Composition of Volatile Oil of *Iris pallida* Lam. from Ukraine

INTRODUCTION

Iris L. is the largest and the most complicated genus of the *Iridaceae*. Genus includes more than 300 *Iris* species. Now the range of the genus extends to all of the continents of the Northern Hemisphere, their distribution covers Europe, the Middle East and northern Africa, Asia and across North America (1-2). *Irises* are used in traditional medicine and aromatherapy. Many of them are common the ornamental plants (3). Sixteen species of *Iris* genus inhabit in Ukraine (4).

Some species of this genus, such as *I. versicolor* L., *I. variegate* L., *I. florentina* L., and *I. germanica* L. are gained a great attention from cosmetic and perfume industries (5-6) due to their violet-like smell caused by irone-type compounds. Essential oil of *Iris* is a part of perfumes and lotions of the higher quality, such as "Iris Ganache" (Guerlain), "Extravagance d`Amarige" (Givenchy), "Dia pour Femme" (Amouage), "Les Exclusifs de Chanel 28 La Pausa" (Chanel), "Ghost summer breeze" (Ghost) and other.

On the pharmaceutical market are several medicines and dietary supplements, which based on the presented *Irises*. Rhizomes of *I. pseudacorus* L. are used as the part of the collecting on M.N. Zdrenko (Ukraine; Russian Federation) and "Pancreophile" ("Fytolynyya SmartMed", Ukraine) (7,8), also, *I. versicolor* is a part of homeopathic medicines "Iris-plus" (Doctor N, Russian Federation) (9) and «Kaliris – ЕДАС-114» (EDAC holding company, Russian Federation), which are used for the treatment of papillomatosis of bladder, chronic pancreatitis, antiacid gastritis, peptic ulcer of stomach. Besides, rhizomes of *I. versicolor* are the part of the complex drug Mastodynon (Bionorica SE, Germany), which is applied at violations of a menstrual cycle, mastopathy and premenstrual syndrome (10). On the base of the rhizome of I. *pallida* was created "Orris (Iris) Herbasol Extract PG" (Lipoid Kosmetik, Switzerland) for use in cosmetology for skin and hair (11). Rhizomes of *I. germanica* are the part of general tonic drug "Original grosser Bittner balsam" (Richard Bittner AG, Austria) (12). From the leaves of *I. lacteal* were created the therapeutic and prophylactic drugs for cancer patients, such as "Vitonk" (multivitamin product) and "Laktir" (to reduce the side effects during chemotherapy and radiation sickness) (Russian Federation) (9, 13).

Iris species have an immense medicinal importance and are used in the treatment of cancer, inflammation, bacterial, and viral infections. Numerous scientific papers of treating were published on the variety their pharmacological activities and evoked of the presence of flavones, flavone C-glycosides (14-15), isoflavones, terpenoids, xanthones, or simple phenolics, stilbenes and quinones in the extracts within recent years (16-17).

Many kinds of *irises* are valued for their medicinal properties in traditional use, especially in India (18) and China (14, 19), where more than 30 species have been used in folk herbal medicines. *Irises* were reported to have various biological properties, including the potent antiulcer, anticancer, antioxidant, piscicidal activities (15, 18), cytotoxic, anti-inflammatory and antibacterial activity (14, 17, 20-22).

Continuing the study of the component composition of essential oil of *Iris* plants (23-29) we chose *Iris pallida* Lam. as an object of the study. "*Orris root*" (*I. pallida, I. germanica, I. florentina*) is used to obtain the essential oil in the Mediterranean countries (6,21,22,30).

I. pallida is a perennial herb; rhizome is creeping and thick. Leaves are broadly-ensiform, 30-60 cm long; perianth is pale – purple with a yellow beard. Rhizomes contain isoflavones – irigenin, iristectorigenin A, nigricin, nigricanin, irisflorentin, iriskumaonin methyl ether, irilone, iriflogenin and other (14,15,17,21); essential oil (about 0.1%), known as "*orris butter*", consisting of about 85% myristic acid, with irone (odiferous constituent with violet-like odour), γ-dihydro-irones, ionone, methyl myristate (22,30); and other substances such as fat, resin, starch, mucilage, bitter principle, glycoside – iridin and small amount of tannin (11,17). Essential oil of rhizome strengthens the immune system, has a regenerating effect (6). Extract of roots of *I. pallida* was formerly used as a diuretic, expectorant, remedy for coughs and chronic diarrhea (18, 21). Chemical composition of essential oil of *I. pallida* from Ukraine is lacking. Studying of the chemical composition of *I. pallida* is interesting in terms of increasing the harvesting of raw materials base.

The objective of the present work was the determination of component composition of essential oil of the rhizomes and leaves of *I. pallida* from Ukraine by chromatography-mass spectrometry method.

MATERIALS AND METHODS

Plant material

The subjects of the study were rhizomes and leaves of *I. pallida* Lam. (*Iridaceae*) (Figure 3), that were prepared in September, 2015 in Luhansk region (village Kremennaya, Ukraine). Analysis and estimation of the results were carried out with the air-dry raw materials. Voucher specimens were deposited in the Herbarium of the Pharmacognosy Department and Botany Department in The National University of Pharmacy, Kharkiv, Ukraine.

Preparation of volatile oils and extracts

Essential oil of the rhizomes and leaves of *I. pallida* was obtained by steam distillation for 12 hours in an apparatus consisting of a 25-mL round-bottomed flask, a reflux condenser and a water bath. The method allows to isolate the essential oil from plant material with the trace quantities of essential oil (23, 31-32).

A weighed sample of material (0.5 g) was placed in 20.0 ml vial. The internal standard tridecane was added in terms of 50 μg of the substance per a certain quantity of a plant sample. At the sample was added 10.0 ml of water and volatile compounds were distilled from the sample by steam during 2 hours under the reflux. In the process of distillation the volatile material was adsorbed on the inner surface of the reflux condenser. After the cooling system the adsorbed material was washed by slow addition of 3.0 ml of ultra-pure pentane Fluka 76869 (content of microimpurities is 1.0 mg by 1.0 l) in a dry vial on 10.0 ml. Washout was concentrated by blowing (100 ml/min) a high-purity nitrogen til the volume of extract was 10.0 μl, and which was fully collected by chromatographic syringe (33).

Chromatographic conditions

The constituent composition of rhizomes and leaves of the plant was studied by a gas chromatography-mass spectrometry method (GC/MS) on an Agilent Technologies 6890 with a 5973 mass-spectrometric detector. Introduction of the sample (2.0 μl) into a chromatographic column was executed according to a *splitless* mode that was without stream splitting. Speed of the sample introduction was 1.2 ml/min within 0.2 minute. The chromatographic column was capillary DB-5 (30 m × 0.25 mm × 0.50 μm). Mobile phase: helium, gas flow rate was 1.2 ml/min. Temperature of the sample introduction heater was 250°C. Temperature of the

temperature-controlled chamber was programmable from 50 to 320°C with the rate of 4 degree/min.

Identification of components

For the identification of component the data from the mass-spectra libraries NIST05 (34, 35) and WILEY 2007 (36) with total number of spectra of more than 470,000 were used combined with the identification programs AMDIS and NIST.

The method was used for quantitative calculations the internal standard (31-32, 37). Calculation of components content C (mg/kg) was carried out by the formula:

$C = P_1 \cdot 50 \cdot 1000 / P_2 M$

Where is: P_1 – a peak area of tested substance; P_2 – a peak area of standard; 50 – mass of internal standard (μg), injected into the sample; m – sample mass (g). Relative component content was defined in per cent from their total amount.

RESULTS

In our previous research work, we reported about the component composition of the essential oil of several *irises* from Ukraine (*I. hungarica, I. pseudacorus, Iris pseudacorus f. alba, I. versicolor, I, germanica*) (23-27) and Azerbaijan (*Iris medwedewii, I. carthaliniae*) (29). Monoterpene ketone *α*-irone and triterpenoid squalene were identified in all samples. These substances can be used as the markers in chemotaxonomy and chemosystematics of plants of the genus *Iris* for further studies. Among other components of essential oil of *Irises* different norterpenoids such as *β*-ionone-5,6-epoxide, *β*-ionone, *trans*-2,6-*γ*-irone, *β*-isometilionone, *β*-damascenone were defined practically in all Iris species. Among other substances in Irises oil were present neophytadiene, eugenol, *α*-terpineol, germacrene D, terpinen-4-ol, hexahydrofarnesylacetone, farnesylacetone, phenylacetaldehyde, geranilasetone, 2-methoxy-4-vinylphenol. Also, in Irises oil contains a large proportion of myristic acid (near 50 – 80%), other fatty acids (caprylic, capric, lauric, palmitic ect.) and their esters.

The component composition of *Irises* of Azerbaijan flora is considerably differed, probably because *I. medwedewii* and *I. carthaliniae* are typical representatives of stony dry steppes; grow in warmer climate, in difference from steppe, marsh and

forest Iris species of Ukraine flora. Sesquiterpenes *β*-farnesene, germacrene D, trans-caryophyllene, *δ*-cadene, spathulenol, caryophyllene oxide, *α*- and *β*-cadinol, *α*-copaene have been identified in the both of Iris species (29). Calamenene was content only in *I. medwedewii,* and *α*- and *β*-bisabolene epoxides were found only in *I. carthaliniae*. Therefore, the component composition of oil of these irises is the similar to oil composition of the rhizomes of *Iris nigricans* of Jordan (38), of the rhizomes of *I. sofarana* and of the flowers of *I. kerneriana* from Turkey (39).

The aim of this work was to determination of the component composition of the essential oil of *I. pallida* by chromatography-mass spectrometry method. 0.03% and 0.20% of the oil yield was obtained from the air-dried leaves and rhizomes of *I. pallida*, respectively, by steam distillation. By GC/MS analysis 26 compounds were found in essential oil from leaves and 18 compounds – from rhizomes of *I. pallida*. The essential oil included terpenoids (12), their oxygenated derivatives (alcohols, ketones, aldehydes, esters), aromatic compounds (11), higher hydrocarbons (6), and higher acids of their esters (8) (40). The constituents of essential oil that have been obtained from the rhizomes and leaves of *I. pallida* are shown with their percentages and relative retention indices (RRI) in the table 1and on the figures 1 and 2.

The essential oil of the leaves of *I. pallida* consists of alkanes (21.37%), aromatic compounds (6.26%), ketones (47.66%), sesquiterpenes (10.06%), diterpenes (5.9%), triterpenes (6.12%), accompanied by relatively smaller amounts of monoterpenes (1.16%) and fatty acids (0.88%).

The dominant terpenes in essential oil of the leaves of *I. pallida* were squalene (6%), hexahydrofarnesylacetone (8%) and neophytadiene (until 6%). Among other compounds of essential oils of the leaves were *β*-ionone (0.86%) and *β*-ionone-5,6-epoxide (0.76%), *β*-damascenone (0.39%), geranylacetone (1.16%), *epi*-maloiloxide (0.84%), ketones megastigmatrienone-1 (0.11%) and megastigmatrienone-2 (0.52%), 4-isobutylacetophenone (15.08%) .

Most of the components composition of essential oil of the rhizomes of *I. pallida* as fatty acids (89%), alkanes (8.29%), aromatic compounds (1.36%), sesquiterpenes (4.53%), triterpenes (0.69%); monoterpenes and diterpenes were not found. The main saturated aliphatic mono-carboxylic acids were caprylic (1.72%), capric (14.50%), lauric (15.42%), tridecanoic (0.21%), palmitic (1.13%) and myristic (56%) acids. Among sesquiterpenes were *α*-irone (2.85%), dihydro-*β*-irone (0.25%), *trans*-

2,6-*γ*-irone (1.22%), *β*-isometilionone (0.21%). Triterpenoids were represented only by squalene.

DISCUSSION

For the study was chosen *I. pallida* Lam. (Fig. 3) of the Ukrainian flora, it had the sufficient resource base. According to the classification of Rodionenko (1987) (2) *I. pallida* 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* of the series *Elatae* Lawrence. *I. pallida* isn't endemic for our region, because it has been entered on the territory of Ukraine through the introduction (4). It is widely cultivated (3, 4).

The eessential oil from "*orris roots*" (*I. pallida, I. florentina* and *I. germanica*) (17, 22) used in perfume industries (5, 6) and in aromatherapy (41, 42). Rhizomes are harvested, dried, and aged for up to three – five years. During this time, fats and oils in the roots undergo degradation and oxidation, which produces many fragrant compounds that are invaluable in perfumery, whose scent is similar to violets (6, 30, 39). The essential oil from *Irises* is received by method of steam distillation (38-39, 43). Volatile components of *I. pallida* from Ukraine weren't studied.

By GC/MS analysis of volatile constituents of the rhizome that are shown in Figure 1 and as a result of it were identified of 18 components, in which fatty acids and their esters predominated with caprylic (1.72%), capric (14.50%), lauric (15.42%), tridecanoic (0.21%), palmitic (1.13%), methyl myristate (0.17%) and myristic (56%) acids, representing the most abundant components. Oil of the leaves is showed only by myristic acids (0.88%). Thus, it's consistent with the literature data, which noted that the myristic acid was the major compound (and/or always present) in the *Iris* species (17, 22-24, 29). Fatty acids are known with antioxidant, antifungal, anti-inflammatory and immunomodulatory properties (43), they are involved in metabolism, positively affect on digestion, and create favorable conditions for beneficial intestinal microorganisms of their life activity (44). On the other hand palmitic, myristic acids are the saturated fatty acids, involved in the synthesis of prostaglandins and stabilize the cellular membranes. It has been found that lauric acid possess antibacterial, antitumour, antimycobacterial and antiviral activities (43,

45). Composition of fatty acids and their esters give us the possibility to investigate the use of *Iris* rhizomes extracts for medical purposes.

Prevalence of fatty acids and their esters, as well as aliphatic hydrocarbons and their derivatives in the volatile constituents of leaves and rhizomes seems to be a common feature among the species of *Iris* (22-29, 38-39, 43).

Monoterpenoid *α*-irone was detected with content 2.85% of the total essential oil of *I. pallida* rhizomes. It is known that ketone *α*-irone is an indicator of the authenticity of irises oil (6). Presence of irones, homologues and ionones, was reported mainly in rhizomes of *I. pallida, I. germanica* and *I. florentina* (21, 30, 46).

For comparison, in essential oil of the dried rhizomes of Syrian wild *Iris germanica* (43) and of the fresh rhizomes of *Iris nigricans* (38) has been established existence of *α*-irone only 0.25% and 1.42% respectively by GC/MS. In essential oil from the fresh rhizomes of *Iris florentina* *α*-irone and *γ*-irone are absent (46), but at that time, content of *α*-irone in the dry rhizome and essential oil of *Iris florentina* were obtained high (4.21%).

Norterpenoids and their derivatives were observed in essential oil of the leaves and rhizomes of *I. pallida* at the first time. Their compositions are differ. The highest percentage of norterpenoids was obtained in the rhizomes (9.06%) with much smaller amounts (2.01%) in the leaves.

The most various compositions of norterpenoids were found in the oil of *I. pallida* rhizomes. *α*-Dihydro-*β*-irone (0.25%), trans-2,6-*γ*-irone (1.22%) and *β*-isomethylionone (0.21%) were identified only in the rhizome. Availability of irones and ionones in the raw material determines characteristic smell of violets of iris and their mucolytic action (22). Various composition of norterpenoids was found in the leaves of *I. pallida*. Among them *β*-damascenone (0.39%), *β*-ionone (0.86%) and *β*-ionone-5,6-epoxide (0.76%) were identified.

The analysis of essential oil of the leaves of *I. pallida* led to the identification of 26 compounds, in which ketones benzophenone and 4-isobutylphenone constituted the major compounds accounting for 31.84% and 15.08% of the total oil composition respectively. Regarding the volatile constituents of the leaves, aromatic compounds, ketones and aldehydes together with other aliphatic hydrocarbon compounds have showed a high prevalence of the total oil composition.

The highest percentage of terpenes of squalene was observed in essential oil of leaves of *I. pallida* (6.12%), and the smallest content of squalene was in essential oil

of rhizomes (0.69%). In addition, sesquiterpenoids hexahydrofarnesylacetone (8.05%) and geranyl acetone (1.16%) were detected in *Iris* leaves, which exhibit an antimicrobial and cytotoxic activity, according to the last pharmacological studies (13, 40, 47).

Moreover, diterpenes neophytadiene and *epi*-manoil oxide with ketones megastigmatrienone-1, megastigmatrienone-2, 4-isobutylphenone were only indicated in oil of the leaves and their content was 5.65, 0.84, 0.11, 0.52 and 15.08%, respectively as illustrated in Figure 2.

The high content of alkanes (21.37%) was detected in essential oil of *Iris* leaves. It existence caused by the fact that they are the part of wax of cuticle that covering leaves and rhizomes of a plant and determinate of volatile components from the raw materials are distilled off by steam together with terpenoids.

There are 2-methoxy-4-vinylphenol, benzophenone, squalene, myristic acid, some saturated hydrocarbons in the leaves and rhizomes of *I. pallida*. The chemical composition of essential oils of the leaves of *I. pallida* was studied at the first. Also, at the first time squalene and *β*-isometilionone were identified from essential oil of *I. pallida* rhizomes. Variable composition of biologically active compounds in essential oil provides a basis for further study of *I. pallida* and as a promising source of raw material for producing valuable essential oil.

CONCLUSIONS

Qualitative and quantitative analysis of the components of the essential oil of *Iris pallida* from Ukraine was conducted by chromatography-mass spectrometry at the first time. Rhizomes of *I. pallida* are characterized by the high content of the essential oil (0.20%), distinguished by a rich chemical composition. 16 volatile components of essential oil of rhizomes and 26 components of leaves were found. There are dihydro-*β*-irone, *α*-irone, *trans*-2,6-*γ*-irone, *β*-isometilionone, benzophenone and other were found in the essential oil of *Iris* rhizomes. The dominant terpenes in essential oil of *Iris* leaves were 4-isobutylphenone, benzophenone, hexahydrofarnesyl acetone, neofitadien, squalene.

Aromatherapy recommends (41-42) to use of iris oil for bronchial inflammation, coughing, and is used as well as in mixtures for care of the skin. Essential oil of iris normalizes the function of a brain, has a detoxifying, diuretic, expectorant, and it strengthens the immune system. Conducted phytochemical studies shown the prospects for further pharmacological study of iris (9). Chemical composition of biologically active substances, including the presence of irones in the rhizomes of *I. pallida* could have the industrial significance (6); irones constitute an expensive natural ingredient of the cosmetic industry. *I. pallida* of Ukraine flora can be recommended as an additional source of the raw materials for essential oil from the rhizomes.

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REFERENCE

1. Goldblatt P, Manning JC. The *Iris* family: natural history and classification. Portland; Timber Press; 2008:336.
2. Rodionenko GI. The genus *Iris* L.: questions of morphology, biology, evolution, and systematics. British Iris Society; London; 1987:222.
3. Karpenko VP. Introduction history of species and varieties of genus Iris L. in Ukraine against the background of global trends. Umans'kiy National'nyi Universytet sadivnytstva visnyk. 2015;2:85-91.
4. Mosyakin SL, Fedoranchuk MM. Vascular plants of Ukraine. A nomenclatural checklist, ed. SL. Mosyakin; Kiev; 1999:346.

5. Lawless J. The illustrated encyclopedia of essential oils. Publ. Element Books Ltd.; Reissue edition; 1995:256.
6. Niir Board. Modern technology of perfumes, flavours and essential oils. 2nd Ed. National Institute of Industrial Research, 2004:282-283.
7. Lekarstvennoe rastytelnoe surie. Pharmacognosy: Posobye. Eds. Yakovlev GP, Holynovoy KF. SPb: SpetsLyt, 2004.765.
8. Medicinal preparations of Ukraine. In. Chernykh VP, Zupanets IA, eds. Kharkiv, Publishing of the NFUU, Golden Pages; 2005:512.
9. Tihomirova LI, Bazarnova NG, Mikushina IV, Dolganova ZV. Pharmacological-biochemical rationale for the practical use of some representatives of the genus iris l. (review). Rastitelnye resursy. 2015;3:25-34.
10. Gordeeva G. Application of the drug Mastodinone with functional deviation in the reproductive system. Reproductive health of a woman. 2002;3(12): 47-51.
11. Henriette's Herbal Homepage: http://www.henriettes-herb.com/eclectic/kings/iris-vers.html
12. Patudin AV, Mishchenko VS, Nechaev NP, Kosmodemyanskiy LV. Homeopathic medicines (reference book for doctors, pharmacists and pharmacists). Moscow, Astral;1999:252.
13. Minina SA, Pryakhina NI, Chemesova II, Chizhikov DV. A pediatric medicinal preparation containing an extract of the milk-white *iris* (*Iris lactea*).Pharmaceutical Chemistry J. 2008;42(1):37-39.
14. Wang H, Cui Y, Zhao C. Flavonoids of the genus *Iris* (*Iridaceae*). Mini Rev. Med. Chem. 2010;10(7):643-661.
15. Tsukasa I, Ootani S. Flavonoids of the genus *Iris*; structures, distribution and function: review. Ann. Tsukuba Bot. Gard. 1998;17:147-183.
16. Kassak P. Secondary metabolites of the chosen genus *Iris* species. Acta Univ. Agric. Silvic. Mendelianae Brun. 2012;60(32):269-280.
17. Kukula-Koch W, Sieniawska E, Widelski J, Urjin O, Głowniak P, Skalicka K, Wozniak. Major secondary metabolites of *Iris* spp. Phytochem. Rev. 2015;14(1):51-80.
18. Khare CP. Indian medicinal plants. Heidelberg: Springer-Verlag; Berlin; 2007:836.
19. Waddick JW, Yu-tang Z. *Iris* of China. Portland, Oregon; Timber Press; 1992:192.

20. Zatylnikova OA., Osolodchenko TP., Kovalev VN. Antimicrobial activity of extracts of *Iris pseudacorus* L. Scientific Journal Annals of Mechnikov's Institute. 2010;4:43-47.
21. Roger B, Jeannot V, Fernandez X, Cerantola S, Chahboun J. Characterisation and quantification of flavonoids in *Iris germanica* L. and *Iris pallida* Lam. resinoids from Morocco. Phytochem Anal. 2012;23(5): 450-455.
22. Deng G, Zhang X, Wang Y, Lin Y, Chen X. Chemical composition and antimicrobial activity of the essential oil of *Iris pallida* Lam. Chemistry and Industry of Forest Products. 2008;28:7-12.
23. Zatylnikova OA, Kovalev VN, Kovalev SV. The study of yellow iris essential oils` components. Rastitelnye resursy. 2013;49(2):233-240.
24. Kovalev VN, Mikhailenko OA, Vinogradov BA. Aromatic compounds and terpenoids of *Iris hungarica*. Chemistry of natural compounds. 2014;50:161-162.
25. Mykchailenko OA, Kovalyov VN, Kovalyov SV. Chromatography-mass spectrometric study of bioactive substances of rhizomes with roots of *Iris pseudacorus f. alba*. Farmaciya of Kazakhstan. 2015;3(166):38-41.
26. Mykhailenko OO. Analysis of essential oil of leaves of *Iris germanica* L. Science and Practice 2015: abstracts of the 6th Int. Pharm. Conf. (November 5-6th, 2015). Kaunas, Lithuania; 2015:30-31.
27. Mykhailenko OA, Kovalev VN. Chemical composition of essential oil from leaves of *Iris versicolor*. Xth Int. symp. on the chemistry of natural compounds (November 21-23th, 2013): abstracts. Tashkent-Bukhara, Republic of Uzbekistan; 2013:280.
28. Mykchailenko OA, Kovalyov VN. Aromatic compounds and terpenoids of essential oil of several *Iris* species. IV Int. Scient. Conf. of Young Researchers: materiallar In 2 vol (April 29-30th, 2016). Azerbaijan, Baku. 2016;1:187-188.
29. Isaev DI, Mikhailenko OA, Gurbanov GM, Kovalev VN. Constituents of essential oils from Azerbaijan *Iris medwedewii* and *I. carthaliniae* rhizomes. Chemistry of Natural Compounds. 2016;52(3):748-750.
30. Bicchi C, Rubiolo P, Rovida C. Analysis of constituents of *Iris* rhizomes. Part II. Simultaneous SFE of irones and iridals from *Iris pallida* L. rhizomes. Flavour and fragrance J. 1993;8:261-267.
31. Chernogorod LB, Vinogradov BA. Essential oils of some *Achillea* L. species, containing fragranol. Rastitelnye resursy. 2006;42(2):61-68.

32. Golembiovska O, Tsurkan A, Vynogradov B. Components of *Prunella vulgaris* L. Grown in Ukraine. J of Pharmacognosy and Phytochemistry. 2014;2(6):140-146.
33. Bicchi C, Brunelli C, Cordero C, Rubiolo P. Direct resistively heated column gas chromatography (Ultrafast module-GC) for high-speed analysis of essential oils of differing complexities. J. Chromatogr. A. 2004;1024(1-2):195-207.
34. NIST Mass Spec Data Center SES. 2005a. Mass Spectra, 6th edn. National Institute of Standards and Technology: Gaithersburg MD.
35. NIST Mass Spec Data Center SES. 2005b. Retention Indices, 6th edn. National Institute of Standards and Technology: Gaithersburg MD.
36. WILEY 2007 http://www.wiley.com/legacy/annual_reports/ar_2007/
37. State Pharmacopoeia of Ukraine. State Enterprise: Scientific and Expert Pharmacopoeial Centre (1ed); Kharkov; 2001:556.
38. Al-Jaber HI. Variation in essential oil composition of *Iris nigricans* Dinsm. (*Iridaceae*) endemic to Jordan at different flowering stages. Arabian J of Chemistry. 2016;9(2):S1190–S1196.
39. Başer KHC, Demirci B, Orhan IE, Kartal M, Sekeroglu N, Sener B. Composition of volatiles from three *Iris* species of Turkey. J of Essential Oil Rese. 2011;23:66-71.
40. Wagner H, Bladt S. Plant drug analysis. Berlin; Springer; 2001:384.
41. Nikolaevskii VV. Aromatherapy. Guide. Moscow; Meditsina; 2000:336.
42. Fischer-Rizzi S. Complete aromatherapy handbook: essential oils for radiant health. Sterling publishing company, Inc.; USA; 1990:240.
43. Almaarri K, Zedan TA, Albatal N. Chemical analysis of essential oils of some Syrian wild *Iris* species. Am J Biochem Mol Biol. 2013;3(1):38-49.
44. Gunstone FD. Fatty acids and lipid chemistry. London; Blackie Academic and Professional; 1996:547.
45. Plant lipids: biology, utilization and manipulation. In Denis J. Murphy, eds. Wiley-Blackwell; 2005:403.
46. Kara N, Baydar H. Scent components in essential oil, resinoids and absolute of iris (*Iris florentina* L.). Anadolu Tarım Bilim. Derg. 2014;29(1):70-74.
47. Wren RC. Potter's new encyclopedia of botanical drugs and preparations. Publ. C.W. Daniel Co. Ltd; 1988:384.