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CHROMATOGRAPHY-MASS SPECTROMETRIC STUDY

OF BIOACTIVE SUBSTANCES OF RHIZOMES WITH ROOTS OF I. PSEUDACORUS F. ALBA

ABSTRACT

Study of component composition of essential oil, fatty acids and sterols of rhizomes with roots of cultivated iris I. pseudacorus f. alba was first conducted by method of chromatography-mass spectrometry. 8 fatty acids were identified in the raw materials, palmitic acid is prevalent (C16:0) 62.7%. Essential fatty acids namely α-linolenic (C18:3ω3, 56 mg/kg) and linoleic (C18:2ω6, 526 mg/kg) are of scientific interest. Total sterol content was 0.04%, 5 substances were identified of which vitamin E (of up to 40%) is prevalent. Essential oil content in the raw materials was 0.04%, 14 substances were detected, 12 of them were identified; triterpenoid squalene with content of up to 32% is prevalent. The results obtained allow assuming presence of antimicrobial, antioxidant action of the raw materials under study.

Key words: chromatography-mass-spectrometry, I. pseudacorus f. alba, rhizomes, fatty acids, sterols, essential oil, component composition.

INTRODUCTION

Development of research in the field of raw materials study in order to obtain new effective and safe herbal medicines is a relevant objective of the pharmacy. Wide variety of wild species is an inexhaustible source for bringing of the most precious ones under cultivation.

Iris genus (Iris L.) belongs to Iridaceae family and includes about 200 species distributed over the Northern hemisphere [1,2]. In the territory of Ukraine 16 species grow [3]. Currently thousands of scientists-introducers are executing even more works on extension of cultivated iris range [1,3-4]. Irises are of a special interest for plant breeders due to their rich potential as ornamental plants since the species of Iris genus take one of the first places in the world among floricultural crops according to number of plant varieties.

I. pseudacorus f. alba (Laevigata Iris) is a variety of Yellow iris with white flower [5] but its form is already widely used in the floriculture. Habitat of I. pseudacorus f. alba is the Eastern Europe, the Northern Africa, China, the South-West Asia. Characteristic feature of the specie is sharply exerting primary rib of the leaf. Colour of the leaves vary from dull green to intense glaucous (due to abundant wax bloom). Yellow iris is easy to keep when planting; it is easily reproduced by autumn seeding. Cultivated forms (variety) can easily reproduced by portions of rhizomes bearing buds [3-4]. For efficient raw materials use it is necessary to have data on the component composition, therefore I. pseudacorus f. alba was selected for study as a new source of bioactive substances (BAS).

The objective of the present paper was determination of component composition of essential oil, fatty acids and sterols of rhizomes with roots of

I. pseudacorus f. alba by chromatography-mass spectrometry method.

MATERIALS AND STUDY METHODS

The subject of the study was rhizomes with root of Iris pseudacorus f. alba (Fig. 1) prepared in May, 2014 in N. N. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, Kiev (Ukraine).

Figure 1 – View of growing plant and underground organs of Iris pseudacorus f. alba

Analysis of methyl esters of fatty acids, sterols as well as component composition of essential oil of rhizomes with roots of Iris pseudacorus f. alba was conducted by method of chromatography-mass-spectrometry 5973N/6890N MSD/DS Agilent Technologies (USA) [6].

Analysis of methyl esters of fatty acids. Internal standard (solution of 50.0 μg of tridecane in hexane) and 1.0 ml of methylating agent (14% of BCl3 in methanol, Supelco 3-3033) were added to weighted amount of the raw materials (50.0 mg) contained in a 2.0 ml vial. This mixture was kept in a hermetically closed vial for 8 hours at 65°C. Reaction mixture was decanted for plant material

remnants elimination and diluted with 1.0 ml of distilled water. Methyl esters were extracted with 0.2 ml of dichloromethane, carefully shacked up several times within an hour then the extract obtained was chromatographed [7].

Analysis of sterols. Weighted amount of the raw materials (50.0 mg) was put into a 2.0 ml vial, then internal standard (solution of 50.0 μg of tridecane) and 0.6 ml of solvent medium (dichloromethane) were added. The sample was kept for 2 – 3 hours at temperature of 50°C within a day. The extract was poured to a 2.0 ml vial and concentrated by means of blowing-off (100.0 ml/min) with especially pure nitrogen to residual extract volume of 10.0 μl and then chromatographed [7].

Analysis of components of essential oil. Essential oil was obtained by means of hydrodistillation allowing its extraction from the plant material in case of essential oil content in traces; analysis conditions are provided in the previously published paper [4].

Chromatographic conditions. Introduction of the sample (2.0 μl) into a chromatographic column was executed according to a splitless mode, that is without stream splitting. Sample introduction speed is 1.2 ml/min within 0.2 minute. Chromatographic column is capillary DB-5 (30 m×250 μm×0.50 μm). Mobile phase: helium, gas flow rate is 1.2 ml/min. Temperature of the sample introduction heater is 250° C. Temperature of the temperature-controlled chamber is programmable from 50 to 320°C with the rate of 4 degree/min. For component identification data from the mass-spectra libraries NIST05 and WILEY 2007 with total number of spectra of more than 470,000 were used combined with identification programs AMDIS and NIST. Substance content was calculated in relation to internal standard [6]. Relative component content was defined in per cent from their total amount. Statistical analysis of the results was conducted in accordance with the requirements of the State Pharmacopeia of Ukraine, the 1st issue, supplement 1, p. 5.3 in Exel XS application [9].

RESULTS AND DISCUSSION

The main function of the rhizomes is nutrient storing. Fatty acids and such their derivatives as mono- and polyhydric alcohol esters with fatty acids, steroids, terpenes, and vitamins are included into plant lipids as storage compounds. Lipids play important role in the biosynthesis of plants and biological activity of plants can be of different nature depending on their component compositions.

Analysis of fatty acid composition of the rhizomes with roots of Iris pseudacorus f. alba showed presence of 6 acids (Table, Fig. 2) and their total content of 0.2%. Content of saturated fatty acids is 68.8% that significantly exceeds content of unsaturated ones that is 31.2%. Palmitic acid (C16:0) in the amount of 62.7% is prevalent among the saturated ones. It is known that palmitic acid takes part in the synthesis of prostaglandins and stabilization of cellular membranes [10]. Unsaturated fatty acids are presented with ω3, ω6 and ω9 acids – linolenic acid (C18:3ω3) of up to 3%, linoleic acid (C18:2ω6) of up to 27%, oleic acid (C18:1ω9) of up to 2%. Such polyenoic acids as linoleic and α-linolenic are not synthetized in the organism and should be obtained from the food due to the fact that they belong to essential ones and are necessary element of the cellular membrane formation process.

Total content of sterols in the raw materials of Iris pseudacorus f. alba was 0.04%. All in all 12 substances were detected of which 5 were identified (Table, Figure 3). Amount of non-identified substances was 17.7%. Vitamin E (tocopherol) is of the highest content of up to 40% as well as triterpenoid squalene of up to 21%. Sterols have anti-inflammatory action and take part in the metabolism of phosphatides and also have physiological significance as the carriers of steroid group similar to steroid hormones and act as inhibitors of radical oxidation. Tocopherol has significant antioxidant action and is able to connect free radicals, regulates normal development and function of reproductive glands [11].

Content of essential oil in the air-dry raw materials is 0.04%. Component content varies from 0.1 to 32%. Analysing the obtained results of the component composition of essential oil of rhizomes with roots of Iris pseudacorus f. alba 14 substances were detected, 12 of them were identified (Table, Fig. 4). Triterpenoid squalene showing immunomodulatory action with content of up to 32% is prevalent among the components identified (and during sterol analysis, as well). Such aromatic compounds as phenylacetaldehyde, 2-methoxy-4-vinylphenol in the amount of up to 2% were detected in the composition of essential oil. Among the components related to monoterpene and sesquiterpene hydrocarbons terpinen-4-ol (7.0%) accumulates in the maximum amount, content of α-terpineol (2.3%), germacrene D (1.0%), geranyl acetate (0.4%) is lower. Essential oils presented with terpenoid and aromatic compounds have antimicrobial, antioxidant and calming action [12].

When analysing components of essential oil another 3 fatty acids were detected: capric (C10:0, up to 30%), lauric (C12:0, up to 10%) and myristic (C14:0, 5.2%) acids. Capric and lauric acids were not detected during analysis of fatty acids. Thus, complex study of the raw materials allowed identifying as much as possible substances in rhizomes with roots of Iris pseudacorus f. alba.

CONCLUSIONS

Qualitative and quantitative analysis of the components of essential oil, fatty acids and sterols of rhizomes with roots of cultivated specie of Iris pseudacorus f. alba by method of chromatography-mass spectrometry was conducted for the first time. 8 fatty acids, vitamin E (tocopherol), 4 phytosterols, 2 aromatic compounds, 4 substances of terpenoid and sesquiterpene nature, 2 alkanes were identified in the raw materials.

Phytochemical studies conducted show perspective of the further study of cultivated forms of iris namely Iris pseudacorus f. alba also as a source of bioactive substances.

Table – Biologically active substances of rhizomes with roots of Iris pseudacorus f. alba

Substances identified	Content		Substances identified	Content	
	mg/kg	%		mg/kg	%
Fatty acids including:	1977.2	100	Essential oil including:	394.28	100
myristic (C14:0)	52.2	2.6	phenylacetaldehyde	4.30	1.1
palmitic (C16:0)	1239.3	62.7	–	3.57	0.9
stearic (C18:0)	67.9	3.4	terpinen-4-ol	28.03	7.1
oleic (C18:1ω9)	35.7	1.8	α-terpineol	8.87	2.3
linoleic (C18:2ω6)	525.8	26.6	–	8.86	2.3
linolenic (C18:3ω3)	56.3	2.9	2-methoxy-4-vinylphenol	5.59	1.5
Sterols including:	404.6	100	capric acid (C10:0)	116.80	29.6
squalene	82.4	20.4	geranyl acetate	1.43	0.4
squalene-epoxide	66.7	16.5	germacrene D	3.75	1.0
ursa-?,12-diene	5.7	1.4	lauric acid (C12:0)	38.78	9.8
stigmast-3,5-diene	16.2	4.0	myristinic acid (C14:0)	20.67	5.2
vitamin E (tocopherol)	161.9	40.0	pentacosane	4.95	1.3
∑ of non-identified sterols	71.7	17.7	squalene	124.84	31.7
			nonacosane	23.84	5.8

Note: "-" means that the substance was not identified.

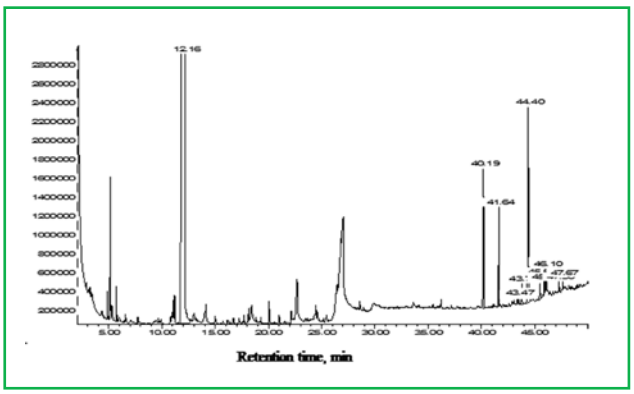

Figure 2 – General view of chromatogram of fatty acids of rhizomes with roots of Iris pseudacorus f. alba. On the X-axis retention time, min is plotted; on the Y-axis relative intensity, CU is plotted

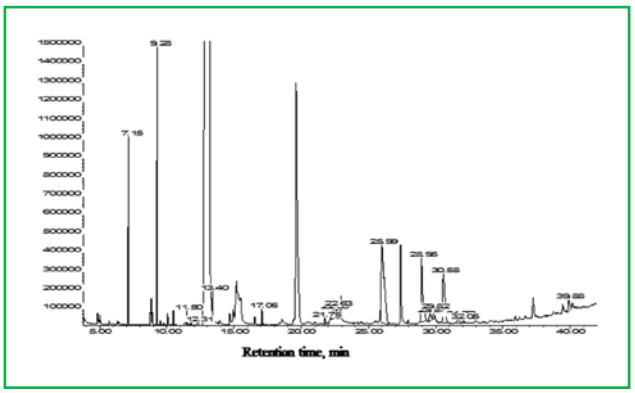

Figure 2 – General view of chromatogram of sterols of rhizomes with roots of Iris pseudacorus f. alba. On the X-axis retention time, min is plotted; on the Y-axis relative intensity, CU is plotted.

of Iris pseudacorus f. alba. On the X-axis retention time, min is plotted; on the

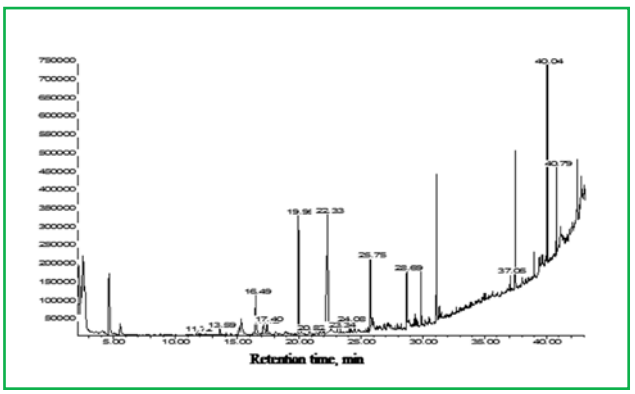

Figure 2 – General view of chromatogram of essential oil of rhizomes with roots
Y-axis relative intensity, CU is plotted

ТҮЙІНДЕМЕ

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I. PSEUDACORUS F. ALBA ТАМЫРЛАРЫ ҚОСЫЛҒАН ТАМЫРСАБАҚТАН ЖАСАЛҒАН БИОЛОГИЯЛЫҚ БЕЛСЕНДІ ЗАТТАРҒА ХРОМАТО-МАСС-СПЕКТРОМЕТРИЯЛЫҚ ЗЕРТТЕУ

Эфир майы, майлы қышқылдар және I. pseudacorus f. Alba мәдени айылқияқ тамырлары қосылған тамырсабақ компоненттік құрамына хромато-масс-спектрометриялық әдіспен алғаш рет зерттеу жүргізілді. Шикізатта 8 майлы қышқыл тенестірілді, 62,7% – пальмитин (С16:0) басымдық көрсетті. α-линоленді (С18:3ω3, 56 мг/кг) және линолді (С18:2ω6, 526 мг/кг) эссенциалді майлы қышқылдар ғылыми қызығушылықты туғызды. Құрамындағы жалпы стерол 0,04% құрады, Е (до 40%) дәрумені басымдық көрсеткен 5 зат теңестірілді. Ал, шикізаттағы эфир май 0,04% құрады, 14 зат анықталып, оның 12 теңестірілді, басымдықты 32%-ға дейінгі тритерпеноид сквален көрсетті. Алынған нәтижелер зерттелген шикізаттың микробқа қарсы, антиоксидантты әсерін болжамдайды.

Түйін сөздер: хромато-масс-спектрометрия, тамырсабақ, I. pseudacorus f. alba, майлы қышқылдар, стеролдар, эфир майы, компоненттік құрам.

РЕЗЮМЕ

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ХРОМАТО-МАСС-СПЕКТРОМЕТРИЧЕСКОЕ ИЗУЧЕНИЕ БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ КОРНЕВИЩ С КОРНЯМИ I. PSEUDACORUS F. ALBA

Методом хромато-масс-спектрометрии впервые проведено исследование компонентного состава эфирного масла, жирных кислот и стеролов корневищ с корнями культурного ириса I. pseudacorus f. alba. В сырье идентифицировано 8 жирных кислот, доминирует пальмитиновая (С16:0) – 62,7 %. Научный интерес представляют эссенциальные жирные кислоты – α-линоленовая (С18:3ω3, 56 мг/кг) и линолевая (С18:2ω6, 526 мг/кг). Общее содержание стеролов составило 0.04%, идентифицировано 5 веществ, из которых витамин Е (до 40%) доминирует. Содержание эфирного масла в сырье составило 0,04%, было выявлено 14 веществ, из них 12 идентифицировано; доминирует тритерпеноид сквален – до 32%. Полученные результаты дают возможность предположить наличие антимикробного, антиоксидантного действия у исследуемого сырья.

Ключевые слова: хромато-масс-спектрометрия, корневища, I. pseudacorus f. alba, жирные кислоты, стеролы, эфирное масло, компонентный состав.

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БЕЗОПАСНОСТЬ ЛЕКАРСТВ

В США одобрен препарат «Пазео» компании Alcon для снятия зуда в глазах

FDA одобрило офтальмологический раствор Пазео/Pazeo (олопатадин гидрохлорид/ olopatadine hydrochloride) 0,7% компании Alcon, предназначенный для снятия зуда в глазах, вызванного аллергическим конъюктивитом.

Лекарственное средство должно вводиться по одной капле один раз в день. Препарат обладает подтвержденной эффективностью в течение 24 часов после применения. В двух клинических исследованиях офтальмологический раствор «Пазео» продемонстрировал статистически значимое уменьшение зуда, связанного с аллергическим конъюктивитом, в течение 24 часов после применения по сравнению с использованием раствора Pataday (olopatadine) 0,2%.

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