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Chemical components of leaf essential oil of *Vitex agnus-castus* L. in the Absheron conditions

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Medicinal plants are used all over the world because of their lower risk of side effects and environmentally friendly, cost-effective production compared to chemical drugs, encouraging researchers to do more research. One of the most popular medicinal plants is *Vitex agnus-castus* L., grown in tropical and subtropical regions, with various health benefits. We have studied the chemical composition of the leaf essential oils of *Vitex agnus-castus* L. introduced to Absheron. Essential oils from fresh leaves of *Vitex agnus-castus* L. were obtained by hydrodistillation and their volatile components were identified by GC/MS. Leaves of *Vitex*, grown at the Institute of Dendrology, have a light yellow liquid with an essential oil concentration of 0.45%. More than 20 components have been identified in the essential oil of the leaves. The leaf oil also contained large amounts of 1,8-cineole (28.31%) and β-pinene (13.1%). The results are given by comparing studies conducted on the same species in our country and in the world.

Keywords: *Vitex agnus-castus* L.*, leaves, essential oil, component composition*

INTRODUCTION

Due to its natural and climatic conditions, Azerbaijan is similar to many regions of the Mediterranean - the main world center for the cultivation of essential oils and medicinal plants. Types and forms of aromatic plants are a source of important groups of biologically active substances, such as essential oils, which have a high content of essential components. Essential oils are used in many industries, including pharmacology, perfumery, and cosmetology. As the properties of essential oils are studied, their range of application expands, and the demand for essential oils and aromatic substances increases year by year. Sacred Vitex (*Vitex agnus-castus* L.) is one of these essential oil plants that have a special place in Azerbaijan. Vitex is a promising tree-like plant with a spherical open crown, 2-4 m tall.

The leaves are large and complex, consisting of 5-7 leaves. Numerous flowers collected in narrow, but large and dense paniculate spike-shaped inflorescences, which appear in large numbers at the ends of almost all shoots. The crown of the flower has two lips, lavender, lilac, or pale purple. This plant blooms from June to October. The plant's odor is very strong, but at the same time, it is pleasant. They are mainly pollinated by insects. The fruits are dry four-celled spherical nuclei, 3-4 mm in diameter, with dark blue flowers, covered with bark, and ripen in October-November.

The literature shows that the chemical components of *Vitex* leaves have terpenes and flavonoids. Their biologically active substances extensively studied in previous years. Our goal is to study the composition of the essential oil of the leaves of this plant grown in Absheron, to apply it in our future research, and expand its use in the Republic of Azerbaijan.

MATERIALS AND METHODS

The research was conducted in 2021 in the laboratory of Essential Oils of Plants at Mardakan

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Institute of Dendrology (Absheron region), in accordance with the pharmacopoeia method. Existing technologies for the processing of essential oil raw materials ensure its preparation. Various methods are used for this: grinding, casting, drying, fermentation, sonication, and infrared radiation. To obtain the essential oil, the leaves of the Institute's collection were used (Fig. 2).

Fig. 1. General view of the plant *V. agnus-castus*

(a)

(b)

Fig. 2. a - flowers and b - fruits of *V. agnus-castus.*

Extraction of essential oil in the laboratory was carried out by hydro-distillation in the Klevenzer apparatus (Jennings, 1980; Tyagi, 2017). Raw material cut into 1-3 cm pieces was used as a sample. Collection of the studied material - calamus leaves was carried out in July during the flowering stage of the plant, in the middle-aged generative state of ontogeny (4th year of life) in natural populations. The essential oil is obtained by hydro-steam distillation for at least 10 hours until the release of the essential oil is stopped. The duration of the hydro-steam distillation process was determined experimentally based on the study of the dynamics of changes in the productivity of essential oils over time. For analysis, we used hexane solutions of the plant's essential oil (1.0-3.0% by weight). The mass fraction of the main components of the essential oil was determined by gas chromatography on a chromatograph "Chromatec-Crystal 2000 M" using the pole column. Capillary column CR is 5 cm, length 30 m, inner diameter 0.25 mm. Phase - 5% phenyl 95% polysylphenylene siloxane, film thickness - 0.25 μm. The thermostat temperature is programmed from 4°C / min to 750°C to 240°C. Evaporator temperature - 250°C. Carrier gas - helium, flow rate 1 ml / min. The temperature of the transition line is 250°C; the temperature of the ion source is 200°C. Electron ionization - 70 eV. The scanning range is 20-450. Scan time - 0.2. The sample volume of the essential oil is 0.2 μL. The normalization method was used to calculate the mass fraction of the components (Tkachev 2008; Adams, 2007). The results of experimental research were developed using mathematical-statistical methods.

The following formula was used for this:

$$\boldsymbol{X = \frac{V \times 100}{m}}$$

V - volume of oil obtained in mm

M - grams of raw material used

The component composition of the separated essential oils was analyzed by chromatography on the gas chromatograph KRISTAL-2000M (Russia), using the computer program Crystal PM-1.

The composition of the components was calculated from the peak areas and the identification of the individual components was based on a comparison of the retention times and total mass spectra with the relevant data for the components of the reference oils and pure compounds (Boelens, 1991). Data from the Wiley 275 library of mass

spectra (275,000 mass spectra) (Hussein, 2014) and atlases of mass spectra and linear capture indices (Shchipitsina, 2010) were also used for identification. Identification was considered final with the complete coincidence of mass spectra and linear capture indices (Chryssavgi, 2008; Ayvaz, 2010).

RESULTS AND DISCUSSION

The essential oil of the common twig is a light yellow liquid. Twenty components were identified in the essential oil of the common twig, of which 19 components were found in quantities exceeding 0.2% and accounting for 80.6% of the whole oil (Table 1). Analysis of the component composition of the essential oil of the sacred Vitex raw material, removed during the flowering phase of plants, showed that its main components are terpene hydrocarbons: α-pinene, sabinene, β-pinene, monocyclic monoterpenes, bicyclic monoterpenes, monoterpene alcohols, monoterpene ketones, and oxides; 1,8-cineole.

Table 1. The main components of *V. agnus-castus* essential oil are obtained under the conditions of Absheron

№	Retention time	Components	Mass fraction of the component, %
1	8.694	α-pinene	6.1
2	9.35	limonene	9.4
3	10.46	1,8-cineole	28.3
4	14.16	linalool	2.8
5	9.46	β-pinene	13.1
6	9.031	sabinene	0.14
7	9.71	carene-3	1.8
8	10.27	α-terpinene	2.6
9	11.10	γ-terpinene	0.5
10	12.94	terpinolene	0.3
11	15.15	terpinene 4-ol	1.04
12	14.61	caryophyllene	0.2
13	15.32	citronellol	2.95
14	15.68	N,N di methyl acetamide	3.84
15	16.21	estragole	2.82
16	16.29	camphene	4.95
17	17.15	camphor	0.4
18	17.25	citronellyl butyrate	0.14
19	19.96	citronellyl tiglate	0.3
20	20.80	geranyl tiglate	5.4

The chemical composition of *Vitex agnus-castus* L. EO and the chromatogram are presented in table 1 and Fig. 3 (Ulukanli, 2015). The oil yield was 1.3% (v/w) based on fresh weight. The main component was 1,8-sineole (28.3%). The combination of bicyclic and monoterpene alcohols (34.81%) had the highest percentage among other constituents, this combination included, α-pinene (6.09%), β-pinene (13.1%), geranyl tiglate (5.36%) and camphene (4.95%).

V. agnus-castus essential oil contains several tens of mono- and sesquiterpenoids. α-pinen and β-pinene, 1,8-cineole, linalool, camphene, geranial tiglate, and terpinen-4-ol are present in the greatest amount (Tkachev, 2008; Senatore, 1996).

The qualitative and quantitative compositions of essential oil have been studied in sacred Vitex growing in Nigeria (Hamid, 2010), Amazonia (Zoghbi, 1999), Italy (Galletti, 1996), Turkey (Hüseyin, 2014), Brazil (Zoghbi, 1999), Morocco (El-Kamari, 2018), Southern coast of Crimea (Bogatyuk, 2015) and in other countries. In addition, the composition of the volatile components of the essential oil is significantly influenced by its genetic, geographical origin, and growing conditions (Chryssavgi, 2008; Boelens, 1991). According to the literature, the main producers are Turkey, Italy, and Brazil (Hüseyin, 2014; Adams, 2007).

The main components of *V. agnus-castus* essential oil from Nigeria are β-pinene (20.0%), viridiflorol (9.8%), α-pinene (9.1%), 1,8-cineole (6.7%), β-farnesene (5.4%), terpinen-4-ol (4.2%), α-terpineol (4.1%), and β-pellandrene (4.1%). The essential oil was extracted from the leaves of *V. agnus-castus* in southern Italy, and the highest component in the leaves was 1,8-cineole (35.2%), sabinene (23.6 %), α-pinene (7.6%); studied the volatile components of the leaves of the *V. agnus-castus* plant growing in the Amazon region. Components with the highest content: in the leaves; β-farnesene (5.2%), 1.8 cineole (33.5%) and sabinene (18.5%); the components of the leaves of the plant of the sacred Vitex native to Brazil were studied, and the highest component in the leaves was 1,8-cineole (23.8%), β-farnesene (14.6%), caryophyllene (12.5%), sabinene (11, 4%), α-terpinyl acetate (7.7%), etc. The highest concentration in the essential oil of *V. agnus-castus* is 1,8-cineole (35.2%), sabinene (23.6%), α-pinene (19.48%).

The essential oil content in Vitex leaves ranges from 0.02% (Hina Zahid Ghazala, 2016) to 1.3% (Katiraee, 2015) (Table 3).

When growing within the Mediterranean region, this figure in plants ranges from 0.2 to 0.8%; outside the natural range (Brazil, Nigeria) is also quite high - 0.3 and 0.8%, respectively. The content of the main component of 1,8-cineole also varies widely - from 6.7 to 50.9%.

Analysis of the available data on the mass fraction of the main components (Table 2) suggests a significant variability in the component composition of Vitex essential oil in different ecological and geographical conditions.

A comparison of the components of Vitex oil shows that the quality and quantity of oil are very different. Thus, even inside the dominant components: sabinene and pinene (predominance of monoterpenes), caryophyllene and farnesene (predominance of sesquiterpenes) (Table 2) the chemical components of Vitex essential oils also differ, the mass fraction of 1,8-cineole is completely absent, the clear predominance of α-pinene and β-pinene allows this oil to be delivered to the pinene component (Hamid, 2010; Zoghbi, 1999; Katiraee, 2015).

Table 2. Qualitative and quantitative compositions of the essential oil of *V. agnus-castus* leaves according to the literature data

Component	Nigeria	Turkey	Crimea	Brazil	Iran	Pakistan	Morocco	Italy
α-pinene	9.1%	26.9%	4.78%	8.9%	19.4%	9.0	9.76%	4.0%
sabinene	1.2%	22.7%	6.8%	18.5%	6.89%	10.8%	14.57%	-
β-pinene	20.0%	1.3%	-	-	-	-	-	-
1,8-sineole	6.7%	14.20%	19.1%	33.5%	-	50.9%	19.61%	15.6%
viridofloral	9.8%	-	15.9%	-	-	-	-	-
β-farnesene	5.4%	8.5%	15.8%	5.2%	-	-	-	8.6%
cisocymene	8.4%	-	-	-	-	-	-	-
transcarifilene	-	9.13%	14.3%	-	-	-	-	-
α-terpinyl acetate	-	-	-	6.4%	-	-	-	-
bicyclogermarkene	-	2.4%	13.7%	3.2%	-	2.4%	-	-
β-caryophyllene	1.1%	8.9%	12.8%	2.8%	8.5%	6.5%	9.5%	8.9%
β-caryophyllene oxide	-	0.6%	-	0.8%	-	-	5.0%	0.5%

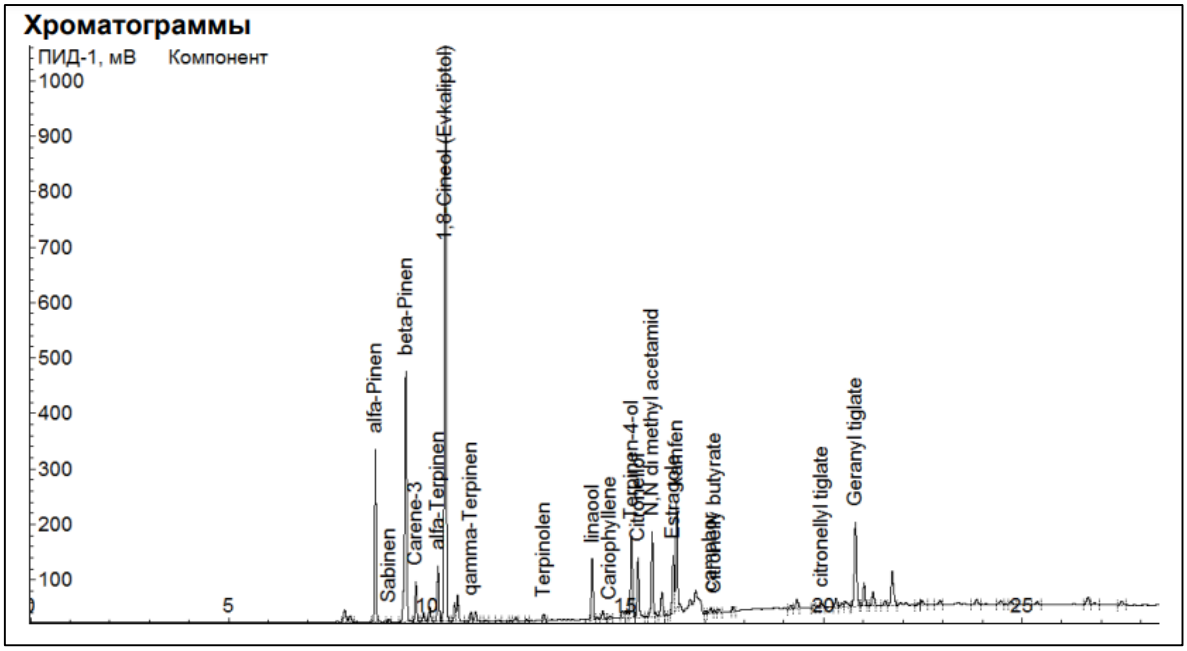

Fig. 3. Chromatogram of *V. agnus-castus* essential oil on SR-5 column.

Table 3. Characteristics of the essential oil of the *V. agnus-castus* leaves from various cultivating areas

№	Cultivation place	Mass fraction of the essential oil for absolutely dry mass, %	Mass fraction of 1,8- cineole, %
1	Pakistan	0.02	50.9
2	Turkey	0.20	24.3
3	Brazil	0.3	33.5
4	Morocco	0.35	8.7
5	Southern coast of Crimea	0.79±0.1	21.8
6	Iran	1.3	13.3
7	Nigeria	0.8	6.7

Most of the studied essential oils can be attributed to 1,8-cineole. This is mainly typical for plants growing in the Mediterranean countries, as well as under cultivation conditions in Brazil (Zoghbi, 1999) and Pakistan (Hina Zahid Ghazala, 2016).

The main features of the sacred Vitex essential oil, which is grown or cultivated in different places, are the predominance of 1,8-cineole or pinene, as well as the presence of an inverse relationship between the group of monoterpenes (sabinene and pinene) and sesquiterpenes (farnesene, caryophylline).

From such changes in the composition of essential oils, Vitex seems to depend on the reserve's climate and growing conditions (El-Kamari, 2018).

CONCLUSIONS

According to research, the main components of the essential oil of the *Vitex agnus-castus* L. during flowering are 1,8-cineol and β-pinene. The components mentioned in the literature were also key components in our study. Considering the natural bioactive compounds that have therapeutic potential, *V. agnus-castus* L. is one of the best-selling and most widely used medicinal plants in the world.

In general, studies of the components of the essential oil of Vitex (although the proportions of the components are different) have shown that they have a similar distribution.

This shows us that the essential oils of *V. agnus-castus* do not differ much even in different geographical regions. It shows that although the plant has a different location, its main components do not change drastically; it was noted that only the coefficients differ.

However, *in vivo* and *in vitro* studies and more clinical experiments are needed to study the composition of the beneficial compounds of this plant and the associated mechanisms of influence.

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Abşeron şəraitində *Vitex agnus-castus* L. növünün yarpaqlarının efir yağının kimyəvi komponentləri

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Dərman bitkiləri bütün dünyada istifadə olunur, çünki onların yan təsirləri riski daha azdır və kimyəvi dərmanlarla müqayisədə ekoloji cəhətdən təmiz, sərfəli istehsal, tədqiqatçıları daha çox araşdırma aparmağa həvəsləndirir. Geniş yayılmış dərman bitkilərindən biri, tropik və subtropik bölgələrdə yetişdirilən, müxtəlif sağlamlıq faydaları olan, *Vitex agnus-castus* L.-dir. Bu tədqiqatda Abşerona introduksiya edilmiş *Vitex agnus-castus* L. yarpaqlarının efir yağlarının kimyəvi tərkibi öyrənilmişdir. *Vitex agnus-castus* L.-nin təzə yarpaqlarından efir yağları hidrodistillə yolu ilə alınmış və onların uçucu komponentləri GC/MS ilə müəyyən edilmişdir. Dendrologiya İnstitutunda yetişdirilən Adi ərgudə yarpaqlarının efir yağının konsentrasiyası 0,45% olan, açıq sarı mayedir. Yarpaqların efir yağında 20-dən çox komponent müəyyən edilmişdir. Yarpaq yağında həmçinin çoxlu miqdarda 1,8-sineol (28,31%) və β-pinen (13,1%) vardir. Nəticələr, ölkəmizdə və dünyada eyni növlər üzərində aparılan tədqiqatların müqayisəsi əsasında, verilib.

Açar sözlər: *Vitex agnus-castus* L., *yarpaqlar, efir yağı, komponent tərkibi*

Химические компоненты эфирного масла листьев *Vitex agnus-castus* L., выращенных в условиях Апшерона

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Лекарственные растения используются во всем мире, потому что риск их побочных эффектов ниже, чем у химических лекарственных средств, а производство более экологично и рентабельно. Одним из наиболее распространенных лекарственных растений является *Vitex agnus-castus* L., который выращивается в тропических и субтропических регионах и обладает рядом преимуществ, способствующих сохранению здоровья человека. В данной работе изучен химический состав эфирных масел листьев *Vitex agnus-castus* L., интродуцированных на Апшерон. Эфирные масла из свежих листьев *Vitex agnus-castus* L. получали гидродистилляцией, а их летучие компоненты определяли методом ГХ/МС. Эфирное масло листьев витекс, выращенных в Институте дендрологии, представляет собой светло-желтую жидкость с концентрацией 0,45%. В этом эфирном масле идентифицировано более 20 компонентов. Оно также содержит большое количество 1,8-синеола (28,31%) и β-пинена (13,1%). Результаты основаны на сравнении исследований одних и тех же видов Vitex *agnus-castus* L, произрастающих в нашей стране и во всем мире.

Ключевые слова: *Vitex agnus-castus* L.*, листья, эфирное масло, компонентный состав*