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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL OF ANTHEMIS PEDUNCULATA AND ANTHEMIS PUNCTATA

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ABSTRACT

The essential oils from aerial parts of Anthemis pedunculata and Anthemis punctata (Asteraceae), obtained by hydrodistillation were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), and evaluated for in vitro antimicrobial activity. α-pinène was the major compound (25.8 %) in the essential oil of A.pedunculata. This oil is characterized by other important constituents as myrcene (8.0 %), trans-β-farnesene (7.8 %) and cadinene (3.0 %). A. pedunculata essential oil has a moderate activity on the E. coli ATCC 25922 (11 mm) and an important activity on S. aureus ATCC 25923 (45mm). The essential oil of A. punctata was composed in first rang by the β-pinène (19.5 %) and other constituent as the α-pinène (7.3 %), the bornéol (5.5 %), the l'anymol (5.4 %) and the 4-aminothiophénol (4.8 %). A. punctata essential oil has a moderate activity on the E. coli ATCC 25922 (11 mm) and an important activity on S. aureus ATCC 25923 (30mm).

Keywords: *Essential Oils, Antibacterial Activity, Asteraceae, Anthemis.*

1. INTRODUCTION

Essential oils are volatile, natural, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. They are known for their antiseptic, bactericidal, virucidal and fungicidal, and medicinal properties and their fragrance; they are used in preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and locally anesthesic remedies. Essential oils are extracted from various aromatic plants generally localized in temperate to warm countries like Mediterranean and tropical countries where they represent an important part of the traditional pharmacopoeia. They are liquid, volatile, limpid and rarely coloured, lipid soluble and soluble in organic solvents with a generally lower density than that of water. They can be synthesized by all plant organs, buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes [1].

The genus Anthemis, comprising 130 species, is a floral element of the Mediterranean [2].

The species of the Anthemis genus are widely used in the pharmaceutics, cosmetics and food industry. The flowers of the genus have well-documented use as antiseptic and healing herbs, the main components being natural flavonoids and essential oils. The antimicrobial activity of the essential oils and different extracts from several Anthemis species has been reported before [3].

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To the best of our knowledge, this is the first report on composition and antibacterial activity of the aerial parts of this species.

2. MATERIAL AND METHODS

The aerial parts of A. pedunculata and A. punctata were collected during the flowering period from Megress Mountain (Sétif) in May 2007 at 1720 m above sea level and were cut into small pieces and subjected to hydrodistillation by a Clevenger Type Apparatus.

The oil was analysed by GC on a Perkin-Elmer 8500 gas chromatograph with FID, fitted with a Supelcowax-10 fused silica capillary column (30 m x 0.32; film thickness, 0.25µm). The column temperature was programmed from 75 °C to 200 °C at a rate of 2.5 °C/min. The injector and detector temperatures were programmed at 230 °C and 300 °C, respectively. Helium was used as carrier gas at flow rate of 0.6 ml/min. The GC-MS analysis was carried out using two different GC-MS systems. The first was a Hewlett Packard 5973-6890 GC-MS operating on EI mode (equipped with a HP 5MS 30m x 0.25mm x 0.25µm film thickness capillary column). Helium (1ml/min) was used as carrier gas. Temperature program: the initial temperature of the column was 60 °C (for 5 min), then raised to 280°C within 3°C/min, and held there for 30 min (total time: 93.33min). The compounds were identified by comparison of their retention indexes (RI), retention times (RT) and mass spectra with those of authentic samples and/or the NIST/NBS, NIST02, Wiley 575 libraries spectra and the literature. The percentage composition of the essential oil is based on peak areas obtained without FID factor correction. The second GC-MS system analysis was a Finnegan trace GC ultra system, operating on EI mode and equipped with AT™ Aquawax 30m x 0,32mm x 0.25µm film thickness capillary column. Helium was used as the carrier gas, at a flow rate of 1,5 ml/min (constant flow) and Split ratio, 1:10. Temperature program: the initial temperature of the column was 60 °C (for 5 min), then raised to 235 °C within 3 °C/min, and held there for 30 min (total time: 93.33min). The MS details were as follows: source temperature, 200 °C; ionization energy, 70eV; emission, 200µA; mass range, 35-650Da; scan time,1,25s., scan rate (amu/s) 500,0; scans per second, 0,7974 [4].

The antibacterial activity of the oils was determined using the disc diffusion method, according to the National Committee of Clinical Laboratory Standards against three of the American Type Culture Collection (ATCC), namely: Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), and Staphylococcus aureus (ATCC 25923), which were obtained from the Microbiology and Parasitology Laboratory of Setif University Hospital [5]. The tests were performed using a 18 h culture growth at 37°C. The density of bacterial suspension was adjusted to a 0.5 McFarland turbidity standard (108 colony forming units [CFU]/ml). The cultures of these bacteria were grown in Mueller-Hinton Agar medium (Fluka). Sterile filter paper disks (Whatman) 6 mm in diameter containing 10 µL of each dilution of the oil (1/2, 1/5 and 1/10 v/v in absolute ethanol) were placed on the surface of the media. The plates were left for 30 min at room temperature to allow diffusion of the oil, and then they were incubated at 37 °C for 24 h. The results were recorded by measuring the zones of growth inhibition. In addition. All the experiments were performed in dublicate [6].

Positive controls were used in the assay as fosfomycin (FOS), gentamicin (GM), and

Amikacin (AN), negative controls (10 µL ethanol) were also included in the test.

3. RESULTS AND DISCUSSION

3.1. ESSENTIAL OILS ANALYSIS

The essential oil obtained by hydrodistillation of aerial parts of A. pedunculata was light green while the one of A. punctata had a bluish colour; both of essential oils had an odour similar to those of the flowers. The yields were 0, 10 % w/w for A. pedunculata and 0, 26% w/w for A. punctata. The chemical composition is summarized in Table 1 and table 2. The identified compounds represented 76, 5 % of all the compounds found in essential oils of A. pedunculata while 84 % of all the compounds of the essential oil of A. punctata were identified. The major compound in A. pedunculata oil included: the α-pinene (25.8 %), the myrcene (8.0 %), the tans-β-farnesen (7.8 %) and the β-pinene (5.7%) (Table 1). The identification of the compounds was based on the comparison of their mass spctra with those of authentic samples which are available at the laboratory.

The main compounds in A. punctata essential oil were the β-pinene (19.5 %), the tricyclene (9.6 %), the α-pinene (7.3 %), the borneol (5.5 %), the anymol (5.4 %) and the 4-aminothiophénol (4.8 %) (Table 2), which concord with the ulterior studies on Anthemis species as Anthemis xylopoda where the major compounds were borneol (31.80%), 1,8-cineole (5.45%) [3] and Anthemis aciphylla where the main compounds were α-pinene (49, 4 %) and terpinen-4 ol (21, 8%) [7].

However the major compounds found in the oil of Anthemis talyshensis were the α-Eudesmol (18.2%), the borneol (13.3%), the hexadecanoic acid (9.5%), the γ-eudesmol (8.6%) and the elemol (7.6%) [8]. The β-Thujone (33.7% and 19.7%, respectively) was found to be the major constituent in the leaf and flower oil of Anthemis altissima [9]. Our results differ also from those obtained by Rezaee [10], where the constituents of the essential oil of the flowers of Anthemis altissima L. var. altissima were spathulenol (18.7%), caryophyllene oxide (9.3%), 1-eicosene (7%) and sabinene (6.2%), while the leafs oil contained spathulenol (18.2%), caryophyllene oxide (9.5%), methyl hexadecanoate (8%) and isocaryophyllene (7.4%).

3.2. ANTIMICROBIAL ACTIVITY ASSAY

Both of the essential oils showed an antibacterial activity against E. coli ATCC 25922 and S. aureus ATCC 25923, while P. aeroginosa ATCC 27853 showed a total resistance. The diameters of inhibition zones showed that the antibacterial activity is inversely proportional to the dilution. (Table 3).

The activity of the two essential oils was higher on S. aureus than the one on E. coli.

The antimicrobial properties of the two Anthemis oils are suspected to be associated with the high terpenoids contents, which has been tested previously and found to have a significant antimicrobial activity [3]. The Gram-positive strains are more sensitive than the Gram-negative bacteria;. Antimicrobial screening of some of the main components of the essential oils demonstrate that α-pinene exhibits a weak activity [11]. Pseudomonas aeruginosa is resistant to both oils witch is reported in many studies by Rojas. [12]. Fifty two plant oils and extracts were investigated for activity against Pseudomonas aeruginosa which was inhibited by the lowest number of extracts (three) [13].

Pseudomonas aeruginosa is less susceptible to the antimicrobial properties of some essential oils than many bacteria and its tolerance is considered to be due to its outer membrane [14,15].

TABLE 1: CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF A. PEDUNCULATA

Chemical composition of the essential oil of A. pedunculata Pic	RT*	Compounds	RI**	%
1	3.70	benzène	-	0.4
2	3.81	cyclohexane	-	0.2
3	5.31	toluène	751	0.9
4	9.93	santolina triène	902	1.7
5	10.96	α-thujène	922	0.3
6	11.34	α-pinène	930	25.8
7	11.94	camphène	942	0.3
8	13.05	sabinène	964	0.3
9	13.29	β-pinène	969	5.7
10	13.71	nd***	977	0.2
11	13.92	myrcène	981	8.0
12	15.14	delta-3-carène	1005	0.8
13	15.36	benzene, 1 methyl-4 -1-methylethyl	1008	0.3
14	15.53	para cymène	1011	0.4
15	15.98	1, 8 cinéole	1018	1.4
16	16.09	limonène	1020	2.7
17	17.74	γ-terpinène	1047	0.5
18	19.80	nonanal	1081	0.5
19	19.91	α-terpinolene	1083	0.5
20	22.29	sabinol	1120	0.5
21	23.91	bornéol	1145	0.7
22	24.78	terpinène-4-ol	1158	1.0
23	25.47	α-terpinéol	1169	1.3
24	26.98	2 methyl-6-methylène 2-7-octadienal	1192	0.6
25	37.01	α-longipinène	1346	0.5
26	37.71	isopornyl propanate	1357	0.3
27	39.04	β-bourbonène	1378	0.2
28	40.33	bornyl ester of n-butanoic acid	1398	0.8
29	40.74	α-zingiberène	1404	0.5
30	41.13	trans β-farnesène	1411	7.8
31	43.11	α-humulène	1443	0.6
32	44.69	γ-cadinène	1469	3.0
33	45.61	bicyclogermacrène	1484	0.8
34	45.71	germacrène D	1486	0.5
35-53	/	nd***	/	29.1

* RT: retention time,
** RI: retention index
*** nd : not determined

TABLE 2: CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF A. PUNCTATA

Pic	RT*	Compounds	RI**	%
1	5.31	toluène	751	0.2
2	5.54	tricyclène	762	9.6
3	8.48	nd***	862	2.0
4	9.67	nd***	895	2.8
5	11.33	α-pinène	930	7.3
6	11.94	camphène	942	2.0
7	12.36	nd***	950	0.5
8	12.55	nd***	954	1.0
9	13.36	β-pinène	970	19.5
10	13.93	myrcène	981	3.2
11	15.99	1,8 cinéole	1019	1.6
12	16.10	limonène	1020	2.5
13	17.74	γ-terpinène	1047	0.5
14	19.91	α-terpinolène	1083	0.3
15	22.06	L-camphor	1117	2.2
16	23.32	3 cyclohexen-1-ol, 4-methyl 1 (1methylethyl)	1136	0.5
17	23.97	bornéol	1146	5.5
18	24.14	β-fenchyl alcohol	1149	0.7
19	24.78	terpinène-4-ol	1158	0.6
20	25.48	α-terpinéol	1169	0.7
21	38.59	α-copaène	1371	1.0
22	40.69	α-gurjunène	1404	0.7
23	40.85	trans β-farnesène	1406	2.8
24	41.12	dehydro-aromadendrène	1411	1.0
25	44.51	nd***	1466	1.2
26	44.69	germacrène D	1469	2.0
27	45.83	α-murolène	1488	0.6
28	46.57	cis γ-cadinène	1500	1.0
29	47.12	delta cadinène	1509	2.3
30	47.57	trans γ-bisabolène	1517	0.5
31	48.36	nd***	1531	2.2
32	48.51	4-aminothiophénol	1533	4.8
33	50.00	caryophellène oxide	1559	1.6
34	51.43	nd***	1584	0.8
35	51.75	α-ionone	1589	1.0
36	52.80	alloocimène	1608	1.5
37	52.86	nd***	1609	1.2
38	53.01	nd***	1612	2.0
39	53.37	nd***	1618	2.0
40	54.06	α-cadinol	1630	1.8
41	55.80	anymol	1662	5.4

* RT: retention time,
** RI: retention index
*** nd : not determined

TABLE 3: ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS OF A. PEDUNCULATA AND A. PUNCTATA AND THE INHIBITION ZONE FORMED BY STANDARD ANTIBIOTIC DISC

	Gram	Oils						Antibiotics		
		A. pedunculata			A. punctata					
		1/2	1/5	1/10	1/2	1/5	1/10	GM	AN	FOS
E. coli ATCC25922	-	11	09	07	11	7.5	07	23	34	28
S. aureus ATCC25923	+	45	36	29	30	27	23	23	34	28
P. aeruginosa ATCC27853	-	-	-	-	-	-	-	23	34	33

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المكونات الكيميائية والنشاط المضاد للبكتيريا للزيوت الطبيعية المشتقة من ANTHEMISPEDUNCULATA وANTHEMISPUNCTATA

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الملخص:

تم تحليل الزيوت العطرية من الأجزاء الهوائية لكل من Anthemis pedunculata و Anthemis pedunculataوالناتجه .من التقطير المائى بأستخدام كروماتوجرافى الغاز وكروماتوجرافى الغاز باستخدام طيف الكتلة، . وتم تقييمه كفترو للأثر المضاد الميكروبي بواسطة طريقة الانتشار على الوسط الصلب، تبين من التحليل الكيميائي للزيوت العطرية تميز زيوت A.pedunculata بوجود التربينات α -pinène (٢٥.٨٪). يتميز هذا الزيت بوجود المكونات الهامة الأخرى myrcene 8.0%، trans- β -farnesene 7.8%، و ٣.٠% cadinene . هذا الزيت لديه نشاط معتدل على E. coli ATCC ٢٥٩٢٢ (١١ملم)، ونشاطا هاما على S. aureus ATCC 25923 (٣٠ ملم)، اما بالنسبه لنوع A. Punctata يتميز هذا الزيت بوجود المكونات الهامة من β-pinène 19.5% ، والمكونات الأخرى مثل α-pinène ٧.٣% ، bornéol 5.5% ، l'anymol 5.4% ، و aminothiophénol 4.8% . هذا الزيت المنقط لديه نشاط معتدل على E. coli ATCC ٢٥٩٢٢ (١١ ملم) ونشاطا هاما على S. aureus ATCC 25923 (٣٠ ملم) .