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# INVESTIGATION ON THE SEEDS OF *IRIS SPURIA L. SUBSP. MUSULMANICA* (FOMIN) TAKHT. *(IRIDACEAE).*

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## Abstract

*40 species and 49 taxa of the genus Iris are growing naturally in Turkey (1-3). I. spuria L. subsp. musulmanica (Fomin) Takht., is one of the the 10 species of Limniris section and grows wildly in Eastern Anatolia. Plant material was collected from Van-Erciş road. Besides the morphological and anatomical investigation; oil content and methyl esters of fatty acids of the seed oils were examined in this study for the first time. Fatty Acid methyl esters were prepared according to the method of Metcalfe et al. (13) from the oil obtained by the Soxhelet apparatus with n-hexane. Analysis of the methyl esters have been carried out with GC-MS. Myristic Acid, Palmitic Acid, Linoleic Acid, Linolenic Acid, Stearic Acid, Oleic and Arachidic Acid were determined as the main fatty acids in the oil. Seeds were rich in Linoleic (40 %) and Oleic Acid (30 %).*

***Key words:*** *Iris spuria subsp. musulmanica, seed, morphology, anatomy, seed oil, fatty acid.*

## *Iris spuria* L. subsp. *musulmanica* (Fomin) Takht. (*Iridaceae*) Tohumları Üzerinde Çalışmalar

*Ülkemizde Iris cinsine ait 40 tür ve 49 taksa doğal olarak yetişmektedir (1-3). Limniris seksiyonunda yer alan on türden birisi olan Irıs spurıa L. subsp. musulmanıca (Fomın) Takht. Doğu Anadolu Bölgemizde doğal olarak ve bol miktarda yetişen bir türdür. Bitki, Van-Erciş arasından toplanmıştır. Türün tohumlarının morfolojik ve anatomik yapısı çalışılmış ayrıca tohumların taşıdığı sabit yağ miktarı tayin edilmiştir. Elde edilen sabit yağdan Metcalfe ve ark. Yöntemi ile metil esterler hazırlanmıştır (13). Yağ asitlerinin metil esterleri GC-.MS ile tayin edilmiş ve yağın başlıca miristik asid, palmitik asid, linoleik asid, linolenik asid, stearik asid, oleik and araşidik asid taşıdığı tesbit edilmiştir. Tohumların linoleik (% 40) ve oleik asid (% 30) içeriği bakımından zengin olduğu belirlenmiştir.*

***Anahtar kelimeler:*** *Iris spuria* subsp. *musulmanica*, *tohum, morfoloji, anatomi, sabit yağ, yağ asiti.*

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## INTRODUCTION

37 *Iris* species naturally grow in Turkey. *Iris spuria* L. subsp. *musulmanica* (Fomin) Takht. is one of the 10 species within the *Limniris* section and grows abundantly in East Anatolia naturally. The plant is 50-100 cm tall with a thick rhizome, clothed with fibrous remains of leaf bases. Leaves (0.5) 0.8–1.7 (-2.3) cm broad, rather tough and rigid, greyish-green. Stem usually with 1 or 2 erect branches, 2-5 flowered. Bracts and bracteoles 6-12 cm, green with membraneous margins. Perianth tube 0.7–1.5 cm. Flowers pale to mid-lilac, veined purple on a whitish or yellowish ground in lower half of falls or white (albinos), signal patch in centre of falls rather small, yellow; falls panduriform, 5.5-7 cm, lamina equal to or slightly shorter than claw, 2.5–3.5 x 2–2.7 cm, claw distinctly winged, 3–3.5 x 0.9–1.2 cm; standards obovate or oblanceolate, 5–6 x 1.3–2.4 cm; style branches 4-4.5 x 0.7–1 cm, with slightly obliquely acute lobes 0.7–1 x 0.3–0.4 cm. Ovary with a slender beak-like apex c. 1.5–2 cm. Capsules oblong, 4-5 cm, with a beak c. 1.5 cm. Fl, 5-7. Damp meadows, salty flats, alluvial plains, 800-1900 m (1-3).

The medicinal parts of the *Iris* (Orris) species are the rhizomes with the roots. They contain volatile oil (α, β, γ, irone) giving in the odor of violets, triterpenes, isoflavonoids, flavonoids, xanthones and starch (4).

Orris has been used for disorders of the respiratory system (4).

*Iris* species have been used as diuretic, laxative and cholagogue traditionally. Volatile oil prepared from the rhizomes is widely used in perfumery. **Rhizoma Iridis pro infantibus** found in European markets is specifically produced for children during teething (5).

In homoepathy this drug has been used to treat disorders of the respiratory tract or thyroid gland, for digestion complaints and headaches ( 4).

It was observed during the literature search that seed oils of some *Iris* species has been studied. A study was carried out in Italy in 1969 on the seed oils of some aquatic plants including *Iris* species. Seed oils of those plants were found to contain fatty acids with C numbers between $C_{16}$-$C_{24}$. It was shown that the major fatty acids are $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ (6). Another study on *Iris* species was conducted in China in 1983 on the seeds of *I. pallasii*. It was found that the oil content of the seeds were 12% and the GC analysis of this oil indicated that decanoic acid (0.02-0.69%), lauric acid (0.02-0.37%), tetradecanoic acid (0.08-1.12%), palmitic acid (5-7.65%), stearic acid (0.89-3.6%), oleic acid (28.51-37.53%) and linoleic acid (50.36-65.35%) were the components of the oil. These seeds are used for their diuretic, antipyrrhetic, hemostatic and inflammation inhibitor properties in the traditional Chinese medicine (7).

There are taxonomical studies on *Iris* species growing Turkey (8-10) but there has been no study on the seeds of these species. It was noticed by us during our East Anatolian trips that the seeds of *I. spuria* subsp. *musulmanica*, abundantly growing in the region and rich in seed yield, were preferred by the wild birds as a food source. It has been known that seeds that are rich in oil are important for the dietary needs of birds especially in winter times (11). Following this field observation, seeds of the plant were collected, morphological and anatomical properties were examined. In addition to that seed oil content was determined and the methyl esters of fatty acids were studied with GC-MS.

## EXPERIMENTAL

*Plant material*

Plant material was collected before the opening of the mature capsules, by *M. Koyuncu* in 10.9.1997, $B_9$ Van-Ercis road, 25$^{th}$ km, roadside, wet areas at 1800m altitude. Seeds were dried at room temperature under shade and were used as the material for the study. Morphological studies were performed on a total of 100 specimens selected randomly from 10 different groups. Measurements were done with a compass. Morphological photographs were taken with a Nikon SMZ1000.

*Anatomical studies*

For the anatomical studies; one seed from each of the 10 different groups were randomly selected. Seeds were soaked in water for some time and the cross-sections prepared by hand from them were processed with Sartur Reagent (12) and chloralhydrate solution separetely. For the anatomical drawings Leitz Weitzlar drawing prism attached to a microscope was used. Photographs from the preparations were taken with a camera adapted to Olympus BX 50 microscope.

*Extraction*

20 grams of seed material were weighed and extracted with 200 ml n-hexane for 8 hours in the Soxhelet apparatus for the extraction of oil. Hexane was evaporated in rotary evaporator (40° C) and the oil content was calculated in percentage. Methyl esters of the fatty acids were prepared according to the method of Metcalfe et. al. (13) and were examined by using GC-MS under following conditions:

*Instruments*

GC/MS System 1: The GC/MS analysis was carried out with a Shimadzu GC/MS QP5000 Quadropol system. Carrier gas was Helium. J & W Scientific Ltd. DB-WAX column (30m x 0.25mm) was used. The temperature of the GC oven was held at 55° C for 10 min and programmed to 240° C with 2° C/min increase. Injector and detector temperatures were kept at 250° C. Split ratio was 50:1. Mass Range was 35-450 M/z and ionization voltage was held at 70 eV. Quantitative results are given with this system.

GC/MS System 2: The GC/MS analysis was carried out with a Agilent GC 6890 MSD 5973 N. Carier gas was Helium. Innowax column (30m x 0.32mm x 0.25 µm ) was used. The temperature of the GC oven was held at 50° C for 10 min and programmed to 150° C with 8° C/min (5 min), and to 200° C with 5° C/min (10min.), and finally to 250° C with 5° C/ min (10 min.) increase. Injector and detector temperatures were kept at 250° C. Split ratio was 20:1. Mass Range was 35-450 M/z and ionization voltage was held at 70 eV. Methylated sample is monitored together with n-alkans in this system.

*Identification*

Identification of individual components was achieved using The Wiley/NBS Registry of Mass Spectral Data, Comparison with reference MS and also with Retantion Indices.

## RESULTS

### 1. Botanical results

*Morphological results*

Morphological studies were carried on 100 randomly selected seeds. Shape is almost reniform. 14 mm x 4. 25 mm x 3 mm. Pale brownish, darker at center. Upper and lower surface is rough, wavy and net-like, mostly at center. Dark brown to blackish hilum is surrounded by a light-coloured ring (Photo. 1,2).

Weight of 1000 seeds were 28.91gr and the number of seeds per capsule were found to be 50-70.

**Photo. 1:** General view of the seeds of *I. spuria* subsp. *musulmanica* (Fomin) Takht

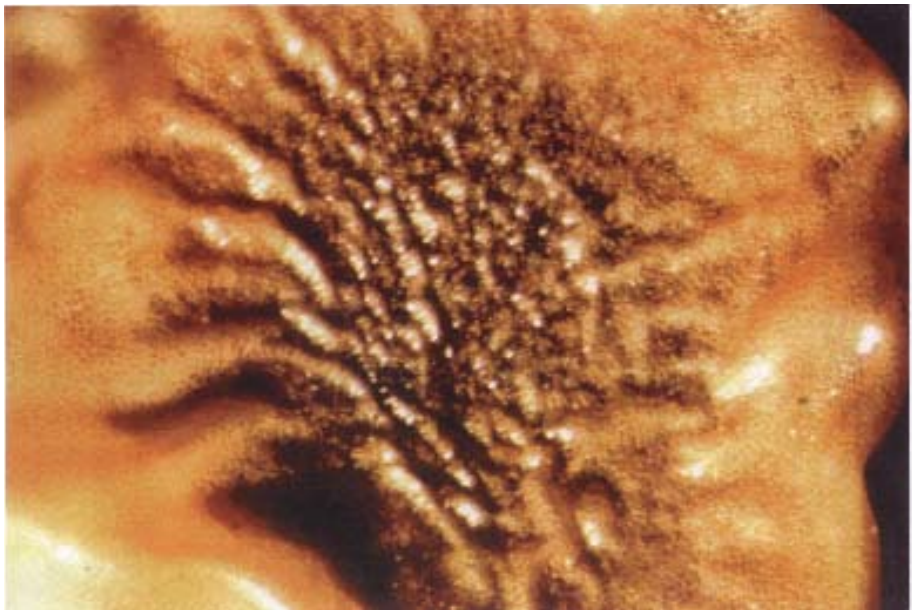


**Photo. 2:** Surface view of the seeds of *I. spuria* subsp. *musulmanica.*

*Anatomical Results*

Cross-section of the seed is ovate in form and cosists of seed coat epidermis, seed coat parenchyma, integument, endosperma and vascular bundle (Fig. 1).

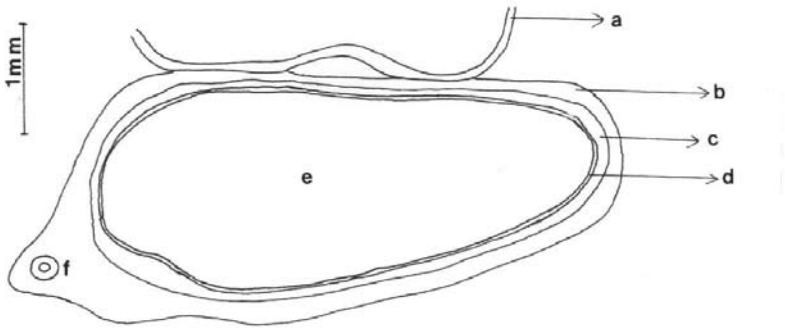



**Figure 1:** Cross-section of seeds of *I. spuria* subsp. *musulmanica* **a-** Seed coat epidermis, **b-** seed coat parenchyma, **c-** upper integument, **d-** lower integument, **e-** endosperma, **f-** vascular bundle.

**Seed Coat Epidermis:** Closely packed, multi angled cells are visible from surface view (Photo. 3). In cross section, outer surface of testa epidermis cells are smooth and thinly, cutinized.

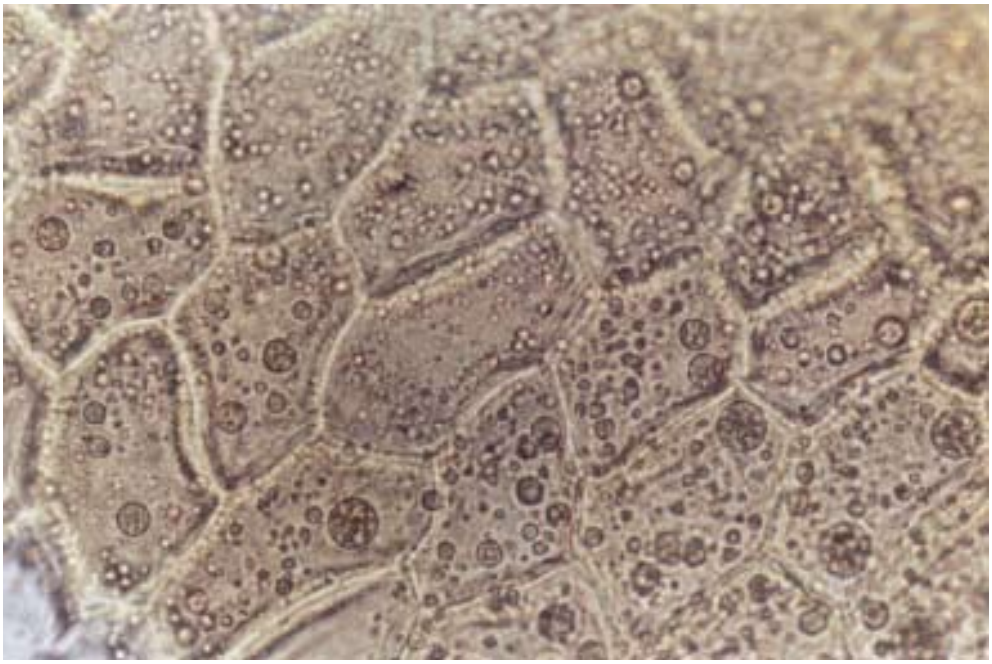


**Photo. 3:** Seed coat epidermis cells seen from surface view.

**Seed Coat Parenchyma:** Composed of 2-5 layer of cells without ergastic matter; single layer beneath the epiderma is regularly packed whereas the layers down beneath are irregularly oriented, isodiametrical, thick walled and have intercellular spaces. Parenchyma is multilayered in the area where vascular bundle exists (dorsal face) (Photo. 4).

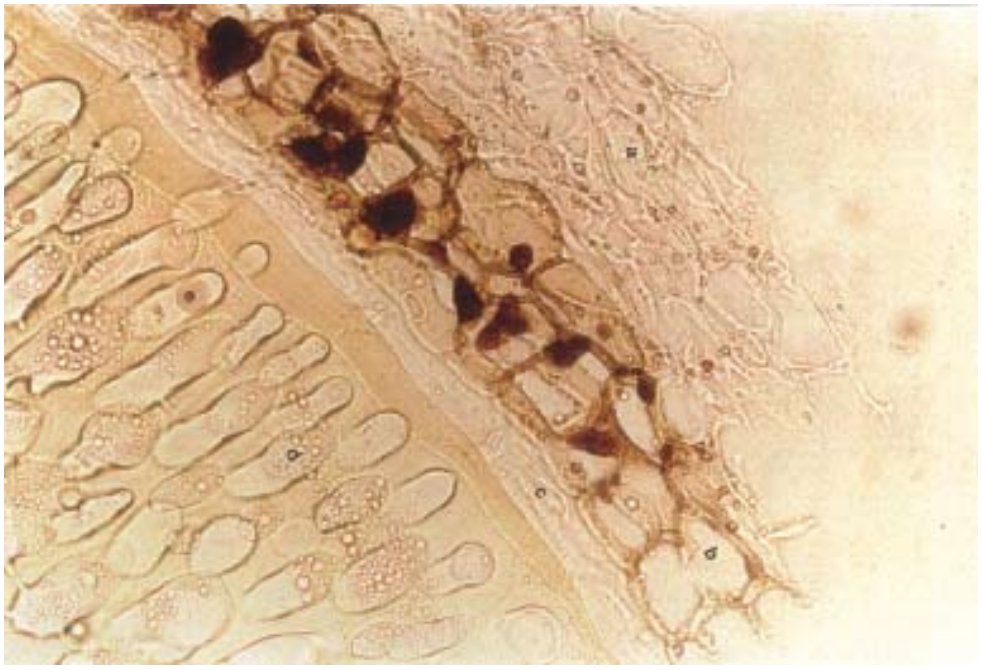


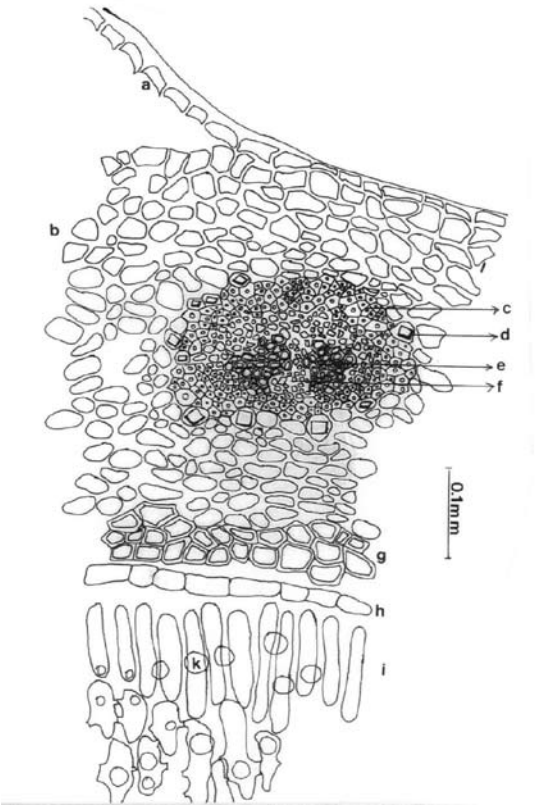
**Photo. 4:** Cross-section of seeds *I. spuria* subsp. *musulmanica* **a-** Seed coat parenchyma, **b-** upper integument, **c-** lower integument, **d-** endosperma, **e-** fixed oil.

**Integument:** Composed of two different cell types. Upper integument cells are isodiametrical, 2 to 3 layered and dark colored. Lower integument cells thin walled, slender, almost rectangular and colorless. Between these two layers there exist a narrow layer formed by crushed cells.

**Endosperma:** Endosperma layer consists of thick with pits walled cells and drops of fixed oil.

**Vascular Bundle:** Surrounded by a thick sclerenchymatic bundle. Some of the parenchymatic cells surrounding the sclerenchyma carry crystals. No crystals have been observed except this location. Xylem is formed by two parts. Phloem is found between these two parts of xylem (Fig. 2; Photo. 5,6).

**Figure 2:** a- Seed coat epidermis,
b- seed coat parenchyma,
c- sclerenchymatic bundle,
d- calcium oxalate crystal,
e- xylem,
f- phloem,
g- upper integument,
h- lower integument,
i- endosperma,
k- fixed oil.



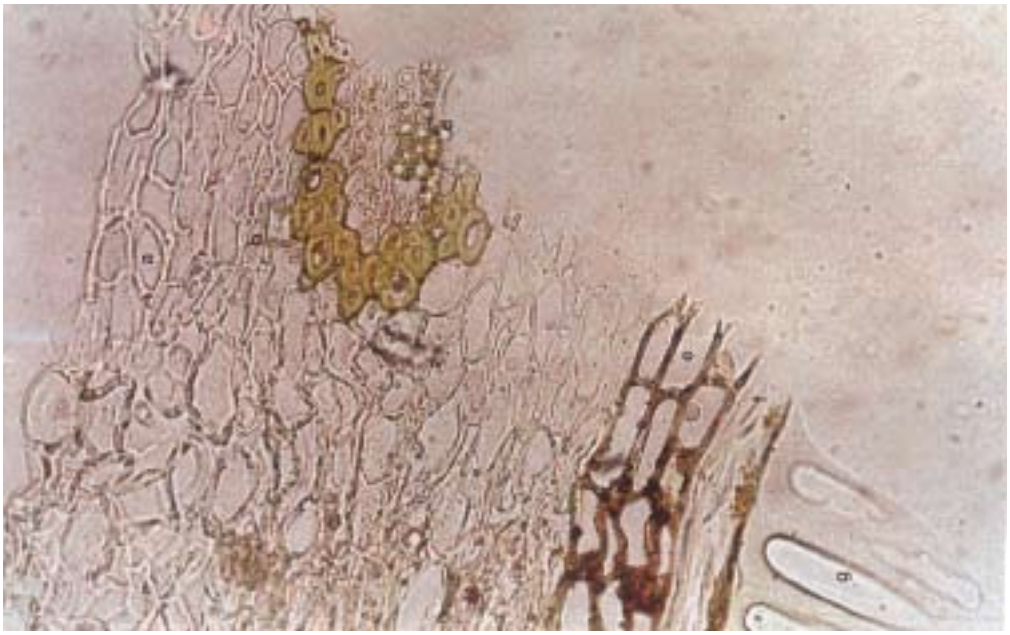



**Photo. 5:** Cross-section of seeds *I. spuria* subsp. *musulmanica* **a-** Seed coat parenchyma, **b-** calcium oxalate crystal, **c-** sclerenchymatic bundle, **d-** xylem, **e-** upper integument, **f-** lower integument, **g-** endosperma.

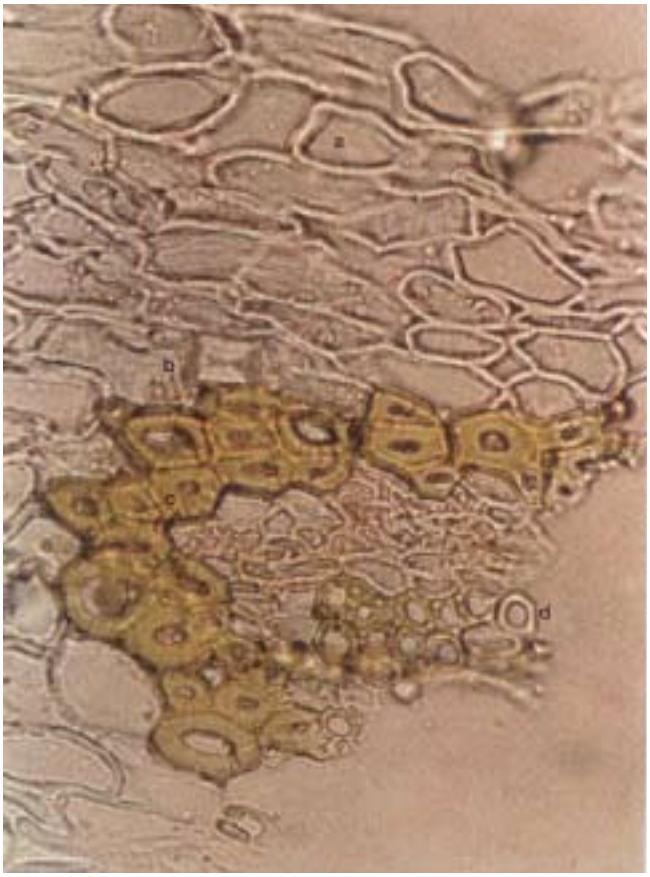


**Photo. 6:** Cross-section of seeds *I. spuria* subsp. *musulmanica* **a-** Seed coat parenchyma, **b-** calcium oxalate crystal, **c-** sclerenchymatic bundle, **d-** xylem,

## 2. Chemical results

*Fatty acid composition*

Following the Soxhelet extraction of 20 grams of seed material, a dark brown and viscous oil was obtained. Oil content is ranging between 5-7% w/w. Fatty acid percent composition of the seed oil is given in (Table 1 and Table 2) two different system.

**Table 1.** Fatty acid composition of *Iris spuria* subsp. *musulmanica* seed oil in the DB.WAX column in system 1. (The peak numbers in the table are given according to the retention time only to the major peaks).

| Peak no | Rt minutes | Compounds | % amount |
|---|---|---|---|
| 1 | 58.384 | Myristic acid | 1.08 |
| 2 | 68.611 | Palmitic acid | 10.66 |
| 3 | 69.617 | Palmitoleic acid | 2.79 |
| 4 | 78.069 | Stearic acid | 6.50 |
| 5 | 78.839 | Oleic acid | 30.71 |
| 6 | 79.030 | Elaidic acid | 3.48 |
| 7 | 80.960 | Linoleic acid | 40.12 |
| 8 | 83.345 | Linolenic acid | 2.28 |
| 9 | 86.337 | Arachidic acid | 1.25 |

**Table 2.** Fatty acid composition of *Iris spuria* subsp. *musulmanica* seed oil in the Innowax column in system 2. (The peak numbers in the table are given according to the retention time only to the major peaks).

| Peak no | Rt minutes | Compounds | RI | % amount |
|---|---|---|---|---|
| 1 | 16,67 | Myristic acid | 1883,90 | 1.23 |
| 2 | 22,27 | Palmitic acid | 2181,27 | 10.61 |
| 3 | 22,83 | Palmitoleic acid | 2204,33 | 2.00 |
| 4 | 26,77 | Stearic acid | 2303,53 | 6.86 |
| 5 | 27,17 | Oleic acid | 2408,06 | 30.17 |
| 6 | 27,28 | Elaidic acid | 2413,95 | 3.73 |
| 7 | 28,13 | Linoleic acid | 2458,65 | 40.47 |
| 8 | 29,53 | Linolenic acid | 2525,74 | 2.12 |
| 9 | 31,45 | Arachidic acid | 2602,35 | 1.04 |

## DISCUSSION

*Iris spuria* subsp. *musulmanica* grows naturally around Van province. Seeds of the plant were used as material in this study. Morphological and anatomical structures were studied in detail for the first time in this work. In the cross sections taken from the bright, pale brown almost reniform seeds show that the testa epidermis is apart from the testa parenchyma. However, testa epidermis covers the testa parenchyma at the centers of two faces of the seed. Starting from hilum vascular bundles are visible all along the dorsal side of the seed covering nearly ¾ of it. Some of the parenchyma cells contain some crystals near the sclerenchyma. Seed surface is wavy and testa epidermis is thinner where the parenchimatic invaginations exist but multi layered around the vascular bundles. Endosperma is rich in oil. No calcium oxalate crystals are observed except the

parenchymatic cells around the vascular bundles and no starch was found in the seed. According to these properties seeds show an interesting character.

It was measured that seed oil content is between 5-7%. It has been observed that the oil content of the seeds are high in linoleic and oleic acids (40.1 and 30.7 respectively). This is comparable with the results of Smith, P. M. (14) which states that the linoleic and oleic acid content of the family Iridaceae is high and also with the results of Zang, J., et.al. on the seed oils of *I. pallasi* (7).

*I. spuria* subsp. *musulmanica* seeds collected from Van province were studied morphologically and anatomically in this study. In addition to that amount of oil in seed and composition of the fatty acids were determined.

## REFERENCES


1. **Mathew, B.,** *Iris* L. pp. 382-411, In: **Davis, P. H.** (ed.), Flora of Turkey and the East Aegean Islands. Volume 8, Edinburgh at the University Press, Edinburgh, 1984.

2. **Mathew, B.,** *Iris* L. pp. 227-228, **Davis, P. H.** (ed.), Flora of Turkey and the East Aegean Islands. Volume 10, Edinburgh at the University Press, Edinburgh, 1988.

3. **Mathew, B.,** *Iris* L. pp. 227-228, **Güner, A., Özhatay, N., Ekim, T., Başer, K. H. C.,** (ed.), Flora of Turkey and the East Aegean Islands. Volume 11, Edinburgh at the University Press, Edinburgh, 2000.

4. **PDR for Herbal Medicines,** Second Edition. Medical Economics Company, Montvale, New Jersey, pp. 561-62, 2000.

5. **Baytop, T.,** Türkiye'de Bitkiler İle Tedavi, Geçmişte ve Bugün. Nobel Tıp Kitabevleri, Istanbul, pp. 342, 1999.

6. **Lotti, G., Averna, V.,** *Riv. Ital. Sostanze Grasse.* 46(12): pp. 668-672, 1969. Ref.: C.A. 32340j vol. 72 1970.

7. **Zang, J., Xia, G., Xueming, L.,** Physiochemical properties of Ma Lin Zi (Seed of *I. pallasii*) oil and identification of its fatty acids. *Zhongcaoyao,* 14 (3): pp. 103-105. Ref C.A. 221681y 1983 vol. 98, 1983.

8. **Koca, F.,** Karyological studies on *Iris attica* Boiss. Et Heldr. and *Iris suaveolens* Boiss. Et Reuter. *J. Fac. of Phar. Istanbul,* 21: pp. 69-79, 1985.

9. **Koca, F.,** Karyological studies on four endemic *Iris* species (sect. *Iris*) in Turkey**.** *J. Fac. Phar. Istanbul***,** 25: pp. 1-16, 1989.

10. **Koca, F.,** Morphological and anatomical studies on some endemic *Iris* L. species (Sect. *Iris*) in Turkey. *Tr. J. of Botany,* 20 (supplementary vol.), pp. 43-58, 1996.

11. **Welty, J.C., Baptista, L.,** The Life of Birds. Fourth ed., Sounders Publishing, New York, pp. 2, 1988.

**12.** **Çelebioğlu, S. & Baytop, T.,** Bitkisel tozların tetkiki için yeni bir reaktif. *Farmakolog,* **19:** 301, Istanbul, 1949.

**13.** **Metcalfe, L.D., Schimita, A.A., Pelka, J.R.,** Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem.,* 38 (3): pp. 514-515, 1966.

**14.** **Smith, P. M., 1976:** The chemotaxonomy of plants**.** Edward Arnold Press. London pp. 71-76, 1966.

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