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[www.revistafarmacia.ro](http://www.revistafarmacia.ro)

ISSN 0014-8237

Romanian Society for Pharmaceutical Sciences

# Farmacia

# 5

Volume 62  
September - October 2014

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ISSN 0014-8237 (for the Printed Edition)  
ISSN 2065-0019 (for the On -Line Edition)

“Printech” Publishing House  
Bucharest-Romania



## COMPARATIVE STUDY ON THE VOLATILES' COMPOSITION OF *IRIS DICHOTOMA* PALL. RHIZOME EXTRACTS

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### Abstract

The current study was performed to examine the qualitative and quantitative composition of the essential oil, n-hexane and ethanol extracts from the rhizomes of *Iris dichotoma* Pall. for their volatile content. The essential oil was particularly found to possess the most rich composition, while the latter extracts (n-hexane and ethanolic ones) consisted of various fatty acid esters. GC and GC-MS analyses of the oil resulted in the identification of 28 components. Among them myristic acid (13.67 %), methyl myristate (9.08 %), and  $\alpha$ -terpineol (2.42 %) were found to be the dominant compounds. The qualitative composition of both n-hexane and ethanol extracts of *Iris dichotoma* Pall., obtained by pressurized liquid extraction, was similar and revealed the presence of 6 components, which were identified as natural products with methyl myristate as the major one (22.47 % and 10.18 % in ethanolic and n-hexane extracts, respectively).

### Rezumat

Studiul prezentat în acest articol a fost efectuat pentru a examina compoziția calitativă și cantitativă a uleiului volatil, a extractelor n-hexanic și etanolic obținute din rizomi de *Iris dichotoma* Pall. în ceea ce privește conținutul lor volatil. S-a constatat că uleiul volatil are cea mai bogată compoziție, deși extractele finale (n-hexan și cele etanolice) constau din diverși esteri ai acizilor grași. Prin analizele GC și GC-MS ale uleiului s-au identificat 28 de compuși. Dintre acestea, acidul miristic (13,67%), metilmiristatul (9,08%) și  $\alpha$ -terpineolul (2,42%) s-au dovedit a fi compusii dominanți. Compoziția calitativă a extractelor n-hexanic și etanolic, obținute prin metoda extracției cu lichide presurizate, a fost similară și a evidențiat prezența a 8 componente. Șase dintre ele au fost identificate ca produși naturali, având miristatul de metil drept component major (22,47% și 10,18% în extractul etanolic și, respectiv, n-hexanic).

**Keywords:** *Iris dichotoma*, volatile oil, terpenes, GC-MS analysis.

### Introduction

*Iris* species belong to the plants widely distributed around the globe. They grow world-wide in tropical and temperate regions of the world, with

the highest diversity occurring in South Africa, followed by South America, Europe and temperate regions of Asia [6]. Approximately 300 species of these colourfully flowering plants have been described so far [7].

*Iris* species have an immense medicinal importance and are used in the treatment of cancer, inflammation, bacterial, and viral infections [10, 18, 19]. Numerous scientific papers treating on the variety of their pharmacological activities and evoking the presence of isoflavones, flavones, terpenoids, xanthenes, or simple phenolics in the extracts have been published within recent years [7, 11, 13, 18].

*Iris dichotoma* Pall. is a perennial herb that is native to the north China. It is an elegant herb, 40–60 cm long with underground rhizomes. The leaves are 1.5–3.5 cm wide, 15–35 cm long, sword-shaped, slightly curved, with no midvein present. The plant has one green flower stalk dichotomously branched rising from a rhizome. The flowers are 12–15 cm in diameter, violet, pale blue, or cream with purplish brown markings [24]. Rhizomes of *I. dichotoma*, called Bai-Sheganare commonly used in Chinese folk medicine for clearing heat and detoxification, phlegm elimination, swelling, and sore throat [25].

The main active components in *I. dichotoma* are phenolic compounds, namely flavonoid or isoflavonoid aglycones and their corresponding glycosides. Previous phytochemical studies indicated that the flavonoids of *I. dichotoma* include wogonin, rhamnazin, isovitexin, hispidulin, rhamnocitrin and tamarixetin-7-glucoside, whereas the isoflavonoids of *I. dichotoma* include irisfloreantin, dichotomitin, irigenin, genistin, tectorigenin, tectoridin, iristectorigenin A, iristectorin A and iristectorin B [11, 14, 16]. Although the polyphenolic compounds of *I. dichotoma* are well described, the chemical composition of its essential oil is lacking. In the recent scientific literature only few studies treat on the composition of essential oil obtained from other *Iris* species. *Iris nigricans* Dinsm. – endemic to Jordan – was found to possess significant amounts of aliphatic hydrocarbons in the essential oil, but also monoterpene hydrocarbons represented by  $\alpha$ - and  $\beta$ -pinenes [4]. Syrian species of iris rhizomes were found to produce myristic acid as the main component of the terpene fractions. Furthermore, lauric acid, decanoic acid, palmitic acid and its methyl ester, octadecanoic acid methyl ester, and elaidic acid methyl ester were identified as sub major compounds. Stearic acid, methyl oleate, palmitic acid and 1,8-cineole belong to the major constituents of *Iris pseudoacorus* essential oil [5]. The above mentioned results confirm the diversity of essential oils' composition depending on the investigated species.

In this paper, the essential oil as well as n-hexane and ethanolic extracts of *I. dichotoma* rhizomes were analysed.

## Materials and Methods

### *Plant material*

Rhizomes of *Iris dichotoma* Pall. (Iridaceae) were collected in Mongolia Dornot province, Bayandun Ulaanbataar and identified by Otgonbataar Urjin. A voucher specimen has been deposited in the Chair and Department of Pharmacognosy with Medicinal Plant Unit (WID10001).

### *Preparation of the extracts*

- Hydrodistillation in a Deryng apparatus

100 g of dried rhizomes of *Iris dichotoma* Pall. were coarsely powdered, transferred to a round-bottomed flask and submitted to steam distillation process with 250 mL of water in a Deryng-type apparatus for 4 hours. The obtained essential oil was dried over anhydrous sodium sulphate and stored in amber glass at 4°C.

- Extraction in a Pressurized Liquid Extractor (PLE)

Pressurized Liquid Extraction was performed on a Dionex 100 apparatus (Dionex, Sunnyvale, USA). 5 g of rhizomes were placed in a 34 mL stainless steel extraction cell and extracted with n-hexane. The detailed extraction conditions were set as follows: extraction temperature: 60°C, static time: 15 min, flush volume: 100%, purge time: 80 sec, number of cycles: 3. The operating pressure was ranging around 105 bar. The same procedure was repeated when ethanol was used as a solvent.

The obtained extracts were subsequently evaporated to dryness under vacuum on a water bath at 25°C, re-dissolved in 3 mL of n-pentane, filtered through a nylon membrane filter (nominal pore size: 45 µm) and transferred to an amber glass vial.

### *Reagents*

All solvents used were of analytical grade and were purchased from the Polish Reagents (POCH, Gliwice, Poland). Helium 5.0 used for GC-MS analysis was 99.999% pure (PGNiG, Poland).

### *GC-MS*

GC-MS was performed with a Shimadzu GC-2010 Plus gas chromatography instrument coupled to a Shimadzu QP2010 Ultra mass spectrometer. Compounds were separated on a fused silica capillary column ZB-5 MS (30 m, 0.25 mm i.d.) with a film thickness of 0.25 µm (Phenomenex). The following oven temperature program was initiated at 50°C [17], held for 3 min, then increased at the rate of 5°C min<sup>-1</sup> to 250°C, held for 15 min. Injector, interface and ion source were kept at 250, 250 and

220°C, respectively. Split injection (1 µL) was conducted with a split ratio of 1:20 and helium was used as carrier gas of 1.0 mL\*min<sup>-1</sup> flow-rate. The spectrometers were operated in electron-impact (EI) mode, the scan range was 40–500 amu, the ionization energy was 70 eV and the scan rate was 0.20 s per scan. The retention indices were determined in relation to a homologous series of n-alkanes (C8-C24) under the same operating conditions. Compounds were identified using a computer-supported spectral library [1], mass spectra of reference compounds, as well as MS data from the literature [2, 3].

## Results and Discussion

A significant number of *Iris* species have been reported for their medicinal properties so far. The plants were used in Asian traditional medicine to treat cough, sore throat or asthma [12, 21, 23]. Currently they have been reported to possess notable antimicrobial, antifungal, chemopreventive, anti-tubercular and antioxidant properties [8, 22] mainly due to the isoflavone content, although Deng *et al.* [8] confirmed marked antibacterial and antifungal properties of the essential oil derived from *Iris bulleyana*. The terpene composition of *Iris dichotoma* has not been reported so far. As the plant is widely used in traditional medicine of Mongolia and China until nowadays, the authors found it reasonable to identify the dominant volatiles, which presence may influence its overall pharmacological activity.

The major constituents of the essential oil obtained from *Iris dichotoma* rhizomes are shown in the Table I. while the composition of n-hexane and ethanol extracts is presented in the Table II. 28 compounds representing 39.52% of essential oil were identified. Myristic acid (13.67%) and methyl myristate (9.08%) were the most abundant components. The others were α-terpineol (2.42%), hexadecanoic acid methyl ester (1.86%), brachyloxide (1.62%), n-decanoic acid (1.22%) and geranyl acetone (1.04%). The following chemical components occurred in the analyzed essential oil in smaller amounts: eudesm-4(15)en-7-ol (0.92%), isoneral (0.82%), 1-epi-cubenol (0.77%), tetradecanal (0.77%), octanoic acid (0.58%), nonanoic acid (0.52%), trans anethol (0.48%), δ-cadinene (0.44%), *trans*-geraniol (0.43%), α-caracorene (0.41%), cymen-9-ol (0.36%), linalool (0.33%), α-muurolene (0.31 %), α-cadinol (0.27%), *cis*-verbenol and 2,4-decadienal (0.2%), capric acid methyl ester and geranial (0.16%) and *m*-eugenol (0.11%) (Table I).

**Table I**

Chemical composition of the volatile compounds of *Iris dichotoma* rhizomes obtained by hydrodistillation of essential oil (RRI – Relative Retention Index).

| No | Compound                                      | Retention time [min] | RRI NIST | RRI MF | Area % |
|----|-----------------------------------------------|----------------------|----------|--------|--------|
| 1  | Linalool                                      | 13.24                | 1105     | 1086   | 0.33   |
| 2  | <i>cis</i> -Verbenol                          | 14.54                | 1148     | 1132   | 0.20   |
| 3  | Isoneral                                      | 14.65                | 1152     | 1140   | 0.82   |
| 4  | Octanoic acid                                 | 15.80                | 1189     | 1156   | 0.58   |
| 5  | Cymen-9-ol                                    | 15.99                | 1196     | 1157   | 0.36   |
| 6  | $\alpha$ -Terpineol                           | 16.19                | 1203     | 1176   | 2.42   |
| 7  | <i>trans</i> -Geraniol                        | 17.74                | 1256     | 1235   | 0.43   |
| 8  | $\alpha$ -Citril                              | 18.19                | 1272     | 1244   | 0.16   |
| 9  | Nonanoic acid                                 | 18.53                | 1284     | 1263   | 0.52   |
| 10 | <i>trans</i> -Anethol                         | 18.72                | 1293     | 1262   | 0.48   |
| 11 | 2,4-Decadienal                                | 19.63                | 1324     | 1270   | 0.2    |
| 12 | Capric acid methyl ester (methyl decanoate)   | 19.72                | 1327     | 1300   | 0.16   |
| 13 | <i>m</i> -Eugenol                             | 20.59                | 1359     | 1346   | 0.11   |
| 14 | n-Decanoic acid                               | 21.29                | 1385     | 1347   | 1.22   |
| 15 | Tetradecanal                                  | 22.05                | 1414     |        | 0.77   |
| 16 | Geranyl acetone                               | 23.02                | 1452     | 1430   | 1.04   |
| 17 | 2-Tridecanone                                 | 24.22                | 1500     | 1477   | 0.3    |
| 18 | $\alpha$ -Murolene                            | 24.37                | 1505     | 1496   | 0.31   |
| 19 | $\delta$ -Cadinene                            | 24.86                | 1526     | 1520   | 0.44   |
| 20 | $\alpha$ -Caracorene                          | 25.44                | 1550     | 1527   | 0.41   |
| 21 | Brachyloxide                                  | 26.80                | 1607     | 1599   | 1.62   |
| 22 | 1-Epi-cubenol                                 | 27.52                | 1638     | 1623   | 0.77   |
| 23 | Eudesm-4(15)en-7-ol                           | 27.88                | 1654     | 1643   | 0.92   |
| 24 | $\alpha$ -Cadinol                             | 28.20                | 1668     | 1643   | 0.27   |
| 25 | Methyl myristate (myristic acid methyl ester) | 29.58                | 1730     | 1700   | 9.08   |
| 26 | Farnesal                                      | 29.88                | 1744     | 1707   | 0.07   |
| 27 | Myristic acid (tetradecanoic acid)            | 30.83                | 1788     |        | 13.67  |
| 28 | Hexadecanoic acid methyl ester                | 33.80                | 1932     |        | 1.86   |

MF = Mass Finder, NIST = National Institute of Standards and Technology

6 compounds representing 15.82 and 35.36 percent of hexane and ethanol extracts from *Iris dichotoma* rhizomes, respectively, were identified. Methyl myristate was the most abundant component in both hexane and ethanol extracts (10.18% and 22.47%). In the case of hexane extract methyl palmitate (3.15%) and methyl linoleate (1.21%) were identified. Presence of methoxyeugenol (0.46%), methyl oleate and methyl stearate (0.41%) was confirmed in hexane extract in small amounts. The other components of ethanol extract were methyl palmitate (6.99%), methyl linoleate (2.76%),

methoxyeugenol (1.23%) and methyl oleate (1.0%). Methyl stearate (0.91%) occurred in ethanol extract in small amounts (Table II).

**Table II**

Chemical composition of the volatile compounds of *Iris dichotoma* rhizomes in hexane and ethanol extracts (RRI – Relative Retention Index)

| No | Compound                                     | Retention time [min] | RRI NIST | RRI MF | Area % (Hexane) | Area % (Ethanol) |
|----|----------------------------------------------|----------------------|----------|--------|-----------------|------------------|
| 1  | 4-Allyl-2,6-dimethoxyphenol (methoxyeugenol) | 26.72                | 1604     | 1561   | 0.46            | 1.23             |
| 2  | Methyl myristate                             | 29.59                | 1730     | 1700   | 10.18           | 22.47            |
| 3  | Methyl palmitate                             | 33.80                | 1932     | 1901   | 3.15            | 6.99             |
| 4  | Methyl linoleate                             | 37.02                | 2099     | 2046   | 1.21            | 2.76             |
| 5  | Methyl oleate                                | 37.14                | 2106     | 2082   | 0.41            | 1.00             |
| 6  | Methyl stearate                              | 37.65                | 2134     | 2104   | 0.41            | 0.91             |

MF = Mass Finder, NIST = National Institute of Standards and Technology

The qualitative composition of both n-hexane and ethanol extracts of *Iris dichotoma* varied from the hydrodistilled essential oil. The former extracts contained significant amounts of long-chained constituents, which prevailed over simple terpenes - so abundant in the essential oil. Both pressurised liquid extraction (PLE) extracts contained methoxyeugenol which was absent in the essential oil. Furthermore, a variety of long-chained esters of fatty acids appeared in the GC spectrum after pressurized liquid extraction.

Composition of *Iris dichotoma* essential oil differed from the one described in the case of other species [4, 5, 20]. 1,8-cineole was not present in the investigated rhizomes. Moreover, pinenes - major components of Jordan's *Iris spp.* - were absent in the studied samples. Myristic acid and its methyl ester were identified in the essential oil of the Mongolian species and constituted the major products of the hydrodistilled oil, similarly to the formerly discussed Syrian species of the same genus [5]. Based on these observations, it can be concluded, that *Iris dichotoma* is characterized by a distinguishing composition of terpenes. Presented results confirmed marked intra-species differences.

Both n-hexane and ethanol extracts contained fatty acids' esters which are common among *Iris* rhizomes. Esters of palmitic, stearic, oleic or linoleic acids were found in *Iris dichotoma*, but were also described in other *Iris* species.

## Conclusions

Rhizomes of *Iris dichotoma* Pall. are characterized by a high content of essential oil, distinguished by a rich chemical composition. Among 28

volatile components of the essential oil - myristic acid, methyl myristate and  $\alpha$ -terpineol were dominant. Chemical composition of volatiles varied depending on the extraction conditions applied. The content of ethanol and n-hexane extracts obtained by means of PLE extraction revealed the presence of long-chained fatty acids' esters with methyl myristate and methyl palmitate as the major ones.

#### Acknowledgements

The authors would like to thank Shim-Pol A.M. Borzymowski Company, sole representative of Shimadzu and Phenomenex in Poland, for GC-MS equipment and ZB-5 MS column.

The authors have declared no conflict of interest.

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*Manuscript received: September 2013*