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Research Article

FATTY ACID COMPOSITION OF ALGERIAN PROPOLIS

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ABSTRACT

The fatty acid composition of eight honey bee (Apis mellifera) propolis load samples acquired in Algeria, were determined. The fatty acids presented a variable composition among these samples. All samples contained oleic, linoleic, stearic, eicosenoic, palmitoleic and palmitic acid. Only two samples did not contain arachidonic acid. Also results indicated palmitic, palmitoleic, linoleic, arachidonic and eicosenoic acid, respectively, accounted for 0.05% to 3.71%, 0.14% to 14.58%, 1.3% to 12.1%, 0.19% to 18.83%, and 0.23% to 12.86%; of the total lipids.The unsaturated fatty acid level varied from 19.72% to 51.85% of the total fatty acid composition, suggesting that propolis is a good source of unsaturated fatty acids to the diet. These data might help the regulatory agencies establish quality parameters for propolis produced in Algeria. There are no additional data available on Algerian propolis fatty acid composition.

Keywords: propolis, fatty acid composition, unsaturated fatty acid, oleic, linoleic.

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1. INTRODUCTION

Propolis, a complex mixture of several resinous materials, it's often called "bee glue", is a natural resinous product that honeybees collect from trees and several plants and mix it with β-glucosidase (salivary enzymes) and beeswax [1]. Propolis is a lipophilic material that is hard and easily broken when cold but soft, elastic, and very viscous when warm; it possesses an enjoyable aromatic smell and different coloration, including red, brown, and green, among others [2]. In terms of chemical composition, it is generally constituted of 50% resin, 30% wax, 10% essential oils, 5% pollen, and 5% other substances which include minerals and organic compounds like phenolic acids (caffeic and chlorogenic acid) or their esters, flavonoids (flavones, flavanones, flavonols, and dihydroflavonols chalcones), aromatic aldehydes, terpenes and alcohols, fatty acids, stilbenes, steroids and stilbenes [3, 4]. In the following years, analysis of a large number of samples from different geographic origins revealed that chemical composition of propolis is highly variable because it depends on factors such as the vegetation, season, and environmental conditions of the site of collection. Marcucci and Bankova [3, 4] registered more than 300 substances in propolis and recent reports showed the presence of compounds never mentioned before [5, 6].

Propolis fatty acids have been investigated previously in Bulgaria [7], Oxford (UK) [8], Turkey [9], Omani [10] and Cameroon [11]. There are no studies related to the fatty acid composition of propolis samples collected in Algeria. The present study aims to provide new information about fatty acid composition of propolis collected by bees in Algeria.

2. MATERIALS AND METHODS

2.1. Chemicals

Fatty acids (FA) standards (Supelco® 37 Component FAME Mix) were all purchased as methyl esters from Sigma-Aldrich (St Louis, MO, USA). These quantitative commercial preparations (Table 1) of fatty acid methyl ester (FAME) included homologous series of normal straight-chain saturated, monounsaturated, and polyunsaturated FA, from 4 to 24 carbon atoms in length. Potassium hydroxide (KOH), sodium chloride (NaCl) and sodium sulphate (Na_2SO_4) were purchased from Sigma-Aldrich (St Louis, MO, USA). Methanol (MeOH) and hexane were obtained from BIOCHEM chemopharma Co (FRANCE). All other

reagents used were of analytical grade. Nitrogen (N_2), helium (He), hydrogen (H_2) and compressed air for operation of the analytical instruments were obtained from National Industrial Gas Company, Algeria (Ouargla).

Table 1. Fatty Acid Methyl Ester Mix

N	Compound	common name	Rt (min)
1	C4:0	Butyric acid	5.972
2	C6:0	Capronic acid	7.365
3	C8:0	Caprylic acid	8.786
4	C10:0	Caprinic acid	10.13
5	C11:0	Undecanoic acid	10.835
6	C12:0	Lauric Acid	11.61
7	C13:0	Tridecanoic Acid	12.476
8	C14:0	Myristic Acid	13.479
9	C14:1	Myristoleic Acid	13.943
10	C15:0	Pentadecanoic Acid	14.636
11	C15:1	Ginkgolic acid	15.18
12	C16:0	Palmitic acid	15.989
13	C16:1	Palmitoleic acid	16.44
14	C17:0	Heptadecanoic Acid	17.516
15	C17:1	cis-10-heptadecenoic acid	18.054
16	C18:0	Stearic acid	19.369
17	C18:1 c+t n-9	Oleic acid + Elaidic Acid	19.891
18	C18:2 c+t n-6	Linoleic acid	20.955
19	C18:3 n-6	γ-Linolenic acid	21.763
20	C18:3 n-3	α-Linolenic acid	22.636
21	C20:0	Arachidic acid	24.411
22	C20:1 n-9	cis-11-Eicosenoic acid	25.173
23	C20:2 n-6	cis-11,14-Eicosadienoic acid	26.903
24	C20:3 n-6 + C21:0	cis-11,14,17-Eicosatrienoic acid + Heneicosylic acid	27.919
25	C20:3 n-3	cis-8,11,14-Eicosatrienoic acid	28.051
26	C20:4 n-6	Arachidonic acid	29.069
27	C20:5	cis-5,8,11,14,17-Eicosapentaenoic acid	29.604
28	C22:0	Behenic acid	32.131
29	C22:1	Erucic acid	32.402
30	C22:2	cis-13,16-Docosadienoic acid	33.64
	Identified components (%)		92
	Nº Identified components		34

2.2. Instruments

Chromatographic analysis was performed using a Shimadzu GC-2014 gas chromatograph with a flame ionization detector (FID), with a LabSolutions, Shimadzu GC Solution, Chromatography Data System, software version 2.3, equipped with a split/splitless injector. The capillary column was a DB-Wax of dimensions 30 m × 0.32 mm i.d. ×0.25 µm df (polyethylene glycol, Agilent). The column temperature was held at 50 ºC (1 min) and then increased at a rate of 25 ºC.min^{-1} up to 200 ºC and then increased at a rate of 3 ºC.min^{-1} up to 230 ºC and held for 18 min. The linear velocity of carrier gas (nitrogen) was constant at 12 cm/s. The temperature of the injector was 250 ºC, and the detector was 280°C, applying the split mode (split ratio was fixed at 1:50) [12].

2.3. Propolis origin

Propolis samples were collected from six different regions of Algeria as shown in Figure 1. The samples from Jijel (1), Tizi Ouzou (2, 7), Boumerdès (3, 4), and Tlemcen (5) Khenchela (6) and El Oued (8). Hand-collected propolis samples were kept desiccated in the dark up to their processing.

Fig 1. Locations (1-8) where propolis samples were collected in Algeria

Table 2. Algerian propolis samples used in this study on the basis of date of harvest, geographical origin.

	Sample code	Date of harvest	Color	Place of production	Geographical origin
1	PR1	April 2012	Brown	Jijel	Mountain
2	PR2	December 2012	Brown	Tizi Ouzou	Mountain
3	PR3	April 2011	Dark green	Boumerdès	Field (Mitidja)
4	PR4	November 2012	Dark green	Boumerdès	Field (Mitidja)
5	PR5	April 2013	green	Tlemcen	Mountain
6	PR6	September 2012	Dark green	Khenchela	Mountain
7	PR7	May 2011	Brown	Tizi Ouzou	Field
8	PR8	May 2012	Dark green	El Oued	Sahara

2.4. Lipid extraction

A known weight (approximately 5 g) of propolis, was extracted with 150 mL of hexane for 3h in a Soxhlet apparatus [13]. Hexane extracts containing lipids were subjected to vacuum distillation at approximately 40◦C using a Rotovap to remove hexane. Extracted lipids were stored at –10◦C under nitrogen until further analysis. Lipid samples were analyzed in duplicate for saturated and unsaturated fatty acid composition. Total fat content and fatty acid analysis were carried out in bulked harvest samples.

2.5. Total fat content

Total fat content was determined in accordance with [13] method 7.061.

% Fat on dry weight basis=(g of fat in sample/g of dried sample)×100 [14]

2.6. Fatty acid methyl ester preparation

0.5g of lipid extracts were converted into fatty acid methyl esters. Methyl esters were synthesized using potassium hydroxide (KOH) as catalyst and methanol at 75 °C. After 1 h, the stirring was stopped and the reaction product was separated into two phases by simple decantation. FAME (upper phase) was further purified by washing several times with water. Then, FAME was dissolved in hexane, kept standing over magnesium sulfate, filtered and the

volatiles removed under reduced pressure. The transesterification reaction utilized a total molar ratio of methanol: oil: KOH of 10:0.5:0.02.

2.7. Analysis of fatty acid methyl esters

Fatty acids were identified by comparing the relative retention times of the samples' FAME peaks with fatty acids methyl esters standards. The samples were spiked with the standard. The peak areas were determined with Star software (GCSolution). The data were expressed as percentages of the normalized area of fatty acids.

2.8. Description of data and variables

The fatty acid composition was identified and quantified in area percentage, being SFA (sum of mean peak area percentages of saturated fatty acids), UFA (sum of mean peak area percentages of unsaturated fatty acids), MUFA (sum of mean peak area percentages of monounsaturated fatty acids), PUFA (sum of mean peak area percentages of polyunsaturated fatty acids), SFA:UFA ratio, MUFA:PUFA ratio, and Hypo:Hyper ratio, hypocholesterolemic fatty acids (MUFA + PUFA) to hypercholesterolemic fatty acids (C14:0 + C16:0) [15].

2.9. Clustering Analysis.

Compounds identified by GC/FID of all eight propolis samples were analyzed using the neighbor-joining method and the software PAST v.2.17.

3. RESULTS AND DISCUSSION

3.1. Gravimetric extraction yield

The yields of dry propolis extracts in the studied propolis samples were found to be; 71.16% for Tizi Ouzou (PR7), 65.3% for Tlemcen (PR5) and El Oued (PR8), 55.2% for Jijel (PR1), 41.16% for Boumerdès (PR3), 37.74% for Tizi Ouzou (PR2), 25.75% for Boumerdès (PR4), 16.19% (w/w) for Khenchela (PR6) using hexane as solvent (Figure 2). The best yield of soluble content was found the propolis collected from Tizi Ouzou region. Different propolis samples collected from different areas showed different solubility in hexane even if the same amount of propolis samples were tried to be dissolved in the same volume of hexane.

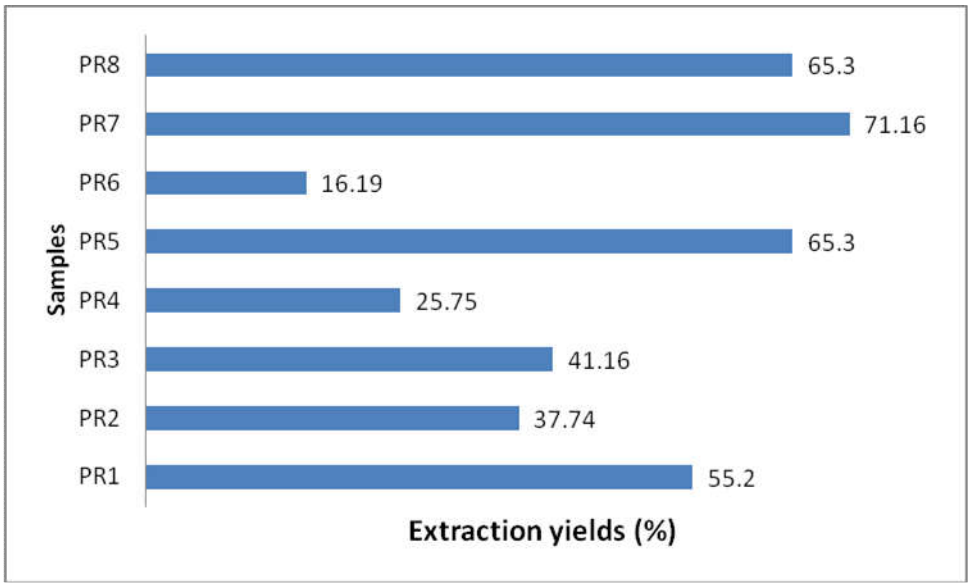

Fig 2. Comparison of lipid recovery by Soxhlet extraction utilizing hexane

3.2. Fatty acid profile

The GC-FID techniques have been proven to be suitable for chemical profiling of food and natural products like propolis. Even though these techniques provide a sufficient profile and identification of the compounds analyzed, the propolis has to be derivatized in order to increase the concentration of volatile compounds for detection. However, not all compounds comprising propolis are able to be derivatized or become volatile after derivatization. Fatty acids are one of the groups that are derivatized to convert to fatty acid methyl esters.

The GC-FID analysis of the fatty acid methyl esters of the lipid extracts led to the identification of over 34 compounds belonging to various groups of fatty acid such as Saturated Fatty Acids; Monounsaturated Fatty Acids; Polyunsaturated Fatty Acids; Omega-3and Omega-6. The most abundant compounds in the hexane extracts are Polyunsaturated Fatty Acids, Monounsaturated Fatty Acids, and Omega-6, respectively. The major compounds present in the different propolis lipid extracts identified by GC-FID analysis are listed in Table 3 respectively. Their percentages are given in the tables and refer to percent of the total area.

Table 3. Comparison of normalized fatty acids (FA) composition between different propolis lipid extracts determined from FAME analysis by GC-FID

Fatty acid profile		PR1	PR2	PR3	PR4	PR5	PR6	PR7	PR8
Saturated fatty acids									
C4:0	Butyric acid	0.05	0.06	0.12	0.11	0.15	0	0.09	0.1
C6:0	Capronic acid	0	0.05	0.06	0	0	0	0	0
C8:0	Caprylic acid	0	0.1	0	0	0	0	0	0.04
C10:0	Caprinic acid	0	0.19	0.08	0.06	0.08	0.06	0	0.04
C11:0	Undecanoic acid	0.21	0.16	0.05	0.15	0.06	0.05	0.1	0.04
C12:0	Lauric acid	0.06	0.2	0	0	0	0	0	0.07
C13:0	Tridecanoic acid	0.06	0.05	0.12	0.08	0	0.06	0.2	0.09
C14:0	Myristic acid	0.06	0.11	0.06	0	0	0.07	0.1	0.06
C15:0	Pentadecanoic acid	0.06	0.27	0.37	1	0.1	0.31	0	0.07
C16:0	Palmitic acid	1.78	0.05	0.07	0.3	2.37	0.12	0.22	3.71
C17:0	Heptadecanoic acid	0.1	0.16	0.17	0.12	0.47	0.24	0.43	0.32
C18:0	Stearic acid	0.09	0.07	0.08	0.22	0.21	0.07	0.19	0.1
C20:0	Arachidic acid	0	0.12	0.31	0.12	0.07	0	0.41	0.04
C22:0	Behenic acid	0.07	0.25	0	0	0.27	0	0.13	0.03
Monounsaturated fatty acids									
C14:1	Myristoleic acid	0.06	0.1	0	0.79	0	0.12	0	0.04
C15:1	Ginkgolic acid	0	0	0.75	0.25	0.1	0	0.09	0.04
C16:1	Palmitoleic acid	6.91	3.45	0.52	0.98	2.5	0.14	14.58	1.92
C17:1	cis-10-heptadecenoic acid	0.06	0.06	0.06	0	0.06	0	0	0.04
C18:1 c+t n-9	Oleic acid + Elaidic acid	0.93	0.04	0.06	0.09	1.42	0.09	0.3	0.08
C20:1 n-9	cis-11-Eicosenoic acid	12.86	13.21	3.85	3.66	22.62	0.23	8.28	0.41
C22:1 n-9	Erucic acid	0.11	1.63	0	0.27	0.05	0	0.1	0.36
Polyunsaturated fatty acids									
C18:2 c+t n-6	Linoleic acid	1.49	6.9	6.2	3.63	1.3	7.92	1.5	12.01
C18:3 n-6	γ-Linolenic acid	0.16	0.05	0.07	0.19	0.06	0.19	0.27	0.28
C18:3 n-3	α-Linolenic acid	0.06	0.22	0.05	2.12	0.14	0.13	0.2	0.29
C20:2 n-6	cis-11,14-Eicosadienoic acid	3.82	16.86	0.15	0.06	3.73	13.58	2.33	19.71
C20:3 n-6 + C21:0	cis-11,14,17-Eicosatrienoic acid + Heneicosylic acid	0.07	0.48	0.09	0.75	0.22	0.29	0.1	0.04
C20:3 n-3	cis-8,11,14-Eicosatrienoic acid	0	0.07	0	0.05	0.3	0.29	0.57	0.04
C20:4 n-6	Arachidonic acid	0	1.07	3.84	0	18.83	0.06	6.51	0.19
C20:5 n-6	cis-5,8,11,14,17-Eicosapentaenoic acid	0.13	2.63	3.99	21.59	0.45	6.62	3.26	0.24
C22:2 n-6	cis-13,16-Docosadienoic acid	0.07	0.19	0.09	0.33	0.07	0.09	0.09	0.96

The total concentrations of fatty acids in the propolis samples ranged from 21.21 to 55.63% (Table 4). The lowest concentration was found in the propolis from the PR3 (Boumerdès) area and the highest concentration was in sample PR5 (Tlemcen). In a previous report, the total fatty acid concentration of Yemeni propolis samples was low, ranging from 0.25 to 20.78% [16].

The fatty acids of flora and fauna have mainly even carbon chain length homologues and usually range from C_{12} to C_{32}. They are generally unsaturated in flora and saturated in fauna. The major fatty acids in plants are the C_{18} monosaturated, disaturated and triunsaturated forms, whereas polyunsaturated fatty acids are more common in algae than higher plants [16, 17].

In total, 14 Saturated fatty acids were determined; four of them are from all propolis samples (Undecanoic; Palmitic; Heptadecanoic and Stearic acid), seven obtained from most samples (Butyric; Caprinic; Tridecanoic; Myristic; Pentadecanoic; Arachidic and Behenic acid), and three obtained from two or three samples (Capronic; Caprylic and Lauric acid) (Figure 3). In a previous report, compounds extracted from Canadain; Turkish and New Zealand propolis also showed a Lauric; Myristic; Palmitic and Stearic acid [18-20].

In total, 16 unsaturated fatty acids were determined; seven of them are monounsaturated fatty acids and nine are polyunsaturated fatty acids, ten of them are from all propolis samples (Palmitoleic; Oleic, Elaidic; Linoleic, γ-Linolenic, α-Linolenic, cis-11,14-Eicosadienoic, cis-11,14,17-Eicosatrienoic, Heneicosylic, cis-5,8,11,14,17-Eicosapentaenoic and cis-13,16-Docosadienoic acid). Myristoleic acid obtained from PR1; PR2; PR4; PR6 and PR8 samples, Ginkgolic acid obtained from PR3; PR4; PR5; PR7 and PR8 samples, Erucic and cis-8,11,14-Eicosatrienoic acid obtained from most samples (Figure 3). The analyzed samples present a level of UFA between 19.72% and 51.85. The profiles obtained for the analyzed propolis from Algeria were similar to the data from a report by Al-Ghamdi, on Yemeni propolis [16].

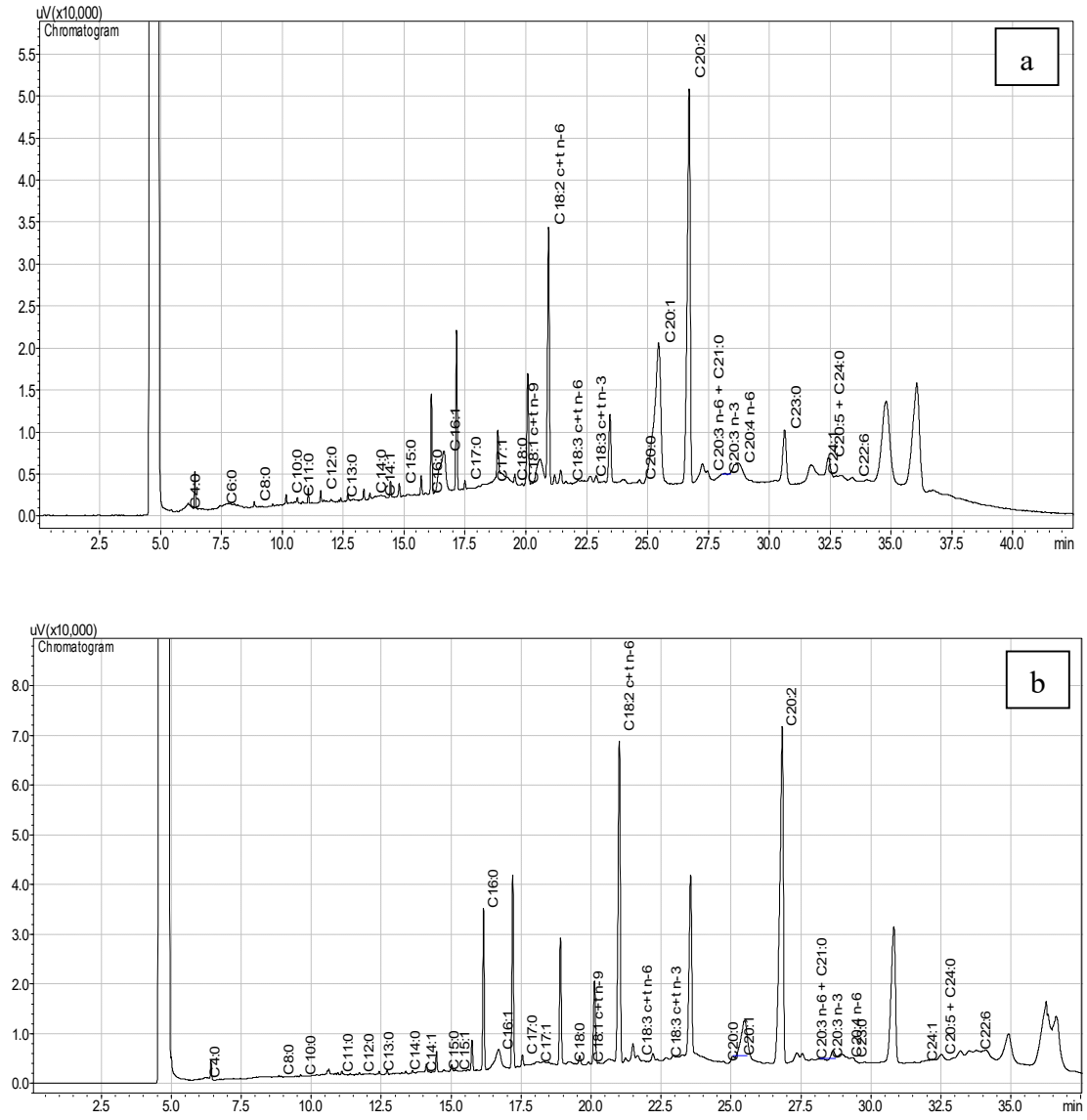

Fig 3. GC−FID profiles of hexane extracts of propolis (a) PR2, and (b) PR8 samples

The hexane extracts are similar in that they all contain Butyric, Caprinic, Undecanoic, Heptadecanoic, Stearic, Oleic, Elaidic, γ-Linolenic, α-Linolenic, cis-11,14,17-Eicosatrienoic, Heneicosylic and cis-13,16-Docosadienoic acid, but PR2 and PR8 contains Caprylic acid exclusively, also PR2 and PR3 contains Capronic acid exclusively, and PR1, PR2, and PR8 contains Lauric Acid exclusively (Table 3).

The Palmitic, Palmitoleic, cis-11-Eicosenoic, Linoleic, and cis-11,14-Eicosadienoic acid were the most abundant in the PR1 sample, while cis-11-Eicosenoic, Linoleic, cis-11,14-Eicosadienoic and cis-5,8,11,14,17-Eicosapentaenoic acid were the most abundant in

the PR1 sample.

The cis-11-Eicosenoic, Linoleic, Arachidonic, and cis-5,8,11,14,17-Eicosapentaenoic acid were the most abundant in the PR3 sample, and the cis-11-Eicosenoic, Linoleic, α-Linolenic and cis-5,8,11,14,17-Eicosapentaenoic acid were the most abundant in the PR4 sample, In relation to the individual fatty acids, PR8 sample showed a higher percentage of linoleic, cis-11,14-Eicosadienoic acid and, in general, of palmitic acid, compared to other samples (Table 3).

PR1 sample showed a significantly higher proportion of palmitoleic and cis-11,14-eicosadienoic acid. PR2 and PR6 samples showed the highest proportion of cis-11,14-Eicosadienoic acid and PR4 samples, the highest levels of cis-5,8,11,14,17-Eicosapentaenoic acid. PR7 sample showed the highest proportion of Palmitoleic; Arachidic and cis-8,11,14-Eicosatrienoic acid.

Table 4. Proportion of different fatty acids groups in the propolis lipid extracts

Fatty acid group	PR1	PR2	PR3	PR4	PR5	PR6	PR7	PR8
SFA	2.54	1.84	1.49	2.16	3.78	0.98	1.87	4.71
UFA	26.73	46.96	19.72	34.76	51.85	29.75	38.18	36.65
UFA/SFA ratio	10.52	25.52	13.23	16.09	13.72	30.36	20.42	7.78
MUFA	20.82	16.86	5.24	5.77	26.7	0.58	23.25	2.53
PUFA	5.78	27.47	10.49	7.4	24.7	22.55	11.67	33.88
PUFA/MUFA ratio	0.28	1.63	2.00	1.28	0.92	38.88	0.50	13.39
ω-3	0.06	0.29	0.05	2.17	0.44	0.42	0.77	0.33
ω-6	5.74	28.18	14.43	26.55	24.66	28.75	14.06	33.43
ω-6/ ω-3 ratio	95.67	97.17	288.6	12.23	56.04	68.45	18.26	101.3
H	1.84	0.16	0.13	0.3	2.37	0.19	0.32	3.77
h	2.84	11.46	14.3	28.42	22.72	15.59	12.71	13.17
h/H ratio	1.54	71.62	110	94.73	9.58	82.05	39.72	3.49
Total FA	29.27	48.8	21.21	36.92	55.63	30.73	40.05	41.36

SFA: Saturated Fatty Acids; UFA: Unsaturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; ω-3: Omega-3; ω-6: Omega-6; H: Hypercholesterolemic Fatty Acids (H=C14:0 + C16:0); h: Hypocholesterolemic Fatty Acids (h=C18:1+C18:2+C18:3+C20:3+C20:4+C20:5+C22:4+C22:5+C22:6).

The analyzed samples present a level of **ω-6** between 5.74% and 33.43% of the total fatty acids. In all cases, **ω-6** is significantly higher than **ω-3** (Table 4). The profiles obtained for the

analyzed propolis from Algeria were opposite to the data from a report by Feás [21]. Fatty acids from ω−6 series are biogenetic precursors of some physiologically important prostaglandins, thromboxanes, and leukotrienes hormones, which are related to the inflammatory response. The nutritional value of essential ω−3 and ω−6 fatty acids is also widely known for its health advantages [22].

A comparison between the results of the present investigation and data from the literature relative to the composition of propolis reveals a general similarity between the latter and Algerian propolis, although notable differences are apparent as to the relative proportions of some constituents (Table 5).

Table 5. Results of previous studies

	Cameroon [11]	**Canada [19]**	**Yemen [16]**	**Turkey [9, 20, 23, 24]**
Caprinic acid				0.02
Lauric acid				0.33
Myristic Acid				0.001-0.13
Palmitic Acid	0.6	+	0.1-4.67	0.33-2.39
Stearic Acid	0.2	0.1	0.013	0.11-1.23
Arachidic Acid	0.2		0.01-0.018	
Oleic Acid	0.8	0.3-0.7	0.002-1.21	0.63-1
Linoleic acid				0.12-3.43
α-Linoleic acid				0.4
9-Octadecanoic acid				2.12-0.36
Behenic acid				1.88-1.02

3.3. Clustering analysis

Obviously, the fatty acids compositions of propolis samples vary between different samples. The major compounds determined in the samples from Algeria include cis-11-Eicosenoic; cis-11,14-Eicosadienoic; cis-5,8,11,14,17-Eicosapentaenoic; Arachidonic; Linoleic; Palmitoleic; Palmitic and α-Linolenic acid. Heptadecanoic; Myristoleic; Ginkgolic; γ-Linolenic and Erucic acid, which were detected in these samples, were not detected in Turkish, Yemeni, Cameroon and Brazilian propolis. The compounds vary between different samples and locations. To investigate the similarity between the different samples,

hierarchical cluster analysis (HCA) by the neighbor-joining method was used. The output of the cluster analysis is shown in Figure 4 where three separate sample clusters are recognized. The first cluster includes three (PR2; PR6 and PR8) propolis samples, the second includes two (PR3 and PR4) propolis samples, and the third includes the rest of the propolis samples (PR1; PR5 and PR7).

The third cluster shows that the sample PR5 deviate from PR1 and PR7. This indicates that sources of propolis components from Tlemcen are different from the samples collected from Jijel and Tezi ouzou.

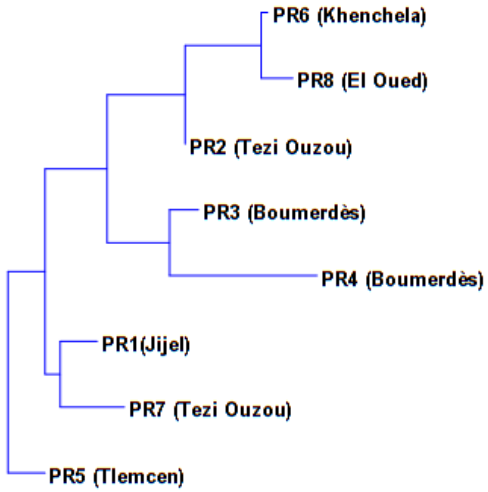

Fig 4. Clustering analysis of identified constituents by GC/FID of samples of Algerian propolis using the neighbor-joining method.

4. CONCLUSIONS

In the present study, propolis from the six regions of Algeria was characterized by identifying and semi-quantifying major fatty acids components. The major compounds were in order: cis-11-Eicosenoic Acid> cis-11,14-Eicosadienoic acid> cis-5,8,11,14,17-Eicosapentaenoic acid> Arachidonic acid> Linoleic acid> Palmitoleic acid> Palmitic acid> α-Linolenic acid.

The results obtained in this study demonstrated that propolis constitutes a good source of healthy compounds, namely, unsaturated fatty acids (ω-6 and ω-3), and suggests that it might be useful in prevention of diseases. Algerian propolis from different regions is nutritionally well-balanced and revealed high levels of healthy fatty acids and good PUFA/SFA ratios.

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