

Microelement Contents and Fatty Acid Compositions of some *Isatis* Species Seeds

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Abstract

Isatis species are important biennial, herbaceous shrubs represented by 40 taxa, of which 24 are endemic to Turkey. The chemical compounds found in leaves and roots of all *Isatis* species have medicinal properties and are used for treatment of a wide range of disorders. No study reports seed characteristics, microelement contents and fatty acid compositions of the seeds of any *Isatis* species except *I. tinctoria*. Therefore the study was carried out to find seed fatty oil, fatty acid compositions protein content, characteristics, and microelement contents of *I. aucheri*, *I. cochlearis*, *I. constricta*, *I. demiriziana* (endemic), *I. glauca* and *I. lusitanica*. The results showed fatty oil and protein content of these species in range of 2 to 10% and 16.07 to 25.17%, respectively. Furthermore, these seeds were rich in microelements Al, Ca, Fe, K, Mg, Na and P. Major fatty oil components of these species were determined as erucic, oleic, linoleic and linolenic acid; such that erucic acid content of these species ranged 16.94 to 30.52%.

Keywords: *Isatis* species, microelements, fatty acids, erucic, oleic and linoleic acid

Introduction

Isatis species are biennial, herbaceous shrubs belonging to family Brassicaceae and is represented by 40 taxa, of which 24 are endemic to Turkey (Davis, 1965; Davis, 1988) with representation of 31 species and 14 sub species in Eastern and South-eastern Anatolia (Misirdali, 1985). *Isatis aucheri*, *I. cochlearis*, *I. constricta*, *I. demiriziana* and *I. lusitanica* are naturally found in the flora of the Southeast Anatolia, Turkey.

The chemical compounds found in leaves of *Isatis* species have antibacterial, anticancer, antiviral, astringent and febrifuge properties. These are also used for treatment of a wide range of disorders including meningitis, encephalitis, mumps, influenza, erysipelas, heat rash etc. Similarly, the roots of these plants are rich in antibacterial and anticancer chemical compounds (Bown, 1995).

Erucic acid, a characteristic, Brassicaceae oil occurs in about three-fourths of these species in range of 3 to 59%. Some oils free of erucic acid contain up to 63% linolenic or up to 58% eicosenoic acid (Mikolajczak *et al.* 1961). Dolya *et al.*, (1972) found that *Isatis tinctoria* contain 16.19%

oleic, 10.49% linoleic, 27.74% linolenic, 10.22% arachidic and 25.55% erucic acids.

No study reports medicinal uses, seed characteristics, microelement contents and fatty acid compositions of the seeds of any *Isatis* species. Therefore, the study aimed to determine some seed characteristics, microelement contents and fatty acid compositions of the seeds of seven *Isatis* species - *I. aucheri* Boiss., *I. cochlearis* Boiss., *I. constricta* Davis, *I. demiriziana* Misirdali, *I. glauca* Auch. ex Boiss., *I. lusitanica* L. and *I. tinctoria* L.

Materials and methods

Plant Materials

Fruits of *I. aucheri*, *I. cochlearis*, *I. constricta*, *I. demiriziana*, *I. glauca*, *I. lusitanica* and *I. tinctoria* were obtained from the Seed Collection unit of Medicinal and Aromatic Plants, Department of Field Crops, Faculty of Agriculture, Dicle University, Diyarbakır, Turkey. Where, *I. tinctoria* and *I. glauca* are found as introduction material and *I. constricta* (collected from around the Hazar Lake at altitude of 1200 m lying in between Diyarbakır and Elazığ prov-

inces), *I. cochlearis* (collected from Nemrut Mountains in Adiyaman province at altitude of 1769 m) *I. aucheri* (collected from Nemrut Mountains, Adiyaman province at latitude of 2056 m); *I. demiriziana* (collected from Ergani, district of Diyarbakir province at altitude of 1480 m) and *I. lusitanica* (collected from Ceylanpinar, district of Şanlıurfa at altitude of 600 m) are found as collection material from the wild of the Southeast Anatolia. Except *I. lusitanica*, an annual, all other species are biennial.

Oil Extraction

Ground samples were extracted in a Soxhlet extraction apparatus with petroleum ether (Merck) as solvent; once the extraction was complete, the ether was removed completely by rotary evaporation.

Ash Content

The ash contents of 7 *Isatis* species were determined according to Kacar (1972).

Protein Content

Protein content of the samples was determined by Kjeldahl method.

Microelement content

Microelement content of plants was determined using Perkin Elmer Optima 2100 DV ICP OEMS apparatus, as per instructions of the manufacturer.

Lipid Extraction

Three gram seeds were used for each analysis. The samples were homogenised in chloroform-methanol (2:1, v/v) (Bligh and Dyer, 1959). Autoxidation of unsaturated components was minimized by adding 50 µl of 2% butylated hydroxytoluene in chloroform. The total lipid extracts were dried under a stream of N₂ and measured gravimetrically. For subsequent analyses, the dry extracts were scraped into reaction vials, and the associated fatty acids were transmethylated by refluxing the fractions in acidified methanol for 90 min at 85°C. The fatty acid methyl esters (FAMES) were extracted from reaction vials three times with hexane (Stanley-Samuels and Dadd, 1983).

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

Fatty oils of *Isatis* species were analysed by GC-MS (Hewlett Packard computerized system comprising a 6890 gas chromatograph coupled to a 5973 mass spectrometer) Chromatographic separations were accomplished with a ZB-5 capillary column (0.25 mm i.d. x 60 m, film thickness 0.25 µm) with injections in the split mode with a split ratio of 40:1. The temperature was set to 60°C for 5 min initially, and then increased to 240°C at rate of 3°C per minute and maintained at 240°C for 10 min. The injection temperature and ion source temperature were both set at 250°C. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The ionization mode used was electronic impact at 70 eV, mass range m/z 35-550 a.m.u. The separated components were identified by matching with National Institute of Standards and Technology (NIST98) mass spectral library data and Wiley/GC/MS library. The quantitative determination was carried out based on peak area integration.

Results and discussions

The results of oil, ash & protein content, microelement content and fatty acid composition of seven *Isatis* species are given in Tab. 1, 2 and 3.

The average oil content, ash and protein content of species ranged 4 to 10%, 3 to 9.5% and 16.07 to 25.17%, respectively (Tab. 1). The oil content of *I. tinctoria* (10%), *I. constricta* (9.0%) and *I. lusitanica* (8.0%) were higher

Tab. 1. Fatty oil, ash and protein content of the seeds of seven *Isatis* species

Species	Fatty oil (%)	Ash (%)	Protein (%)
<i>Isatis aucheri</i>	4	9	19.58
<i>Isatis cochlearis</i>	5.4	5.5	18.87
<i>Isatis constricta</i>	9	7	25.17
<i>Isatis demiriziana</i>	5.5	7	22.43
<i>Isatis glauca</i>	5	7	18.37
<i>Isatis lusitanica</i>	8	9.5	16.07
<i>Isatis tinctoria</i>	10	3	23.69

Tab. 2. Microelement analysis of the seeds of seven *Isatis* species

Species	Microelements (mg kg ⁻¹)													
	Al	Ca	Cd	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	Zn
<i>I. aucheri</i>	178.3	2515	0.348	0.788	1.532	124.3	4355	621.1	5.104	83.34	5.155	533.2	2.225	5.681
<i>I. cochlearis</i>	259.3	2842	0.221	1.147	2.194	160.0	4452	695.8	14.27	165.7	5.735	636.5	2.050	11.67
<i>I. constricta</i>	112.4	2372	0.249	0.539	1.558	78.54	>6000	557.7	9.402	75.62	4.537	428.7	1.673	14.69
<i>I. demiriziana</i>	172.3	2181	0.520	0.843	2.036	130.8	>6000	583.7	9.970	75.13	5.053	1157	3.231	11.08
<i>I. glauca</i>	78.18	3339	0.317	0.633	11.97	77.51	>6000	713.9	7.658	66.77	5.066	772.7	3.514	12.64
<i>I. lusitanica</i>	223.8	2451	0.246	1.141	2.187	158.7	3420	558.3	9.915	121.2	5.154	991.3	3.199	19.83
<i>I. tinctoria</i>	74.78	2173	0.250	0.630	1.888	77.54	3493	593.0	7.383	66.31	4.789	625.6	2.493	11.01

Tab. 3. Fatty acid compositions (%) of the seeds of seven *Isatis* species

Fatty acids	RT*	I**	II	III	IV	V	VI	VII
Lauric acid	42.54	0.14	-	-	-	0.12	0.89	-
Azelaic acid	43.66	0.22	0.24	-	-	0.10	0.18	-
Tetradecanoic acid	50.27	0.29	-	-	0.10	0.24	1.29	-
Pentadecanoic acid	53.85	0.13	-	-	-	0.14	0.32	-
1H-indole-3-acetic acid	54.89	0.35	-	-	-	-	-	-
9-Hexadecenoic acid (Palmitoleic acid)	56.46	0.21	-	-	-	-	-	-
7-Hexadecenoic acid	56.62	0.22	0.08	0.11	0.11	0.32	-	-
Palmitic acid	57.28	8.74	6.29	5.45	6.59	11.08	20.39	11.18
14-methyl-hexadecanoic acid	60.51	0.13	-	-	0.11	0.11	0.24	-
Linoleic acid	62.75	5.93	7.16	5.13	6.52	12.78	6.60	2.74
Oleic acid	62.95	24.91	22.01	15.61	22.04	22.92	14.16	14.64
Linolenic acid	63.01	18.65	20.39	29.08	19.11	11.79	19.56	14.05
Linolenic acid ethyl ester	63.35	-	-	-	2.19	-	-	-
Stearic acid	63.62	1.08	1.53	1.14	1.85	2.11	2.58	2.17
11-Eicosenoic acid	68.78	6.51	8.50	8.04	9.79	6.07	4.23	10.40
Arachidic acid	69.43	1.15	0.85	1.06	1.60	1.12	1.23	1.22
Erucic acid	74.23	21.84	26.07	30.52	20.82	14.69	16.94	26.48
Behenic acid	74.78	0.81	0.45	0.52	0.89	1.00	-	0.66
Erucic acid ethyl ester	75.83	-	0.38	0.13	-	-	-	-
15-tetracosenoic acid	79.26	2.42	1.64	1.39	2.17	1.79	1.19	2.22
Tetracosanoic acid	79.78	0.61	0.30	0.35	-	0.90	1.02	1.08
Total		94.96	95.89	98.53	93.89	87.28	90.82	86.84

*RT; Retention Time, **I- *Isatis aucheri*, II- *I. cochlearis*, III- *I. constricta*, IV- *I. demiriziana*, V- *I. glauca*, VI- *I. lusitanica*, VII- *I. tinctoria*

compared to other species. The highest ash content was recorded in *I. lusitanica* due to high hull content/seed ratio. Protein content of species varied between 16.07 to 25.17%, with the highest in *I. constricta* among all species. *I. tinctoria* seeds contained 10% fatty oil, which is the highest among all species, 3% ash and 16.07% protein.

Al, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb and Zn content of seven *Isatis* species ranged 74.78-259.3 mg kg⁻¹, 2173-3339 mg kg⁻¹, 0.221-0.520 mg kg⁻¹, 0.539-1.147 mg kg⁻¹, 0.630-2.194 mg kg⁻¹, 77.51-160 mg kg⁻¹, 3420->6000 mg kg⁻¹, 557.7-713.9 mg kg⁻¹, 5.104-14.27 mg kg⁻¹, 66.77-165.7 mg kg⁻¹, 4.537-5.735 mg kg⁻¹, 428.7-1157 mg kg⁻¹, 1.673-3.514 mg kg⁻¹ and 5.681-19.83 mg kg⁻¹, respectively (Tab. 2).

The highest Al (259.3 mg kg⁻¹), Cr (1.147 mg kg⁻¹), Cu (11.97 mg kg⁻¹) Fe (160 mg kg⁻¹), Mn (14.27 mg kg⁻¹), Na (165.7 mg kg⁻¹) and Ni (5.735 mg kg⁻¹) content were obtained from *I. cochlearis*. The highest Ca (3339 mg kg⁻¹) and Zn (14.69 mg kg⁻¹) contents were obtained from *I. glauca*. Similarly, the highest Cd content of 0.520 mg kg⁻¹ and P content 1157 mg kg⁻¹ of were recorded from *I. demiriziana*. The highest Mg content of 713.9 mg kg⁻¹ and Pb content of 3.514 mg kg⁻¹ was found in *I. glauca*. In general K content of *I. constricta*, *I. demiriziana* and *I. glauca* species was higher than 6000 mg kg⁻¹ (Tab. 2). Geographical

origin of plants belonging to the same species can result in different concentrations of elements, depending on soil features and environmental condition. The Cd contents of *I. aucheri*, *I. demiriziana* and *I. glauca* found in this study are higher than the maximum permissible Cd content of 0.3 mg kg⁻¹ by the World Health Organization (WHO).

The quality and oil composition of species depend on genotype. There is considerable variation in the types and proportions of fatty acids present in different species. Oil of seeds of plants belonging to family Brassicaceae mainly contains erucic acid. *Isatis* species also contain erucic, oleic, linolenic, palmitic and linoleic acid. *I. cochlearis*, *I. constricta* and *I. tinctoria* species contain erucic acid as dominant component of oil. The highest erucic acid percentage (30.52%) among all species was determined from *I. constricta*. However, *I. lusitanica*, had the highest palmitic acid (20.39%). Oleic, linoleic, linolenic and erucic acid were the four abundant fatty acids. Total oil of *I. aucheri*, *I. cochlearis*, *I. constricta*, *I. demiriziana*, *I. glauca*, *I. lusitanica* and *I. tinctoria* seeds was 71.33, 75.63, 80.34, 68.49, 62.18, 57.26 and 57.91%, respectively (Tab. 3). This is in agreement with Shewry (1997), who noted that oleic and linoleic acids together account for over 60% of the total fatty acids in most species. The results clearly indicate that *Isatis* species contain oleic, linoleic, linolenic and erucic acid

as major components with chemical variations in major components among species. Similarly Bagci and Ozcelik (2009) recorded linolenic and erucic acid as major components of *I. cappadocica* subsp. *steviani*, *I. kotschyana*, *I. candolleana*, *I. spectabilis*, *I. glauca* subsp. *glauca* and *I. kozlowskyi*. They found that the total oil yield of these species reached 22.2-31.8 % by seed weight. They also found that major extracted seed oil fatty acids of these *Isatis* species were α -linolenic acid oleic acid and linoleic acid with range of 26.6-32.62%, 13.3–18.8% and 8.94-15.86%, respectively. Similarly α -linolenic, linolenic and oleic acid, were reported as major fatty acids of *I. aleppica*, *I. tinctoria*, *I. tinctoria*, and *I. glauca* by Miller (1965), Scrimgeour (1976), Iba, (1992) and Blatger, (1993), respectively.

Conclusions

This study compared seed oil, fatty acid compositions, protein and 14 microelement composition contents of *I. aucheri*, *I. cochlearis*, *I. constricta*, *I. demiriziana* (endemic), *I. glauca* and *I. lusitanica*. The results showed that fatty oil and protein contents of these species ranged 2 to 10% and 16.07 to 25.17%, respectively. Furthermore, the seeds of these species were rich in microelements Al, Ca, Fe, K, Mg, Na and P with a large variation among species. Major oil components of these species were determined as erucic, oleic, linoleic and linolenic acid; with range of 14.16 to 24.91%, 14.64 to 24.91% and 11.79-29.08%, respectively. High percentage of fatty acids in seven *Isatis* species may have practical importance to facilitate genetic engineering or plant breeding for renewable lipid resources. It may also attract significant importance with respect to natural product chemistry, plant chemotaxonomy and evolution.

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