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Short Communication

COMPARATIVE COMPOSITIONS AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OILS OF *ANTHEMIS NOBILIS* L. AND *ANTHEMIS MIXTA* L. (ASTERACEAE)

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ABSTRACT

Objective: To evaluate the chemical composition of essential oils from aerial parts of *Anthemis nobilis* (*An*) and *Anthemis mixta* L (*Am*). and investigate their antibacterial property.

Methods: The essential oils were isolated by hydro distillation and analyzed by GC and GC-MS. The disc diffusion and Agar dilution methods were used to screen the antibacterial activity against referenced and/or clinically isolated (HS) strains of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella enterica, Klebsiella pneumonia* and *Shighuelle sonnei.*

Results: The main constituents of *An* essential oil were 3-methyl-2-buten-1-yl cyclopropane carboxylate (29.3%), vinyl-2,2-dimethylbutanoate (24.2%), glycidyl methacrylate (9.9%), 2-methylbutyl-2-mehylbutyrate (9.1%), isobutyl isobutyrate (7.8%) and 3,3,4-trimethylhexane (6.2%), while *Am* essential oil was mainly characterized by *α*-thujone (51.8%), *β*-thujone (14.6%), borneol (7.3%) and 3-hexen-1-ol (4.9%). *An* and *Am* essential oils exhibited the best antibacterial activity against the following strains of *Escherichia coli* ATCC 25922 (25±1.20, 32±1.00 mm), *Pseudomonas aeruginosa* ATCC 27853 (23±0.87, 32±0.76 mm) and *Staphylococcus aureus* ATCC 43300 (21±1.44, 25±1.24 mm inhibition zone diameters, respectively). Minimum inhibitory concentration (MICs) values of these oils were ranged from 32-128µg/ml.

Conclusion: From this study, it can be concluded that the chemical composition of the essential oils was related to their antibacterial activity.

Keywords: *Anthemis nobilis* L., *Anthemis mixta* L., Chemical composition, Antibacterial activity

Medicinal plants have been used in developing countries as alternative treatments to health problems. Many plant extracts and essential oils isolated from plants have been shown to exert biological activities *in vitro* and *in vivo*, which justified research on traditional medicine focused on the characterization of antimicrobial activity of these plants [1]. China, Turkey, India, Brazil, Cuba, and Mexico are examples of countries that have a diverse flora and a rich tradition in the use of medicinal plants for both antibacterial and antifungal applications [1-5]. The genus *Anthemis* L. (tribe *Anthemideae* Cass.), is the second largest in the Asteraceae family consists of more than 210 species [6]. Previous investigations had been carried out to determine the chemical composition of the essential oils of plants of this genus. These studies led to further research into their pharmacological properties [7] and their possible connection with specific components.

The information concerning the *in vitro* antibacterial activity features and the composition of *Anthemis* essential oils from the Algerian flora has not been reported earlier. In continuation of our works on Asteraceae essential oils [8-13], we present here, for the first time, the GC and GC–MS analyses and the antibacterial activity of the essential oils from the aerial parts of *Anthemis nobilis* (*An*) and *Anthemis mixta* (*Am*).

An and *Am* were collected, respectively in June 2014 at the full flowering stage at Constantine-Algeria. Voucher samples (ZKLOST An06/14 and ZKLOST Am07/1, respectively) were deposited at the herbarium of the faculty of sciences, university of Mentouri-Constantine.

The hydro distillation of fresh aerial parts (200 g) of *An* and *Am*, for 3h in a Clevenger-type apparatus, according to the British Pharmacopeia [14], yielded yellow, good smell essential oils (0.67%, 0.45%, respectively) which were stored at 4 °C until tested and analyzed.

The composition of essential oils was determined by GC and GC-MS that was performed using an HP (Agilent technologies) 6800 plus chromatograph coupled with an HP (Agilent technologies) MSD 5973 selective detector, using an hp-INNOWAX column (30m×0.25 mm, film thickness 0.25 µm). The oven temperature was programmed at 120 °C for 2 min, then raised to 200 °C at 10 °C/min and held at this temperature, for 15 min, then raised to 240 °C for 2 min.

Helium was used as the carrier gas at a rate 0.5 ml/min. 0.1 µL oil was introduced directly into the source of the MS, *via* a transfer line (280 °C) with a split ratio of 1:50 and a linear velocity of 30.0 cm/sec. Ionization was obtained by electron impact (70eV, source temperature 230 °C, resolution 1000). The identification of the volatile constituents was accomplished by the visual interpretation, comparing their retention indices and mass spectra with literature data [15, 16].

The essential oils were individually used against a range of bacteria, namely *Escherichia coli* (*E. coli*) ATCC 25922, *Staphylococcus aureus* (*S. aureus*) ATCC 43300, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853, *Salmonella enterica* (HS), *Klebsiella pneumonia* (HS) *and Shighuelle sonnei* (HS). The reference strains were obtained from the Pasteur Institute (Algiers). The other strains (HS) were obtained from the laboratory of bacteriology, Benbadis Hospital, Constantine, using conventional methods. Susceptibility of the bacterial strains to the essential oil was investigated using the disk diffusion method and minimum inhibitory concentration (MIC) methods and by comparing their antibiogram inhibition zones to those reported by NCCLS [17].

Each oil was dissolved in ethanol to a final concentration of 512 µg/ml. This was serially diluted 2 fold with MH medium to obtain a concentration of 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 µg/ml. All experiments were performed in triplicate. The data were recorded as mean±standard error meaning (SEM). Significant differences between means were determined by student's-t test.

Comparison of the calculated retention indices (RI) and mass spectra with literature data [15, 16] together with authentic samples

of major components permitted us to identify the components of the studied essential oils (fig. 1A-1B, table 1). It appeared that *An* oil was mainly characterized by 3-methyl-2-buten-1-ylcyclopropane - carboxylate (29.3%), vinyl-2,2-dimethylbutanoate (24.2%), glycidyl methacrylate (9.9%), 2-methylbutyl-2-mehylbutyrate (9.1%), isobutyl isobutyrate (7.8%) and 3,3,4-trimethylhexane (6.2%), while the major components of *Am* essential oil were *α*-thujone (51.8%), *β*-thujone (14.6%), borneol (7.3%) and 3-hexen-1-ol (4.9%).

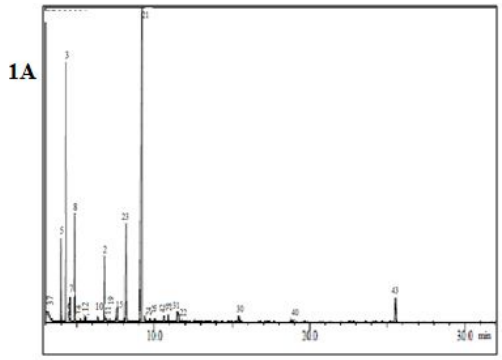

Fig. 1A: GC of *An* essential oil

The results showed that the composition of An essential oil was dominated by esters, and it was similar to the composition of the essential oils of *A. segetalis* from Montenegro [18] and *An* from Italy [19] but it was different with the exclusive content of 3-methyl-2-buten-1-ylcyclopropane-carboxylate and vinyl-2, 2-dimethyl butanoate, as main components. However, similarly to the present essential oil of *Am*, α-and β-thujone were mainly found in the essential oil of the turkish species *A. xylopoda* [20].

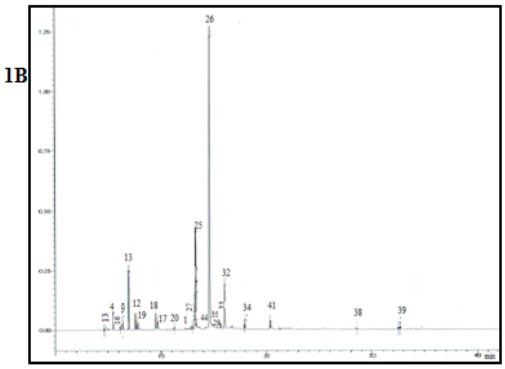

Fig. 1B: GC of *An* essential oil

Table 2 reported the inhibition zones of *Anthemis* essential oils against the tested microorganisms. The minimum inhibitory concentration (MICs) determination was performed by the serial dilution method. Ampicillin was used as a positive control in these tests. *An* and *Am* essential oils inhibited the growth of the referenced strongly and clinically isolated bacterial strains namely, *E. coli* ATCC 25922 (25±1.20, 32±1.00 mm), *P. aeruginosa* ATCC 27853 (23±0.87, 32±0.76 mm) and *S. aureus* ATCC 43300 (21±1.44, 25±1.24 mm inhibition zone diameters, respectively). MICs of the essential oils were also determined by an agar dilution method; the values were ranged from 32-128 µg/ml. However, *Am* essential was more effective compared to *An* oil.

The effectiveness of Am essential oil compared to An essential oil can be attributed to the high content of thujone which was known to be a powerful antibacterial agent [21].

Table 1: Chemical composition of *An* and *Am* essential oils

Compounds	%		
	RRI[a]	1[b]	2[c]
2,4-Hexadiene	637	-	0.3
3,3,4-Trimethylhexane	851	6.2	-
Vinyl-2,2-dimethylbutanoate	889	24.2	-
Santolinatriene	909	-	0.4
Isobutyl isobutyrate	925	7.8	-
Isobutylmethacrylate	928	0.9	-
α-Pinene	938	2.3	-
Glycidyl methacrylate	942	9.9	-
Camphene	954	-	1.8
Isobutylbutyrate	958	0.4	-
Allylvalerate	965	0.9	-
β-Pinene	979	0.2	2.1
3-Hexenol	1005	-	4.9
α-Methylbutylisobutyrate	1014	0.1	-
3-Methylbutylcyclopropanecarboxylate	1021	1.3	-
Isoamyl-2-methylbutyrate	1023	-	0.2
Santolina alcohol	1024	-	1.1
o-Cymene	1025	-	1.4
β-Phellandrene	1030	0.2	0.8
1,8-Cineole	1031	-	0.3
3-Methyl-2-buten-1-ylcyclopropanecarboxylate	1070	29.3	-
3-Methyl-2-buten-1-ylpivalate	1083	0.7	-
2-Methylbutyl-2-mehylbutyrate	1103	9.1	-
Nonanal	1104	0.2	-
β-Thujone	1114	-	14.6
α-Thujone	1122	-	51.8
p-Menth-2-enol	1125	-	0.4
trans-Pinocarveol	1139	0.7	-
Camphore	1146	-	0.2
Prenylsenecioate	1153	0.5	-
Pinocarvone	1165	0.9	-
Borneol	1169	-	7.3
cis-Isopuleone	1177	-	1.0

Terpinen-4-ol	1178	-	0.2
α-Terpineol	1189	-	0.3
2-Methylbutyl-2-mehylbutanoate	1190	0.3	-
Perhydro geraniol	1196	1.1	-
cis-3-Hexenylisovalerate	1235	-	1.2
Isobornylacetate	1289	-	0.3
Tridecane	1300	0.1	-
α-Terpinylacetate	1349	-	0.7
N-(tetrahydro-2-furanylmethyl) cyclopropane carboxamide	1449	0.7	-
β-Himachalene	1505	1.3	-
1,5-*Z*,7*E*-Dodecatriene	1571	-	0.3
Total		99.3	91.6

[a]Relative Retention Indices as determined on DB-5MS column, [b]*An* essential oil, [c]*Am* essential

Table 2: Inhibition zone diameters (IZD) and Minimum inhibitory concentration (MIC) (µg/ml) of *an* and *Am* essential oils against gram positive and gram negative bacteria

Microorganismes	1[a]		2[b]		3[c]	
	IZD (mm)	MIC (µg/ml)	IZD (mm)	MIC (µg/ml)	IZD (mm)	MIC (µg/ml)
E. coli ATCC 25922	25±1.20	32±1.00	32±1.15	32±2.00	18±1.50	8±0.40
S. aureus ATCC 43300	21±1.44	64±2.00	25±1.24	64±1.00	30±0.44	4±0.10
P. aeruginosa ATCC 27853	23±0.87	64±2.50	32±0.76	32±2.50	-	-
Salmonella enterica (HS)	14±1.31	128±1.50	14±0.80	128±1.50	-	-
Klebsiella pneumonia (HS)	16±1.04	128±2.00	16±0.64	128±1.00	14±1.12	32±0.40
Shighuelle sonnei (HS)	32±0.53	32±1.00	17±1.20	32±1.50	-	-

Values are mean±SD (n=3), [a]: *An* essential oil (128 µg/ml, [b]: *Am* essential oil (128 µg/ml), [c]: Ampicillin (30 µg/ml)

The composition and the *in vitro* antibacterial activity of the essential oils of Algerian *Anthemis* have not been reported earlier. Our results revealed that the essential oils of *An* and *Am* showed a good antibacterial activity against the tested bacteria. It is not possible to establish a relationship between oil composition and biological activity, due to the synergistic action between certain components.

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CONFLICT OF INTERESTS

Declare none

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