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## ***Development method of Ziziphora Clinopodioides Lam. Standardization by flavonoids***

**Abstract:** At first the specific absorption coefficient was given to flavonoid 7-methyl sudahitin by the spectrophotometric method  $E_{1\%}^{1\text{cm}} = 920$ . A modified method for the quantitative determination of the sum of flavonoids in raw Ziziphora Clinopodioides Lam. in terms of flavonoid 7-methyl sudahitin was made by the spectrophotometry method.

It has been shown that the plant Ziziphora Clinopodioides Lam. collects the flavonoids - significant total amount from 2,57 up to 4.18%.

**Keywords:** Ziziphora Clinopodioides, flavonoid, spectrophotometric method.

### **Introduction**

The herbal medicines have been widely used for the last decade in the pharmaceutical market. Along with the research for the new medicinal plants, it becomes relevant a deep physico-chemical study of the raw materials plant, which have already been applied in the traditional medicine.

Each herb before becoming officinal, must undergo the scientific trial stages, the final one is biological active compounds standardization.

One of the most promising plants as a valuable raw material for the obtaining of essential oils, as well as a raw material source of the flavonoids is a wild species of Ziziphora Clinopodioides Lam., which is widespread in the floras of Armenia and Artsakh [4,5,6,7].



In recent years, the interest of the phenolic plant compounds is not random and associated with a wide range of physiological activities and their low toxicity [1].

The research for the new flavonoid-containing plant-derived sources, have showed that the main biological active substances of the herb *Ziziphora Clinopodioides* Lam. are phenolic and flavonoid compounds [2,3,4,5].

Previously, with the help of the complex physical and chemical research methods (UV and IR spectroscopy, NMR  $C^1$  and  $C^{13}$  spectroscopy) flavonoid composition of the extracts of the herbs *Ziziphora Clinopodioides* Lam., containing flavonoids 7-methyl sudahitin, timonin and flavonoid glycosides, rutosides chrysin, linarin and diosmin have been studied [3,4,5,6].

## MATERIAL AND METHODS

The aim of this work is to develop the method of quantifying the amount of the flavonoids in the extracts obtained from the overground parts of the grass *Ziziphora Clinopodioides* Lam. with the help of spectrophotometric method.

In order to determine the sum of the flavonoids in the extracts of herbs *Ziziphora Clinopodioides* Lam., there were collections from the aerial part of natural populations of wild species *Ziziphora Clinopodioides* Lam. plants in the flowering stage (i.e. in the phase of greatest accumulation of flavonoids) in June – July, 2013 from the mountain villages Voghjaberd, Hankavan, Arzakan (the mountain range Teksari), adjacent to the eastern and south-western slopes of the ridge Geghama and mountain district villages of Artsakh (Nakhijevanik Surenavan, Berdadzor), as well as the grass *Ziziphora Clinopodioides* Lam. grown up in the conditions of soils and of hydroponics [6,7,8].

After the collection of raw materials, the samples of *Ziziphora Clinopodioides* Lam. was re-identified by the registry to determine the species (*Z. clinopodioides* Lam 1791, Tabl. Encycl. Meth. Bot, fig. 1: 63).

To quantify the sum of flavonoids in the grass *Ziziphora Clinopodioides* Lam., the modified method was used [10,11].

An analytical sample of the raw material is crushed to a particle size passing through a sieve with 1.2 mm diameter holes. Approximately 1.0 g (accurately weighed) crushed material was placed in a flask with microsection capacity of 250 ml, adding 250 ml of 50% ethyl alcohol, the flask was connected to a reflux

condenser and heated in a boiling water bath about 30 min. The flask was cooled to the room temperature and filtered through the filter paper into a volumetric flask with 250 ml and 50% alcohol filtrate volume adjusted to 250 ml tags. Then 2 ml of the solution was placed in a volumetric flask of 50 ml, and the solution volume was adjusted to 50% alcohol to the mark.

Using the standard (the standard sample) - 7-methyl flavonoid sudahitin (SIGMA-ALDRICH), by spectrophotometry absorption, the specific absorption coefficient was determined ( $E_{1cm}^{1\%}$ ) at the analytical wavelength (207 nm) 920.

The optical densities of the solutions were determined by the UV spectrophotometer company SPECORD UV VIS, Germany; at a wavelength of 207 nm, the cuvette with thickness of 10 mm, using as a control solution of 50% alcohol.

Spectrophotometric method of the sum of flavonoids determination without prior separation of the components is based on the property of absorbance values additives (optical density) of the components of the mixture at the same wavelength  $\lambda = 207$  nm [9].

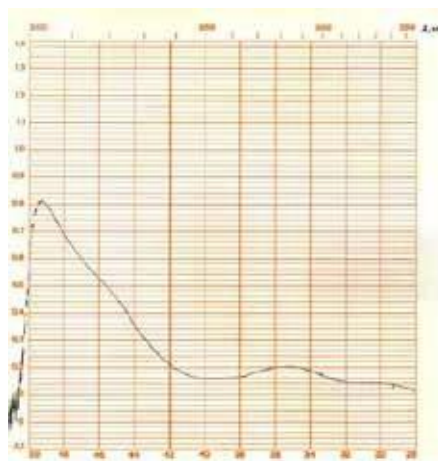
In the formula for calculating the value of the specific absorption coefficient ( $E_{1cm}^{1\%}$ ) for the flavonoid 7-methyl sudahitin was incorporated  $E_{1cm}^{1\%} = 920$  [3,7,8,9] (Figure 1).

The percentage of the flavonoids amount in absolutely dry raw material in terms of the 7-methyl sudahitin is calculated by the formula:

$$x = \frac{D_x \cdot 250 \cdot 50}{920 \cdot 2m}$$

where m – the mass of the sample materials,  $D_x$  - an optical density of the test solution at  $\lambda = 207$  nm; 250 - a solution volume, (ml); 50 - the aliquots volume is taken from solution (ml); 920 - the specific absorption coefficient flavonoid 7-methyl sudahitin at  $\lambda = 207$  nm [10,11].

Statistical data processing was carried out using the program MS «Excel 2007».



**Fig. 1. The absorption spectrum of the 7-methyl sudahitin solution**

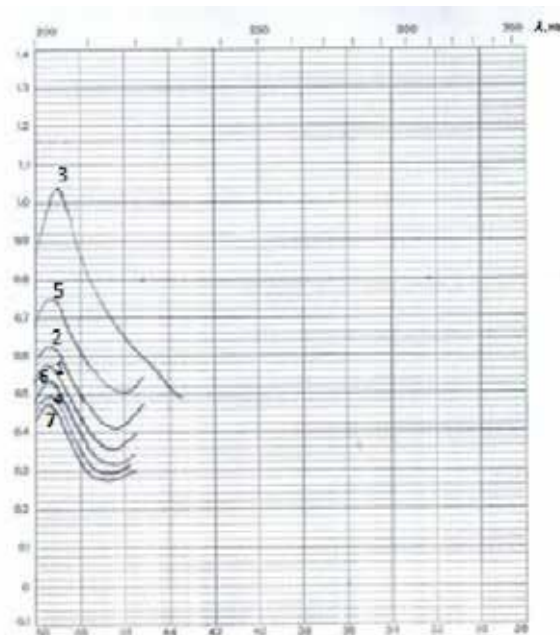
### Results and discussion

The development method was tested on the samples of the raw materials from the different climatic zones, as well as different growth conditions (in the conditions of the soil and hydroponics). These results lead to the conclusion that the content of the sum of flavonoids in the grass *Ziziphora Clinopodioides* Lam. ranges from 2.57 to 4.18% (Table 1, Fig. 2).

**Table 1**

**The sum of the flavonoids based on 7-methyl sudahitin depending on the raw material from different climatic zones and different growth conditions**

	The name of the sample ***	Absorbance of the test solution, $D_x$	Content of flavonoids amount, % x
1	Hydroponics	0,63	3,30±0,02
2	Soil culture	0,60	3,35±0,025
3	Hankavan (Armenia)	0,76	4,18 ± 0,015
4	Vohchabert (Armenia)	0,51	2,91±0,025
5	Surenavan (Artsakh)	0,62	2,84±0,015
6	Nakhijevanik (Artsakh)	0,62	3,39±0,025
7	Berdadzor (Artsakh)	0,52	2,57±0.02



**Fig. 2. Absorption spectra of Ziziphora Clinopodioides Lam. plant extract solutions from different regions; Gidroponika-1, Soil Culture-2, Hankavan-3, Vohchabert-4, Surenavan-5, Nakhijevanik-6, Berdadzor-7**

The results of statistical processing of the data showed that the error of a single determination with the confidence probability of 95% is within 1.21%.

Thus, the modified development method for calculating the amount of flavonoids, can be used to assess the quality of grass Ziziphora Clinopodioides Lam. by the content of the basic group of biological active compounds, flavonoids.

Our results indicate that all the samples of extracts both the qualitative composition and the quantitative content of the total flavonoids are close. Differences in the quantitative content of the certain components are not only the climatic factors, as well as the growth conditions.

#### **Conclusions:**

✓ at first the specific absorption coefficient was given to flavonoid 7-methyl sudahitin by the spectrophotometric method  $E_{1\%}^{1\text{cm}} = 920$

✓ a modified method for the quantitative determination of the sum of flavonoids in raw Ziziphora Clinopodioides Lam. in terms of flavonoid 7-methyl sudahitin was made by the spectrophotometry method.

The relative error in the determination of the sum of flavonoids by the developed method at the confidence probability 95% does not exceed 1.21%.

✓ it has been shown that the plant *Ziziphora Clinopodioides* Lam. collects the flavonoids - significant total amount from 2,57 up to 4.18%.

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