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COMPOSITION OF THE ESSENTIAL OIL OF SALVIA OFFICINALIS L., GROWN IN UZBEKISTAN

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Abstract

The paper presents the results of qualitative and quantitative analysis of essential oil obtained from Salvia officinalis L. by hydro-steam distillation. The essential oil obtained from given plant is a light yellow, easily mobile liquid with a characteristic specific odor. The qualitative composition and quantitative content of essential oil components were determined by GC/MS. The composition of essential oils is represented mainly by mono- and sesquiterpene compounds. The yield of essential oils of the aerial part of Salvia officinalis L. was 0.76% of the weight of air-dried raw materials. The content of monoterpene compounds was 44.00%, sesquiterpene compounds - 44.47%.

Keywords: Salvia officinalis L., essential oil, chromatograph mass-spectrometry, terpenes, State Pharmacopoeia of Uzbekistan.

Introduction

The flora of Uzbekistan includes 4,500 plants belonging to 138 families. Of these, about 750 plants have medicinal properties and more than 180 are used in folk medicine to treat various diseases. Currently, 110 species of medicinal plant raw materials, as well as preparations from them, are officially used in medical practice.

The development of herbal medicines that meet modern requirements of international standards and their introduction into medical practice is of particular relevance. In accordance with the Resolution of the President of the Republic of Uzbekistan "On measures for organization the cultural cultivation, processing and widespread use of medicinal plants in treatment" and the Decree "On measures for creation of value added chain by means of effective use of source of raw materials and support of conversion of herbs", current tasks for the efficient use of the raw materials base of medicinal plants, the widespread use of medicinal plants in the prevention and treatment of various diseases have been identified.

In total the **State Pharmacopoeia** of the Republic of Uzbekistan contains 304 names of medicinal plant raw materials and medicines produced on their basis. In "**The State Register of Medicines and Medical Devices and Medical Equipment Allowed for Use in Medical Practice of the Republic of Uzbekistan**" for 2022 the total number of domestic and foreign medicinal plant raw materials and/or medicines obtained by primary and deep processing of medicinal plant raw materials is 88 and 216 respectively [1].

In connection with the above, it is especially important to conduct phytochemical researches on study the composition of medicinal plant raw materials for the development and introduction into medical practice of a herbal medicine based on it.

About 300 species of medicinal plants are used in modern domestic scientific medicine. The flora of our country includes 1700 higher flowering plants, of which more than 500 are recognized as medicinal.

Some of the species of sage - Salvia L. are the promising plants. [State Pharmacopoeia of the Republic of Uzbekistan. 2023. 1st ed. 3rd volume, 1st part. p. 6139 (medicinal) and p. 6140 (leaves)].

The genus Salvia - sage includes more than 1000 species worldwide [1] and has different names, including Common Sage, Kitchen Sage, Golden Sage, Culinary Sage, etc. Salvia officinalis L. belongs to the family Lamiaceae and grows in the Middle East and the Mediterranean [2], as well as in Estonia, Georgia, Hungary, England, Belgium, France, Austria, Ukraine [3], southern Albania [4] and Uzbekistan [5,6]. The medicinal plant is a subshrub with a strong aroma.

In Nepal, China, India and Europe, this plant has been used in folk medicine to treat bronchitis, cough, asthma, sore throat, inflammation of oral cavity and throat, depression, excessive sweating, skin diseases and many other ailments [2].

Salvia officinalis has demonstrated antioxidant property for the prevention and treatment of various neurodegenerative diseases [6].

It is used in folk medicine as antibacterial, antitumor, antioxidant and anti-inflammatory drug, as well as drug for the treatment of a number of diseases, including diseases of the nervous system, heart and circulatory system, respiratory, digestive, metabolic and endocrine systems [7, 8, 9,10,11].

In England, a decoction of sage leaves with wine was used for rinsing of oral cavity to relieve toothache; in Germany, sage was taken internally for the treatment of gastrointestinal disorders and excessive sweating, and applied topically for the treatment of inflammation of the mucous membranes of the mouth and throat; Native Americans used an infusion of the plant for the treatment of colds and coughs, and as an antidiarrheal drug [12].

Sage essential oils have been used for the treatment of a wide range of diseases, such as diseases of the nervous system, heart and blood circulation, respiratory system, digestive system, as well as for the treatment of metabolic and endocrine disorders. In addition, sage essential oil has been shown to have carminative, antispasmodic, antiseptic, and astringent properties [13,14].

It should be noted that the essential oil of Salvia species has different composition depending on genetic, climatic, seasonal and environmental factors [15,11].

The essential oil of this species is marketed as anticholinesterase [16], antidiabetic [17], anti-inflammatory [18], antispasmodic, antiseptic and astringent [19], antioxidant, antimicrobial, anticancer [20,21], antimutagenic [22] and antiviral [23] medicine. It is also traditionally used as a flavoring agent in herbal teas and as fragrances in perfumes and cosmetics [24].

The phytochemical composition of sage leaves has been studied quite well. For example, in [25] it was shown that sage leaves contain such biologically active substances as phenolic compounds (caffeic, dihydrocaffeic (monomers), rosmarinic (dimer), salvianolic (trimer), sage (tetramer) acids, their esters and other oligomers of caffeic acid), flavonoids: apigenin, luteolin, salvigenin, rutin, kaempferol , astragalin in glycoside form, as well as phenolic carboxylic acids (ferulic, p-coumaric, protocatechuic). The aerial part of sage officinalis contains triglycerides: linoleic, oleic and linolenic acids.

The work [26] presents the results of a study of the amino acid and vitamin composition of leaves of sage officinalis. medicinal. The raw material was found to contain 20 amino acids, of which 10 are essential. It was also found to contain vitamins C, PP, B6, B2, B1, B9, which play an important role for the body.

Among the biologically active compounds of common sage, special place occupy terpenoids . Essential oils mainly contain monoterpenoids: thujone and isothujone , camphor, 1,8-cineole, borneol.

The authors [2] studied the chemical components of the essential oil of Salvia officinalis L. leaves, growing in Nepal. The major chemical compounds identified were camphor (65.18%), camphene (9.73%), eucalyptol or 1,8-cineole (4.72%), 2-nephthalenemethanol (3.85%) and α-pinnen (2.33%), whereas the minor chemical components were borneol (1.45%), eucarvone (1.44%), benzenemethanol (1.36%), viridiflorol (1.23%), o- cymene (1.22%), limonene (1.07%), trans- pinocarveol (0.90%), 2-(3-oxobutyl) -cyclohexonone (0.84%), β- caryophyllene oxide (0.78%), β- pyene (0.78%), m- cymene (0.72%), β- Ocymene (0.71%), 8-hydroxy-p-cymene (0.67%), bornyl acetate (0.49%), valerenal (0.40%) and tricylene (0.11%). The essential oil of Salvia officinalis L. leaves showed strong antimicrobial potential against both Gram-positive Staphylococcus aureus and gram-negative bacteria.

A comparative study of the composition of essential oils of Salvia officinalis L. from commercial samples and their main chemotypes was conducted. The main components (>5%) among the 25 main identified compounds were 1,8-cineole (8.3%–45.3%), α-thujone (3.0%–34.0%), camphor (11.3%–29.3%), β-thujone (1.5%–12.9%), viridiflorol (1.1%–10.4%), camphene (2.6%–7.1%) and α- pinene (1.3%–5.8%). At the same time, α-thujone dominated in the Estonian, English, French, Hungarian, Belgian, Ukrainian and Georgian samples. Four samples (Estonia, Georgia, England, Hungary) belong to the most common chemotype α-thujone > camphor > 1,8-cineole. Eight chemotypes of essential oils of Salvia officinalis L. have been found [3,4].

The influence of climatic conditions - temperature and precipitation at the selected location on the change in the components of essential oils of Salvia officinalis L. in Southern Albania over a period of 5 years was studied. The analysis of sage essential oil from this site showed the presence of higher concentrations of alpha-thujone, beta-thujone, camphor, cineole and camphene in it. The most variable components for a analyzed period (5 years) were camphor, α- pinene, cineole and beta-thujone. It was noted that bicyclic monoterpenes and sesquiterpenes were more affected by climate change than other groups of compounds [4].

Comparison of the compositions of sage oil from different European countries showed some differences in the amounts of the main components. The highest content of camphor (29.8%), borneol (11.8%) and bornyl acetate (7.8%) was found in the oil of Scottish origin. Unlike other samples, Scottish sage contained terpinen-4-ol, a-terpineol, tyrnol, carvacrol, caryophyllene oxide and humulene epoxide from 2.1 to 4.3 % (other samples contained less than 1.8% of these compounds). The sample from Greece was rich in 1,8-cineole (45.3%) and the monoterpenes c-pinel, campheter, β-pinene and myrceter (23%). The preparation from Moldova contained less monoterpenes (0.7%) and oxygenated monoterpenes (54.9%) than the other samples studied, but was rich in (E)-β- caryophyllene (7.5%), humulene (7.5%) and viridiflorol (15.7%). The concentrations of the main compounds of three sage samples from Estonia grown in 2000-2002 were very similar to those of samples from France, Hungary, Belgium, Russia and Ukraine. The total content of toxic thujone (α- and β-thujone) in samples from Estonia was 28.1, 29.3, 30.2 and 36.9%, respectively, while in samples from other countries their concentration was comparatively low: (3.4-14.2% in samples from Ukraine, Greece, Scotland and Belgium) or average (in samples from Russia (23.3%) and Hungary (25.2%). [3,27].

In this study [28], the chemical composition and biological activity of essential oil of Salvia officinalis grown in Laghouat (Algerian Sahara) were investigated. Thirty-nine components representing 96.41% of the essential oil were detected by gas chromatography/mass spectrometry (GC/MS), of which the main compounds were camphor (16.41%), thujone (15.68%), manool (15%), viridiflorol (11.69%) and 1,8-cineole (10.06%).

In the essential oil obtained from Salvia officinalis growing in Bulgaria, twenty-eight components were identified, the main ones being the following: α-thujone (26.68%), (E)-β-caryophyllene (7.47%), 1,8-cineole (7.19%), α- humulene (6.11%), β-pinene (5.44%), β-thujone (5.35%), camphor (4.84%), allo- aromandendrene (4.55%), borneol (3.69%) and α-pinene (3.58%). The identified compounds belonged to three groups.

Among them, oxygen-containing monoterpenes accounted for the highest percentage of essential oil components, amounting to 59.15%, followed by sesquiterpene hydrocarbons (24.37%) and monoterpene hydrocarbons (14.66%) [29].

Considering the high content of biologically active compounds in the leaves of sage officinalis, their undoubted importance in the manifestation of the biological activity of this raw material, it seemed appropriate to study the component composition of the volatile fraction for the development of a natural combined dental medicine based on it.

The aim of the study was to investigate the chemical composition of Salvia officinalis L. essential oil, grown in the botanical garden of Tashkent, Republic of Uzbekistan using the GC-MS method.

The object of the study was leaves of Salvia officinalis L. and essential oils obtained from its raw materials.

The leaves of Salvia officinalis L. were collected in the botanical garden of Tashkent, Republic of Uzbekistan, in 2023. The raw materials of the sage samples were dried in a ventilated place in the shade.

The essential oil of Salvia officinalis L. was obtained by distilling it with water steam in a special device under the conditions described in the State Pharmacopoeia of the Republic of Uzbekistan [30].The distillate was collected in a graduated tube using xylene to absorb the essential oil; the aqueous phase was automatically returned to the distillation flask. From 200 g of crushed product, 1 liter of purified water was used to obtain essential oil.

Extraction of essential oils

The essential oil was isolated from dried sage herb by the distillation method described in the State Pharmacopoeia of the Republic of Uzbekistan by distilling it with water vapor from plant material followed by measuring the its volume according to the method [30]. The specified volume of water for distillation is poured into the flask and connected to the condensing system. Water is poured into the tube until it reaches the level of junction B. Xylene is added. The liquid is heated and the distillation rate is adjusted so that it corresponds to 2-3 ml/min. Distillation is carried out for 30 min. Then heating is stopped and after 10 min the volume of xylene in a graduated tube is measured. 200 g of the sample is placed into the flask and the distillation process is continued as described above for 30 min. After the required distillation time has passed, the heating is stopped and after 10 minutes the volume of liquid collected in the graduated tube is measured and the previously found volume of xylene is subtracted from the obtained value. The resulting difference corresponds to the amount of essential oil in the mass of the sample taken. The result was recalculated in milliliters per 1000 g of medicinal plant raw material. The process was carried out for 2 hours.

The obtained essential oil of the Salvia officinalis L. herb was kept in anhydrous sodium sulfate to remove water and filtered for storage in tightly sealed dark bottles at a temperature of 4ºC. The obtained essential oil (light yellow with a distinctive odor) was 0.078 ml. The content of essential oil in volume-weight percent, recalculated to absolutely dry raw materials, was 0.39%.

Analysis of essential oils of Salvia officinalis by gas chromatography coupled with mass spectrometry (GC/MS).

The qualitative and quantitative composition of the isolated essential oil was studied by GC-MS using an Agilent 7890A GC chromatograph equipped with an Agilent 5975C quadrupole mass spectrometer with an inert MSD detector. Separation of substances in the essential oil was carried out on a quartz capillary column HP-5MS (30 m×250 μm × 0.25 μm) at a temperature of 50 ^{0}C (2 min) - 10^0 C/min up to 200 ^{0}C (6 min) - 15 ^{0}C/min up to 290 ^{0}C (15 min). The sample volume was 1 μl (chloroform), the flow rate of the mobile phase was 1.3 ml/min. Injector temperature was 220°C. EI-MS spectra were obtained in the m/z range of 10-550 amu. The isolated substances were identified by comparing their mass spectra with the electronic library data (W9N11.L Wiley Registry of Mass Spectral Data-9th Ed. and NIST08.L Mass Spectral Library), as well as using mass spectra from the literature. A 1% methanol solution of oils was injected into the chromatograph.

Qualitative analysis of essential oil components was based on their retention indices and comparison of full mass spectra with those of pure compounds, as well as using the NIST 02 electronic library database. The content of components was calculated from the areas of chromatographic peaks without using correction factors. All analyses were carried out in three repetitions.

Results and their Discussion

Yield of essential oil from the aerial parts of Salvia officinalis L. was 0.39% (v/w dry weight basis) and the density of the concentrated oil was 0.067 g/ml. The oil was light yellow in color, soluble in methanol, and had a characteristic strong sage aroma. The results of analysis obtained by GC/MS are shown in Fig. 1 and Table 1.

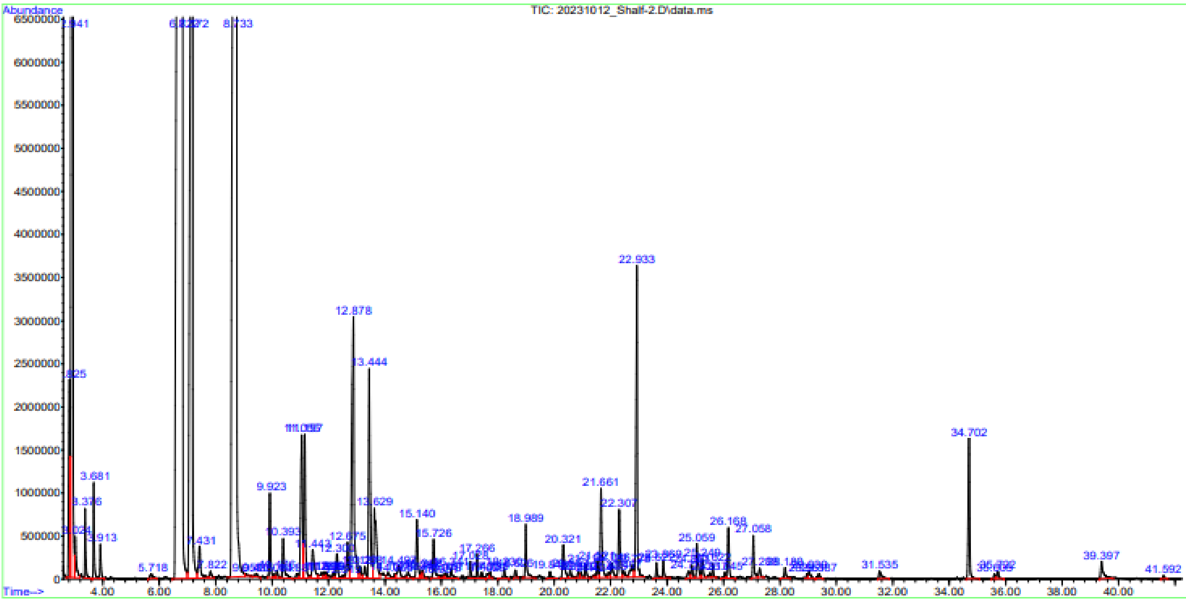

Fig. 1. Gas chromato-mass spectrum of Salvia Officinalis L. essential oil

Table 1 Retention times and percentages of identified compounds in Salvia Officinalis L. essential oil

No.	Compounds	Retention times	Percentages %
1	(+/-)- Limonene	2.826	1.44
2	1,8-Cineol (Eucalyptol)	2.943	10.67
3	γ-Terpinene	3.376	0.43
4	o - Tsimen	3.680	0.52
5	Terpinolene	3.913	0.23
6	β-Thujone	6.824	30.75
7	α-Thujone	7.173	11.65
8	Camphene	7.820	0.15
9	Camphor	8.732	20.76
10	Isocaryophyllene	9.437	0.04
11	α- Ocimene	9.922	0.53
12	trans -β- Ocimene	10.084	0.03
13	β- Phellandrene	10.175	0.06
14	(+)- m -Menta-1,8-diene	10.395	0.39
15	α-Terpinene	10.880	0.05
16	Bornylene	11.158	1.16
17	(+)- Aromadendren	11.443	0.4 2
18	1(7),5,8- o -Mentathriene	11.766	0.09
19	Mentha-1,4,8-triene	12.303	0.56
20	Isosilveterpinolene	12.678	0.36

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21	α- Bisabolene	12.879	3.03
22	p -Mentha-1,5,8-triene	13.124	0.1 6
23	Cosmen	13.286	0.11
24	(-)- Isoborneol	13.441	1.86
25	α - Pinene	13.629	1.05
26	α -Elemen	14.108	0.13
27	α- Muurolene	14.496	0.19
28	δ- Cadinene	15.143	0.58
29	β -Bisabolene	15.285	0.04
30	3-Ethylidene-1-methyl-1,4-cycloheptadiene	15.343	0.06
31	Artemisiatrien	15.951	0.02
32	(-)-α- Cedren	16.081	0.03
33	p - Cymen	16.165	0.05
34	Carvacrol	16.339	0.59
35	Cosmen	17.025	0.12
36	Dehydro - p - cymene	17.264	0.17
37	Allyl benzyl ether	17.627	0.05
38	Kalamennen	18.629	0.06
39	γ- Muurolene	19.845	0.23
40	6-Oxocamphora	20.570	0.09
41	(-)- Alloaromadendren	21.113	0.22
42	β- Selinen	21.663	0.86
43	α- Guayane	21.935	0.40
44	(C)-β- Bisabolene	22.310	0.60
45	Kubenen	22.465	0.08
46	(+)-γ- Guryunen	22.931	2. 7 2
47	9,10-Dehydro isolongifolene	23.869	0.14
48	α-Amorphene	24.884	0.51
49	2-Methoxy-4-vinylphenol	25.059	0.33
50	D- Carvone	25.622	0.0 6
51	(+)- Aromadendrena	26.042	0.05
52	3-(2,4-Dimethylpenta-1,4-dien-3-ylidene)-6,6-dimethylcyclohex-1-ene	28.177	0.09
53	3,7,11-Trimethyl-1,3,5,8,10-dodecapentaene	29.037	0.09
54	Decene-1	31.534	0.10
55	n -Dodec-1-ene	35.603	0.04
56	[4aC,8aC]-1,2,3,4,4a,5,8,8a-Octahydro-1,1,4a,6-tetramethyl-5-methyl	35.719	0.07
57	n -Tetradec-1-ene	39.394	0.24
58	1-Phenoxynaphthalene	41.593	0.03
	Σ		**97.99**

As a result of the GC-MS studies, 58 major and minor compounds were identified, which constitutes 97.99% of the total oil composition. The percentages and retention times of the identified compounds of the obtained essential oil are summarized in Table 1. As can be seen, the main components of the essential oil are β -thujone - 30.75%, α -thujone - 11.65%, camphor - 20.76%, 1,8-cineole - 10.67% and borneol (1.86%). Also, other compounds such as bornylene, isoborneol, limonene were present in an amount of about 1%.

According to literature research, the composition of essential oil can be influenced by various factors, such as geographical origin, harvest season, growing conditions of the plant, and the method of extracting the oil from plant raw materials.

Table 2 Chemical composition of essential oils of Salvia Officinalis L. from different countries of the world analyzed by GC-MS

Name of substances	Uzbe-kistan	Ukrai-ne	Estonia	Georgia	Bolga ria	Russia	Czech Republic	Türkiye
α-thujone	11.65	22.4	23.9	23.8	26.7	25.3	0.1	12.60
β-thujone	30.75	11.6	6.3	11.4	5.35	2.5	5.4	34.59
camphor	20.76	12.9	19.3	16.1	4.84	12.3	20.6	10.09
1,8-cineole	10.67	13.7	11.7	13.0	7.19	12.5	12.1	4.2
α- humulene	1.50	2.1	5.3	0.8	6.11	5.6	-	5.72
β- pinene	-	1.6	2.5	1.0	5.44	21.1	1.9	4.10
borneol	1.86	3.0	1.8	3.2	3.69	2.6	3.6	-
camphene	0.15	3.60	5.2	4.7	-	4.1	2.0	4.22
α- pinene	1.05	3.7	5.1	4.6	3.58	-	3.1	3.38
viridiflo -rol	1.00	7.9	4.0	0.8	-	3.3	-	6.24
β-caryophyllene	0.04	-	-	-	7.5	5.9	5.2	3.48
bornyl acetate	-	1.9	2.1	1.9	-	1.8	1.9	1.08
bornylene	1.16	-	-	-	-	-	-	4.45
isoborneol	1.86	-	-	-	-	-	-	
limonene	1.44	-	-	-	-	-	-	1.09
(+)-γ-Guryunen	2.72	-	-	-	-	-	-	-
O- cymene	0.52	-	-	-	-	-	-	-
terpinolene	0.23	-	-	-	-	-	-	-
carvacrol	0.55	-	-	-	-	-	-	-
bisabolene	3.03	-	-	-	-	-	-	-
8-hydroxy-p-cymene	0.05	0.5	1.7	1.0	-	-	1.7	-
		[3,27]	[27]	[3,4]	[29]	[27,34]	[3, 36]	[7,27,35]

A review of studies on the chemical composition of the essential oil of Salvia Officinalis L., conducted in some countries of the world, in particular in Ukraine, Estonia, Georgia, Bulgaria, Russia, the Czech Republic and Turkey, showed the following: almost all essential oils, including the sample obtained in Uzbekistan, are characterized by the presence of β-thujone, α-thujone, camphor, 1,8-cineole, β-pinene, borneol, camphene, α-humulene, α-pinene and viridiflorol. Sage oils are generally characterized by the presence of thujone, with β-thujone usually predominating (2.5–34.59%) over α-thujone (0.1–26.7%), camphor (4.84–20.76%), 1,8-cineole (4.2–13.7%), α-humulene (0.8–6.11%), α-pinene (1.05–5.1%), camphene (0.15–5.2%), and bornyl (1.8–3.69%) [23]. The highest amount of α-thujone was found in the essential oil from sage grown in Bulgaria. The essential oil of sage from Turkey contains significantly more β-thujone than other essential oils of sage. The essential oils of sage grown in Uzbekistan and the Czech Republic contain a lot of camphor. The essential oil of Salvia Officinalis L.

obtained in Ukraine is rich in 1,8-cineole (13.7%) compared to other oils. Bulgarian essential oil is characterized by the highest content of α-humulene (6.11%). Significant differences are found in the composition of Salvia Officinalis L. essential oils in terms of the content of α-pinene, borneol, camphene, β-pinene, viridiflorol and bornyl acetate (Table 2).
The reported differences in the composition of Salvia Officinalis L. essential oil from different geographical regions can be explained by various factors such as diverse plant growing environment, environmental factors, plant growth phase, harvest season, temperature and extraction methods [31-33].

Conclusion

This article presents the results of research on the study of chemical composition of essential oil obtained from Salvia Officinalis L. raw material collected in the botanical garden of Tashkent, Republic of Uzbekistan. Isolation of Salvia Officinalis L. essential oil was carried out by distilling it with steam in a special device under the conditions described in the State Pharmacopoeia of the Republic of Uzbekistan. Using the method of gas chromatography in combination with mass spectrometry, 58 compounds, representing 96.41% of the essential oil, were identified. The main compounds of the essential oil were β-thujone (30.75%), camphor (20.76%), α-thujone (11.65%), 1,8-cineole (10.06%), borneol (1.86%) and α- humulene (1.5%). The obtained data will provide the opportunity to use them in the development of quality control methods for the standardization of sage-based medicinal products.

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