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Chemical investigations of volatile constituents of *Inula viscosa* (L.) Aiton (Asteraceae) from different areas of Apulia, Southern Italy

Nicolino De Laurentis[1], Vincenzo Losacco[1], Maria Antonietta Milillo[2], Olimpia Lai[2]

[1]*Department of Medicinal Chemistry, Faculty of Pharmacy, University of Bari, Via Orabona 4, I-70100 Bari, Italy;* [2]*Department of Animal Health and Welfare, Faculty of Veterinary Medicine, University of Bari, S.P. Casamassima km 3, I-70010 Valenzano (BA), Italy.*

Riassunto. Inula viscosa *(L.) Aiton è diffusa nell'Europa Meridionale ed è usata nella medicina popolare nell'area mediterranea. Gli autori indagano sulla composizione chimica degli olii essenziali di questa pianta erbacea perenne proveniente da 4 diverse località della Puglia.*

Abstract. Inula viscosa *(L.) Aiton is found in Southern Europe and used in folk medicine in the Mediterranean area. The authors investigated the chemical composition of essential oils of this herbaceous perennial plant from four different areas in Apulia.*

Key words: *Essential oil,* Inula viscosa *(L.) Aiton*

Introduction

Inula viscosa (L.) Aiton (Tribe Inulae, Asteraceae) is a herbaceous perennial plant, widespread in Southern Europe (Ball, 1976). It is common on road edges in the Iberian Peninsula (Rigual Magallòn, 1972) and in the Italian Levante Region (Imbesi, 1964). It is used in folk medicine in the Mediterranean area to treat injuries, sprains, and bruises (Fernandez-Ocana *et al*., 1996), and to combat intestinal disorders and jaundice (Lopez, 1982; Font Quer, 1978).

Previous researches on *Inula viscosa* (=*Drittrichia viscosa* (L) W. Greuter) have revealed in this species the presence of flavonoids (Grande *et al.*, 1985; Wollenweber *et al.*, 1991; Benayache *et al.*, 1991), sesquiterpenes (Ceccherelli *et al.*, 1985; Bohlmann & Guptar, 1982), triterpenes (Oksuz, 1976) and essential oil (Chamaco *et al.*, 2000).

With the first work on essential oil (Chiarlo, 1968), seven azulenes were separed by gas-chromathography from a high-boiling fraction of the essential oil. The major component (49%) was identified as 1,4-dimethylazulene, followed by chamazulene (32%). The presence of azulenes supported the antiphlogistic properties ascribed to the plant by the folk medical tradition in a previous pharmacological investigation (Susplugas *et al.*, 1980).

Antimicrobial and antifungal activity of ethanolic extracts of this plant were also investigated (De Laurentis *et al.*, 2000; Cafarchia *et al.*, 1999).

The aim of this work is to investigate the chemical composition of essential oil of *I. viscosa* collected from four different areas in Apulia (South Italy).

Materials and methods

Plant Material

Aerial parts of wild *I. viscosa* in the flowering stage were collected in October 1999 from four different areas of Apulia:

Sample A. Martina (Taranto province)
Sample B. Putignano (Bari province),
Sample C. Bari
Sample D. Otranto (Lecce province).

Voucher specimens have been deposited in the Herbarium at the Department of Botany, Faculty of Science, University of Bari, Italy.

Isolation of the essential oil

Fresh samples of leaves and flowers (1 kg) were hydrodistilled for 3 h using a modified Clevenger-type apparatus (Perez-Alonso *et al.*, 1996). The oils obtained were recovered with hexane, dried over anhydrous sodium sulfate, concentrated with nitrogen and stored at -4 °C until analysis.

The essential oil of sample C (300 mg) of leaves was chromatographed over silica gel 60 (230-400 mesh, Merck), using hexane, followed by hexane-ethyl acetate mixtures of increasing polarity (0-40% ethyl acetate). Thirty fractions were taken and combined based on TLC monitoring, with sampling of 10 fractions for each analysis. The chemical composition of the fraction was identified by GC/MS.

12-carboxyeudesma-3,11(13)-diene was isolated in its pure state (65 mg). 1H-NMR, 13C-NMR and MS data of this compound were identical to values form literature (Perez-Alonso, 1996)

100 g of flowers of sample C were set in a glass column and extracted by percolation with dichloromethane for 1 h. 100 ml of extract were recovered and evaporated to dryness under vacuum in a rotavapor.

300 g of fresh flowers of sample A were set in a glass column and extracted by percolation with dichloromethane for 1 h and 600 ml of extract were recovered and evaporated to dryness under vacuum in a rotavapor.

The residue was redissolved in methanol/water (8:2) and extracted with hexane to remove chlorophyll. The aqueous phase was evaporated under vacuum.

The yellowish column residue (85 g) was dissolved in methanol and chromatographed on a silica gel column using chloroform/methanol (9:1) as eluent. The fractions containing cycloheptafuranone were recovered and purified by preparative TLC with petroleum ether/ethyl acetate (6:4). The chemical composition of the fraction was investigated by GC/MS. Pure tomentosin (45 mg) was isolated and confirmed by 1H-NMR, 13C-NMR and MS.

Quantitative and qualitative analyses

Quantitative and quantitative analyses (area %) were performed by Capillary GC-MS, Hewlett Packard 6890-5973 MSD controlled by chemstation software and equipped with an HP 6890 Series Injector autosampler. Capillary column: HP5MS 30 m x 0.25 mm i.d.x 0.23 µm film thickness phase of 5% phenylmethylsilaxane. The amount injected was 1 µl. Injector: 250 °C, carrier gas helium 1 ml/min constant flow; split ratio 60:1. Oven temperature program: 4 min at 40 °C, then 4 °C/min to 280 °C and additional 60 min at 280 °C. Mass spectra were acquisition at 70 eV in scan mode from 40 to 550 u.m.a.; source 230 °C, quadrup. 150 °C.

Components were identified by comparison with the computerised NIST 98 software from the NIST Library, by their relative retention times and by comparison with authentic samples and literature.

Results and Discussion

Steam distillation of leaves and flowers of *I. viscosa* yielded light yellow-coloured

essential oils (0,35% for leaves and 0,37% for flowers) whose qualitative and quantitative determination is given in Tab. 1. Components are listed in order of elution from a SE-30 fused-silica capillary column and were identified by means of GC/MS by comparison of both their fragmentation patterns and retention indices with those of authentic samples. Examination of the oils indicated the presence of more than 80 components, 52 of which were identified, accounting for ca. 80% of the oil.

Samples A, B, C, and D constituted a complex mixture of 45, 48 , 41, 44 compounds for leaves and 43, 45, 49, 50 for flowers respectively. The compounds identified by GC/MS represented 76%, 79%, 78%, 77% for leaves and 84%, 80%, 81%, 73% for flowers of the total oils.

Tab. 1 shows the compounds listed in order of elution.

All samples were characterised by a very high content of 12-carboxyeudesma-3,11(13)-diene. This constituted 44% of sample A, 53% of B, 62% of C, 56% of D in the leaves and 20% of A, 42% of B, 48% of C, and 27% of D in the flowers.

The results of our analysis revealed that flower extracts contain larger amounts of monoterpenes, sesquitepene hydrocarbons, oxygenated aromatic compounds and fatty acid esters. Leaf extracts contained oxygenated sesquiterpene compounds to a greater extent.

Sample A contained eucalyptol (10%), trans-nerolidol (7%), hexahydrofarnesyl acetone (5%) in the flowers and selina α–6-en-4-ol (7%), caryophyllene oxide (3%), α–eudesmol (3%) in the leaves.

Sample B contained eugenol (7%), α-terpineol (4%), nerolidol (3%) in the flowers, and selina-6-en-4-ol (3%), α-caryophyllen-5-ol (3%), α-eudesmol (2%) in the leaves.

Sample C contained α-terpineol (3%), nerolidol (2%), eucalyptol (2%) in the flowers and selina-6-en-4-ol (3%), nerolidol (2%), tomentosin (2%) in the leaves.

Sample D contained ethyl palmitate (6%), ethyl linoleate (4%), nerolidol (3%), eucalyptol (2%), α-caryophyllen-5-ol (2%) in the flowers and selina-6-en-4-ol (2%), α-caryophyllen-5-ol (2%), α-eudesmol (1%) in the leaves.

It is notable that sample A (from Martina Franca) and sample B (from Putignano), both collected in mountainous areas, have a high percentage of monoterpene compounds, such as eucalyptol (sample A) and terpineol (sample B), oxygenated sesquiterpenes such as nerolidol and hexahydrofarnesyl acetone (sample A) and oxygenated aromatic compounds such as eugenol (sample B). The samples close to the sea (Bari, sample C, and Otranto, sample D) demonstrated a higher content of oxygenated sesquiterpenes: 12-carboxyeudesma-3,11(13)-diene (60%) in sample C and fatty acid esters (10%) in sample D.

In conclusion, the major constituent of essential oils of *Inula viscosa* from the Apulia region is an oxygenated carboxylated bicyclic sesquiterpene (12-carboxyeudesma-3,11-diene). This contrasts with the composition reported for *I. viscosa* oil from Turkey, where the main component was a monoterpene alcohol (borneol, 38%) (Perez-Alonso *et al.*, 1996), and from Spain, where the main components was an allylic tertiary alcohol (fokienol) (Chamaco *et al.*, 2000).

Tab. 1- Essential oils of leaves and flowers of *Inula viscosa* Aiton

		Leaves				Flowers			
RT	Components	A	B	C	D	A	B	C	D
	Monoterpenes								
9,50	α-Pinene	-	-	-	-	0,29	-	0,10	-
11,10	ß-Phellandrene	-	-	-	-	0,75	-	0,10	-
13,35	Eucalyptol	0,11	0,12	0,15	-	9,61	0,32	2,31	2,27
16,14	ß Linalool	t	0,17	t	0,14	1,37	0,89	0,74	0,60
17,99	Nonanal	0,91	0,53	-	0,33	0,50	0,45	0,85	0,99
18,94	l-Terpineol	0,15	0,14	0,14	0,27	1,76	2,00	1,28	0,74
19,44	(+) α-Terpineol	t	0,34	0,10	0,32	3,10	3,86	2,57	1,74
20,78	2-Bornene	-	t	-	-	-	0,10	0,13	0,12
21,93	α-Ionone	0,17	0,41	-	0,30	0,21	0,14	0,12	0,14
23,02	Edulan I dihydro	0,23	0,39	0,17	0,23	0,75	0,25	0,49	0,83
24,97	Dehydro α-ionene	-	0,12	0,10	0,14	0,86	0,37	0,10	0,47
25,19	Eugenol	t	0,11	-	0,14	-	7,27	0,43	1,21
25,75	Copaene / α-cubebene	0,11	0,15	t	t	0,58	0,13	0,22	0,54
26,05	E ß-Damascenone	t	0,17	-	0,16	0,20	0,16	0,14	0,21
26,74	Methyl eugenol	0,11	0,18	-	0,16	-	0,16	0,12	0,30
27,11	ß-Caryophyllene	0,67	0,54	0,27	0,15	1,45	0,91	0,87	1,05
27,89	Guaiene	0,45	0,21	0,22	0,10	0,51	-	0,36	0,48
28,22	α-Caryophyllene	0,13	0,11	-	-	0,25	0,13	0,23	0,34
28,42	Allo aromadendrene	0,14	0,12	0,12	-	0,25	-	0,12	0,14
29,03	α-Muurulene	0,82	0,33	0,35	0,17	0,91	1,20	0,90	1,36
29,19	α-Patchoulene	0,71	0,55	0,29	0,46	0,83	0,44	0,37	0,45
29,37	δ-Selinene	0,85	0,41	t	0,19	0,99	0,72	0,82	0,63
29,48	Valencene	0,29	0,28	0,12	0,15	1,64	1,12	1,44	1,04
29,64	ß- Cadinene	0,14	0,15	0,10	0,10	0,50	0,13	0,20	0,31
29,86	α-Cadinene	0,53	0,12	0,11	0,14	0,17	0,42	0,64	0,92
30,34	δ-Cadinene	0,27	0,26	t	0,17	0,67	-	0,28	0,43
30,85	α-Copaen-11ol	0,25	0,25	t	0,10	0,31	0,12	0,11	0,25
31,31	1,10-Guaiadiene	0,12	0,12	t	0,13	-	0,12	0,14	0,14
31,56	Trans Nerolidol	1,94	1,47	2,09	0,58	6,93	3,35	2,35	2,91
32,06	Caryophyllene oxide	3,27	1,77	0,84	0,91	2,62	0,63	1,31	2,19
32,35	Ylangene	0,38	0,10	0,11	0,11	-	-	0,11	0,13
32,69	α-Elemene	0,17	0,11	t	0,10	0,30	-	0,22	0,14
33,12	Selina6en4 ol	7,52	3,09	2,95	2,29	3,31	1,35	2,10	2,02
33,60	α-Caryophyllen-5-ol	1,82	2,80	1,49	1,60	1,66	1,02	1,07	1,90
33,97	ß-Eudesmol	2,06	0,91	0,22	0,41	1,01	0,44	0,59	0,55
34,11	α-Eudesmol	3,46	1,65	0,15	1,43	2,72	2,19	1,35	1,55
34,38	α-Guaiene	0,14	-	1,37	0,29	-	0,21	0,17	0,16
34,57	Isoaromadendrene epoxide	0,40	0,22	0,15	0,17	-	0,14	-	0,19
37,03	Benzyl benzoate	0,76	0,51	0, 27	0,39	0,16	0,48	0,43	0,32
39,12	Hexahydrofarnesyl acetone	0,13	0,46	t	0,30	5,14	0,94	1,80	2,07
40,97	12-Carboxyeudesma-3,11(13)diene	43,97	52,54	62,37	56,23	20,24	42,13	47,97	27,14
41,12	Methyl palmitate	-	-	0,10	-	0,72	0,57	-	0,44
42,78	Ethyl palmitate	0,42	0,42	-	1,07	0,62	0,32	0,86	6,25
43,10	Cyclohexadecane	-	0,22	0,35	0,14	1,21	0,39	0,35	0,22
45,50	Phytol	0,62	2,65	0,18	1,48	1,74	0,28	0,54	0,81
46,52	Tomentosin	0,38	0,18	1,64	-	-	0,55	0,31	0,27
46,52	Linoleic acid ethyl ester	t	0,15	t	1,29	-	0,32	-	3,78
47,78	1-Octadecene	-	t	t	-	0,64	0,32	0,13	0,11
49,45	Nonadecane	0,28	0,45	-	0,46	0,83	0,31	0,44	0,41
51,48	Tetracosane	0,14	0,16	0,10	0,20	0,32	0,14	0,15	0,20
53,44	Docosane/tricosane	0,60	1,33	0,65	1,65	4,61	2,01	1,54	1,39
57,13	Heptacosane	0,45	1,06	0,30	1,42	0,95	0,53	0,71	1,03
	Monoterpenes	0,26	0,77	0,39	0,73	16,88	7,17	7,23	5,47
	Hydrocarbon sesquiterpenes	5,92	3,68	3,16	2,40	9,91	5,90	7,19	8,30
	Oxygenated sesquiterpenes	66,22	68,96	72,25	66,19	46,84	53,69	60,25	43,03
	Oxygenated compounds	1,78	1,33	0,27	1,02	0,66	8,36	1,83	2,82
	Fatty acids	0,42	0,57	0,10	2,36	1,44	1,21	0,86	10,47
	Waxes	1,47	3,22	1,40	3,87	8,56	3,70	3,32	3,36
	Total	**76,07**	**78,53**	**77,57**	**76,57**	**84,29**	**80,03**	**80,68**	**73,45**

Literature Cited

Adams R. P. 1991. Essential oils, waxes In:. Linskens H. F, Jackson J. F. Modern Methods of plant Analysis. Pag. 159-173. Springer-Verlag, Berlin.

Alonso P. 2000. Composition of the essential oil of the *Dittrichia viscosa* (L.) W.Greuter. Rivista Italiana Eppos, XXIX, Giugno: 3-8.

Ball P. W. 1976. *Dittrichia* W. Greuter. In: Tutin T.G., Heywood V. H., Burges N. A., Moore D. M., Valentine D. H.,. Walters S. M & Webb D. A. (Eds.). Flora Europaea. Vol. 4: 136-137. University Press, Cambridge.

Benayache S., Benayache F., Dendouguy H. & Jay M. 1991. Les flavonoides d'*Inula viscosa* L. Plantes Med. Phytother, XXV (4): 170-176.

Bohlmann F. & Guptar K. 1982. Naturally occurring terpene derivatives. Part 434. Ineupatorolide-like sesquiterpene lactones from *Dittrichia viscosa*. Phytochemistry, 21 (6): 1443-1455.

Cafarchia C., De Laurentis N., Milillo M. A., Losacco V. & Puccini V. 1999. Ricerche sull'attività fungistatica di fiori e foglie di *Inula viscosa* (Asteraceae). Parassitologia, (41): 579-82.

Ceccherelli P., Curini M., Marcotullio M. C. & Menghini A. 1985. Sesquiterpene acids from D*ittrichia viscosa.* Phytochemistry, 24 (12): 2987-2989.

Chamaco A., Fernandez A., Fernandez C. & Altarejos J. 2000. Compositions of essential oil of *Dittrichia viscosa*. Eppos, XXIX : 3-8.

Chiarlo B. 1968. Sui costituenti dell'*Inula viscosa* Ait. Contenuto in azuleni dell'olio essenziale. Boll. Chim. Farm, 107 (6): 370-382.

De Laurentis N., Losacco V., Corrente M. L., Milillo M. A. & Stefanizzi L. 2000. Attività antibatterica dei flavonoidi di *Inula viscosa* Ait. Acta Phytoterapeutica, III: 129-133.

Fernandez-Ocana A. M., Irtuno-Moja I., Martos-Gilabert A. I. & Fernandez-Lopez C. 1996. Boletin del Istituto de Estudios Giennenses, Diputacion Provincial de Jaen, (161): 223-224.

Font Quer P. 1978. Plantas Medicinales: el Dioscorides Renovado. Pag. 778-790. Labor, Barcelona.

Grande M., Piera F., Cuenca A., Torres P. & Bellido 1985. Flavonoids from *Inula viscosa.* Planta Medica, 24 (9): 414-419.

Imbesi A. 1964. Index Plantarum. Pag. 418. Principato, Messina.

Lopez G. 1982. La Guia de Incafo de los Arboles y Arbustos de la Peninsula Iberica. Pag. 957-959. Incafo, Madrid.

Oksuz S. 1976. Psi-taraxasterol acetate from *Inula viscosa*. Planta Medica, 29 (4): 343-345.

Perez-Alonso M. J., Velasco-Neguerela A., Duru M. E., Harmandar M. & Garcia-Vallejo M. C. 1996. Flavour Fragr. J., 11: 349.

Rigual-Magallòn A. 1972. Flora e vegetaciòn de la Provincia de Alicante, I.E.A. Excma. Pag. 220-224. Disputaciòn Provincial de Alicante.

Shtacher G. & Kashman Y. 1971. Chemical investigation of volatile constituents of *Inula viscosa* Ait. Tetrahedron, 27: 1343-1349.

Susplugas C., Balansard G. & Julien J. 1980. Evidence of anthelmintic action of aerial parts from *Inula viscosa* Ait. Herba Hung., 19: 19-33.

Wollenweber E., Mayer K. & Roitman J. N. 1991. Exudate flavonoids of *Inula viscosa*. Phytochemisty, 30 (7): 2445-2446.

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