Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2012, 4 (6):1878-1882
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Antibacterial Activity and Chemical Composition of Essential Oils of *Inula viscosa* (L.) Ait. (Asteraceae) from Constantine, Algeria

Berhail Boudouda H[1], Benmerache A[1], Chibani S[1], Kabouche A[1], Abuhamdah S[2], Semra Z[1,3], Kabouche Z[1*]

[1]*Université de Constantine 1, Département de chimie, Laboratoire d'Obtention de Substances Thérapeutiques (LOST), 25000 Constantine, Algeria.*
[2] *Natural Products Laboratory Room 253, chemistry department, university of Jordan, Amman 11942, Jordan*
[3]*CHUC Benbadis-Constantine, service de Bactériologie, 25000 Constantine, Algeria*

ABSTRACT

The hydrodistilled essential oils of fresh aerial parts of I. viscosa (L.) Ait., collected at Ain El-Bey (Southern Constantine), was characterized by nerolidol (25.3%), isocostic acid (10.1%), costic acid (8.0%), neo-intermedeol (6.4%) and caryophyllene oxide (5.5%), as major components. The essential oil of hydrodistilled fresh aerial parts of I. viscosa (L.) Ait., collected at Hamma-Bouziane (Northern Constantine), was mainly represented by isocostic acid (25.1%), costic acid (15.2%), nerolidol (9.6%), linoleic acid (9.1%), neo-intermedeol (7.5%) and fokienol (7.2%). The present compositions are quite different from those reported for species growing over the world. The essential oil of the plant collected at Ain El-Bey inhibited considerably the growth of K. pneumoniae (24mm), P. aeruginosa ATCC (21mm), P. aeruginosa HS (21mm), E. coli ATCC (20mm) and E. coli HS (20mm) while the essential oil of the species growing at Hamma Bouziane exhibited a good antibacterial activity against P. aeruginosa ATCC (21mm), P. aeruginosa HS (21mm) and K. pneumoniae (20mm).

Keywords: *Inula viscosa* (L.) Ait., Asteraceae, Essential oil, Antibacterial activity

---

INTRODUCTION

Inula viscosa (L.) Ait. (Asteraceae) (syn. *Dittrichia viscosa)*, locally called *magramen*, is a herbaceous perennial plant widely distributed in the Mediterranean area. It's used, in North African folk medicine, to treat diabetes and inflammations [1]. Sesquiterpene acids and various biological properties have been reported from extracts of powdered dried aerial parts of *Inula viscosa* (L.) Ait [2, 8]. The composition of essential oils of *I. viscosa* (L.) Ait, growing in different countries of the Mediterranean area, have been investigated [9, 17]. In continuation of our works on Asteraceae [18, 28], we report here the GC/MS analyses and antibacterial activity of the essential oils of the Algerian species *I. viscosa* (L.) Ait [29] growing in Hamma-Bouziane (Northern Constantine) and Ain El-Bey (Southern Constantine).

MATERIALS AND METHODS

Plant material

Fresh aerial parts of *I. viscosa* (L.) Ait., growing in Hamma-Bouziane (Northern Constantine) and Ain El-Bey (Southern Constantine) were collected in November 2011. Voucher specimen were deposited at the herbarium of Mentouri-University, Constantine, Algeria (LOST Iv ham/11/11 and LOST Iv ain/11/11, respectively).

Extraction

The hydrodistillation of fresh aerial parts (200 g) of *I. viscosa* (L.) Ait; growing in Hamma-Bouziane (Northern Constantine) and Ain El-Bey (Southern Constantine) for 3 h in a Clevenger-type apparatus, according to the British Pharmacopeia, yielded 8.0 and 10.0 % (w/w) of yellow essential oils, respectively.

Gas chromatography: GC analysis was performed on a Varian CP3800 gas chromatograph equipped with a cross-linked VF5-MS column (30 m × 0.25 mm, film thickness 0.25 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.

Gas Chromatography-Mass spectrometry

GC/MS was performed using a Saturn 2200 mass selective detector. Operating conditions were the same as for the analytical GC. The MS operating parameters were as follows: 0.1 µL of crude oil was mixed with diethyl ether (40%); ionization potential, 70 ev; ionization current, 2 A; ion source temperature, 200ºC; resolution, 1000. scan time, 5 s; scan mass range, 40–400 u; split ratio, 1:50; linear velocity, 30.0 cm/sec. Relative percentage amounts were calculated from peak area without the use of correction factors.

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 [30, 31] and with authentic compounds.

Antibacterial activity

Tested microorganisms: 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*, *Proteus mirabilis* and *Streptococcus a-hemolytic*. The reference strains were obtained from the Pasteur Institute (Algiers). The other strains from hospital (HS) were obtained from the laboratory of bacteriology, Benbadis Hospital, Constantine, using conventional methods (clinical isolation).

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 [32]. 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.

RESULTS AND DISCUSSION

GC/MS analyses: The yellowish hydrodistilled essential of fresh aerial parts *I. viscosa* (L.) Ait., collected at Ain El-Bey (Southern Constantine), was obtained with 10% yield and characterized by 34 compounds, representing 85.2% of the essential oil with nerolidol (25.3%), isocostic acid (10.1%), costic acid (8.0%), *neo*-intermedeol (6.4%) and caryophyllene oxide (5.5%) as major components (Table 1). Hydrodistillation of fresh aerial parts of *I. viscosa* (L.) Ait. collected at Hamma-Bouziane (Northern Constantine) furnished 8% of a yellowish essential oil. 23 compounds, representing 84.5% of the essential oil were identified. Table 1 shows the percentage composition of this oil mainly represented by isocostic Acid (25.1%), costic acid (15.2%), nerolidol (9.6%), linoleic acid (9.1%), *neo*-intermedeol (7.5%) and fokienol (7.2%). From these analyses, it appeared that nerolidol was more abundant in the essential oil of the plant collected at Ain El-Bey (Southern Constantine) while costic acid, isocostic acid, linoleic acid, *neo*-intermedeol and fokienol were found in higher percentages in the essential oil of the plant collected at Hamma-Bouziane (Northern Constantine). Caryophyllene oxide was found to be a main component only in the essential oil of Ain El-Bey's plant. It's the first time that isocostic and costic acids are found to be major components of essential oil of *Inula* species. These sesquiterpene acids have been isolated from the hydroalcoholic extract of the Turkish powdered dried aerial parts of *Inula viscosa* (L.) Ait [7]. Fokienol (38.8, 8.6%) and nerolidol (7.6, 2.6%) have also been detected in reported essential oils of *I. viscosa* (L.) Ait., growing in Spain [11] and France [9], respectively. The present compositions are different from those reported from the essentials of the leaves of the Algerian species *I. viscosa* growing at Sidi Rezine village (South of Algiers) and extracted by two methods (hydrodistillation and steam distillation) which were mainly represented by 12-carboxyeudesma-3,11(13)-diene (28.9 and 56.8%,

respectively) [15]. The hydrodistilled oil from Sidi Rezine was also characterized by the main presence of linoleic acid (7.8%), mainly found in the present essential oil *I. viscosa* collected at Hamma-Bouziane (9.1%).

Table 1. Chemical composition of *I. viscosa* (L.) Ait. essential oils

N°	Compounds [a]	RI [b]	Percentage composition [c]	Percentage composition [d]
1	2-Methylpentanene-2-thiol	862	1.5	-
2	α-Terpinene	913	0.1	-
3	Fenchyl alcohol	1122	0.1	-
4	Isomenthone	1159	0.3	-
5	Borneol	1180	1.6	-
6	Dihydroedulan II	1284	0.1	-
7	Dihydroedulan I	1286	0.9	-
8	Bornyl acetate	1289	0.9	-
9	Theaspirane B	1302	0.1	-
10	α-Ionone	1408	0.5	-
11	Isocaryophyllene	1421	-	0.1
12	Aromadendrene	1441	0.4	-
13	Cabreuva oxide B	1466	0.1	-
14	β-Selinene	1489	-	0.1
15	α-Selinene	1498	0.4	-
16	γ-Cadinene	1514	-	0.1
17	δ-Cadinene	1523	-	0.1
18	Nerolidol	1531	25.3	9.6
19	Selena-3,7(11)-diene	1540	-	0.1
20	*cis*-Dracunculifoliol	1541	-	0.9
21	Spathulenol	1578	0.2	-
22	β-Humulene	1579	-	2.2
23	Caryophyllene oxide	1583	5.5	0.1
24	α-Copaen-11-ol	1588	0.6	-
25	Viridiflorol	1590	0.2	-
26	Fokienol	1596	4.4	7.2
27	Humulene epoxide II	1604	0.3	0.4
28	*trans*-Longipinocarveol	1618	-	0.1
29	Epiglobulol	1629	0.1	-
30	τ-Cadinol	1640	-	0.1
31	Alloaromadendrene oxide	1641	0.2	-
32	Cubenol	1647	1.0	-
33	α-Eudesmol	1654	-	0.9
34	*neo*-Intermedeol	1658	6.4	7.5
35	Davanol acetate	1689	2.0	-
36	*cis*-Lanceol	1760	-	0.4
37	6,10,14-Ttrimethyl pentadecan-2-one	1846	1.1	-
38	Isocostic acid	1925	10.1	25.1
39	Costic acid	1930	8.0	15.2
40	Linoleic acid	2105	3.1	9.1
41	9-Hexadecenoic acid	2144	1.8	-
42	Tricosane	2300	1.1	1.0
43	Tetracosane	2400	2.2	1.9
43	Pentacosane	2500	2.1	0.4
44	Hexacosane	2600	2.3	1.9
	Identified compounds	**Total**	**85.2**	**84.5**

[a]Compounds listed in order of their RI
[b]RI (retention index) measured relative to n-alkanes (C6-C24) using VF-5MS column
[c] Essential oil of fresh aerial parts of I. viscosa collected at Ain El-Bey (Southern Constantine)
[d]Essential oil of fresh aerial parts of I. viscosa collected at Hamma Bouziane (Northern Constantine)

Table 2. Antibacterial activity of the essential oils of *Inula viscosa* (L.) Ait.

Microorganism	Inhibition zone (mm)		MIC (µg/ml)	
	Oil[c]	Oil[d]	Oil[c]	Oil[d]
Escherichia coli ATCC 25922	18	16	40	40
Escherichia coli	20	13	40	80
Staphylococcus aureus ATCC 43300	21	10	40	80
Staphylococcus aureus	11	09	80	80
Streptococcus α-hemolitic	15	15	80	80
Pseudomonas aeruginosa ATCC 27853	21	21	20	20
Pseudomonas aeruginosa	21	15	20	20
Enterobacter aerogenes	18	16.5	20	80
Klebsiella pneumonia	24	20	32	40
Proteus mirabilis	19	16	-	40

[c] Essential oil (128□g/ml) of fresh aerial parts of I. viscosa collected at Ain El-Bey (Southern Constantine)
[d] Essential oil (128□g/ml) of fresh aerial parts of I. viscosa collected at Hamma Bouziane (Northern Constantine).

Antibaterial activity: The essential oil of the plant collected at Ain El-bey inhibited considerably the growth *K. pneumoniae* (24mm), *P. aeruginosa* ATCC (21mm), *P. aeruginosa* HS (21mm), *E. coli* ATCC (20mm) and E. coli HS (20mm) while the essential oil of the species growing at Hamma Bouziane exhibited a good antibacterial activity against *P. aeruginosa* ATCC (21mm), *P. aeruginosa* HS (21mm) and *K. pneumoniae* (20mm). It seems that the first oil is more antibacterial againt a larger number of microorganisms. That may be explained by the difference of the compositions of the tested oils (Table 2).

CONCLUSION

The hydrodistilled essential oils of fresh aerial parts of *I. viscosa* (L.) Ait., collected at Ain El-Bey (Southern Constantine), was mainly represented by nerolidol, isocostic acid, costic acid, *neo*-intermedeol and caryophyllene oxide. The essential oil of hydrodistilled fresh aerial parts of *I. viscosa* (L.) Ait., collected at Hamma-Bouziane (Northern Constantine), was characterized by the major components namely, isocostic acid, costic acid, nerolidol, linoleic acid, *neo*-intermedeol and fokienol. From this study, isocostic and costic acids and *neo*-intermedeol seem to be exclusive to the present essential oils of the plant growing in Northern and Southern Constantine while caryophyllene oxide was found to be exclusively a main component of the plant collected at Ain El-Bey (Southern Constantine). The latter exhibited a considerable antibacterial activity against a larger number of microorganisms. That should be due to the difference of the compositions of the present essential oils which are also different from the previously reported compositions of the oils obtained from the species collected from Algiers province or from other countries; which may be explained by the influence of the soil nature and of the environment.

Acknowledgments
We are grateful to the ANDRS (Algeria) and MESRS-DG/RSDT (Algeria) for financial support and to Mr. Abaza (Amman University, Jordan) for technical help.

REFERENCES

[1] Bellakhdar J. Pharmacopée marocaine. Ibis press, France, **1997**.
[2] Abu Zarga M.H, Hamed E.M, Sabri S.S, Voelter W, Zeller K.P. *Journal of Natural Products*, **1998**, 61, 798-800.
[3] Abu Zarga MH, Sabri SS, Hamed E.M, Khanfar MA, Zeller KP, Atta-Ur- Rahman. *Natural Product Research*, **2002**, 17(2), 99-102.
[4] Alarcon De La Lastra C, Lopez A, Motilva V. *Planta medica*, **1993**, 59, 497-501.
[5] Maoz M, Meeman I. *Journal of Ethnopharmacology*, **2000**, 3, 479-482.
[6] Susplugas C, Balansard G, Julien J. *Planta medica*, **1979**, 36, 223-252.
[7] Ulubelen A, Goren S. *Phytochemistry*, **1986**, 26, 1223-1224.
[8] Zeggwagh N.A, Ouahidi M.L, Lemhadri A, Eddouks M. *Journal of Ethnopharmacology*, **2006**, 108, 223–227.
[9] Blanc MC, Bradesi P, Goncalves MJ, Salgueiro L, Casanova J. *Flavour and Fragrance Journal*, **2006**, 21, 324–332.
[10] Cafarchia C, De Laurentis N, Milillo MA, Losacco V, Puccini V. *Parasitologia*, **2002**, 44, 153-156.
[11] Camacho A, Fernandez A, Fernandez C, Altarejos J, Laurent R. *Greuter. Rivista Italiana*, **2000**, 29, 3–8.
[12] Chahboun J, Casabianca , Jeannot V. *Rivista Italiana EPPOS*, **1998**, 701-707.
[13] Chiarlo B. *Chimica Farmatsia*, **1968**, 107(6), 370–382.
[14] De Laurentis N, Losacco V, Milillo MA. *Delpinoa n.s*, **2002**, 44, 115–119.
[15] Haoui I E, Derriche R, Madani L, Oukali Z. *Arabian Journal of Chemistry*, **2012**, in press, online.
[16] Perez-Alonso M.J, Velasco-Negueruela A. *Flavour and Fragrance Journal*, **1996**, 11, 349–351.
[17] Rovesti P. 1930. *Rivista Italiana essenze e profumi*, **1963**, 12, 202-6.
[18] Nacer A, Bernard A, Boustie J, Touzani R Kabouche Z. *Chemistry of Natural Compounds*, **2007**, 42(2), 230-231.
[19] Benkiki N, Kabouche Z, Bruneau C. *Chemistry of Natural Compounds*, **2007**, 43(5), 612-613.
[120] Bensouici C, Kabouche A, Kabouche Z. *Chemistry of Natural Compounds*, **2012**, 48(3), 510-511.
[21] Boutaghane N, Kabouche A, El-Azzouny AM, Kabouche Z. *Chemistry of Natural Compounds*, **2008**, 44(6), 817-818.
[22] Boutaghane N, Kabouche A, Touzani R, Maklad YA, El-Azzouny AM., Bruneau C, Kabouche Z. *Natural Product Communications*, **2011**, 6(2), 251-252.
[23] Gherboudj O, Benkiki N, Seguin E, Tillequin F, Kabouche Z. *Chemistry of Natural Compounds*, **2012**, 48(3), 470-471.
[24] Kabouche A, Kabouche Z, Touzani R, Bruneau C. *Chemistry of Natural Coumpounds*, **2010**, 45(6), 966-967.
[25] Khalfallah A, Berrehal D, Kabouche A, Kabouche Z. *Chemistry of Natural Compounds*, **2012**, 48(3), 482-483.
[26] Lakhal H, Kabouche A, Kabouche Z. *Chemistry of Natural Coumpounds*, **2011**, 46(6), 964-965.

[27] Mokaddem-Daroui H, Touafek O, Kabouche A, Kabouche Z, Calliste CA, Duroux JL. *Chemistry of Natural Compounds*, **2012**, 48(3), 498-499.
[28] Nacer A, Merza J, Kabouche Z, Rhouati S, Boustie J, Richomme P. *Biochemical Systematics and Ecology*, **2012**, 43, 163-165.
[29] Quezel P, Santa S. Nouvelle Flore de l'Algérie et des Régions Désertiques Méridionales. C.N.R.S., Paris, France, **1963**.
[30] Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, Allured Publishing Co. Carol Stream, Illinois, , **2007**, 4th Ed.
[31] Mc Lafferty FW, Stauffer DB. The Important Peak Index of the Registry of New York. Mass Spectral Data. John Wiley & Son, **1991**.
[32] NCCLS. Performance standards for antimicrobial disk susceptibilities tests; Villionova, PA, USA: Approch Standard NCCLS: Publication M2-A5, **1993**.