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Volatile Components and Antioxidant Effect of Essential Oil of *Anthemis mauritiana* Maire & Sennen Flowers

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ABSTRACT

The volatile components isolated from flowers of *Anthemis mauritiana* have been studied. The essential oil was obtained by hydrodistillation and by micro steam distillation and analysis were performed by GC/MS. The main constituents of the essential oil obtained by both methods were α-pinene, β-pinene, β-myrcene, γ-terpinene respectively by hydrodistillation and micro steam distillation.

The essential oil obtained by hydrodistillation was also subjected to screening for its possible antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The best result was obtained at a dose of 100µg/ml with an inhibition of 37.80 ± 0.24 %, this activity was less effective than the synthetic BHT with 56.59 ± 0.43% at the same dose.

Key words: *Anthemis mauritiana*, Essential oil, Antioxidant activity, GC/MS.

INTRODUCTION

The genus *Anthemis* (Asteraceae, syn. Compositae) is the second largest genus in this family, with more than 210 species that occur in the Mediterranean region, southwest Asia and eastern Africa[1]. *Anthemis mauritiana* Maire & Sennen is an endemic specie distributed in Morocco and Algeria. The species of this genus are widely used in the pharmaceutic, cosmetic and food industries. The flowers of the genus have been well-documented. Their main components are natural flavonoids and essential oils[2,3]. In the Mediterranean region, tinctures, tisanes, and salves of this genus are widely used as anti-inflammatory, antioxidant, antibacterial, and antispasmodic agents[4-7].

In the present study, we were interested in evaluating the composition and the possible antioxidant activity of *Anthemis mauritiana* Maire & Sennen flowers (EOAM) essential oil. Indeed, many pathological conditions are associated with oxidative stresses which are responsible for the development of numerous diseases such as cancer and cardiovascular complications[8,9,10]. Nowadays, there is considerable evidence that the Mediterranean diet, rich in fruits, vegetables and natural anti-oxidants is able to prevent the risk of oxidative stress related diseases[11].

MATERIAL AND METHODS

Chemicals

Butylated hydroxytoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich , and homologous series of C7_C30 n-alkanes and various reference chemicals used for identification were obtained from Supelco Analytical (Bellefonte, USA). Diethyl ether and methanol used in this study were purchased from Merck (Darmstadt, Germany).

Plant material

The flowers of *Anthemis mauritiana* Maire & Sennen were collected locally in May 2009 from the North eastern area of Morocco; the botanical identification was done by Professor Benyounes Haloui at the department of Biology, Faculty of Science University Mohammed I Oujda, Morocco. A voucher specimen (N° 64666) was previously deposited at the Scientific Institute of Rabat.

Isolation of the essential oil
Hydrodistillation

The air-dried and ground flowers (100g) of *Anthemis mauritiana* were submitted for 3 h to water distillation using a Dean stark type apparatus (yield: 0.2%).

Micro-steam distillation-extraction method

35 g of *Anthemis mauritiana* Maire & Sennen flowers were subjected to a simultaneous distillation-extraction method for 1 hour using a Likens-Nickerson apparatus with 1 ml of diethyl ether as solvent.

Gas Chromatography Mass Spectrometry (GC/MS) analysis

GC/MS analysis of the essential oil was performed using Agilent-Technologies 6890N Network gas chromatographic (GC) system (Agilent-Technologies, Little Falls, CA, USA) equipped with a flame ionization detector (FID), a Varian CP-99927 capillary column (30 m _ 0.25 mm, film thickness 0.25 µm) and an Agilent Technologies 5975 inert XL mass detector (electron impact mode). The injector and detector temperatures were set at 230 and 250 °C, respectively. Column temperature was programmed from 40°C to 260 °C at a rate of 10 °C/min. Helium was used as carrier gas at a flow rate of 1.2 ml/min. A sample of 1.0 µL of the essential oil diluted 2000 times with diethyl ether was injected using the splitless mode

Compounds identification

The identification of the essential oil constituents was based on a comparison (i) of their retention factors to those of (C7_C30) n-alkanes and (ii) of their Kovats indices to published data and to those of reference compounds. The molecules were further identified and authenticated using their MS data and the Wiley 275.L mass spectral library.

DPPH scavenging activity

The scavenging activity of essential oil against DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was measured according to the method of Blois (12). 4 ml of a 0.1 M DPPH methanolic solution was added to 1 ml of essential oil solubilized in methanol. A series of concentrations ranging from 10 to 100 mg/ml of essential oil extracted by hydrodistilation were tested. The mixtures were shaken vigorously and incubated in the dark for 30 min. The reduction of DPPH absorption was then measured at 517 nm. The scavenging activity of DPPH radical (%) was calculated following the equation:

$$100 \times (A517_{blank} - A517_{sample}) / A517_{blank}.$$

Where $A517_{blank}$ is the absorbance of the control reaction (a reaction with all the reagents except the essential oil tested) measured at 517 nm, and $A517_{sample}$ is the absorbance of the essential oil sample measured at 517 nm against the

Table 1: Phytochemical composition (%) of essential oil obtained by hydrodistillation from flowers of *Anthemis mauritiana* Maire & Sennen

Pics	Retention time (min)	Composition (%)	Compounds	KI*
1	4.21	28,14	α-Pinene	1020,88
2	5.29	0,11	Camphene	1063,07
3	6.47	15,57	β-Pinene	1100,00
4	6.85	0,94	Sabinene	1118,76
5	7.99	4,75	βMyrcene	1169,38
6	8.21	0,38	β-Terpinene	1178,71
7	8.61	0,59	Limonene	1193,95
8	9.57	4,28	γ-Terpinene	1246,61
9	9.74	0,3	Trans β-Ocimene	1255,89
10	10.02	2,23	*p*-Cymene	1270,83
11	10.24	0,23	α-Terpinolene	1282,28
12	11.21	8,27	Heptenone-6-Methyl	1341,40
13	12.06	0,16	Nonyl Aldehyde	1394,59
14	13.15	0,25	Δ-Elemene	1471,83
15	14.2	0,07	Nonenol	1550,75
16	14.84	3,64	β-Caryophylene	1600,00
17	14.93	0,63	Lavandulyl Acetate	1607,78
18	15.48	0,65	Sabinyl Acetate	1654,30
19	15.73	0,77	α-Humulene	1674,90
20	15.79	0,19	Lavandulol	1679,80
21	15.96	0,09	α-Copaene	1693,57
22	16.01	0,16	α-Terpineol	1697,59
23	16.07	0,28	Cis-Sabinol	1702,72
24	16.20	2,84	D-Germacrene	1713,56
25	16.35	0,11	trans-α-Bergamolene	1727,88
26	16.48	0,23	Bicyclo-Germacrene	1739,41
27	16.56	0,14	Geranyl Acetate	1746,47
28	16.61	0,5	Farnesene	1750,86
29	16.70	2,48	Linalyl-isovalerate	1757,86
30	16.75	0,15	β-Cadinene	1763,08
31	16.83	0,28	Lynalyl methylbutanouate	1770,02
32	18.41	0,8	Phenol	1900,00
33	18.45	0,28	Neryl Acetate	1904,55
34	19.2	0,48	oxid Caryophylene	1988,03
35	20.53	0,19	Spathulenol	2125,70
36	20.94	0,56	Nonanoic acid	2168,91
37	20.98	0,11	Δ-Cadinene	2172,04
38	21.35	0,71	α-Longipinene	2211,20
39	21.54	0,41	β-Eudesmol	2233,43
40	21.76	5,76	α-Curcumene	2257,66
41	21.91	0,28	Decanoic acid	2274,03
42	22.15	0,53	Tricosane	2300,00
43	23.88	0,33	Pentacosane	2500,00
44	25.25	0,3	Heptacosane	2700,00
45	26.86	0,69	Hexadecanoic acid	2908,22
Total		90,84		

* KI: Kovats Retention Index relative to n-alkanes.

Table 2: Phytochemical Composition of essential oil obtained by micro-steam distillation from flowers of *Anthemis mauritiana* Maire & Sennen

Pics	Retention time (min)	Composition (%)	Compounds	KI*
1	4,24	15,36	α-Pinene	1021,75
2	4,31	0,59	α-phellandrene	1024,78
3	6,23	7,81	β-Pinene	1093,00
4	6,61	0,2	Sabinene	1107,04
5	7,86	0,34	β-Myrcene	1163,98
6	8,71	0,29	Cineole	1197,74
7	9,53	0,78	γ-Terpinene	1243,85
8	10,02	2,19	p-Cymene	1270,31
9	10,23	0,18	α-Terpinolene	1281,25
10	11,26	9,22	Methyl heptenone	1343,99
11	12,72	0,27	α-Thujene	1441,32
12	12,96	0,15	Acetic acid	1458,47
13	13,04	0,47	Heptenol methyl	1464,12
14	13,07	0,28	furancarboxaldehyde	1466,23
15	13,76	0,1	Camphor	1516,11
16	13,87	0,32	Benzaldehyde	1524,87
17	14,19	0,25	Nonenol	1549,97
18	14,77	0,18	Methyl heptadienone	1594,06
19	14,84	0,72	β-Caryophylene	1599,26
20	15,48	1,23	Sabinyl acetate	1653,47
21	15,79	2,31	Lavandulol	1678,98
22	16,01	0,25	α-Terpineol	1696,79
23	16,1	6,04	sabinol acetate	1704,53
24	16,21	0,54	D-Germacrene	1757,86
25	16,71	1,46	Linalyl isovalerate	1758,73
26	16,82	0,17	linalyl methylbutanoate	1768,29
27	17,12	0,11	Myrtenol	1794,05
28	18,41	0,85	Phenol	1912,93
29	19,2	0,51	Caryophylene Oxide	1989,58
30	19,9	2,04	Octanoic acid	2060,20
31	20,92	1,16	Nonanoic acid	2165,78
32	20,98	0,19	Δ-Cadinene	2172,04
33	21,54	0,37	β-eudesmol	2232,33
34	21,78	3,27	α-Curcumene	2258,75
35	21,9	0,77	Decanoic acid	2271,85
36	22,17	1,06	nonadecane	2301,17
37	23,04	0,2	Tetracosane	2401,20
38	23,73	0,28	Dodecanoic acid	2482,61
39	23,82	0,4	Octadecadienoic acid	2493,06
40	23,9	1,89	Pentacosane	2501,39
41	24,35	0,26	Octadecatrienoic acid	2564,73
42	25,02	0,25	Hexadecenolide	2664,36
43	25,2	0,62	Tetradecanoic acid	2692,28
44	25,27	2,06	Heptacosane	2701,41
45	25,46	0,3	β-fenchene	2729,46
46	25,97	0,53	Octadecanol	2901,37
47	26,86	5,93	Hexadecanoic acid	2907,19
48	29,62	0,56	Octadecenoic acid	
49	30,46	2,25	Linoleic acid	
Total		77,56		

·Kovats RI: Kovats Retention Index relative to n-alkanes.

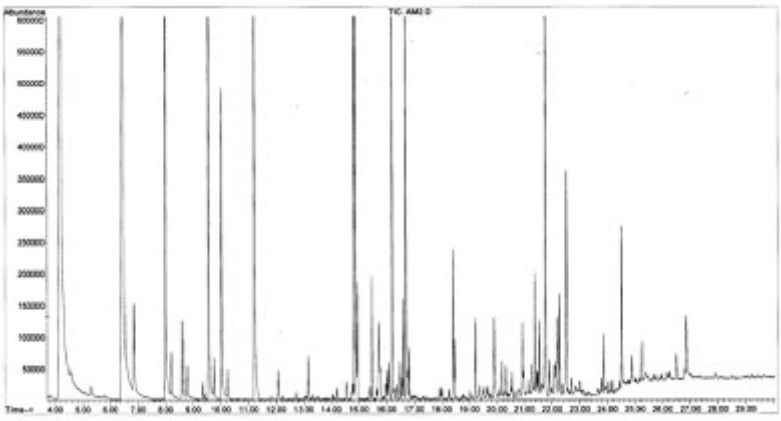

Fig. 1: Typical chromatogram of *Anthemis mauritiana* Maire & Sennen flowers essential oil obtained by hydrodistillation

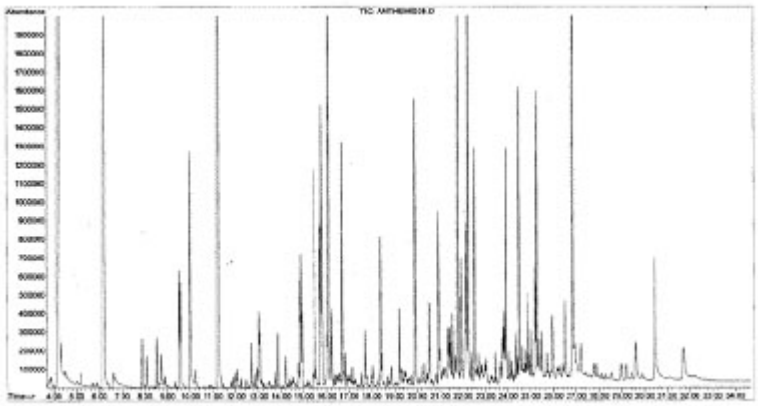

Fig. 2: Typical chromatogram of *Anthemis mauritiana* Maire & Sennen flowers essential oil extracted by micro-steam distillation

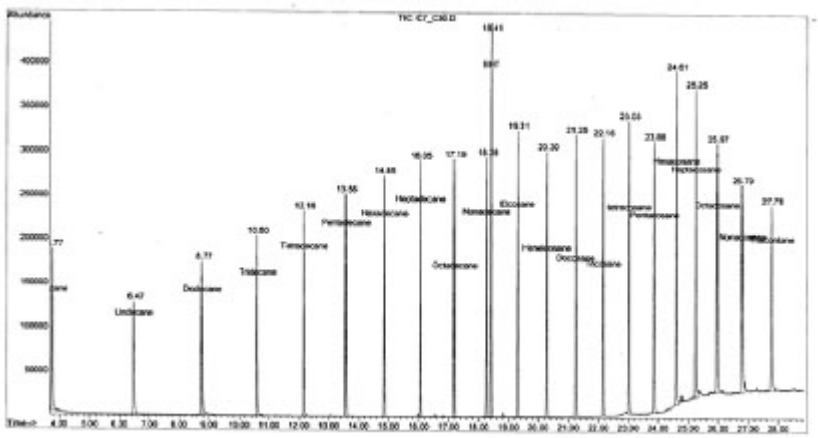

Fig. 3: Typical chromatogram of hydrocarbures mixture.

corresponding blank. Tests were carried out in triplicate.

RESULTS

The essential oil samples obtained by both methods were compared. In the case of hydrodistillation, 45 compounds were identified representing, the 90.84% of the total oil, while in the micro-steam distillation method, 49 compounds were identified representing 77.56% of the oil. The results show that the percentage of each compound depend of the extraction method. Some molecules are more abundant in the essential oil extracted by hydrodistillation than in the essential oil extracted by micro-steam distillation with respectively 28.14 % and 15.36 % for α-Pinene, 15.57 % and 7.81% for β-Pinene, 4.75 and 0.34% for β-Myrcene, 4.28 and 0.78% for γ-Terpinene, 5.76 and 3.27 % for α-Curcumene, 3.64 and 0.72% for β-Caryophylene, and 2.84 and 0.54% for D-Germacrene. On the other hand, some compounds are found most abundantly in the oil extracted by micro-steam distillation than in the oil extracted by hydrodistillation. For example, this is the case of lavandulol with 2.39% and 0.19%, sabinol acetate (acetyl) 6.04% and 0.65%, heptacosane 2.06% and 0.3% respectively. Some molecules are only found in essential oils extracted by micro-steam distillation such as octanoic acid and linoleic acid, others such as camphene and limonene are found only in the oils extracted by hydrodistillation. The chromatograms of the 2 samples and a mixture of hydrocarbons, used to calculate the kovats indexes are shown in Figs. 1, 2 and 3. The results are summarized in the Tables 1 and 2.

Table 3: Scavenging effect of *Anthemis mauritiana* Maire and Sennen flowers essential oil and BHT against DPPH radical

	10 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
AMEO (% inhibition)	9,04 ± 0,15	23,28 ± 0,24	31,50 ± 0,15	37,80 ± 0,24
BHT (% inhibition)	47,19±2,24	43,30 ± 0,43	44,71 ± 0,99	56,59 ± 0,43

Values are expressed as mean ± SEM of triplicates assays.

The EOAM exhibit a dose-dependent scavenging activity against DPPH activity. In fact, the essential oil yielded percentage scavenging activities of 9%, 23%, 31% and 37.8% at concentrations of 10, 25, 50 and 100 µg/ml respectively. It is noted that the Butylated Hydroxytoluene (BHT) used as a known synthetic antioxidant has more efficient scavenging activity than the EOAM with 56.59 ± 0.43 at 100 µg/ml (Table 3).

DISCUSSION AND CONCLUSION

The analysis of the essential oil obtained from flowers of *Anthemis mauritiana* by hydrodistillation allowed 45compounds to be identified representing,91% of the total oil, while by micro-steam distillation, 49 compounds were identified representing 78% of the oil.

It has been reported that the chemical composition of essential oils varies according to the climatic, seasonal, geographical, and geological conditions that reigns where the plant is collected[13-15]. Previous studies by GC-MS have shown that the composition of the essential oils of Anthemis differs from one species to another and also depends of the collection year[3].

Free radicals are the most common initiators of oxidative reactions[16]. When these radicals react with unsaturated fatty acids, the chain reaction of lipid oxidation starts and become a risk factor for development of many diseases especially atherosclerosis and related arterial complications[17]. Indeed, free radicals oxidize the LDL-cholesterol which is converted to oxidized LDL (ox-LDL) having a very atherogenic action[18]. Reactive radicals also

cause a deleterious action to the skin structure[19]. Natural antioxidants can scavenge and react with free radicals, and hence terminate the free radical reaction. The suppression of the oxidative effect of free radicals by anti-oxidants constitute one of the major targets of many therapeutic agents and the preferable strategy to prevent the cardiovascular diseases[20,21] and skin damages. Essential oil may help to provide protection against oxidative stress by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of the human body. In fact, many experimental investigations have demonstrated that a number of essential oils isolated from medicinal and aromatic plants possess a high anti-oxidant potential due to their radical scavenging activity and protect very efficiently against some cardiovascular and degenerative diseases[22,23]. Our experimental study has demonstrated that EOAM has an antioxidant power which may contribute to the potential capability of its compounds, added to foods or given therapeutically, to prevent oxidative stress and related events. Many investigators have proposed some mechanisms to explain the antioxidant activity of the natural antioxidants. Firstly, these compounds may directly scavenge free radicals and consequently break chain reaction of lipid peroxidation[12, 9]. Secondly, they may also chelate pro-oxidant metal ions such as iron and copper that stimulate free radical formation[12,9,24].

α-pinene and β-pinene are the major components of the studied oil. These compounds can be responsible for the radical scavenging effect observed. Our result is in accordance with the work of Wang[25] demonstrating that these components exhibited remarkable antioxidant activity in the DPPH test system. On the other hand, according to Bektas Tepe[26], γ-terpinene showed important antioxidant activity. However, the anti-radical effect of our oil can be also attributed to other minor compounds which can react individually or in synergy with major compounds. The radical scavenging effect of the studied oil can play a pivotal role in the prevention of oxidative stress and related diseases.

In conclusion, our results showed that the EOAM contains two major compounds, the α-pinene and β-pinene which are very probably responsible for the observed radical scavenging effect. More experimental researches must be designed to confirm this hypothesis. EOAM exhibits antioxidant activity and can be used as alternative medicine to prevent or treat oxidative stress and related complications.

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