http://www.ejchem.net

ISSN: 0973-4945; CODEN ECJHAO
E-Journal of Chemistry
2012, **9(2)**, 796-800

Antibacterial Activity and Chemical Composition of Essential Oil of *Athamanta sicula* L. (Apiaceae) from Algeria

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Received 1 October 2011; Accepted 3 December 2011

Abstract: Essential oil extracted from fresh aerial parts of *Athamanta sicula* L. (syn. *Tingara sicula*) was analysed by gas phase chomatography coupled to mass spectrometry (GC-MS). The main constituents were: germacrene B (88.5%) and apiol (4.9%). Comparing with the tested bacteria, the growth of *Escherichia coli* and *Klebsiella pneumoniae* strains was more inhibited by the essential oil of *A. sicula*.

Keywords: *Athamanta sicula* L., Apiaceae, Essential oil, Antibacterial.

Introduction

The genus *Athamanta* L. (Apiaceae) comprises about nine species, which are distributed mainly in southeastern Europe and North-Africa. In Algeria, there is only one species, *Athamanta sicula* (Syn. *Tingara sicula*)[1]. In South-Italy, fresh roots of this plant are used as a diuretic and to dissolve kidney stones (rock splitters)[2,3]. Essential oil composition of *Athamanta* species have been reported[4-7]. In continuation of our works on Apiaceae essential oils[8-12], We report here the GC/MS analysis and the antibacterial activity of the essential oil of the Algerian *Athamanta sicula* L.

Experimental

Plant material

Athamantha sicula L. was collected at Didouche Mourad (Constantine - North Eastern Algeria) in June 2009. A voucher specimen was deposited at the herbarium of the

Laboratory of Therapeutic Substances, Faculty of Sciences, Mentouri-University, Constantine, Algeria (LOST ZKAK As/ 06/09).

Essential Oil extraction

The hydrodistillation of 200 g of fresh leaves of *Athamanta sicula* L. for 3 h using a Clevenger-type apparatus, yielded 1.2 % (w/w) of a yellowish oil which was stored until tested and analyzed.

Gas chromatography

GC analysis was performed on a Shimadzu GC17A gas chromatograph equipped with a split/splitless injector (250°C) and a flame ionization detector (250°C). Retention times for comparison with authentic compounds were measured using a cross-linked DB5-MS column (40 m × 0.18mm, film thickness 0.18 μm). The oven temperature was programmed as isothermal at 60°C for 5 min, then raised to 275°C at 5°C/min and held at this temperature for 5 min. Helium was used as the carrier gas at a rate of 1 ml/min. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A column without the use of correction factors.

Gas Chromatography-Mass spectrometry

GC/MS was performed using a Shimadzu QP5050 mass selective detector using a cross-linked DB5-MS column (40 m × 0.18mm, film thickness 0.18 μm). The oven temperature was programmed as isothermal at 60°C for 5 min, then raised to 275°C at 5°C/min and held at this temperature for 5 min. Helium was used as the carrier gas at a rate of 1 ml/min. 0.1 μl of the 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 (70 eV, source temperature 200°C, resolution 1000).

Identification of components

Essential oil components were identified based on their retention indices (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature[13,14] and with authentic compounds.

Antibacterial activity

Tested microrganisms

The essential oils were individually used against a range of bacteria, namely *Escherichia coli ATCC 25922*, *Escherichia coli, Staphylococcus aureus ATCC 25923, Staphylococcus aureus, Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa, Enterobacter aerogenes, Klebsiella pneumoniae,* and *Morganella morganii*. The reference strains were obtained from the Pasteur Institute (Algiers). The other strains were obtained from the laboratory of bacteriology, Benbadis Hospital, Constantine, using conventional methods (clinical isolation).

Table 1. The composition of the volatile oil of *Athamanta sicula* L. from Algeria.

Compound	Percentage	Retention index
α-Thujene	0.1	930
α-Pinene	0.7	939
Sabinene	0.2	975
Isobutyl isobutyrate	0.7	1004
α-Copaene	0.4	1377
Germacrene D	1.7	1485
β-Bisabolene	0.1	1506
γ-Cadinene	0.1	1514
Myrsticin	0.4	1519
β-Elemicin	0.1	1557
Germacrene B	88.5	1561
τ-Muurolol	0.1	1646
α-Cadinol	0.2	1654
Apiol	4.9	1678
Identified compounds (%)		**98.3**

Susceptibility tests

Susceptibility of the bacterial strains to the essential oil was investigated using the disk diffusion method and by comparing their antibiogram inhibition zones to those reported by the National Committee for Clinical Laboratory Standards (NCCLS). Disks containing freshly prepared essential oil were used for antibacterial activity assays. The diameters of inhibition zones were measured and compared with those suggested by NCCLS (sensitive P≥15 mm). The susceptibility of the strains to the essential oil was further evaluated by agar dilution method; different concentrations of the essential oil were included in Mueller-Hinton agar plates (sensitive MIC ≤ 32 lg/ml). The minimum inhibitory concentration (MIC) was defined as the concentration at which no colony was observed after incubation[15]. The agar plates were prepared and inoculated with bacterial suspension. After incubation at 37°C for 18–24 h, the inhibition zones were measured and averaged. The essays were performed in triplicate. MICs of the essential oils were also determined by an agar dilution method.

Table 2. Antibacterial activity of the essential oil of *Athamanta sicula* L.

Microorganism	Inhibition zone (mm)		MIC (μg/ml)	
	Ampicillin (10 μg/ml)	Oil (128 μg/ml)	Ampicillin (10 μg/ml)	Oil (128 μg/ml)
Escherichia coli ATCC 25922	18	18	10	16
Escherichia coli		15		32
Staphylococcus aureus ATCC 43300	30	18	5	16
Staphylococcus aureus		10		32
Pseudomonas aeruginosa ATCC 27853	-	15	-	16
Pseudomonas aeruginosa		13		16
Enterobacter aerogenes	-	18	-	16
Klebsiella pneumonia	14	20	32	16
Morganella morganii	-	15	-	16

Results and discussion

GC/MS analyses

The hydrodistillation yielded 1.1 % of a yellowish oil. 14 components were identified representing 98.3% of the essential oil with germacrene B (88.5%) and apiol (4.9%) as the major components (Table 1). The composition of our oil is different from the reported oils of the essential oil of leaves and fruits of *A. sicula* L. collected in the Madonie area of central Sicily[6] which was mainly represented by myristicin (97.6 and 41.3 %, respectively) as the main component and from the oil of fresh flowers, stems, and leaves of *A. sicula* growing at Palermo[7] (Italy) mainly represented by apiol (87.8, 85.2, and 80.4 % respectively). It's the first time that an *Athamanta* oil is mainly represented by germacrene B since the essential oils of the flowers and fruits of *A. haynaldii*[4] were found to contain myristicin (39 % ca. of the total oil) in addition to monoterpene and sesquiterpene compounds while essential oil aerial parts of *A. macedonica*[6] was mainly represented by sabinene (50.4%).

Antibacterial activity

The essential oil exhibited the best antibacterial activity against *Klebsiella pneumoniae, Escherichia coli ATCC 25922*, *Staphylococcus aureus ATCC 43300* and *Pseudomonas aeruginosa* ATCC 27853 with 20 mm, 18 mm, 18 mm, 18 mm inhibition zone diameters, respectively (Table 2).

Conclusion

Germacrene B (88.5%) and apiol (4.9%) were found to be the major components of the essential oil of *Athamantha sicula* L. This oil exhibited the best antibacterial activity against *Klebsiella pneumoniae, Escherichia coli ATCC 25922*, *Staphylococcus aureus ATCC 43300* and *Pseudomonas aeruginosa* ATCC 27853.

Acknowledgments

We are grateful to ANDRS (Algeria) and MESRS-DG/RSDT (Algeria) for financial support.

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