УДК 615.322:582.949.27:543.061/062

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СРАВНИТЕЛЬНЫЙ КАЧЕСТВЕННЫЙ И КОЛИЧЕСТВЕННЫЙ АНАЛИЗ ФЕНОЛЬНЫХ СОЕДИНЕНИЙ В СЫРЬЕ ШАЛФЕЯ ЛЕКАРСТВЕННОГО И ШАЛФЕЯ СУХОСТЕПНОГО

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Резюме

Химический состав сырья шалфея лекарственного (листья) сравнивался с сырьем шалфея сухостепного (листья и трава), который произрастает в большом количестве на территории Оренбургской области. Особый интерес для авторов представляли фенольные соединения, такие как кофейная кислота, розмариновая кислота и гиперозид. Были проведены как качественный, так и количественный анализы данных фенольных соединений. Качественный анализ производился с помощью тонкослойной хроматографии (ТСХ). Диагностически важные вещества, включая кофейную кислоту (R_f=0,93), розмариновую кислоту (R_f=0,88) и гиперозид (R_f=0,37), были обнаружены во всех образцах сырья как шалфея лекарственного, так и шалфея сухостепного. Количественный анализ суммы фенольных соединений в пересчете на галловую кислоту проводился с использованием метода УФ-спектрофотометрии при длине волны 276 нм. Содержание данных соединений варьировало от 2,034 % до 3,034 %. Результаты статистической обработки проведенных опытов показывают, что ошибка единичного определения суммы фенольных соединений в анализируемых образцах с доверительной вероятностью 95 % варьирует от ±3,70 % до ±4,20 %. Принимая во внимание высокое содержание фенольных соединений в листьях и траве шалфея сухостепного, произрастающего на территории Оренбургской области, считаем, что данный вид шалфея перспективен для дальнейших исследований.

Ключевые слова: **шалфей лекарственный, шалфей сухостепной, количественный анализ, фенольные соединения.**

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COMPARATIVE QUALITATIVE AND QUANTITAVE DETERMINATION OF PHENOLIC COMPOUNDS OF RAW MATERIAL OF SALVIA OFFICINALIS AND SALVIA TESQUICOLA

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Summary

The chemical composition of medical raw material of Salvia officinalis (leaves) was compared with raw material of Salvia tesquicola (leaves and herbs), which grows in the sufficient amount on territory of the Orenburg region. Phenolic compounds such as rosmarinic acid, caffeic acid and hyperoside were the subject of a special interest of the authors. The daignostically important components such as rosmarinic acid (Rf=0,88), caffeic acid (Rf=0,93) and hyperoside (Rf=0,37) were identified during thin layer chromatography analysis (TLC). All these substances were identified in both raw materials of Salvia officinalis and Salvia tesquicola. The quantitative analysis of the amount of phenolic compounds when recalculating on gallic acid in raw material of Salvia officinalis and Salvia tesquicola was made by using the method of spectrophotometry at a wavelength of 276 nm. The content of phenolic compounds in samples of raw materials varies from 2,034 % to 3,034 %. The statistical error according to the results of the metrological characteristics of the methodology of quantitative measurement of the amount of phenolic compounds in raw material of different species of Salvia varies from ±3,70 % to ±4,20 %. The content of phenolic compounds is large enough in leaves and herbs of Salvia tesquicola, which grows in the territory of Orenburg region, this type of Salvia is requires further studies.

Key words: **Salvia officinalis, Salvia tesquicola, quantitative analysis, phenolic compounds.**

Introduction

Salvia officinalis is a hoary subshrub with the grey-green stalks and leaves and height under 500 mm. Stalks are numerous, branchy, tetrahedral with lignification in the foundation. Leaves are stalked, opposite, oblong with dull top and with cut lappet on foundation. Flowers 6 – 8 are gathered in the whorl. Bells are bilabiate and hoary. Crowns are bilabiate and blue-violet, 2 stamens which covered with upper labium. Salvia tesqicola is a perennial herbaceous plant with height 300-600 mm. Apical inflorescence has 1 – 2 side branch (es). Bells are bilabiate, hoary violet or reddish color. Crowns are bilabiate and blue-violet 10 – 13 mm. Leaves are folded, oblongly-oval, toothed on the sides, hoary. Stalk is also covered with filaments (fig. 1) [Sankov, 2001; Tichonov, 2004].

The genus Salvia includes more than 1000 species but only some of them are used in the official medicine. Salvia officinalis possess anti-inflamatory, astringent, bactericidal, gastroprotective and some other properties due to the chemical composition, which involves essential oil, diterpen acids, tannins and flavonoids [Golovkin, 2001; Kurkin, 2007; Kurkin, 2009; Muravyova ee al., 2002; State Pharmacopeia of Belarus Repablic, 2009; State Pharmacopeia of Russian Federation, 2015; European pharmacopeia, 2014; Adzet, 1986]. Unfortunately, the cultivation area of Salvia officinalis is limeted.

However the central part of Russia, including the Orenburg region, is a habitat for some closely related species. Salvia tesquicola is one of them. Leaves of Salvia officinalis are well researched in contrast to raw material of Salvia tesquicola. However, there is no methods for qualitative and quantitative analysis of phenolic compounds in the officinal medicinal agent – Leaves of Salvia offcinalis, despite it is one of leading group. Due to the closeness of these species, we decided to compare their chemical compositions, using methods described in literature [Gavrilin et al., 2010; Zapesochnya, 1996; Trease, 2009].

A B

Рис. 1. Объекты исследования: А – Шалфей лекарственный, В – Шалфей сухостепной

Fig. 1. The objects of present research: A – Salvia officinalis, B – Salvia tesquicola

Aim

The aim of present work was the comparative qualitative and quantitative determination of phenolic compounds from raw material of Salvia officinalis and Salvia tesquicola.

The tasks of the present research were following:

1) The qualitative analysis of the samples of raw material of Salvia officinalis (leaves) and salvia tesquicola (leaves and herbs) using thin layer chromatography.

2) The quantitative analysis of the samples of raw material of Salvia officinalis (leaves) and salvia tesquicola (leaves and herbs) using the method of spectrophotometry at a wavelength of 254 nm.

Materials and Methods

Raw material of Salvia tesquicola was prepared during 2016 in the territory of Orenburg region. Herbs and leaves as the raw material were air-dried in the shade. Industrial samples of medical raw material of Salvia officinalis (leaves) were used.

Thin layer chromatography was performed using an aluminium coated plate «Sorbfil PTLC-AF-A-UV» and the eluent system – ethylacetate : formic acid : water=90:6:6. The plate was kept for one hour at the temperature 100°C. After that it was spotted with 70 % ethanolic sample solutions and was examined in UV light (λ=365 nm, λ=254 nm) [Gird et al., 2014; Kirchner, 1991; Minina, 2009; Dean, 1985].

The technique of quantitative determination of gallic acid in medical raw material of Salvia officinalis (leaves) and raw material of Salvia tesquicola (herbs and leaves). Crushed samples of one gram were placed in flasks with a grinding capacity of 100 ml, water-ethanol mixture (1:1) was added. Flasks were attached to reverse refrigerator and heated on a water bath within 60 minutes. The received extractions were filtrated through paper filters. 5 ml of the filtrates were placed in flasks with a grinding capacity of 100 ml, water-ethanol mixture (1:1) was added. The optical density of the solution was measured on the UV-spectrophotometers «UNICO 2800» at the wavelength of 276±2 nm [Gavrilin et al., 2010; Zapesochnya, 1996; Bagur, 1997.].

The content amount of gallic acid was calculated by the chemical formulation:

$$X\%=\frac{Dx\times Vxd1\times Vxd2}{Est\times mx\times Vax\times l},$$

Where D_x – optical density of the working solution, $V_{x\ d1\ (d2)}$ – volume of dilution (first and second) of the working solution, E_{st} – specific absorption of the standard sample, m_x – the mass of raw material, g, $V_{a\ x}$ – volume of aliquot of the working solution, l – the thickness of the absorbing layer of the cell.

Results and Discussion

Thin layer chromatography analysis of phenolic compounds showed the presence of spots with the same R_f (Retardation factor) for rosmarinic acid (R_f=0,88), caffeic acid(R_f=0,93) and hyperoside (R_f=0,37) Red fluorescent spots correspond to chlorophyll (fig. 2).

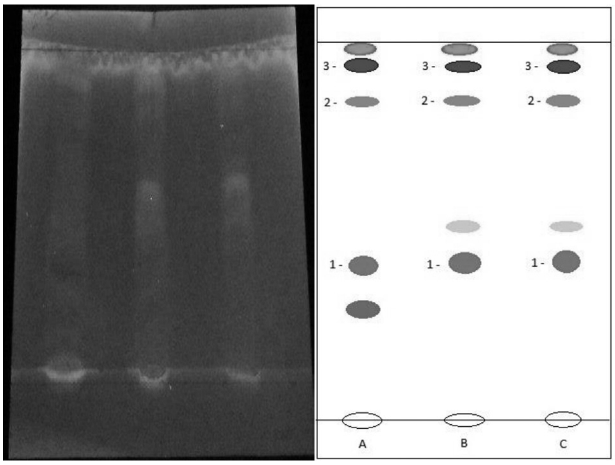

Рис. 2. ТСХ фенольных соединений из листьев шалфея лекарственного (A), травы шалфея сухостепного (B), листьев шалфея сухостепного (C): 1 – гиперозид (R_f=0,37), 2 – розмариновая кислота (R_f=0,88), 3 – кофеиновая кислота (R_f=0,93)

Fig. 2. TLC of phenolic compounds from leaves of Salvia officinalis (A), herbs of Salvia tesquicola (B), leaves Salvia tesquicola (C): 1 – hyperoside (R_f=0,37), 2 – rosmarinic acid (R_f=0,88), 3 – caffeic acid (R_f=0,93)

According to spectrophotometric results, the peaks indicate the presence of gallic acid at the wavelength of 276±2 nm (fig. 3). The optical density of the standart sample of gallic acid was taken from scientific literature [Onishchenko, 2003].

The amount of phenolic compounds when recalculating on gallic acid was calculated by using the afore-mentioned formula. This content variates from 2,034 to 3,034 %. In accordance with the obtained data, the quantity of phenolic compounds is higher in leaves and herbs of Salvia tesquicola than in leaves of Salvia officinalis (table 1).

Таблица 1
Table 1

Содержание фенольных соединений при пересчете на галловую кислоту в листьях шалфея лекарственного и траве и листьях шалфея сухостепного

The content amount of the amount of phenolic compounds when recalculating on gallic acid in leaves of Salvia officinalis and leaves and herbs of Salvia tesquicola

Research objects	The mass of raw material m_x	Optical density of the solution D_x	Content amount X %
Leaves of Salvia officinalis	1,0064	1,0332	2,034
Leaves of Salvia tesquicola	1,0020	1,5056	2,964
Herbs of Salvia tesquicola	1,0035	1,5411	3,034

Metrological characteristics of the methodology of quantitative measurement of the amount of phenolic compounds when recalculating on gallic acid in raw material of different species of Salvia presented in table 2.

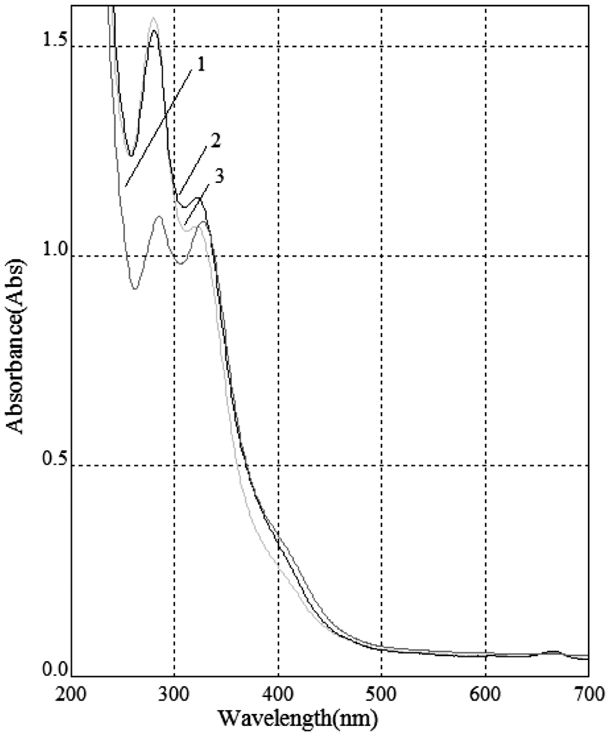

Рис. 3. Спектрофотометрический анализ галловой кислоты, полученной из листьев Шалфея лекарственного – 1, листьев шалфея сухостепного – 2, травы шалфея сухостепного – 3

Fig. 3. Spectrophotometric analysis of gallic acid in leaves of Salvia officinalis – 1, leaves of Salvia tesquicola – 2, herbs of Salvia tesquicola – 3

Таблица 2
Table 2

Метрологическая характеристика методики количественного определения содержания фенольных соединений при пересчете на галловую кислоту в сырье различных видов шалфея

Metrological characteristics of the methodology of quantitative measurement of the amount of phenolic compounds when recalculating on gallic acid in raw material of different species of Salvia

Research objects	f	$\bar{x}$	S	P, %	*t (P, f)*	ΔX	*E, %*
Leaves of Salvia officinalis	10	2,034	0,0335	95	±2,23	±0,0749	±3,7
Leaves of Salvia tesquicola	10	2,964	0,0563	95	±2,23	±0,1256	±4,2
Leaves of Salvia tesquicola	10	3,034	0,0544	95	±2,23	±0,1213	±3,9

Conclusions

1. The daignostically important components such as rosmarinic acid (R_f=0,88), caffeic acid (R_f=0,93) and hyperoside (R_f=0,37) were identified during thin layer chromatography analysis.

2. The quantitative analysis of the amount of phenolic compounds, when recalculating on gallic acid, in raw material of Salvia officinalis and Salvia tesquicola was made by using the method of spectrophotometry at a wavelength of 276 nm. The content of the amount of phenolic compounds, when recalculating on gallic acid, in samples of raw materials varies from 2,034 % to 3,034 %.

3. The content of phenolic compounds is large enough in leaves and herbs of Salvia tesquicola, which grows in the territory of Orenburg region, respectively this type of Salvia is perspective for further researches.

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