

Comparative Quantitative Analysis of Diterpene Acids in Raw Material of *Salvia officinalis*, *Salvia tesquicola* and *Salvia nutans*

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ABSTRACT

The quantitative analysis of diterpene acids in raw materials of closely related species of genus *Salvia* was carried out. The objects of present research were *Salvia officinalis* L., *Salvia tesquicola* Klokov & Pobed., *Salvia nutans* L., which grow in neighboring south (Samara and Orenburg) regions. During analysis different raw materials, such as herbs and leaves, were studied. It was determined that total amount of diterpene acids varies from 1,04% to 1,32% in leaves of different species of *Salvia* and from 1,13% to 1,67% in herbs of different species of *Salvia*. The raw material of *Salvia nutans* contains minimal amount of diterpene acids, but in the raw material of *Salvia tesquicola* the quantitative analysis shows different results, which depend on the region. The medical raw material of *Salvia officinalis*, which is cultivated in Samara region, includes sufficient amount of diterpene acids.

Keywords: *Salvia officinalis* L., *Salvia tesquicola* Klokov & Pobed., *Salvia nutans* L., spectrophotometry, diterpene acids, carnosic acid.

INTRODUCTION

Salvia officinalis has been used in medicine since the dawn of centuries till our days^{1,2}. However other closely related species of genus *Salvia* such as *Salvia nutans* and *Salvia tesquicola* were unreasonably avoided. Biologically active substances of *Salvia officinalis*, which determine its therapeutic effects, are essential oil, flavonoids, tannins and some others³. Today diterpens, which are also a part of chemical composition, become a field of especial interest. Diterpene acids are biologically active substances, which belong to a group of diterpenes, and represent condensed structure, which includes three rings; one of them has aromatic nature. The most studied representatives are carnosic acid and its metabolites such as carnosol, which is more stable than the original substance. Carnosic acid possesses a variety of therapeutic effects such as antimicrobial, antioxidant, anti-inflammatory and wound healing⁴. It widely used in food industry and medicine. Due to this facts the aspect of finding herbal medical raw material, which includes diterpene acids in sufficient amount, is actual and acute issue. It is known, that plants from genus *Salvia* hold carnosic acid in a chemical composition⁵.

The aim of present work is a comparative quantitative analysis of diterpene acids in different species of *Salvia* (*Salvia officinalis* L., *Salvia tesquicola* Klokov & Pobed., *Salvia nutans* L.), which grow in southern part of Russian Federation (Samara and Orenburg regions).

RESULTS AND DISCUSSION

Materials

Raw material of *Salvia officinalis* was prepared in 2016 at the botanical garden, which located in Samara. Raw material of *Salvia tesquicola* was prepared in 2016 in the Samara and Orenburg regions and raw material of *Salvia nutans* was prepared in 2016 in Samara region. Electronic spectra were measured on the UV-spectrophotometers "UNICO 2800", Specord 40 (Analytik Jena).

The UV spectra of the extract from raw material of different species of genus *Salvia* were studied during the research of the amount of diterpene acids.

The procedure, which was developed earlier, was used for the determination of diterpene acids in samples of different species of genus *Salvia*⁴.

The comparative study of the electronic spectra showed that the electronic spectra of different species of genus *Salvia*, such as *Salvia officinalis* L., *Salvia tesquicola* Klokov & Pobed., *Salvia nutans* L. have the maximum absorption at 282±3 nm, which is typical for the solution of dominant carnosic acid (Fig. 1, 2, 3, 4, 5). Consequently, as analytical wavelength may be used a value of 282±3 nm⁴. Carnosic acid is the dominant diterpene acid and can be used as the standard sample.. In the case of the absence of this standard in the calculation formula can be used the theoretical value of the specific absorption index (= 40,92)⁴.

The technique of quantitative analysis of diterpene acids in raw material of genus Salvia

Analytic sample of two grams of raw material is placed in a flask with a grinding capacity of 100 ml and 30 ml of

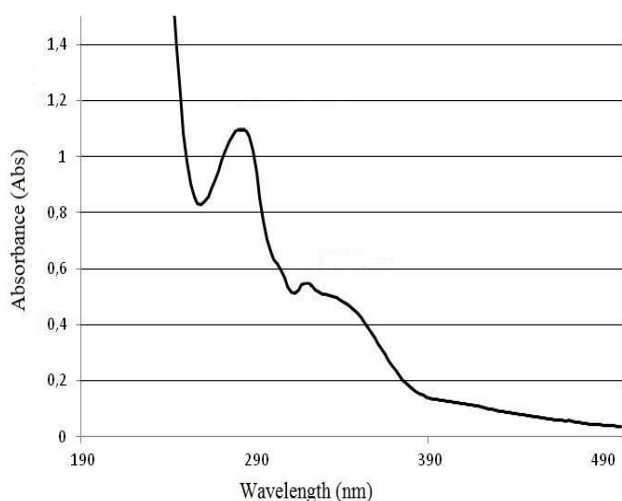


Figure 1: Electronic spectrum of the extract from leaves of *Salvia officinalis*.

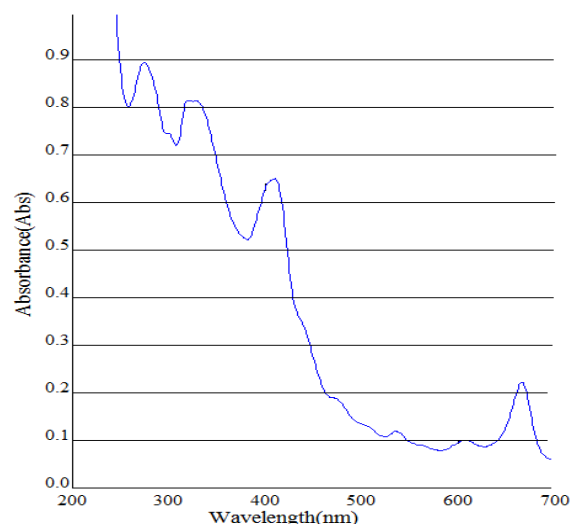


Figure 2: Absorption spectrum of the extract from leaves of *Salvia tesquicola*.

Table 1: Metrological characteristics of the methodology of quantitative measurement of the of total diterpene acids in raw material of different species of *Salvia*.

Research objects	f	\bar{x}	S	P,%	$t(P,f)$	ΔX	E, %
Folia of <i>Salvia officinalis</i> (Samara region)	10	1,32	0,0204	95	$\pm 2,23$	$\pm 0,0457$	$\pm 3,46$
Folia of <i>Salvia tesquicola</i> (Orenburg region)	10	1,04	0,0202	95	$\pm 2,23$	$\pm 0,0451$	$\pm 4,33$
Herbs of <i>Salvia tesquicola</i> (Orenburg region)	10	1,67	0,0264	95	$\pm 2,23$	$\pm 0,0590$	$\pm 3,53$
Herbs of <i>Salvia tesquicola</i> (Samara region)	10	1,37	0,0200	95	$\pm 2,23$	$\pm 0,0446$	$\pm 3,26$
Herbs of <i>Salvia nutans</i> (Samara region)	10	1,13	0,0209	95	$\pm 2,23$	$\pm 0,0467$	$\pm 4,14$

Table 2: The content of diterpene acids in various species of genus *Salvia*.

№	Characteristics of the sample materials	Contents of diterpene acids calculated on carnosic acid and absolutely dry raw material (in%)
1.	Folia of <i>Salvia officinalis</i> (Samara region)	1,32 \pm 0,02
2.	Folia of <i>Salvia tesquicola</i> (Orenburg region)	1,04 \pm 0,01
3.	Herbs of <i>Salvia tesquicola</i> (Orenburg region)	1,67 \pm 0,04
4.	Herbs of <i>Salvia tesquicola</i> (Samara region)	1,37 \pm 0,03
5.	Herbs of <i>Salvia nutans</i> (Samara region)	1,13 \pm 0,02

acetone is added. Flask is attached to reverse refrigerator and heated on a water bath during 30 minutes. The received extraction is filtrated through paper filter. The received filtrate and 30 ml of acetone are added to the analytic sample. The filtered acetone extraction evaporated to dryness and the residue is processed by 10 ml 5% solution of ammonia. The aqua-ammonium solution is filtered through paper filter. This operation is repeated one more time. 20 ml of diluted hydrochloric acid is added to the filtrate with a stirring. The precipitate is quantitatively collected on the Schott filter with pores 16 micrometers in diameter. After this operation Schott filter is rinsed with 10 ml of water. The precipitate on the filter is quantitatively transferred in a flask with a grinding capacity of 100 ml. The volume of the solution is brought to the mark 95% solution of ethyl alcohol. The optical density of the solution is measured on the wavelength at 282 nm⁴.

The content of total diterpene acids is calculated by the chemical formulation:

$$X = \frac{Dx * Vx}{E\% * mx * \ell}$$

Where D_x - optical density of the solution,

V_x - the volume of the solution,

$E\%$ - specific absorptivity (specific absorbance),

m_x - the mass of raw material, g,

ℓ - the thickness of the absorbing layer of the cell

Metrological characteristics of the methodology of quantitative measurement of the of total diterpene acids in raw material of different species of *Salvia* presented in table 1.

Using the method of quantitative determination of diterpene acids in different species of *Salvia* we analyzed a number of sample practical (Table 2) and determined that the content of the of total diterpene acids in folia varies from 1,04% to 1,32%, in herbs varies from 1,13% to 1,67%.

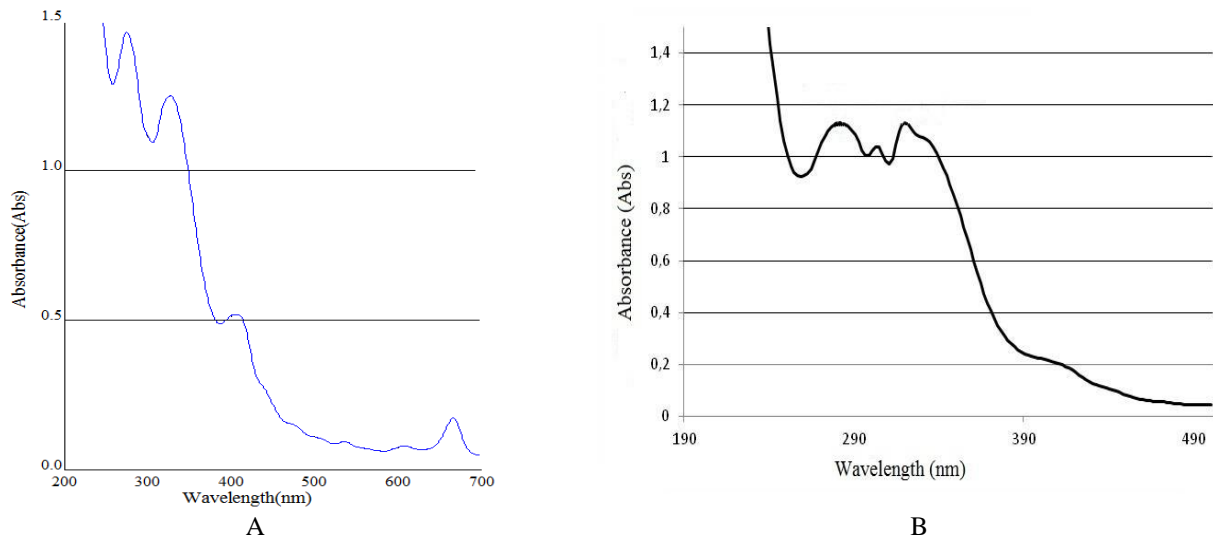


Figure 3: Absorption from Orenburg region; B - Raw material from Samara region) spectrum of the extract from herbs of *Salvia tesquicola* (A - Raw material.

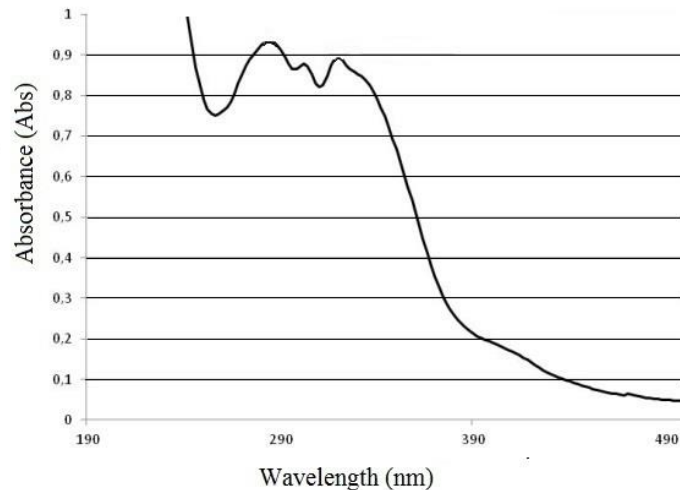


Figure 4: Absorption spectrum of the extract from herbs of *Salvia nutans*.

CONCLUSIONS

The research method of quantitative amount of diterpene acids of different species of genus *Salvia*, which was taken out from the literature information, was carried out by using the standard sample of carnosic acid. The method of quantitative determination of the content of diterpene acids in different species of genus *Salvia* was carried out by using UV-spectrophotometer at the analytical wavelength 282 nm. The research results allow to say that the content of diterpene acids in herbs of *Salvia tesquicola*, which is growing on the territory of an Orenburg region, is maximum and amount 1,67%.

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