ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2024, Vol. 50, No. 7, pp. 2847–2852. © Pleiades Publishing, Ltd., 2024.
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Study of the Chemical Composition of *Stachys Palustris* L. Essential Oil Producing in the Astrakhan Region

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Received September 13, 2022; revised November 2, 2022; accepted November 2, 2022

Abstract—Objective: The aim of this work is to study the component composition of essential oil obtained from the above-ground parts of wild-growing *Stachys Palustris* L. in the Astrakhan region and the dependence of the yield of essential oil on the vegetation period of the plant. **Methods:** Essential oil samples were obtained by hydrodistillation. The GC-MS method was used to perform a quantitative analysis of the main components of the essential oil of *Stachys palustris* L. in the flowering phase. The quantitative content of essential oil components was calculated from the areas of gas chromatographic peaks without using correction coefficients. Qualitative analysis was performed by comparing linear retention indices. The essential oil yield was determined in % based on the weight of air-dry raw materials. **Results and Discussion:** In the results, it is found that the composition of the essential oil of *Stachys palustris* L. turned out to be very specific. 47 compounds belonging to different classes have been identified. The main classes of compounds are fatty acids and acid esters (36.23%), carbonyl compounds (14.25%), and oxygenated sesquiterpenes (12.90%), phenols (5.85%). Among sesquiterpenoids, the predominant component of essential oil is hexahydrofarnesyl acetone (7.5%). The oil also contains (*Z*)-phytol (6.78%), thymol (4.2%), β-ionone (3.36%), and β-caryophyllene (2.87%). The non-specific components of the essential oil of the taxon *Stachys palustris* are coumarin (10.27%) and coumaran (0.36%). The highest yield of essential oil was obtained from plants in the flowering phase (0.19–0.20%). **Conclusions:** The work obtained data on the features of the chemical composition of the essential oil of *Stachys palustris* L., growing wild in the Astrakhan region, which is probably due to both its taxon species affiliation and the climatic conditions of growth and the allelopathic effect of biocenoses.

Keywords: *Stachys palustris* L., hydrodistillation, essential oil, coumarin, β-ionone, (*Z*)-phytol, β-caryophyllene, caryophyllene oxide, carbonyl compounds, fatty acids and acid esters

DOI: 10.1134/S1068162024070033

INTRODUCTION

The genus *Stachys* includes more than 270 plant species [1] distributed in all regions of the world, with the exception of Australia and New Zealand. Only 9 species of *Stachys* are found in the European part of Russia, 7 species in Western Siberia and 5 species in Altai.

Among them, *Stachys officinalis* L. (Medicinal stachys), *Stachys palustris* L. (Marsh Stachys), *Stachys lavandulifolia* Vahl. (Stachys lavandulifolia), *Stachys tibetica* Vatke. (Tibetan Stachys), *Stachys betoniciflora* Rupr. (betonica foliosa rupr.), *Stachys sylvatica* L. (Stachys sylvestris), and others.

Stachys palustris L. is distributed throughout Europe, Turkey and temperate regions of Asia from Iran to China. In Russia, this species is found in the European part, Siberia and Altai.

Stachys palustris L. grows in damp meadows, along river banks, through forests among shrubs. In the Astrakhan region, this plant grows mainly along the banks of rivers.

Stachys palustris—is a perennial herbaceous plant with a creeping rhizome and tuberous thickened underground shoots at the ends. The stem is pubescent with stiff, downward-facing hairs, 30–100 cm high. The leaves are dark green opposite, short-stemmed, lanceolate, pointed. The inflorescence—is long, intermittent in the lower part. The corolla is 14–18 mm long, dark purple, its lower lip with a white pattern. The inflorescence is spike-shaped, the whorls consist of six to ten flowers. The bracts are linear, equal to or longer than the pedicels;

the calyx is clearly double-edged; the corolla is purple or purplish-purple, twice as large as the calyx. The fruit—is a dark brown naked nut of oval shape. Blooms in June–September. The fruits ripen in August–September.

92 compounds have been identified in the *Stachys palustris* L. essential oil, obtained by hydrodistillation from ground parts of a plant growing in Southern Italy: caryophyllene oxide (7.8%), hexahydrofarnesylacetone (7.4%), hexadecanoic acid (6.8%), (*Z*,*Z*,*Z*)-9,12,15-octadecatrienoic acid (6.7%), (*Z*)-phytol (6.4%), thymol (5.8%), *p*-methoxyacetophenone (5.1%), 4-vinyl guaiacol (3.8%), tetradecanoic acid (3.8%), (*E*)-caryophyllene (3.6%), β-ionone (3.3%) and β-damascenon (3.0%). The oil yield was 0.21% [2].

In the essential oil isolated from *Stachys serotina*, which grows in Croatia, the phytol content is only 0.1%, and the main components were (*E*)-β-caryophyllene (22.6%), δ-kadinen (9.6%), α-humulene (7.5%), and germacrene D (6.0%) [3].

The authors of the article [4] studied the chemical composition of *Stachys pilifera* Benth.—an endemic species of Iran, for the first time, the variability of the composition of essential oil in eleven wild populations of *S. pilifera* was shown. The most common components were *cis*-chrysanthenyl acetate (19.1–48.2%), viridiflorol (1.4–19.1%), *trans*-caryophyllene (2.3–11.9%), caryophyllene oxide (1.9–11.0%), limonene (2.0–5.9%), and spatulenol (0.0–9.5). This allowed to identify four main chemotypes with cluster analysis: chemotype I (*cis*-chrysanthenyl acetate), chemotype II (*cis*-chrysanthenyl acetate acetate/viridiflorol), chemotype III (*cis*-chrysanthenyl acetate/viridiflorol/spatulenol), chemotype IV (*cis*-chrysanthenyl acetate/*trans*-caryophyllene/α-pinene).

It was found that the major components of the essential oil *Stachys koelzii* Rech.f., which grows in Iran, are α-pinene (36.71%), 1,8-cineol (20.53%) and *trans*-caryophyllene (12.34%) [5]. Comparative study of the chemical composition of essential oils of *Stachys atherocalyx* C. Koch. and *Stachys sylvatica* L., which grows in Iran, showed that the main components in the first case are spatulenol (22.08%), linalool (9.14%), linalyl acetate (8.86%), and β-borbonene (5.65%), then in the second case—β-caryophyllene (19.64%), δ-kadinen (13.41%), spatulenol (12.51%), and α-humulene (11.17%) [6].

It was established [7] that the main volatile components of the essential oils of three *Stachys* species growing in Turkey are α-pinene (11.2%), *p*-cymol (18.2%), and carvacrol (28.8%) in *S. macrantha*, γ-muurolene (10.2%), α-cedrene (11.2%) and limonene (37.0%) in *S. sylvatica* and α-pinene (11.4%), β-pinene (23.1%), and (*Z*)-β-ocimene (24.8%) in *S. annua* ssp *annua* var. *Annua.*

Significant differences were also found in the chemical composition of six *Stachys* species (*Stachys scardica*, *S. officinalis*, *S. germanica*, *S. sylvatica*, *S. plumosa*, and *S. recta)* growing in Serbia [8].

The chemical composition of essential oils of seven *Stachys* taxa, growing in Croatia, has been studied: *S. alpina* L., *S. officinalis* (L.) Trevis, *S. palustris* L., *S. recta* L. subsp. recta, *S. recta* L. subsp. subcrenata (Vis) Briq., Ten., and *S. sylvatica* L. [9]. It has been established that in all taxa except *S. alpina*, the main components are sesquiterpene hydrocarbons. *S. alpina* essential oil contains a significant amount of oxygenated sesquiterpenes. In addition, the essential oils of *S. alpina* and *S. palustris* contain significant amounts of carbonyl compounds and alcohols. Some differences were also found in the composition of the essential oil of two subspecies of *S. recta* (*S. recta* subsp. recta and *S. recta* subsp. subcrenata) growing under almost identical conditions.

The authors of the work [10] studied, using GC and GC-MS methods, the chemical composition of essential oils of six species of *Stachys*, *S. cretica* L. ssp vacillans Rech., *S. germanica* L., *S. hydrophila* Boiss., *S. nivea* Labill., *S. palustris* L., and *S. spinosa* L. obtained by hydrodistillation. It was found that all oils contain significant amounts of fatty acids and esters (24.2–58.5%), a large number of sesquiterpenes (16–35.9%), with the exception of *S. palustris* oil, which contains a significant amount of carbonyl compounds (25.4%). 92 compounds were identified in *S. palustris* essential oil: oxygenated monoterpenes (3.1%), monoterpene hydrocarbons (0.5%), oxygenated sesquiterpenes (10.6%), sesquiterpene hydrocarbons (5.4%), carbonyl compounds (25.4%), phenols (11.2%), fatty acids, and esters (24.2%). The antiproliferative activity against a number of human cancer cell lines (C32, amelanotic melanoma and ACHN, renal cell adenocarcinoma) was studied for all oils. *S. germanica* oil showed the greatest antiproliferative activity against the C32 cell line with 77% inhibition at the concentration of 100 mcg/mL, and *S. palustris* and *S. spinosa* showed the greatest activity on the ACHN cell line at the concentrations of 100 mcg/mL with inhibition of 81, 77, and 73%, respectively.

It was also found that the essential oils of *S. palustris*, *S. cretica*, and *S. hydrophila* showed a high antiradical effect with IC_{50} values of 0.482, 0.652, and 0.664 mg/mL, respectively [10]. The ability of alcohol extracts of *Stachys palustris* L. to inhibit the DPPH radical (IC_{50} = 92.08–105.42 mcg/mL) was shown in the study [11].

It was found that the essential oil of *Stachys koelzii* Rech.f. inhibits the periodontal pathogen *Prevotella intermedia* (MIC = 0.1 mg/mL, MBC = 0.2 mg/mL), and also exhibits cytotoxic effects on HeLa cells (IC_{50} = 0.06 mg/mL) [5].

In traditional medicine, the roots and the ground part of plants of the genus *Stachys* are used. *Stachys palustris* L. is used as an antitumor, wound healing, antiulcer, soothing, anti-inflammatory agent, in the treatment of sore throat, cough, spleen sclerosis, allergies, and gynecological bleeding [12]. In herbal medicine, tea from plants of the genus *Stachys* is used as a sedative, antispasmodic, diuretic and menstrual stimulant [13–16].

The purpose of this work is to study the chemical composition of samples of *Stachys palustris* L. essential oil, quantification of its main components.

RESULTS AND DISCUSSION

We have studied the dependence of the essential oil yield obtained in the phases of budding, mass flowering and fruit ripening. It was found that the highest yield of essential oil is observed from the plant in the flowering phase (Table 1).

Table 2 shows the Stachys palustris compounds identified in the essential oil, as well as their quantitative content.

Table 1. The release of essential oil from the ground part at different periods of vegetation of the *Stachys palustris*

Terms of vegetation	Essential oil yield, %*
June (budding phase)	0.16/0.17
July—early August (mass flowering phase)	0.19/0.20
End of August—beginning of September (fruit ripening phase)	0.17/0.18

* The numerator and denominator indicate the essential oil yield from fresh and dried plant material, respectively.

Table 2. The quantitative composition of the *Stachys palustris* essential oil in the flowering phase

Component	Retention index RI	Content, % of whole oil
1	*2*	*3*
(*E*,*E*)-2,4-Hexadienal	905	0.10
1-Octen-3-one	969	0.70
1-Octen-3-ol	977	0.50
β-Pinen	978	0.20
α-Tuyen	1019	0.06
Phenylacetaldehyde	1041	1.50
γ-Terpinen	1045	0.09
Heptanic acid	1049	0.09
Linalool	1084	1.11
2-Ethylhexanoic acid	1102	0.21
Methylnicotinate	1115	1.72
Octane acid	1161	0.38
α-Terpineol	1187	0.50
3-Decanol	1198	0.63
γ-Terpineol	1199	0.36
Kumaran	1201	0.36
Verbenon	1210	0.50
4-Methoxybenzaldehyde	1235	0.28

Table 2. (Contd.)

Component	Retention index RI	Content, % of whole oil
1	*2*	*3*
Geraniol	1255	0.50
Nonanoic acid	1277	1.03
Thymol	1290	4.20
Carvacrol	1297	0.92
4-Methoxyacetophenone	1302	4.90
4-Vinylguayacol	1313	1.65
Decanoic acid	1351	1.95
β-Elimen	1387	0.27
Coumarin	1392	10.27
Methyl cinnamate	1394	0.75
β-Caryophyllene	1420	2.87
Undecanoic acid	1449	3.61
α-Selinen	1472	1.20
Germacrene D	1477	1.00
β-Ionon	1485	3.36
Dodecanoic acid	1547	3.66
(*E*)-Nerolidol	1560	0.92
Megastigmatrienon	1577	1.23
Caryophyllene oxide	1585	1.84
Benzophenone	1590	4.02
τ-Cadinol	1633	0.20
Benzyl Benzoate	1762	0.49
Tetradecanoic acid	1769	3.47
6,10,14-Trimethyl-2-pentadecanone	1829	2.75
Hexahydrofarnesyl acetone	1845	7.50
1-Nonadecen	1890	0.50
(*Z*)-Phytol	1949	6.78
Hexadecanoic acid	1952	14.87
(*Z*,*Z*)-9,12-Octadecadienoic acid	2122	4.00
Total identified compounds	47	
Monoterpene hydrocarbons	0.62	
Oxygenated monoterpenes	6.04	
Diterpenoids	6.78	
Sesquiterpene hydrocarbons	5.07	
Oxygenated sesquiterpenes	12.90	
Kumaran and coumarin	10.63	
Carbonyl compounds	14.25	
Fatty acids and esters	36.23	
Phenols	5.85	
Other compounds	1.63	
Total	100	

As follows from the above data, the chemical composition of the *Stachys palustris* essential oil, which grows in the Astrakhan region, is very specific. 47 compounds (100%) have been identified in *Stachys palustris* L. oil. The main components of the essential oil are fatty acids and acid esters (36.23%), carbonyl compounds (14.25%), oxygenated sesquiterpenes (12.90%), phenols (5.85%), as well as coumarin and coumaran (10.63%).

The major components of *Stachys palustris* L. essential oil obtained in the flowering phase are hexadecanoic acid (14.87%), coumarin (10.27%), hexahydrofarnesyl acetone (7.50%), (*Z*)-phytol (6.78%), 4-methoxyacetophenone (4.90%), and thymol (4.20%).

EXPERIMENTAL

Raw materials of *Stachys palustris* L. (ground part) were collected during period from June until September 2022 in the Kamyzyaksky district of the Astrakhan region, in the vicinity of Verkhnekalinovsky village (46°56′16.53″ n.l. and 47°87′40.64 e.l.). The raw materials were analyzed fresh and dry. Dry raw materials were obtained according to the rules for collecting and drying medicinal plants [17]. In order to avoid the destruction of biologically active substances and to remove excess moisture, the raw materials were dried immediately after collection by the most common method—air drying, based on free air access to plant material decomposed in a darkened place.

The extraction of essential oil from the crushed ground part was carried out by hydrodistillation at atmospheric pressure in a stainless steel apparatus from air-dry raw materials and fresh material weighing 5 kg, the distillate was selected for 3 h. The oil was dried with anhydrous sodium sulfate, separated from the desiccant by decantation. The steam distillation process duration has been established experimentally based on the study of the dynamics of changes in the essential oil yield over time. The essential oil yield was determined in % based on the weight of air-dry raw materials.

Qualitative and quantitative compositions of essential oil samples were carried out on a Shimadzu GS 2010 chromatograph with a GCMS-QP 2010 mass-selective detector. The NIST 14 mass spectrum library was used to identify the components.

The essential oil sample was dissolved in benzene in a ratio of 1 : 150 by volume. OPTIMA-1 non-polar column (methylsilicon, solid-bonded) 25 m, diameter 0.25 mm. Chromatography mode: injector—180°C; detector—200°C; interface—205°C; carrier gas—helium (99.99999%), 1 cm^3/min with a flow division of 1 : 25; thermostat—60°C 1 min, 2°C/min to 70°C, 5 deg/min to 190°C, then 30 deg/min to 280°C, isotherm 2 min. Mass spectrum registration mode is 39–550 *m/z*. To determine the linear indices, essential oil and normal paraffins (nonan, monodecane, tridecane, pentadecane, heptadecane, and nonadecane) were dissolved in benzene. *n*-paraffins were diluted to a concentration of 0.007% by volume, Stachys palustris essential oil—1 : 30000 by volume. The quantitative content of essential oil components was calculated from the areas of gas chromatographic peaks without using correction coefficients. The qualitative analysis was carried out by comparing the linear retention indices [18] and the total mass spectra of the components with the corresponding data of pure compounds. Linear retention indices were calculated using the formula given in [19, 20].

CONCLUSIONS

Thus, the conducted studies have revealed the qualitative and quantitative chemical composition of the essential oil of *Stachys palustris* L., which grows wild in the Astrakhan region. The specific composition of this plant essential oil is probably related both to its taxon species affiliation (hexahydrofarnesylacetone, phytol, hexadecanoic acid, thymol, 4-methoxyacetophenone), and the peculiarities of soil and climatic growing conditions and the allelopathic influence of biocenoses (coumarin, coumaran).

FUNDING

This work was not supported by any funding, and no additional grants were obtained.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies involving patients or animals as test objects.

Informed consent was not required for this article.

CONFLICT OF INTEREST

No conflicts of interest was declared by the authors.

AUTHOR CONTRIBUTION

The authors AVV, LVM—conceptual, methodology, data collection, and editing original draft. The authors SBN, TAN—data interpretation and visualization. The authors AVV, APL—project administration, approval of final draft.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Duru, M.E., Cakir, A., Harmandar, M., Izumi, S., and Hirata, T., *Flavour Fragr. J.*, 1999, vol. 14, pp. 12–14.
2. Senatore, F., Formisano, C., Rigano, D., Piozzi, F., and Rosselli, S., *Croat. Chem. Acta*, 2007, vol. 80, pp. 135–139.
3. Jerkovic, I., Gugic, M., Males, Z., and Pilepic, K.H., *Chem. Nat. Comp.*, 2012, vol. 48, pp. 508–509. https://doi.org/10.1007/s10600-012-0292-3
4. Jahantab, E., Morshedl, M.R., and Maggi, F., *Nat. Prod. Res.*, 2019, pp. 1–5. https://doi.org/10.1080/14786419.2019.1682580
5. Ramak, P. and Talei, G.R., *Microb. Pathogenesis*, 2018, vol. 124, pp. 272–278. https://doi.org/10.1016/j.micpath.2018.08.010
6. Dowlatabadi, R. and Mirza, M., *Chem. Nat. Comp.*, 2009, vol. 45, pp. 742–744.
7. Renda, G., Bektaş, N.Y., Korkmaz, B., Çelik, G., Sevgi, S., and Yayli, N., *Marmara Pharm. J.*, 2017, vol. 21, pp. 278–285. https://doi.org/10.12991/marupj.300353
8. Grujic-Jovanovic, S., Skaltsa, H.D., Marin, P., and Sokovic, M., *Flavour Fragr. J.*, 2004, vol. 19, pp. 139–144. https://doi.org/10.1002/ffj.1275
9. Vundac, V.B., Pfeifhofer, H.W., Brantner, A.H., Males, Z., and Plazibat, M., *Biochem. Syst. Ecol.*, 2006, vol. 34, pp. 875–881. https://doi.org/10.1016/j.bse.2006.04.010
10. Conforti, F., Menichini, F., Formisano, C., Rigano, D., Senatore, F., Arnold, N.A., and Piozzi, F., *Food Chem.*, 2009, vol. 116, pp. 898–905. https://doi.org/10.1016/j.foodchem.2009.03.044
11. Venditti, A., Frezza, C., Bianco, A., Serafini, M., Cianfaglione, K., Nagy, D.U., Iannarelli, R., Caprioli, G., and Maggi, F., *Chem. Biodiversity*, 2017, vol. 14, Article ID: e1600401. https://doi.org/10.1002/cbdv.201600401
12. Zvezdina, E.V., Daironas, Zh.V., Bochkareva, I.I., Zilfikarov, I.N., Babaeva, E.Yu., Ferubko, E.V., Huseynova, Z.A., Serebryanaya, F.K., Kaibova, S.R., and Ibragimov, T.A., *Farmatsiya Farmakol.*, 2020, vol. 8, pp. 4–28. https://doi.org/10.19163/2307-9266-2020-8-1-4-28
13. Hartwell, J.L., *Plants Used against Cancer. A Survey*, Quarterman Publications, Inc., Massachusetts, 1982, pp. 274–278.
14. Lewis, W.H. and Elvin-Lewis, M.P.F., *Medical Botany: Plants Affecting Man's Health*. Wiley, New York, 1977, pp. 389–390.
15. Duke, A.J., *Handbook of Medicinal Herbs*, CRC Press, Boca Raton, FL, 1986, 457 p.
16. Tamkovich, T.V., *Pharmacognostic Study of Large-Flowered Chistetsa*: Diss.…Cand. Farm. Sci., Pyatigorsk, 2008.
17. *Rules for the Collection and Drying of Medicinal Plants*, Moscow, 1985.
18. Tkachev, A.V., *Study of Plant Volatiles*, Novosibirsk, 2008.
19. Velikorodov, A.V., Kovalev, V.B., Tyrkov, A.G., and Degtyarev, O.V., *Khimiya Rastitel'nogo Syr'ya*, 2010, no. 2, pp. 143–146.
20. Velikorodov, A.V., Pilipenko, V.N., Pilipenko, T.A., and Tyrkov, A.G., *Khimiya Rastitel'nogo Syr'ya*, 2017, no. 4, pp. 117–120. https://doi.org/10.14258/jcprm.2017042041