Original Article

Thai Journal of Pharmaceutical Sciences

Chemical composition of the essential oil of several *Iris* species

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Received: Jul 07, 2019
Accepted: Mar 09, 2020
Published: Jul 13, 2020

ABSTRACT

The essential oil was obtained by a steam distillation from the dried leaves of *Iris germanica* L., *Iris versicolor* L., *Iris graminea* L., and *Iris halophila* Pall. (*Iridaceae*) and was investigated by a gas chromatography-mass spectrometry (GC/MS) method. In *I. germanica*, *I. versicolor*, *I. graminea*, and *I. halophila* were determined 0.06%, 0.10%, 0.06%, and 0.02% of oil yields, with a total of 28, 31, 25, and 23 compounds, respectively. In leaves are predominate essential oil triterpenoids, fatty acids, their esters, aliphatic hydrocarbons, and their derivatives. The highest content of α-irone and *trans*-2,6-γ-irone was obtained in the oil of *I. graminea* leaves with 2.71% and 1.00%, respectively. Contents of α-irone and γ-irone are accepted as the most significant commercial quality criteria of *Iris* essential oil. Such compounds as β-damascenone (1.11, 0.50% in *I. versicolor* and *I. germanica,* respectively), phytol (only in *I. versicolor*, 22.40%), and squalene (in all species, 5.95–22.84%) were identified for the 1st time in *Iris* genus plants. This is the first report about the isolation and identification of oil from *Iris* leaves by GC-MS.

Keywords: Essential oil, gas chromatography-mass spectrometry analysis, *Iridaceae*, iris species, leaves

INTRODUCTION

Iridaceae is a large family of perennial herbaceous plants with rhizomes or corms comprising nearly 1750 species in 82 genera.[1] Plants belonging to this family are distributed worldwide in tropical and temperate regions of the world, with the highest diversity occurring in South Africa, followed by South America, Europe, and the temperate regions of Asia.[2] In Ukraine, *Iridaceae* family is represented by four genera, including *Gladiolus*, *Crocus*, *Sisyrínchium*, and *Iris*. There are 16 *Iris* species[3] occurring wild in Ukraine. *Iris germanica* and *I. versicolor* are not endemic for our region because they are entered on the territory of Ukraine through the introduction.[4]

Iris genus plants have been used in traditional medicines to treat cold, flu, malaria, toothache, bruises, and burns.[5] The use of rhizomes of several *Iris* plants in traditional medicine dates far back in time, especially in European communities. The dried rhizomes of several *Iris* species, particularly those of *I. germanica*, were collectively used as the ingredients of the toothpowders and as an enema or topically rubbing the oil on arthritic limbs.[6] Drug *Iridis rhizoma*, which is composed of small selected rhizome species, enjoyed popularity due to its emetic, cathartic, diuretic, stimulant, expectorant, and errhine properties, and in addition to its use as a masticatory drug in relieving pain for teething in children.[7] Aromatherapy recommends[8] to use of *Iris* oil for treating bronchitis and whooping cough. Oil of *Iris* root has also a regulating effect on nervous disorders and is helpful for emotional shock, stress, and depression.[5,9] Cosmetically, the oil helps to maintain healthy skin and nourishes sensitive skin.[10]

Plants *Iris* genus are rich sources with secondary metabolites: Flavonoids,[11] isoflavonoids and their glycosides,[12] xanthones,[7,13] quinones,[14] triterpenoids,[15-21] carboxylic acids,[22] and stilbene glycosides.[7,13] The clinical studies of biologically active compounds of *Iris* species have given positive results in the treatment of cancer, bacterial, and viral infections.[7,12,13,20] The compounds were isolated from different *Iris* spp. and were reported to have piscicidal, antineoplastic, antioxidant, antitumor, antiplasmodial, antituberculosis, anti-inflammatory, anticancer, and other properties.[7,18,21]

Phytochemical investigations of *Iris* spp. were very limited, most of them were dealt with a determining of secondary metabolites only in rhizomes. However, *Iris* species and varieties have a large phytomass due to their leaves, which are production waste during the harvesting of *Iris* rhizomes and are thrown away. In addition, the biological feature of the development

of *Iris* requires the mandatory pruning of leaves, which makes it possible to collect raw materials every vegetative period. However, leaves and flowers of *Iris* species accumulate different classes of blood alcohol concentrations such as flavonoids, hydroxycinnamic acids, anthocyanins, carboxylic acids, and aromatical compounds which exhibit various pharmacological effects and can be used as functional components in food products, medicinal products, and food supplements. The chemical composition of *Iris* leaves did not be studied. For the study were chosen four *Iris* species (*I. germanica*, *I. versicolor*, *Iris graminea*, and *Iris halophila*) [Figure 1] of cultivated flora, they had the sufficient resource base and belonged to different sections. According to the classification of Rodionenko,[23] *I. halophila* and *I. gramineae* belong to the subgenus *Xyridion* (Tausch) Spach em Rodion. (sect. *Xyridion*), series *Spuria* (Diels) Lawrence em Rodion.), and series *Graminea* Rodion. ser. nov., respectively. *I. germanica* belongs to the group of Bearded *Irises* (*Barbatae* are the species with flowers, bearing on the outer perianth lobes a beard of multicellular hairsprings) from section *Iris* to the series *Elatae* Lawrence. *I. versicolor* belongs to the subgenus *Limniris* (Tausch) Spach em Rodion., sect. *Limniris*, series *Laevigatae* (Diels) Lawrence.

The aim of the present work was the determination of the essential oil composition of *I. germanica* L., *I. versicolor* L., *I. graminea* L., and *I. halophila* Pall. leaves by gas chromatography-mass spectrometry (GC/MS) method.

MATERIALS AND METHODS

Plant Material

Germanica L., *I. versicolor* L., *I. graminea* L., and *I. halophila* Pall. leaves (*Iridaceae*) [Figure 1] were collected from Kharkiv Botanical Garden's territory of V.M. Karazin Kharkiv National University (Kharkiv, Ukraine), in spring, 2017 and were identified and authenticated by one of the authors (Dr. Orlova). The plant material complies with the description of Ukraine Flora.[3] All voucher specimens (CWU 0056545, CWU 0056540, CWU 0056551, CWU 0056552) verified by Yu. G. Gamulya and deposited at Herbarium of V.M. Karazin Kharkiv National University (CWN), Ukraine. Analysis and estimation of the results were performed with air-dried raw materials.

Preparation of Volatile Oils and Extracts

A sample of the dried raw material (50 mg) was placed in a 20.0 mL vial and 50 μg of tridecane (internal standard) and 10.0 mL of water were added. The volatile compounds were distilled from the samples with steam for 2 h. In the distillation process, the volatile material was adsorbed on the inner surface of the reflux condenser. After the cooling, the adsorbed material was washed off by slow addition of 3.0 mL of pentane (content of micro impurities is 1.0 mg by 1.0 L) in a dry vial on 10.0 mL. Washout was concentrated with nitrogen until the volume of the extract was 10.0 μL, and which was fully collected by a chromatographic syringe.

Chromatographic Conditions

GC/MS analyses of the chemical composition of *Iris* leaves were carry out using an Agilent Technologies 6890 with a mass-spectrometric detector (model 5973) (USA) equipped with fused-silica capillary column 5% phenyl-poly-dimethyl-siloxane (DB-5 capillary column) (30 m x 0.25 mm i.d., 0.25 μm film thickness).[24] Samples were injected in the split mode, using helium as the carrier gas (flow rate 1.2 mL/min); the injection volume was 2.0 μL. The oven temperature was programmed from 50 (3 min hold) to 320°C at 4°C/min; injector temperatures 250°C.

Identification of Components

For the identification of components, data from the mass-spectra libraries NIST-MS Library 05[25,26] and WILEY GC-MS Library 2007[27] were used. The volatiles content was defined as the signal-concentration ratio between volatile compound and internal standard.[24,28] Relative component content (%) of the separated compounds was defined automatically from peak areas of the total ion chromatograms. Calculation of components content C (mg/kg) was carried out by the formula:

$$C = \frac{P1 \times 50 \times 1000}{P2 \times m}$$

Where: P1 – a peak area of tested compound; P2 – a peak area of standard compound; 50 – mass of internal standard (μg), injected into the sample; m – sample mass (mg).

RESULTS

The essential oil was obtained by steam distillation of the air-dried *Iris* leaves. Oil yield was 0.06%, 0.10%, 0.06%, and 0.02% in *I. germanica*, *I. versicolor*, *I. graminea*, and *I. halophila*, respectively.

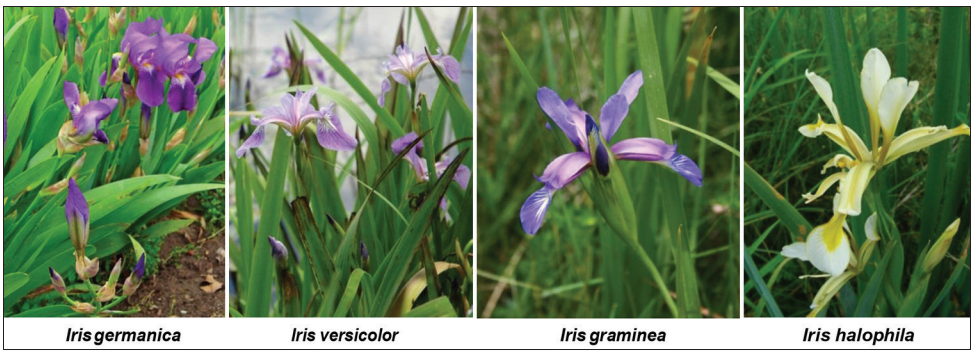

Figure 1: General view of the Irises living plants

By GC/MS analysis has been identified from 23 to 31 compounds in different *Iris* species [Figure 2a-d]. The constituent contents varied from 0.03% to 32% of essential oil. Some compounds were found in all species tested, other compounds were observed in one or two of species by variable quantities. The constituents of the essential oil that have been obtained from *Iris* leaves are shown with their percentages and relative retention indices (RRI) in Table 1. The essential oil included terpenoids, their oxygenated derivatives (alcohols, ketones, aldehydes, and esters), aromatic compounds, and triterpenoids [Table 2].[21,29]

DISCUSSION

We present the results of the study of the volatile components of *I. germanica*, *I. versicolor*, *I. graminea*, and *I. halophila* leaves of Ukraine origin by GC/MS method for which no such similar studies have yet been conducted. The essential oil of the dried *Iris* leaves was obtained individually by steam distillation (50 mg each) for 12 h in an apparatus consisting of a 25-mL round-bottomed flask, a reflux condenser, and a water bath.[28,30] The oil was collected over *n*-hexane and then dried over anhydrous sodium sulfate and kept in separated sealed vials at –30°C for further analyses. The yield in % (w/w) was determined in the triplicate and based on the initial plant of dry weight. The method allows to isolate the essential oil from plant material with the trace quantities of essential oil.

The GC/MS analyses of *Iris* leaves revealed the existence from 23 to 31 constituents. According to the results on *I. graminea*, a sum of 25 identification components was established and the highest content was at the monoterpene ketone – α-irone (2.71%) in the comparison between in all samples. Figure 3 shows the MS spectrum of α-irone identified. It is known that ketone α-irone to be an indicator of the authenticity of *Iris* rhizome oil.[31] Thanks to the process of the enzymatic disintegration of an iridin (7-glucoside of irigenin) for 2–3 years, the dried *Iris* rhizomes get a characteristic violet-like odor, which is lucratively used in perfumery industry.[23,24] The presence of irones, homologs to the ionones in *I. pallida* (4.8%),[31] *I. germanica* (20%),[32] and *I. florentina* (4.21%) rhizomes[33] were reported and therefore, these *Iris* species are successfully used as raw materials in the perfume industry.[34]

Norterpenoids and their derivatives were observed in all *Iris* species leave for the 1st time. Their compositions differ. The highest percentage of norterpenoids was obtained in *I. graminea* (5.71%), there was no significant difference between *I. halophila* and *I. germanica* (2.90% and 3.13%), respectively; with a much smaller amount (1.61%) was in *I. versicolor*. Among them were identified β-damascenone (1.11% and 0.50% at *I. versicolor* and *I. germanica,* respectively), β-ionone-5,6-epoxide (0.41–1.41%), and β-ionone (0.09–1.30%). The most various composition of norterpenoids was found in *I. graminea* leaves. α-Ionone (0.21%), *trans*-2,6-γ-irone (1.00%), and β-isomethyl ionone (0.26%) were identified only in this *Iris* species. Earlier, we identified norterpenoids in *I. pallida*[15] and *I. hungarica* rhizomes.[16] Availability of irones and ionones in the raw material determines of the presence of mucolytic, antimicrobial, and antiviral actions.[34]

The highest percentage of terpenes squalene was observed in *I. halophila* (22.84%), no significant differences were detected in *I. germanica* (17.01%), and *I. versicolor* (16.17%),

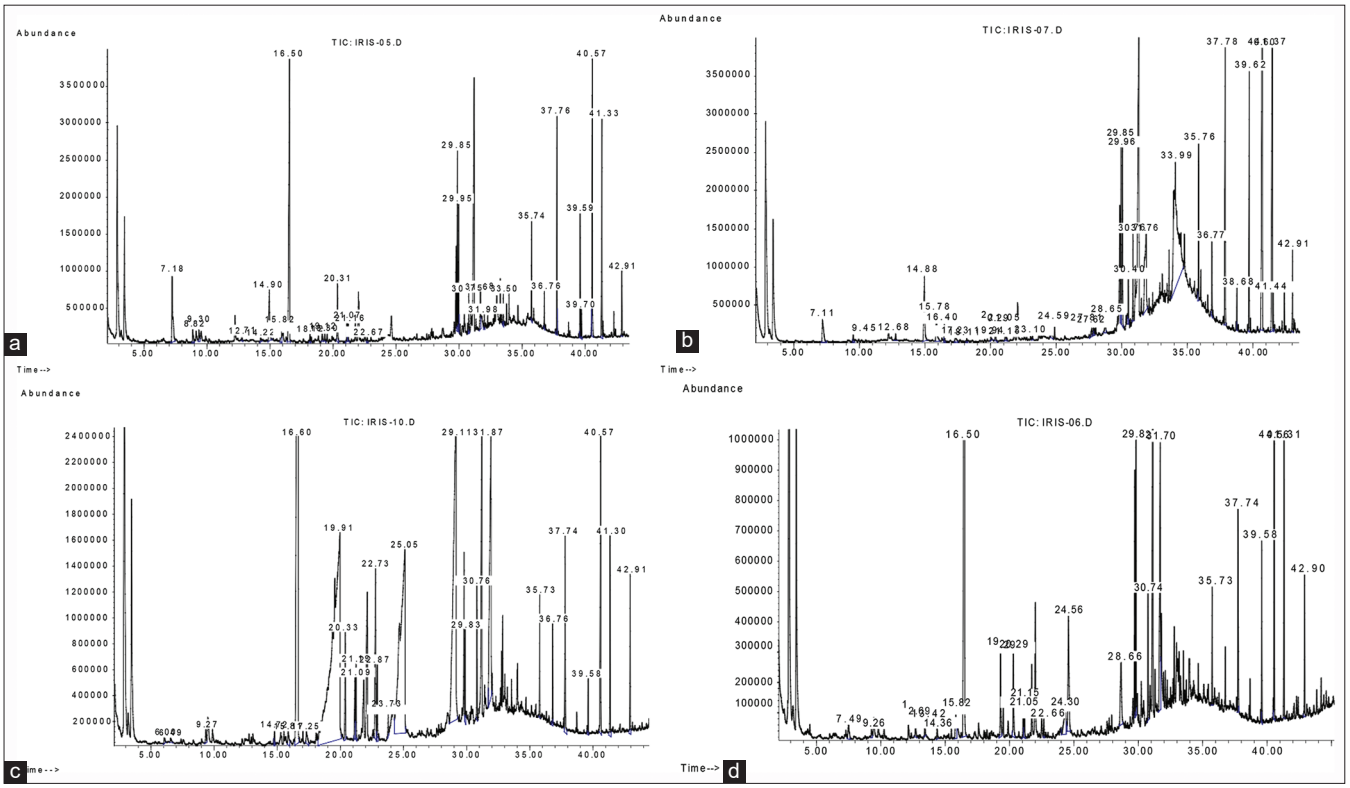

Figure 2: Chromatograms of gas chromatography-mass spectrometry analysis of the essential oil of Iris species leaves (a) *Iris germanica*, (b) *Iris versicolor*, (c) *Iris graminea*, **(**d) *Iris halophila*

Table 1: Chemical composition of the essential oil of *Iris species* leaves in % of the total oil content

RRI	Compound	*Iris germanica*	*Iris versicolor*	*Iris graminea*	*Iris halophila*
973	6-Methyl-5-hepten-2-one	-	-	0.13	-
986	2,4-heptadienal	-	-	0.13	-
1001	Phenylacetaldehyde	7.48	1.47	-	-
1011	2-Acetyl-1,4,5,6-tetrahydropyridine	-	-	-	1.00
1052	2,3,5,6-Tetramethylpyrazine	1.12	-	-	-
1067	6-Methyl-3,5-heptadien-2-one	1.08	-	0.24	0.40
1075	Octanal	-	0.27	-	-
1174	Decanal	0.40	0.24	-	0.76
1197	3-Methyl tin pyridinol-3	-	-	-	0.55
1221	α-Ethylidene-phenylacetaldehyde	0.38	-	-	-
1226	*trans*-2-Decenal	-	-		0.47
1237	α-Ionone	-	-	0.21	-
1242	Indole	5.10	2.94	-	-
1271	2-Methoxy-4-vinylphenol	1.12	1.52	0.34	1.62
1315	Eugenol	-	0.34	0.37	-
1343	β-Damascenone	0.50	1.11	-	-
1374	Dodecanal	0.72	-	-	-
1380	4′-Isobutylacetophenone	0.62	-	-	3.33
1399	Capric acid	-	-	32.27	-
1400	3-Phenylpyridine	-	0.11	-	-
1411	Geranyl acetone	3.86	0.50	1.39	3.15
1435	β-Ionone-5,6-epoxide	1.41	0.41	0.86	0.95
1438	β-Ionone	1.19	0.09	0.93	1.30
1485	α-Irone	0.03	-	2.71	0.65
1491	*trans*-2,6-γ-Irone	-	-	1.00	-
1499	Megastigmatrienone	-	0.10	-	-
1518	β-Isomethyl ionone	-	-	0.26	-
1545	Benzophenone	-	0.66	-	6.60
1560	Lauric acid	-	-	18.29	2.85
1691	2,3,6-Trimethyl naphthoquinone	-	0.43	-	-
1700	Benzyl benzoate	-	0.11	-	-
1742	Myristic acid	-	0.62	17.44	4.47
1802	Hexahydrofarnesylacetone	9.10	4.38	0.87	7.26
1807	Neophytadiene	5.53	3.93	-	-
1872	Farnesyl acetone	1.95	0.99	1.16	3.20
1872	Farnesyl acetone C	-	1.68	-	-
1941	Palmitic acid	2.77	5.66	7.86	12.97
1964	Palmitic acid, methyl ester	0.91	-	-	-
2077	Eicosane	1.46	-	-	-
2114	Phytol	-	22.40	-	-
2300	Tricosane	4.01	3.01	0.94	2.40
2400	Tetracosane	1.44	1.33	0.82	
2500	Pentacosane	10.14	8.56	2.06	5.48
2600	Hexacosane	-	0.68	-	-
2700	Heptacosane	5.19	5.64	0.48	3.92
2714	Lignoceric acid, methyl ester	1.25	-	-	-
2758	Squalene	17.01	16.17	5.95	22.84
2800	Nonacosane	11.00	13.26	1.86	10.58
2856	Cerotic acid, methyl ester	-	0.70	-	-
2906	Untriacontane	2.89	1.60	1.45	3.28

RRI: Relative retention index, "–" means that the compound was not identified

Table 2: General chemical composition of the essential oil of *Iris species* leaves, content in % of the total oil content

Compounds	*Iris germanica*	*Iris versicolor*	*Iris graminea*	*Iris halophila*
Alkanes	53.14	34.08	7.61	25.66
Aromatic compounds	19.97	8.19	1.21	14.73
Monoterpenes	3.68	0.50	1.39	3.15
Sesquiterpenes	9.10	8.66	8.0	13.36
Diterpenes	5.53	26.33	-	-
Triterpenes	17.01	16.17	5.95	22.84
Fatty acids	4.94	6.98	57.57	20.29
Oil yield, %	0.06	0.10	0.06	0.02

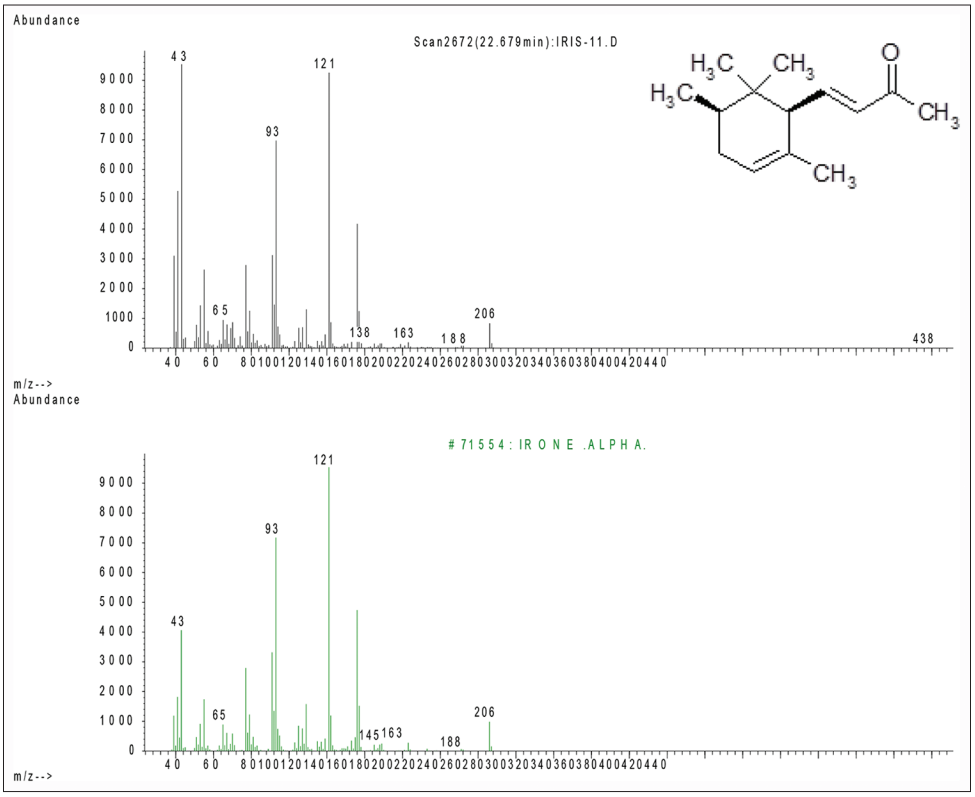

Figure 3: Mass spectrometry fragmentation patterns and structure of the α-irone (experimental data – at the top, the library data – below)

the smallest contents of squalene was in *I. graminea* (5.95%). According to our data, squalene was not identified by other authors in plants of *Iris* genus, there are only a few of our previous studies in which we identified squalene in *I. hungarica, I. pallida, I. carthalinia,* and *I. medwedewii.*[15-17] It is important that squalene has an emollient and antioxidant effect for the skin, it has a hydration and antitumor activities,[35] and it can be used as part of cosmetics.[10]

Furthermore, sesquiterpenoids hexahydrofarnesylacetone (contents from 1 to 9%), farnesyl acetone (1–3%), and geranyl acetone (0.5–4%) were detected in all *Iris* species, which exhibited antimicrobial and cytotoxic activity, according to the last pharmacological studies.[36] At the same time, farnesyl acetone C (1.68%) and megastigmatrienone (0.10%) were identified only in *I. versicolor*. According to authors,[19] geranyl acetone (1.08%) has been identified early only in *I. nigricans* flowers. The results obtained to permit the investigations of *Iris* leaves extracts as immunostimulatory and antibacterial remedies.

It is known that myristic acid (83–96%), it is a second important component of iris concrete received by a steam distillation.[34] The highest contents of myristic acid were obtained in the leaves of *I. graminea* (17.44%), their lowest values of myristic acid were determined in *I. versicolor* and *I. halophila* (0.62% and 4.47%, respectively), in *I. germanica* myristic acid was absent. For leaves of *I. graminea* oil predominate fatty acids, aliphatic hydrocarbons, and their derivatives (57.57%). Capric acid (32.27%) represented the major component of oil in *I. graminea* leaves, followed by lauric acid (18.29%), myristic acid, and palmitic acid (7.89%). Composition of fatty acids and their esters gives us the possibility to investigate the extracts of *Iris* leaves as antifungal, antitumor antioxidant, anti-inflammatory, and immunomodulatory properties,[37] as well as myristic acids

involved in the synthesis of prostaglandins and stability of cellular membranes.

The chemical composition of the essential oil of *I. versicolor* leaves was presented of 31 compounds and the dominant component was diterpene alcohol phytol (22.40%), which was a part of chlorophyll. It has been found only in this *Iris*. Diterpene neophytadiene was indicated in *I. versicolor* and *I. germanica* (3.93% and 5.53%, respectively). For comparison, neophytadiene was identification in the areal part of *I. taochia*[18] and antioxidant activity of plant extract was established. Neophytadiene is a good analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant compound.[37] Ketone megastigmatrienone (0.10%) was identified only in *I. versicolor*. The aromatical compound eugenol (by 0.34%) was found only in *I. versicolor* and *I. graminea*. One of the functions of eugenol is a stimulator of cell proliferation. According to literature analysis, eugenol is found in *I. planifolia* whole part (0.1%)[20] and *I. dichotoma* rhizomes (0.11%)[21] by GC/MS method too.

Common of *Iris* leaves was 2-methoxy-4-vinylphenol (0.34–1.62%), geranyl acetone, *β*-ionone-5,6-epoxide, *β*-ionone, hexahydrofarnesylacetone, farnesyl acetone, squalene, and some saturated hydrocarbons. In the chemical composition of the essential oils from *Iris* leaves, tricosane, tetracosane, pentacosane hexacosane, and other alkanes were found, the presence of which is due to the fact that they are a part of the wax of the cuticle covering the leaves of the plant and are also the volatile compounds and fly with terpenoids in the analysis.

Considering of the total content of different groups of terpenoids in the essential oil of *Iris* species, in particular of oxygenated derivatives (alcohols, phenols, ketones aldehydes [not less than 8%], sesquiterpenoids [not less than 8%], fatty acids [5–60%, depending on *Iris* spp.], and other), it is possible to assume antimicrobic, mucolytic, immunostimulatory, anti-inflammatory activity, etc., that are characteristic of plants. All obtained results give us the possibility to investigate the use of *Iris* leaves extracts for medical purposes. In general, the definition of an orientation of pharmacological effects of essential oils of *Iris* species assumes a continuation of our researches.

Chemical composition of the biologically active compounds, including the presence of irones in leaves of *I. germanica*, *I. versicolor*, *I. graminea*, and *I. halophila* could have the industrial significance.[9,34] To the best of our knowledge, the qualitative and quantitative analysis of the essential oil of *I. germanica*, *I. versicolor*, *I. graminea*, and *I. halophila* leaves from Ukraine has not been previously reported by the method of GC/MS.

CONCLUSIONS

Qualitative and quantitative analysis of the essential oil of *I. germanica*, *I. versicolor*, *I. graminea*, and *I. halophila* leaves were conducted by GC/MS method for the 1st time. It revealed that essential oil contained such compounds as alkanes (7–53%), aromatic compounds (1–20%), monoterpenes (0.5–4%), sesquiterpenes (8–13%), diterpenes (5–26%), triterpenes (5–23%), and fatty acids (5–50%). Since *Iris* essential oil has healthy aromatic compounds, terpenoids, and fatty acids compositions, the oil is favorable for the food and pharmacological and cosmetic industry. The *Iris* genus plants are a source of bioactive substances, so further studies should be carried out to evaluate other healing properties of the oil. It must be remembered that the biological properties depend not only on the total extract yield or on the content of an individual component but also on its complete phytochemical composition.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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