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CHROMATOGRAPHIC ANALYSE oF VOLATILE ORGANIC COMPOUNDS IN ESSENTIAL OIL oF ACORUS CALAMUS L. RHIZOME

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ABSTRACT :

Rhizomes of sweet flag (*Acorus calamus* L.) were collected from Astrakhan state, Russia to define the volatile organic compounds in their essential oil. Rhizomes were washed, dried and subjected to simultaneous distillation – extraction (SDE) technique to extract the essential oil (4 ml/kg rhizome) then analyzed by gas chromatography–mass spectrometry (GC-MS) to isolated the volatile organic compounds. Chromatographic analysis of samples showed 14 bioactive compounds; Asarone, Beta-Pinene (β-pinene), Camphene, 1,2,3,4-tetramethyl-5-methylene-1,3-cyclopentadiene, Linalool, Camphor, γ-muurolene, Shyobunone, Dehydroxy-isocalamendiol, 1,2-Dimethoxy-4-methoxy-l-propenylbenzene, Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester, 9-Acetamido-1-methyl-3,6-diazahomoadamantane, 1,8-dimethyl-8,9-epoxy-4-isopropyl-spiro[4.5]decan-7-one,6-[1-(Hydroxymethyl)vinyl]-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-2(1H)-naphthalenone.

Key Words: *Acorus calamus L, Sweet flag, Rhizome, Volatile organic compounds, Asarone.*

INTRODUCTION:

Essential oils are hydrophobic liquids that contain complex mixtures of volatile organic compounds. These oils are the end product of secondary metabolism, and most of their components, terpenoids, monoterpenes, sesquiterpenes, diterpenes, derivatives and aromatic components. Oil content of the standard components must contain certain chemicals that determine the therapeutic qualities. The molecular structure of essential oils is extremely small and can be absorbed in different parts of the body. Due to the lipophilicity of essential oil compounds, it is

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easy to enter into the cell membrane and, therefore, are absorbed through the skin and lungs [5]. Each component of essential oils contributes to the beneficial or adverse effects of these oils as components of each essential oil has different properties and bioavailability [2]. The pharmaceutical use of essential oils, they are also a part of air fresheners, candles, cosmetics and industrial cleaners, masking agents, soaps and detergents, sauces, beverages and dairy products.

Sweet flag *(Acorus calamus* L.), family Araceae, is a perennial plant. Homeland of sweet flag is the south-east of Asia. In the wild, it grows in the temperate zone basins in Europe, Asia and North America. Sweet flag is widely cultivated in Russia, Europe, India, USA and Japan. In Russia, grows in the basins of the Sura, Don, Volga delta, flooded areas of the Kuban and others [8]. The rhizomes of *Acorus calamus* contain useful components. These compounds are used for medicinal purposes as an analgesic to relieve dental or headache, as an anti-inflammatory, analgesic and antiseptic for oral hygiene, to clean and disinfect the teeth, as an effective tool [5].

Essential oil of *Acorus calamus* rhizomes are used for fragrance in perfumes and alcoholic beverage industry for the preparation of various liqueurs and essences. It is used in syrups, as a spice in small quantities instead of bay leaves for cooking different foods [1]. *Acorus calamus* root has a long history of medicinal use as a water decoction, tincture of alcohol and powder. The plant is mentioned in many works of great scholars in classical medicine [7].

MATERIALS AND METHODS:

Sweet flag (*Acorus calamus* L.) rhizomes were collected from Tre Pratoka, Volga region, Astrakhan state, Russia during 2009 and 2010 seasons. Samples were dried at room temperature and stored in a well ventilated area.

Extraction of essential oils

Rhizome samples (100 g) were subjected to hydro-distillation for 2 hours using the Clevenger apparatus for essential oils [3]. Currently, the most popular method of extraction is steam distillation, in which water is heated to produce steam, which carries the most volatile chemicals and aromatic material. Essential oils are usually float on the surface Hydrosol (a component of distilled water). Extracted essential oils are stored in a clean glass bottle, in the dark at 4 ° C.

Chromatographic analysis

Chromatographic analysis was performed using Shimadzu GC-MS in electron impact mode. The ionization voltage was 70e V as well as temperature of the ion source and injector were 300° C and 250°C, respectively. Capillary column used was a DB-WAX (60 × 0,2 mm ID and 0.25 micron film thickness; J & W, USA). The temperature of the furnace, to be held at 40°C (isothermal for 1 min) was increased to 180°C and 220°C in 20°C/min in 1°C/min then 300° C to 20º C / min and held 10 min. Helium was used as carrier gas at a flow rate of 1 μl/min, with injector volume of 1 μl 1:20 split ratio. 1 μl mixture of alkanes were analyzed to determine retention time (RT) standards for GC-FID. To preserve the index of each peak, the main program was established, which replaced the RT of each peak of *n*-alkanes confirmed at GC chromatogram. Qualitative analysis of volatile compounds was carried out by identification of mass spectra with a spectral reference. The spectrum of each analyzed volatile component has been agreed in the library mass spectrum WILLY 139, NIST 12 and NIST 62.

RESULTS AND DISCUSSION

Acorus calamus essential oil was obtained by distillation for 2 hours using Clevenger apparatus and was analyzed by GC / MS (Fig. 1). Identified volatile organic compounds are consistent with their elution on DB-WAX column (Table 2). The total of 14 previously identified volatile organic compounds belonging to chemical classes: monoterpenes (2), sesquiterpenes (5), ketones (1), alcohols (2), amides (1), alkenes (1), and esters (2) were identified (Fig. 2).

In the essential oil of *A. calamus* two esters; Asarone and 1,2-Dimethoxy-4-methoxy-l-propenylbenzene were detected. Linalool and Acetic acid, 3 - hydroxy-6-isopropenyl-4, 8a-dimethyl-1, 2,3,5,6,7,8,8 a-octahydronaphthalen-2-yl were detected. Five sesquiterpenes compounds were detected; -γ-muurolene, -shyobunone, -dehydroxy-isocalamendiol, -1,8-dimethyl-8,9-epoxy-4-isopropyl-spiro [4.5] ecan-7-one, and 6 - [1 - (Hydroxymethyl) vinyl] -4,8 a-dimethyl-4a, 5,6,7,8,8 a-hexahydro 2 (1H)-naphthalenone. Exactly the same compounds associated with the group of monoterpenes are beta-pinene (β-pinene), and camphene. In addition to these terpene hydrocarbons other compounds from other groups were also found, such as a ketone (camphor), amides (9-Acetamido-1-methyl-3,6-diazahomoadamantane)and alkenes(1,2,3,4-tetramethyl-5-

methylene - 1,3-cyclopentadiene). This analysis shows that asarone is the dominant compound.

Qualitative research of chemical components of essential oils gives an estimate for the composition of these oils. The percentage of essential oil provides perhaps the most important parameters to characterize it. Therefore, our discussion will focus on the characteristics of individual components and their pharmacological properties, based on previous studies. Asarone as ether is contained in some plants, such as *Acorus calamus* and *Asarum europaeum*, as the aroma of essential oils (Fig. 3). It is used to kill pests and bacteria. Some studies showed that asarone can be used as a repellent against insects [11]. It is also used in the production of alcoholic drinks and food at a lower level. Asarone injection of emulsions can be used to counter bacteria, reducing inflammation [13].

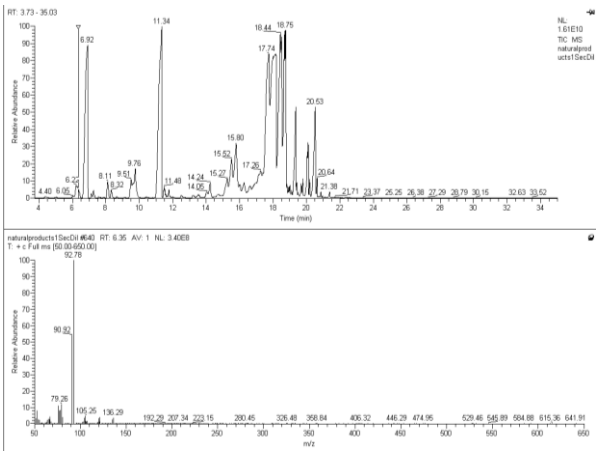


Fig. (1) - G / MS chromatogram of volatile organic components derived from *A. calamus*.

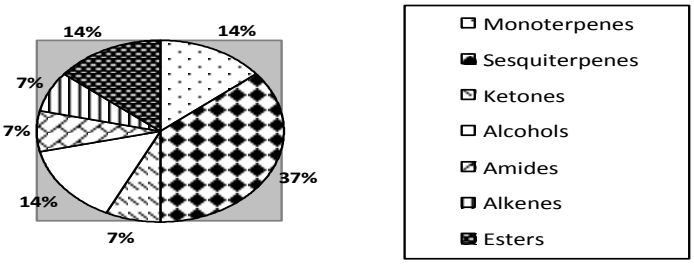
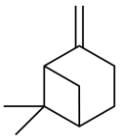
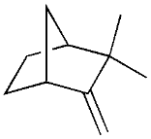
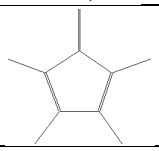
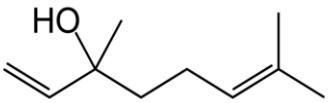
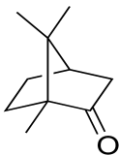
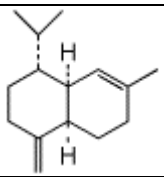
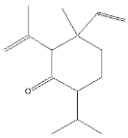
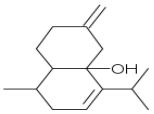
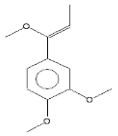
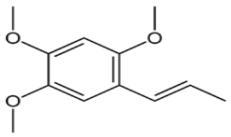
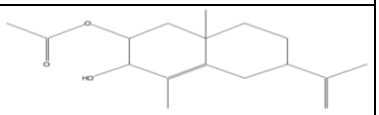
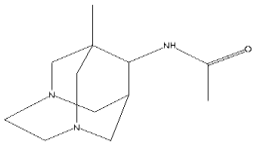
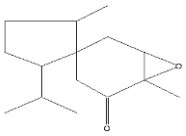
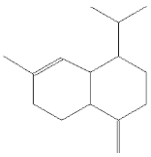


Fig. (2). The relative abundance of functional groups of volatile organic compounds of *A. calamus* .

Table (1). Volatile organic compounds of *A. calamus*

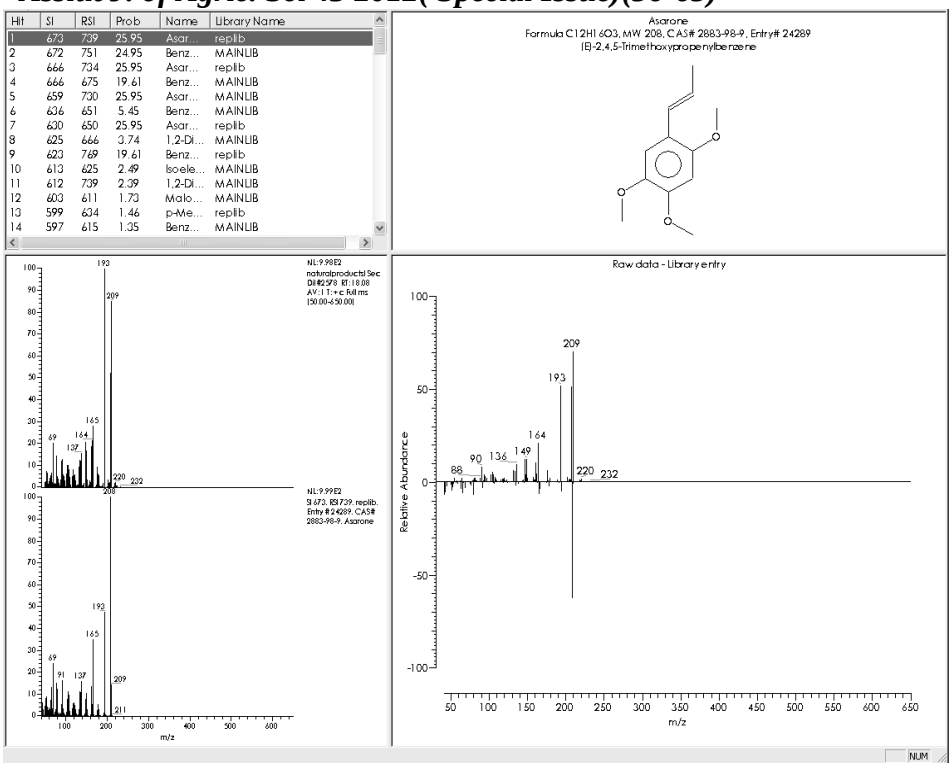
№	Retention time, min	Compound name	Functional group	Molecular formula	Molecular weight	Chemical identification
1	6.22	β-pinene	Monoterpene	$C_{10}H_{16}$	136	
2	6.82	Camphene	Monoterpene	$C_{10}H_{16}$	136	
3	8.28	1,2,3,4-tetramethyl-5-methyl-1 ,3-cyclopentadiene	Alkine	$C_{10}H_{14}$	134	
4	9.60	Linalool	Alcohol	$C_{10}H_{18}O$	154	
5	11.31	Camphor	Ketone	$C_{10}H_{16}O$	152	
6	14.24	γ-Muurolen	Sesquiterpene	$C_{15}H_{24}$	204	
7	15.48	Shyobunone	Sesquiterpene	$C_{15}H_{24}O$	220	

8	17.58	Dehydroxy-isocalamendiol	Sesquiterpene	$C_{15}H_{24}O$	220	
9	18.00	1,2-Dimethoxy-4-methoxy-l-propenylbenzene	Ester	$C_{12}H_{16}O_3$	208	
10	18.08	Asarone	Ester	$C_{12}H_{16}O_3$	208	
11	18.40	Acetic acid, 3-hydroxy-6-isopropenyl-4, 8a-dimethyl-1, 2,3,5,6,7,8,8 a-octahydronaphthalen-2-yl ester	Alcohol	$C_{17}H_{26}O_3$	278	
12	19.31	9-Acetamido-1-methyl-3,6-diazahomoadamantane	Amide	$C_{12}H_{21}N_3O$	223	
13	20.61	1,8-dimethyl-8,9-epoxy-4-isopropyl-spiro [4.5] decane-7-1	Sesquiterpene	$C_{15}H_{24}O_2$	236	
14	20.05	6 - [1 - (Hydroxymethyl) vinyl] -4,8 a-dimethyl-4a, 5,6,7,8,8 a-hexahydro-2 (1H)-naphthalenone	Sesquiterpene	$C_{15}H_{22}O_2$	234	

Linalool is an important compound used in foods as food additives [6], in pharmacology as a sedative effect of the inductor and an inhibitor of glutamatergic neurons [9]. Linalool is a member of 60-80% perfume products, hygiene and cleaning products, including soaps, shampoos, and lotions. It is also used as a chemical intermediate. One of the common products of linalool is vitamin E. [12]. Camphor is a well known chemical compound with pronounced antimicrobial potential [10]. Camphor is a waxy, white or transparent color with a strong, aromatic odor. This is a terpenoid with the chemical formula $C_{10}H_{16}O$. It is also used in medicine. Camphor is easily absorbed through the skin and creates a feeling of cooling similar to menthol and acts as a little local anesthetic and antimicrobial substance. Camphor is the active ingredient (along with menthol) [12].

Monoterpenes belong to a class of terpenes that consist of two isoprene units and the molecular formula of $C_{10}H_{16}$. Monoterpenes can be linear (acyclic) or contain rings. Biochemical changes such as oxidation or rearrangement produce the related monoterpenoids. In this study sensor, monoterpene compounds - beta-pinene and camphene were detected. Camphene, a bicyclic monoterpene hydrocarbon, is nearly insoluble in water but soluble in organic solvents. It evaporates easily at room temperature and has a sharp smell. [12].

Sesquiterpenes are a class of terpenes that consist of three isoprene units $C_{15}H_{24}$. Like monoterpenes, sesquiterpenes may be acyclic or contain rings, including a number of unique combinations. Biochemical changes such as oxidation or rearrangement produce the related sesquiterpenoids. Sesquiterpenoids are defined as a group of 15 carbon compounds produced by assembling three isoprene units, and they are found mainly in higher plants. Monoterpenes and sesquiterpenes, are an important part of the essential oils in plants. They are the most diverse group of isoprenoids.



igure (3). Mass spectra of major compounds asarone.

5-Sesquiterpenes are primarily used in cosmetics, perfumes and fragrances. Five sesquiterpenes were detected and identified in this study; (γ-muurolene, Shyobunone, Isocalamendiol, 1,8-dimethyl-8,9-epoxy-4-isopropyl-spiro [4.5] decane-7-1, and 6 - [1 - (Hydroxymethyl) vinyl] -4,8 a-dimethyl-4a, 5,6,7,8,8 a-hexahydro-2 (1H)-naphthalenone), which have been confirmed by previous studies.

CONCLUSION:

It was confirmed that the healing power of essential oil of sweet flag (*Acorus calamus* L.) is related to its individual components working together synergistically. Some volatile organic compounds that are found in sweet flag may be useful for the pharmaceutical (taste, aroma), and cosmetic industries. It would be advisable to use one or two specific compounds, but not all of the essential oil. In addition, it was shown that asarone is a major component of essential oil, which determines its quality. With the use of chromatographic methods of distillation and the crushing of

the oil, biologically active compounds that have medicinal and commercial importance can be produced.

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تحليل كروماتوجرافى للمركبات العضويه الطياره بالزيت الطيار فى ريزومات قصب الذريرة

عصام يوسف عبدالحفيظ[1]، ميخائيل اليكسيفيتش ييجورف[2]

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أجريت الدراسة بمعمل البيوتكنولوجى و البيوايكولوجى بجامعة استراخان الحكوميه بروسيا الاتحاديه خلال موسمى 2009، 2010 حيث تم جمع ريزومات قصب الذريرة (*Acorus calamus* L.) من منطقة ترى براتوكا باقليم استراخان، و تم غسل و تجفيف العينات ثم استخلاص الزيت الطيار منها بالتقطير بالبخار و تم تقدير كمية الزيت الناتج (4 مل/كجم ريزومات)، تم تحليل الزيت باستخدام جهاز التحليل الكروماتوجرافى GC-MS بهدف تحديد محتوى الزيت الطيار من المواد الفعالة.

و قد أوضحت النتائج ما يأتى:

- يحتوى الزيت الطيار على 14 مركب فعال تم فصلها هى:

*أزارون، *بيتا-بينين،* كامفين، *1،2،3،4-تيترا ميثيل-5-ميثيلين-1،3-سيكلوبنتادين، *لينالول، *كامفور، *ميرولين، *شابينون، *ديهيدروكسى ايزوكالامنديول، *1،2-دايميثوكسى-4-ميثوكسى-|-بروبينايلبنزين، *حامض الخليك' 3-هيدروكسى-6-ايزوبروبينايل-4،8أ-دايميثيل-1،2،3،5،6،7،8،8أ-اوكتاهيدرونافثالين-2-يل استر، *9-اسيتاميدو-1-ميثيل-3،6-دياز اوموادامانتان، *1،8-دايميثيل-8،9-ايبوكسى-4-ايزوبروبايل-سبيرو[4.5]ديكان-7-وان، * 6-[1-(هيدروكسيميثيل)فينيل-]-4،8أ-دايميثيل-4أ،5،6،7،8أ-هكساهيدرو-2(1انش)-نافثالينون.

- اتضح من دراسة المركبات الفعالة السابقة ان مركب أزارون هو المركب الرئيسي في محتوى زيت قصب الذريرة و الذى يتميز باستعماله على نطاق واسع فى الصناعات الدوائيه و كذلك كمطهر بكتيرى و فى مقاومة حشرات المخازن.