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Annals of Biological Research, 2010, 1 (2) :103-107
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233

Volatil constituents of *Algerian propolis*

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Abstract

Hydrodistillation of three Algerian propolis collected in different locations of east of Algeria (El-malha, Benibelaîd and Kaous) afforded a yellowish oil in a yield of approximately 0.03% to 0.11%. A total of 54 compounds were identified across all the samples representing about 74% of total content of each sample. Components were mainly monoterpenes, sesquiterpenes, acids and alkanes. GC-MS analysis indicated that the predominant components of the essential oil of propolis of El-malha were 2-hexenal (4.85%), myristic acid (2.03%), linoleic acid (1.86%) and (-)-spathulenol (1.6%). Nevertheless the predominant components of the essential oil of propolis of Benibelaîd were isooctane (3.89%), linoleic acid (2.18%), undecane (2.03%), myristic acid (1.65%), hexadecane (1.14%), p-cymene(1.21%), palmitic acid (1.05%) and 4-terpineol (1.03%). The major constituents of the essential oil of propolis of Kaous were 2-hexenal (11.15%), myristic acid (5.66%), linoleic acid (5.16%), carvacrol (4.47%), alpha-cedrol (2.57%) and p-cymene (1.27%).

Keywords: *Algerian propolis*, volatiles compounds, GC-MS.

INTRODUCTION

Propolis is a resinous hive product collected by honeybees from parts of plants, buds and exudates, and has been used in folk medicine since around 300 BC [1]. It was reported to possess several bioactivities such as: antibacterial [2,3], antifungal [4,5], antiviral [6], local anaesthetic [7], anti-inflammatory [8], hepatoprotective [9], antioxidant and antitumor [10].

Propolis is a very complex mixture and its chemical composition varies according to its location. Hundreds of chemical compounds have been identified from Propolis involving important

chemical families such as flavonoid aglycone, phenolics and diverse aromatic compounds. It also contains some volatiles components and bee wax [11].

The identification of secondary plant metabolites in Propolis are of great importance in the development of the pharmacognosy. Its volatile compounds were reported to be of a considerable biological effect in spite of their low composition.

Propolis Essential oils have been investigated previously in Brazil [12, 13, 14], Croatia [15] and Greece [16]. In the present study we aim to investigate the composition of volatile oils of Propolis from eastern Algeria.

MATERIALS AND METHODS

2.1. Essential oils isolation:

Algerian propolis harvested in 2006 was used to carry out the present work. The collection sites are abbreviated as follows**:** sample P_1 from El-malha in Mila, sample P_2 from Benibelaîd in Jijel and sample P_3 from Kaous in Jijel.

The essential oil of Propolis was extracted by hydrodistillation and collected in diethyl ether after 4 hours of distillation, prior to be separated in a funnel. The aqueous fraction was extracted twice with diethyl ether. The organic phase was dried on anhydrous sodium sulphate, filtered and evaporated under vacuum. Oil yield was then estimated and the oil composition was analysed by GC-MS.

2.2. Gas chromatograph-mass spectrometry (GC-MS) analysis:

Analysis of essential oil was performed using a Perkin Elmer Clarus 500 gas chromatograph, equipped with an Elite-5MS capillary column (60m X 0.255mm i_d., 0.25µm film thickness, made in USA) and a mass spectrometer detector. The carrier gas was helium at a flow rate of 1ml/min. Oven temperature was initially 40°C for 10 min and was then gradually increased to 270°C at 3°C/min. For GC-MS detection, an electron ionization system was used with ionization energy of 70 ev.

Each extract was diluted in 1: 100 (V/V) with n-hexane to obtain solutions A, B and C (from P_1, P_2 and P_3). From each originally diluted sample, 40µl was diluted 1:4 (V/V) with n-hexane and placed in headspace vials with bevelled tops. The solutions, together with a blank sample, were loaded on a headspace auto sampler and, according to a predominated batch sequence; the samples were injected automatically in scan mode. The scan repetition rate was 0.5 s over a mass range of 50-450 atomic mass units (amu). Injector and detector temperature were both set at 250°C.

Identification of the components of the volatiles oils was based on retention indices and computer matching with Wiley libraries and the National Institute of Standards and Technology (NIST). The components of Propolis essential oils were determined by considering their area as percentage of the total ion current.

RESULTS AND DISCUSSION

The hydrodistillation of Propolis gave yellowish oil with a yield of 0.03% for P_1 and P_3 and 0.11% for P_2. 37, 26 and 30 compounds were identified in the essential oils of propolis from El-malha, Benibelaîd and Kaous respectively. The compositions of volatile oils are given in table1.

Table 1: Constituents of the essential oil of *Algerian propolis*

Compounds	R_t	P_1 (%)	P_2(%)	P_3(%)
2-nonanoe	17,42	0,55	-	-
cyclopentanol, 3-methyl	17,92	1,25	-	-
2-hexenal	24,61	4,85	1,14	11,15
1-octanal	26,82	-	0,64	-
Cyclohexane, diethyl	27,32	1,01	0,50	-
1,2,3,5 tetramethyl cyclohexane	28,02	0,04	1,86	Traces
Isooctane	30,33	0,23	3,89	-
Undecane	30,92	0,46	2,03	0,34
Trans-alpha dihydroterpineol	31,81	-	0,09	-
DL- limonene	32,72	0,03	0,09	0,09
Decane	33,73	-	0,39	0,95
1-phenyl-2-butanone	34,10	0,02	-	-
Dodecanal	34,57	0,01	0,96	0,89
Prehnitol	35,32	-	0,32	0,02
p-cymene	35,86	-	1,21	1,27
Nonanol	36,85	-	-	0,34
1H indene 2,3 dihydromethyl	36,96	0,01	-	0,08
1-methyl indene	36,98	-	0,06	-
1(-)-indene,1 methylene	36,98	-	0,20	-
D-isomenthol	37,11	0,03	-	-
+(R)-*p* -mentha-1,8 dien-4-ol	37,63	0,10	-	-
Trans pinocarveol	39,09	0,12	-	0,05
p-mentha-1,5 dien-8-ol	39,57	0,03	-	-
p-menth-1-ene-3,8 diol	40,55	0,22	-	0,12
Menthol	40,93	0,36	1,03	0,12
4-terpineol	41,03	0,33	-	0,05
p-cymene-8-ol	41,30	-	-	0,16
Naphthalene	41,43	-	-	0,16
1-H indene	41,53	0,07	-	-
p-menth-1-ene-8-ol	41,84	0,06	-	-
Dodecanal	42,15	0,02	-	-
1-dodecane	44,89	0,05	0,18	0,01
Thymol	46,06	-	-	4,47
Carvacrol	46,48	-	-	0,30
Tridecane	46,56	0,63	0,82	0,07
2,4 decadienal (E,E)	46,58	0,14	0,17	Traces
Cis-salvene	47,47	-	-	0,25
Camphene	48,87	Traces	-	-
Nerolidol	52,41	0,02	-	0,09
Germacrene D	54,58	Traces	-	-
(-)-spathulenol	58,88	1,6	-	-
Alpha-cedrol	60,26	0,15	-	2,57
Alpha-gurjunene	60,98	0,07	-	-
Hexadecane	61,21	0,28	1,14	-
Alpha-eudesmol	61,92	0,40	-	-
Palmitic acid	70,84	0,38	1,05	0,53
Myristic acid	71,92	2,03	1,65	5,66
9,12 octadecanoic acid(Z,Z)methyl ester	76,01	-	0,44	0,40
6-octadecanoic acid, methyl ester	76,21	0,07	-	0,55
Octadecane	76,34	1,01	0,07	-
1-dotricontanol	76,41	-	2,33	-
Methyl 13-methyltetradecanoate	77,19	-	0,36	-
Linoleic acid	77,51	1,86	2,18	5,16
Cyclotetradecane	80,65	-	6	0,30
Octacosane	83,65	0,32	-	-
Others	--	56,89	48,7	35.87

The components are listed in the order of their elution.
R_t : Retention time.; P_1 : Propolis of El-malha.; P_2 : Propolis of Benibelaid.; P_3 : Propolis of Kaous.

Twelve compounds were common between the oils of the samples, namely 2-hexenal, 1,2,3,5 tetra methyl cyclohexane, 2,4-decadienal (E,E), DL-limonene, dodecanal, menthol, 1-dodecane, tridecane, undecane, palmitic acid, myristic acid and linoleic acid; but with different rates. For example the undecane was 2.03 % in P_2 oil but only 0.46% and 0.34% in P_1 and P_3.

The major constituents of the essential oil of P1 were 2-hexenal (4, 85%), myristic acid (2.03%), linoleic acid (1.86%) and (-)-spathulenol (1.6%).

The major constituents in P_2 were isooctane (3.89%), linoleic acid (2.18%), undecane (2.03%), myristic acid (1.65%), hexadecane (1.14%), p-cymene (1.21%), palmitic acid (1.05%) and 4-terpineol (1.03%).

The major constituents in P_3 were 2-hexenal (11.15%), myristic acid (5.66%), linoleic acid (5.16%), carvacrol (4.47%), alpha-cedrol (2.57%) and p-cymene (1.27%).

In all the analysed oils, acids represent 4.34%, 5.32% and 12.3% in P_1, P_2 and P_3 respectively). Monoterpene hydrocarbons and oxygen containing monoterpenes represent 1.55%, 2.74% and 6.90% respectively (Table 2).

Table 2: composition of particular classes of compounds in Propolis essential oil

Grouped components	**P_1 (%)**	**P_2(%)**	**P_3(%)**
Monoterpene hydrocarbons	0,03	1,30	1,61
Oxygen-containing monoterpenes	1,25	1,44	5,26
Sesquiterpene hydrocarbons	0,07	-	-
Oxygen-containing sesquiterpenes	2,17	-	2,66
Aliphatic hydrocarbures	5,04	11,38	1,68
Aromatic hydrocarbures	0,08	0,26	0,24
Acids	4,34	5,32	12,3
Alcohols, phenols,aldehydes and cetones	6,84	5,60	12,38
Others	56,89	48,70	35,87

Propolis of Kaous is rich in oxygenated monoterpenes (carvacrol 4.47%) while propolis of Benibelaîd contains monoterpene hydrocarbons (menthol 1.03%). Sesquiterpenes represent 2.24% of the oil of P_1 (0.02% nerolidol, 1.6% (-)-spathulenol, 0,41% alpha eudesmol, 0.15% alpha-cedrol and 0.07% alpha-gurjunene), but only traces of sesquiterpenes hydrocarbons were detected in P_3. Oxygen containing monoterpenes represent 2.66% of the total oil of P_3. These classes of compounds were absent in P_2.

It is interesting to note that *Algerian propolis* is very rich in acids, hydrocarbons, alcohols, aldehydes and ketones. Compared to previous reports on Propolis essential oils from other localities [12, 13, 14, 15, 16], the compositions of present analyses are quite different. It is possible that this difference is due to the botanical origin of the tested Propolis. Other research will be pursued to determine the plant origin of *Algerian propolis* and its chemical composition especially volatile compounds and flavonoids contents.

Acknowledgement
Partial financial support by MESRES (Ministère de l'Enseignement Supérieur et de la Recherche

Scientifique) are gratefully acknowledged.

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