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Characterization of bioactive compounds in South-Western Algeria propolis samples

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Abstract
The prepared aqueousextract of propolis samples, which are harvested in Rebahia (Saida, Algeria), were analyzed by gas chromatography-mass spectrometry (GC / MS) to provide 50 volatile bioactive compounds. The major compoundshad been found, to know: Terpenoids (spathulenol 2.01 %, 1-dotrontanol 1.25 and trans-2-hexen-1-ol 1.03 %), aromatic alcohols(carvacrol 0.71 % and thymol 0.49 %) Hydrocarbons (1,4-diethyl cyclohexane 8.22 % and 1-docecane 12.13 %) and organic acids (9,12-octadecadienoic acid 1.27 %).
Keywords: Propolis;GC-MS; Terpenoids.

1.Introduction

Various plant species, abound in the mountains and forests of South-Western Algeria, are the most frequented sites by honeybees (Apismellifera L.) to collect a natural resinous substance released by buds and exudations of plants and to produce the propolis after have been mixed it with pollen and enzymes secreted by bees [1]. Propolishad been used in the beehive to smooth out the internal walls of the hive and as a protective barrier against their predators [2]. The content of propolis may have differences depending on the plant kinds visited by honeybee, the environment and the honey storage conditions[3,4].Propolis includes 50 % resin, composed of flavonoids and phenolic acids, 30 % wax, 10 % essential oils, 5 % pollen and 5 % other organic compounds[3,5].Propolis, as an antioxidant product, includesflavonoids, phenolic acids, enzymes (glucose oxidase, catalase,...), vitamins, organic acids, free amino-acids and proteins[3,5].Propolis, as a natural product, have various biological activities, including antimicrobial, anti-inflammatory and antitumoral effects[3,6].

The local population of South-Western Algeria used propolis as phytotherapeutic remedy against diseases. Studies have been published on the anti-inflammatory, anti-oxidant and antimicrobial activities of propolis [2,7]. Algerian propolis samples extracted inTigzirt, Yennarou, AinOuassara and Ksar El Hiran areas, located in Eastern and Center od Algeria, showed various chemical compositions in which it was found different polyphenol levels [8]. Other studies also have revealed the changes of chemical composition propolis[9,10]. Bioactive compounds as; polyphenols, flavonoids and terpenoids have been detected in propolis from different sources which had been influenced by climatic, botanical and geographical factors [10,11]. The South-Western Algeria flora presents high biodiversity with many endemic plants, which is expected to differentiate the composition of Algerian propolis from that of typical African and European ones [10,12]. The aims of this study were to identify the chemical composition of the South-Western Algeria propolisethanolic extract by gas chromatography / mass spectrometry (GC/MS) analysis.

2.Experimental

2.1.Chemicals

Dimethyl Sulfoxide (DMSO), diethyl ether, distilledwater and hydrochloric Acid (HCl)were provided by Biology Department, Faculty of Science, University of Saida (West Algeria) and have been used to prepare the propolis aqueous extract solution.

2.2.Preparation of propilsethanolic extract

Fifty grams of the resinous material of South-Western Algeria propolis (harvested from Rebahia area, province of Saida, located in Western Algeria) were powdered and extracted with 500 ml of diethyl ether at 35° C for 35 min. After extraction, the mixture was centrifuged and the supernatant was evaporated to complete dryness at 40 °C [13]. The dried residue has been kept at 4°C for further use.

2.3.Gas chromatography-mass spectrometry

The propolis sample was analyzed in GC – MS – Varian-saturn two-dimensionnal instrument GC 2200 series equiped with two columns; column 1 (capillary HP-1, dimension: 50 m × 0.32 mm, 0.26 µm thickness) and column 2 (capillary, dimension: 60 m × 0.32 mm, 0,50 µm thickness). The mobile phase consists of Helium gaswith a flow rate of 1.2 ml / min.

GC conditions: spliteless injection mode (1.5 s), injector temperature 280 °C, temperature program: initial temperature 50 °C (1 min hold) and up to 280 °C (5°C / min) and ionization voltage 70 eV. The instrument is connected to a computer system managing a mass spectral database (NIST, NBS and WILE).

Table 1: Chemical composition of South-Western Algeria propolisaqueousextract.

N°	Compound name	Category	%	RT (min)
01	b-menth-ene-8-oil	Terpenoid	0.13	17.08
02	Do decanal	Terpenoid	0.02	17.68
03	1-docecane	Alkane	12.13	24.22
04	Thymol	Aromatic alcohol	0.49	26.65
05	Carvacrol	Aromatic alcohol	0.71	27.11
06	Tridecane	Alkane	0.91	27.89
07	2,4-decadienal	Terpenoid	-	29.49
08	Cis-Salvene	Terpenoid	0.09	30.25
09	Camphene	Terpenoid	Trace	31.99
10	Nerolidol	Terpenoid	0.07	32.90
11	Germacrene	Terpenoid	Trace	34.60
12	Spathulenol	Terpenoid	2.01	34.78
13	Alpha-cedrol	Terpenoid	0.20	35.07
14	Alpha-Gurjunene	Terpenoid	0.04	35.70
15	Hexadecane ketone	Terpenoid	0.33	36.04
16	Alpha-Eudesmol	Terpenoid	0.40	36.91
17	Hexadecanoic acid	Organic acid	0.99	36.99
18	Tetradecanoic acid	Organic acid	0.51	37.00
19	9,12-Octadecadienoic acid	Organic acid	1.27	37.18
20	Octadecanoic acid	Organic acid	-	37.55
21	Octadecane	Alkane	0.61	38.85
22	1-Dotrontanol	Terpenoid	1.25	39.25
23	Tetra-decanoate methyl	Organic acid	0.59	39.64
24	lionoic acid	Organic acid	6.09	40.15
25	Heptyl methyl-ketone	Terpenoid	0.71	40.80
26	Trans-2-Hexen-1-ol	Terpenoid	1.03	41.17
27	1-octanol	Aliphatic Alcohol	5.07	41.25
28	1,4-diethyl cyclohexane	Alkane	8.22	41.63
29	1,2,3,5-Tetramethyl Cyclohexane	Alkane	0.71	41.95
30	2,2,4-Trimethyl Pentane	Alkane	1.01	41.99
31	N-Undecane	Alkane	0.63	42.00
32	Limonen	Terpene	0.06	43.59
33	N-decane	Alkane	0.21	46.01
34	Benzyl Ethyl ketone	Flavonoid	0.01	46.35
35	Lauraldehyde	Flavonoid	2.01	46.45
36	1,2,3,4-tetramethyl benzene	Aromatic hydrocarbon	0.17	46.60
37	p-Cymene	Terpenoid	0.63	47.29
38	Nonane-1-ol	Terpenoid	0.02	48.90
39	2,3-dihydro-1-methyl-1H-indene	Terpenoid	0.17	52.20
40	1-Methyl-Indene	Terpenoid	0.64	54.01
41	1-methylene-1H-indene	Terpenoid	0.04	59.61
42	D-Isomenthol	Terpenoid	0.44	60.34
43	p-Mentha-1,8-diene-4-oil	Terpenoid	2.11	60.79
44	Menthol	Terpenoid	0.89	61.33
45	4-Terpineol	Terpenoid	0.79	61.85
46	p-Cymene-8-Oil	Terpenoid	0.65	70.26
47	Naphthalene	Aromatic Hydrocarbon	0.20	71.80
48	1-hindene	Terpenoid	0.05	76.10
49	Cyclo-Tetradecane	Terpenoid	0.55	81.63
50	Octacosane	Terpenoid	1.84	84.01
51	Others	-	42.3	-

RT: retention time

3.Results and discussions

Chemical composition analysis of the South-Western Algeria propolis aqueousextract, collected from Rebehia area located in the province of Saida, were determined by GC/MS analysis and the results are showed in Table 1 and figure 1. According to the results of GC/MS analysis, propolis contains about 50 volatile compounds identified. The main chemical compounds identified are listed in Table 1. Among these organic compounds, it has been found different amounts of bioactive compounds which29 terpenoids, 2 aromatic alcohols, 2 flavonoids, 8 alkanes, 6 organic acids, 2 aromatic hydrocarbons and only one compound of aliphatic alcohol. The relative amount of terpenoids, in the propolisethanolic extract, was higher. Major compounds were terpenoids such as spathulenol (2.01 %), 1-dotrontanol (1.25 %), trans-2-hexen-1-ol (1.03 %) and aromatic alcohols as carvacrol (0.71 %), thymol (0.49 %) and hydrocarbons as 1,4-diethyl cyclohexane (8.22 %), 1-docecane (12.13 %) and organic acids as 9,12-octadecadienoic acid (1.27 %). In addition, trace amounts of camphene and germacrenewere detected.Phenolic compounds of the propolisare affected by the botanical and geographical origins and climatic conditions of the area[3,14]. The results of this study join the Turkish studies [3]. Flavonoids have been used in various medicines and foods due to their health benefits. They may reduce the risk of health problems through their antioxidant activities[15].These secondary metabolites have biological and pharmacological importance as scavenging free radicals and the toxic metal-chelating[15].

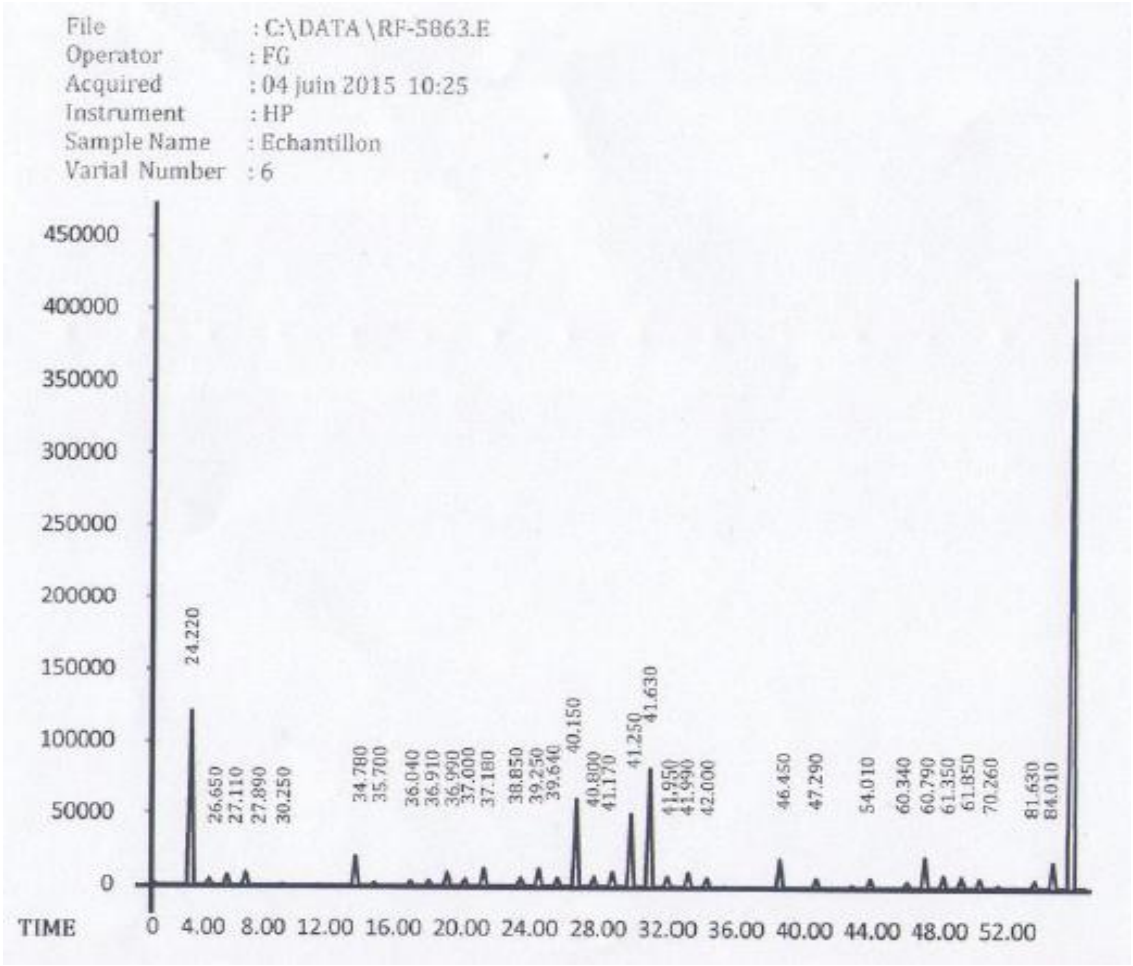

Figure 1: GC-MS of South-Western Algeria propolis aqueousextract.

24.22: 1-Docecane, 26.65: Thymol, 27.11: Carvacrol, 27.89: Tridecane, 30.25: Cis-Salvene, 34.78: Spathulenol, 35.70: Alpha-Gurjunene, 36.04: Hexadecane ketone, 36.91: Alpha-Eudesmol, 36.99: hexadecanoic acid, 37.00: Tetradecanoic acid, 37.18: 9.12-Octadecadienoic acid, 38.85: Octadecane, 39.25: 1-Dotrontanol, 39.64: Tetra-decanoate methyl, 40.15: Lionoic acid, 40.80: Heptyl methyl-1-ketone, 41.17: Trans-2-Hexen-1-ol, 41.25: 1-Octanol, 41.63: 1,4-Diethyl Cyclohexane, 41.95: 1,2,3,5-Tetramethyl Cyclohexane, 41.99: 2,2,4-Trimethyl pentane,42.00: N-Undecane, 46.45: Lauraldehyde, 47.29: p-Cymene, 54.01: 1-Methyl-Indene, 60.34: D-Isomenthol, 60.79: p-Mentha-1,8-Diene-4-Oil, 61.35: Menthol, 61.85: 4-Terpineol, 70.26: p-Cymene-8-Oil, 81.63: Cyclo-Tetradecane, 84.01: Octacosane.The last peak, with a major intensity, represents other compounds of propolis chemical composition.

In the South-Western Algeria, propolis is modestly used by beekeepers and very little is known about its biological activities. A study of Boufadi et al. (2014) reported that propolis harvested, from central and eastern areas of Algeria, contained various high concentrations of polyphenolic compounds known as the main active molecules exhibited free radical scavenging activity [8]. In the present study, GC/MS analysis of the aqueousextract of propolis showed the presence of terpenoids, alkanes, polyunsaturated fatty acids, alcohols and flavonoids. Terpenoids such as spathulenol, 1-dotrontanol and trans-2-hexen-1-ol are compared to the terpenoids of propolis collected from Greece [16]and Turkey [17]. The South-Western Algeria propolis presentshigher levels of polyunsaturated fatty acids such as lionoic acid and 9,12-octadecadienoic acid whereas it's reported lower levels of flavonoids such as benzyl ethyl ketone. These results could be compared to the chemical composition of the red propolis extract reported in the Brazilian studies, which in the main flavonoids were formononetin, isoliquiritigenin, liquiritigenin, medicarpin, and biochaninA [18-22]. The results obtained are in disagreement with the results of propolis collected from China [23] and Malaysia [24].

4.Conclusion

The South-Western Algeria propolis, for the first time, was analyzed by GC/MS. The analysis revealed that this propolis is rich in terpenoids because of its high level. This propolis, one type among the different Mediterranean propolis types, need further research. The South-Western Algeria propoliscould be used in the food as well as pharmaceutical industry and will become an important source of terpenoids.

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