91	3.77 ± 1.24	-	-	-
EC_{50} (DPPH)[5] mg/mL	0.055 ± 0.001	0.205 ± 0.007	0.065 ± 0.003	0.59 ± 0.001	0.27 ± 0.002	0.34 ± 0.011	0.124 ±0.001	0.0042 ±0.0001	0.0025 ±0.0002
EC_{50} (ABTS) mg/mL	0.0306 ± 0.0014	0.088 ± 0.0041	0.0033 ± 0.001	0.354 ± 0.007	0.106 ± 0.0081	0.158 ± 0.010	0.004 ± 0.0001	0.0058 ±0.0001	0.0043 ±0.0005
EC_{50} **(FRAP)** mg/mL	0.109 ± 0.01	0.178 ± 0.026	0.109 ± 0.012	0.311 ± 0.072	0.294 ± 0.009	0.377 ± 0.062	0.0072 ± 0.001	0.0056 ± 0.001	0.013 ± 0.003
EC_{50} (Phosphomolybdenum) mg/mL	0.071 ± 0.0014	0.078 ± 0.0035	0.055 ± 0.0077	0.064 ± 0.0028	0.125 ± 0.007	0.47 ± 0.014	0.023 ±0.0014	0.027 ±0.0021	0.155 ±0.0012

[1]Results are (means ± S.D.) (n = 3)
[2]GAE: Gallic acid equivalent
[3]QE: Quercetin equivalent
[4]CE: Catechin equivalent
[5]EC_{50}: Half maximal effective concentration

The antimicrobial activity of propolis extracts

The antimicrobial activity of the extracts was assessed against *Staphylococcus aureus* (G+ve bacteria), *Escherichia coli* (G-ve bacteria), *Candida albicans* (yeast), and *Aspergillus niger* (fungus) through the measurement the diameter of inhibition zone, the results in Table2 indicated that the hydro-alcoholic extracts of propolis are positively effective against the *Staphylococcus aureus*, *Candida albicans,* and non-effective considering *Escherichia coli* and *Aspergillus niger* except for the sample P4 which is effective against the fungus.

Table 2: The antimicrobial activity of propolis extracts compared to standard antibiotics

Samples	Clear zone (ϕmm)			
	Staphylococcus aureus	***Escherichia coli***	***Candida albicans***	***Aspergillus niger***
P1	9.83 ± 0.76	0	13.33 ± 0.58	0
P2	8.17 ± 0.29	0	8.16 ± 0.29	0
P3	9.33 ± 0.57	0	8.33 ± 0.58	0
P4	7.50 ± 0.50	0	6.97 ± 0.06	7.33 ± 0.58
P5	10.92 ± 0.14	0	8.0 ± 0.0	0
P6	8.67 ± 0.58	0	8.50 ± 0.50	0
Neomycin50ug/ml	23.50 ± 0.50	19.83 ± .76	19.17 ± 0.29	0
Cyclohexamide 50ug/ml	0	0	0	22.17 ± 0.76

P: Propolis. mm: Millimeter.

MIC and MBC determination

Results in Table 3 explained that extract P5 exhibited the lowest MIC and MBC against *S. aureus* with values of 62.5 and 125 µg/ml, respectively followed by extracts P1 (125&250 µg/ml) and P3 (250 &325 µg/ml). For *E. coli* the MIC and MBC value for all extracts were high but extract P5 had moderate values of MIC and MBC (250 and 500 µg/ml, respectively).

Table 3: The minimum inhibitory concentrations (MICs), and minimum bactericidal concentrations (MBCs) of the most active selected extracts

Extracts	Pathogenic microorganisms			
	***S. aureus* ATCC 6538**		***E. coli* ATCC 25922**	
	MIC (µg/ml)	**MBC (µg/ml)**	**MIC (µg/ml)**	**MBC (µg/ml)**
P1	125	250	250	750
P3	250	325	500	750
P5	62.5	125	250	500

MIC: Minimum Inhibitory Concentration.

ATCC: American Type Culture Collection.

HPLC-fingerprint analysis of propolis samples

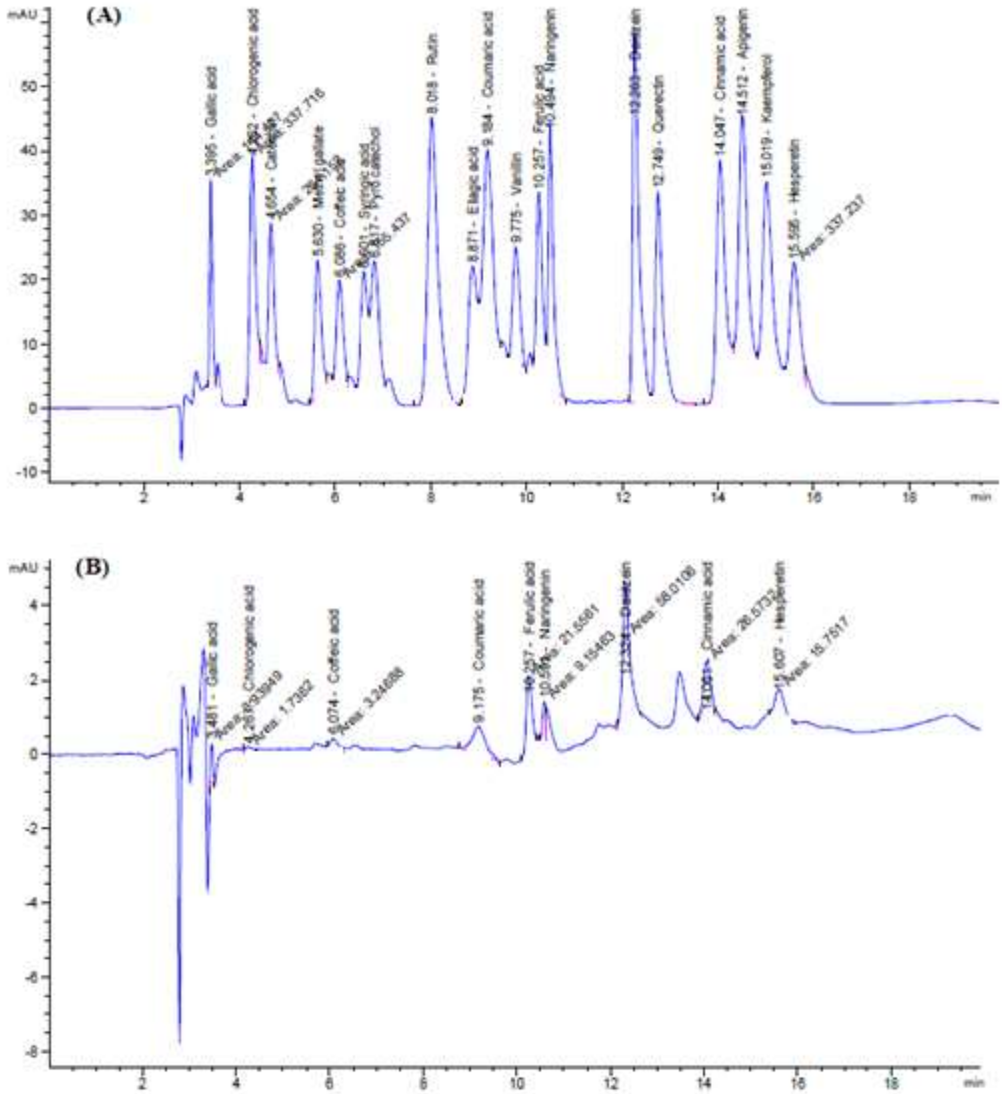
In this research work, a proper HPLC-fingerprint approach has been established to determine the chemical components in the most bioactive Algerian propolis samples (P1, P4, and P5). The obtained HPLC chromatograms of the investigated extracts were compared to nineteen standard phenolic compounds (Table 4 and Figure1).

Table 4: Areas under peaks and concentrations of the identified phenolic compounds in three propolis samples (P1, P4, and P5) compared to nineteen standard phenolic compounds

Polyphenol STD			P1				P4				P5			
Standards	Conc. (µg/ml)	Area%	R_t (min)	Area %	Conc. (µg/ml= µg/200mg)	Conc. (µg/g)	R_t (min)	Area%	Conc. (µg/ml= µg/200mg)	Conc. (µg/g)	R_t (min)	Area %	Conc. (µg/ml= µg/200mg)	Conc. (µg/g)
Gallic acid	15	2.1465	3.48	3.94	0.41	2.05	3.48	3.64	0.38	1.89	3.48	3.75	0.39	1.95
Chlorogenic acid	50	5.0193	4.26	1.74	0.26	1.29	4.25	2.35	0.35	1.74	4.28	1.60	0.24	1.19
Catechin	75	3.9259	4.65	ND	ND	ND	4.65	ND	ND	ND	4.65	ND	ND	ND
Methyl gallate	15	2.9489	5.63	ND	ND	ND	5.72	9.88	0.75	3.73	5.49	1.46	0.11	0.55
Caffeic acid	18	2.3102	6.07	3.25	0.38	1.88	6.08	8.52	0.99	4.94	5.85	3.59	0.42	2.08
Syringic acid	17.2	2.6503	6.60	ND	ND	ND	6.60	ND	ND	ND	6.60	ND	ND	ND
Pyrocatechol	40	3.9140	6.81	ND	ND	ND	6.83	2.32	0.35	1.76	6.81	ND	ND	ND
Rutin	61	10.1941	8.01	ND	ND	ND	8.01	ND	ND	ND	8.01	ND	ND	ND
Ellagic acid	120	3.6625	8.87	ND	ND	ND	8.51	2.85	1.39	6.93	8.87	ND	ND	ND
Coumaric acid	20	7.900	9.17	14.93	0.56	2.81	9.18	4.85	0.18	0.91	9.18	9.77	0.37	1.84
Vanillin	12.9	2.7036	9.77	ND	ND	ND	9.74	1.54	0.11	0.55	9.77	ND	ND	ND
Ferulic acid	20	3.6901	10.25	21.56	1.74	8.68	10.25	ND	ND	ND	10.25	ND	ND	ND
Naringenin	30	4.5775	10.59	9.15	0.89	4.46	10.59	39.84	3.88	19.41	10.59	12.75	1.24	6.21
Daidzein	35	8.1338	12.32	58.01	3.71	18.55	12.31	61.95	3.96	19.81	12.34	29.29	1.87	9.36

Polyphenol STD			P1				P4				P5			
Standards	**Conc. (µg/ml)**	**Area%**	**R_t (min)**	**Area %**	**Conc. (µg/ml= µg/200mg)**	**Conc. (µg/g)**	**R_t (min)**	**Area%**	**Conc. (µg/ml= µg/200mg)**	**Conc. (µg/g)**	**R_t (min)**	**Area %**	**Conc. (µg/ml= µg/200mg)**	**Conc. (µg/g)**
Quercetin	40	5.2241	12.74	ND	ND	ND	13.15	1.41	0.16	0.80	12.63	40.36	4.59	22.96
Cinnamic acid	10	7.5052	14.06	26.57	0.53	2.63	14.06	42.60	0.84	4.22	14.08	20.99	0.42	2.08
Apigenin	50	10.0262	14.51	ND	ND	ND	14.52	29.99	2.22	11.12	14.49	28.66	2.12	10.62
Kaempferol	60	8.4556	15.01	ND	ND	ND	15.0	11.57	1.22	6.10	15.01	ND	ND	ND
Hesperetin	20	5.0122	15.60	15.75	0.93	4.67	15.62	80.57	4.78	23.89	15.58	16.65	0.99	4.94

R_t: Retention time. ND: Not Detected.



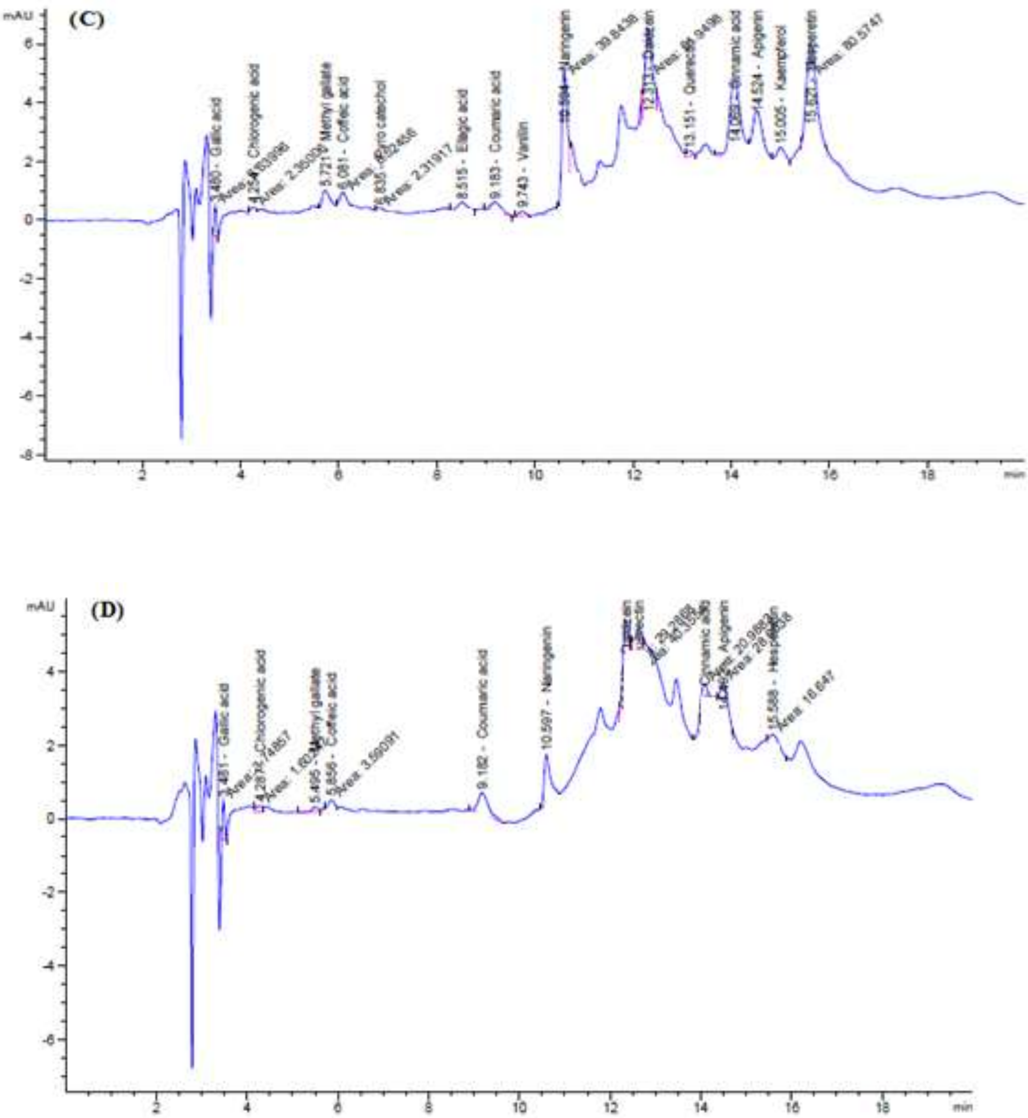


Fig.1. (A) HPLC chromatogram of standard phenolic compounds; (B) HPLC chromatogram of P1; (C) HPLC chromatogram of P4 and(D) HPLC chromatogram of P5.

DISCUSSION

Regarding the extraction procedures, the ethanol 70% solvent is commonly used for the phenolic extraction considering the solubility concept and it is more effective than water, less toxic than methanol with advantage for dewaxing purposes [33]. Based on previous studies the extraction was carried out in darkness at the room temperature to reduce possible degradation of the matter that may result from agitation.

The phenolic results of Algerian propolis (45.37- 210.93 mg GA/g propolis) are similar to the range of Morocco (77.89-241.66 mg GAE/g) [34], Kashmir Himalaya region (180-260 mg GAE/g) [35], Poland (150.05 to 197.14 mg/g GAE) [36], and Indian propolis (159.10-269.10) [37]. As for the flavonoids, the amounts (7.32-34.33 mgQE/g propolis) at most are in the same range of west Algeria and Ethiopia reported values [25,38] but mainly are less than many other countries. The tannins content in propolis was not widely analyzed by researchers probably due to their low abundance. This large variation in phenol, flavonoid and the tannins amounts, whether between Algerian regions or comparing with other parts of the world suggests that the geographical locations including the botanical floral affect the quantification of propolis [12], in addition to the climate and the harvesting time factors [22].

The highest value in DPPH free radical-scavenging activity (P1) among these samples is close to the findings of the south of Portugal, Kashmir Himalaya and India [22,39]. The synthetic radical $ABTS^{\bullet+}$ with the blue-green color becomes pale after turning it into a stable form and gaining an electron from the antioxidant agent [40]. The FRAP test is similar to ABTS except that it done under acidic pH instead of neutral conditions, the FRAP process reduces ferric-tripyridyltriazine $[FeIII(TPTZ)]^{3+}$ to a ferrous complex $[FeII(TPTZ)]^{2+}$ with a blue color. It is known that the antioxidant activity is related to the phenolic compounds including the flavonoids [10], therefore we report the diversity of the capacity between locations and in the activity type as well, which explains why the extracts with the highest amounts in phenolic P1, P3 have more potent antioxidant properties relative to the other investigated samples.

Regarding the antimicrobial activitity of the tested propolis samples, the current findings come in good agreement with many published reports that have demonstrated the effectiveness of propolis against Gram-positive bacteria and *Candida albicans* while inactive against Gram-negative bacteria [41].

HPLC-fingerprint approach is a well-known method was utilized for the determination of phenolic profiles in many plant extracts [31,32]. In the current study, the tested propolis samples showed a variable content of phenolic compounds, this is due to several factors, including Ecological conditions. Reviewing the literature revealed that *HPLC-UV analysis of Algerian propolis led to identification of six phenolic compounds including pinostrombin chalcone (38.91%), galangin (18.95%), naringenin (14.27%), tectochrysin (25.09%), methoxychrysin (1.14%) and suberosin (1.65%)* [42]*. The ethanolic extract of Uruguayan propolis was investigated for its phenolic composition via using RP-HPLC. The results revealed the presence of gentistic and p-coumaric acids as well as 8 flavonoidal compounds namely fisetin, myricetin, luteolin, quercetin, kaempferol, pinocembrin, chrysin and tectochrysin* [43]. RP-HPLC analysis of water extract of Brazilian propolis revealed the presence of phenolic acids like caffeic acid, *p*-coumaric acid and *trans*-cinnamic acid [44]. Eight polyphenolic compounds were detected by HPLC-UV in the 80% methanol extract of Chinese propolis *viz.* caffeic acid, isoferulic acid, 3,4-dimethoxycinnamic acid, pinobanksin 5-methyl ether, pinocembrin, benzyl caffeate, chrysin and galangin [45]. Rutin, quercetin, apigenin, kaempferol, chrysin and caffeic acid were detected in different aqueous ethanolic extracts of Romanian propolis using HPLC analysis [46,47] reported that 21 flavonoidal compounds and two caffeic acid esters were identified by HPLC in the 70% ethanol extract of Egyptian propolis and its sub-fractions including luteolin, apigenin, chrysin, acacetin, chrysin-7-methylether, luteolin-3'-methylether, myricetin, galangin, naringenin, hesperetin, genistein, dimethylallylcaffeate, and phenylethylcaffeate. Our current findings are matched with study of Shashikala and his Co-workers, which stated that HPLC-fingerprint analysis of the 70% ethanol extract of Indian propolis led to identification of p-coumaric acid, ferulic acid, epicatechin, gallic acid, caffeic acid and quercetin [48]. HPLC-UV/DAD analysis of Italian propolis hydroalcoholic extract revealed the presence of phenolic acids and their derivatives including caffeic acid, *p*-coumaric acid, ferulic acid, isoferulic acid, 3,4-dimethyl-caffeic acid, cinnamic acid, caffeic acid prenyl ester, caffeic acid benzyl ester, caffeic acid phenylethyl ester, *p*-coumaric prenyl ester, *p*-coumaric benzyl ester, caffeic acid cinnamyl ester, p-coumaric cinnamyl ester, and pmethoxy cinnamic acid cinnamyl ester. Also, the results revealed the presence of flavonoides like quercetin, quercetin-3-methyl-ether, chrysin-5-methyl-ether, apigenin, kaempferol, isorhamnetin, galangin-5-methyl-ether, quercetin-7-methyl-ether, chrysin, and galangin [49]. HPLC analysis of ethanolic extract of Croatian propolis allowed the identification of caffeic acid, naringenin, chrysin, pinocembrin, and galangin [50]. HPLC-UV/DAD investigations of Chinese propolis 80% methanol extract led to characterization of rutin, quercetin, luteolin, genistein, galangin and curcumin [51]. UHPLC-DAD analysis of the Indian propolis extract allowed the quantification of caffeic acid, *trans*-ferulic acid, *p*-coumaric acid, quercetin, luteolin, naringenin, apigenin, kaempferol, pinocembrin, CAPE, pinobanksin-3-*O*-acetate, acacetin, and galangin [52-54].

In conclusion, propolis, natural resins produce by bees, is considered as a promising source for the isolation of different groups of compounds such as phenolics, flavonoids as well as tannins with clinical value for the treatment of certain medical conditions.

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التركيب الكيميائي والتقييم البيولوجي للبروبوليس الجزائري من ست مناطق مختلفة

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ملخص

يعتبر البروبوليس راتينجًا طبيعيًا ينتجه النحل ولا يزال يستخدم في الطب الشعبي. تم فحص ست عينات بروبوليس من نحل العسل الغربي (P1-P6) تم جمعها من مناطق مختلفة في الجزائر لمعرفة محتوياتها وأنشطتها البيولوجية. أظهرت النتائج المتحصل عليها أن البروبوليس P1 أظهر أعلى نسبة من الفينولات الكلية (210.93 مجم من GAE / جم بروبوليس)، وفلافونويد كلي (34.33 مجم QE / جم بروبوليس)، والتانين (23.36 مجم / جم بروبوليس). بالنسبة للأنشطة المضادة للأكسدة، أظهر P1 نشاطًا قويًا في إزالة الجذور الحرة بقيم EC_{50} تبلغ 0.055 و 0.0306 و 0.109 و 0.071 مجم / مل على التوالي لمقايسات DPPH و ABTS و FRAP و phosphomolybdenum. من ناحية أخرى، أظهرت جميع أنواع البروبوليس نشاطًا مضادًا للبكتيريا ضد بكتيريا (*S. aureus*) G + ve مع أنشطة أعلى قليلاً مرتبطة بعينات P1 و P5 (9.83 و 10.92 مم، على التوالي). أظهر P5 أدنى MIC وMBC ضد *S. aureus* بقيم 62.5 و 125 ميكروجرام / مل على التوالي. علاوة على ذلك ، كان لجميع عينات البروبوليس أنشطة متوسطة إلى منخفضة كمضادات للميكروبات ضد*C. albicans* (الخميرة) مع أنشطة متوسطة لعينات P1 و P6 (8.50 و13.33مم، على التوالي). كما أدى التنميط الكيميائي لعينات البروبوليس الأكثر نشاطًا بيولوجيًا (P1 و P4 و P5) بإستخدام تحليل بصمات الأصابع (HPLC-fingerprint) بشكل أساسي إلى إكتشاف الأحماض الفينولية والفلافونويدات بنسب متغيرة.

الكلمات الدالة: البروبوليس، مضادات الأكسدة، مضادات الميكروبات، عديد الفينولات، الجزائر.

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