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ОПРЕДЕЛЕНИЕ АНТИОКСИДАНТНОЙ АКТИВНОСТИ СЫРЬЯ ZIZIPHORA CLINOPODIOIDES LAM. ДИКОРАСТУЩЕГО ВО ФЛОРАХ АРМЕНИИ И АРЦАХА И ВЫРАЩЕННОГО В УСЛОВИЯХ ГИДРОПОНИКИ

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Аннотация. Целью настоящего исследования является исследование антиоксидантной активности экстрактов, полученных из сырья Ziziphora clinopodioides Lam. дикорастущего во флорах Армении и Арцаха и выращенного в условиях гидропоники.
Определение антиоксидантной активности проводили спектрофотометрическим методом, при котором природный антиоксидант взаимодействует со стабильным хромоген радикалом ДФПГ(2,2-дифенил-1-пикрилгидразил). Результаты исследования показали, что экстракты разных образцов Z.clinopodioides обладают различной антиоксидантной активностью.

Ключевые слова: *Ziziphora clinopodioides, антиоксидантная активность, сухой спиртовый экстракт, ДФПГ, спектроскопический метод.*

DETERMINATION OF ANTIOXIDANT ACTIVITY OF RAW MATERIAL OF ZIZIPHORA CLINOPODIOIDES LAM. CULTIVATED AND WILDLY GROWING IN THE FLORAS OF ARMENIA AND ARTSAKH

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Abstract. The aim of the current study was to determine antioxidant activities of the extracts obtained from the raw material of wild growing Ziziphora clinopodioides Lam. in the floras of Armenia and Artsakh, and cultivated in the hydroponic conditions.
The antioxidant activity determination was carried out by means of the spectrophotometric method, in which the natural antioxidant interacts with the stable chromogen radical of DPPH (2,2-diphenyl-1-picrylhydrazyl). The results of the study showed that the extracts of the different samples of Z.clinopodioides were revealed in diverse antioxidant activity.
The study results show the future prospects of the use of Z.clinopodioides not only as the source of flavonoids as well as antioxidant agents.

Keywords: *Ziziphora clinopodioides, antioxidant activity, dry alcohol extract, DPPH, spectrophotometric method.*

ՀԱՅԱՍՏԱՆԻ ԵՎ ԱՐՑԱԽԻ ՖԼՈՐԱՅՈՒՄ ՎԱՅՐԻ ԱՃՈՂ ԵՎ ՀԻԴՐՈՊՈՆԻԿ ՊԱՅՄԱՆՆԵՐՈՒՄ ԱՃԵՑՎՈՂ ZIZIPHORA CLINOPODIOIDES LAM. ԲՈՒՍԱՀՈՒՄՔԻ ՀԱԿԱՕՔՍԻԴԱՆՏԱՅԻՆ ԱԿՏԻՎՈՒԹՅԱՆ ՈՐՈՇՈՒՄԸ

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Ամփոփագիր**:** Հետազոտության նպատակն է Հայաստանում և Արցախում վայրի և հիդրոպոնիկ պայմաններում աճեցվող Ziziphora clinopodioides հումքից ստացված լուծամզվածքների հակաօքսիդանտային ակտիվության հետազոտումը:
Հակաօքսիդանտային ակտիվությունը որոշվել է սպեկտրոֆոտոմետրիկ մեթոդով, որի դեպքում բնական հակաօքսիդանտը փոխազդում է ԴՖՊՀ (2,2-դիֆենիլ, պիկրիլ հիդրազիլ) կայուն քրոմոգեն ռադիկալի հետ:Հետազոտության արդյունքները ցույց տվեցին, որ Ziziphora clinopodioides տարբեր նմուշների լուծամզվածքները ցուցաբերում են տարբեր հակաօքսիդանտային ակտիվություն:

Հիմնաբառեր՝ Ziziphora clinopodioides, հակաօքսիդանտային ակտիվություն, չոր սպիրտային լուծամզվածք, ԴՖՊՀ, սպեկտրաչափական մեթոդ:

Introduction

Along with the research for the new medicinal plants a deep physicochemical study of the raw materials which have already been applied in the traditional medicine becomes relevant.

Nowadays, the interest of the plants phenolic compounds is not random and associated with a wide range of their physiological activities and low toxicity (Krivchenkov *et al*. 2012; Voronkova and Mochalova 2015).

In the prestigious international scientific journals, we often find studies of flora of different countries, mainly related to the discovery of phenolic substances in plants and the study of biological activity caused by them. Special attention is paid to some endemic plants of different floras, the study of which is currently promising. From this point of view, the study of Lamiaceae family species, such as *Ziziphora* found in different floras became relevant.

Plants of the family Lamiaceae have the special interest as the source of flavonoids and essential oils, including the genus *Ziziphora clinopodioides* Lam. that contains bioflavonoids and terpenic compounds as a main group of biologically active substances (Ulikhanyan *et al*. 2019).

From *Ziziphora tenuoir* (Lamiaceae) gathered from the west of Iran were investigated flavonoids, anthocyanins, total phenolic compounds for polar and non-polar sub-fraction in different stages of growth (pre-flowering and flowering) (Gholiv *et al.* 2014).

Due to gathered information about four Kazakh *Ziziphora* species, their traditional utilization confirmed the following compounds: monoterpenic, essential oil, phenolic substances belong to the flavonoids and phenolic acids, and triterpenes, which are identified in extracts obtained from these plants (Šmejkal *et al.* 2016). In some reports, significant antioxidant activities of *Ziziphora* species have been documented for both essential oil and extracts using a wide array of antioxidant assays. This comprehensive survey reveals that the majority of essential oil from Ziziphora plants contains considerable amounts of pulegone (Majid 2017).

The research done by Tian *et al.* (2011) investigated the total polyphenolic and flavonoid content as well as the antioxidant activity of *Ziziphora clinopodioides Lam*. extracts of different polarity and revealed ethyl acetate extracts containing a large number of polyphenolic compounds (19.27%) and flavonoids (65.61%) which have good antioxidant capacity.

In Mojtaba *et al.* (2016) study showed that the extract of the *Z.tenuior* and its ethanol and petroleum ether fractions could have anti-inflammatory properties.

The species *Ziziphora* is one of the most common plants in the floras of Armenia and Artsakh. The study of the raw material resources indicated that the populations of the wild-growing *Ziziphora clinopodioides* met in Armenia and Artsakh has a form of the small scattered semi shrubs in small populations which alternate from the rocky slopes of mountain belts to subalpine elevations (Chichoyan *et al*. 2015).

Ziziphora clinopodioides Lam. is one of the most promising plant as a valuable raw material for obtaining the essential oils and as a source of the flavonoids (Galstyan and Hovanisyan 1990; Chichoyan *et al*. 2015; *Guo et al*. 2015), which is widespread in the floras of Armenia and Artsakh (Ulikhanyan *et al*. 2019).

Earlier, with the help of the complex physicochemical research methods (UV and IR spectroscopy, NMR ^{1}H and ^{13}C spectroscopy) the flavonoid content of the *Ziziphora* extracts were investigated and, in the result, flavonoid glycosides; chrysin-7-rutinoside, linarin, flavonoid aglicones; diosmin, 7-methyl sudahitin, timonin were revealed (Oganesyan *et al*.1990; 1991).

The aim of this study is determination of the antioxidant activities of the extracts obtained from the raw material of *Ziziphora clinopodioides* of Armenian and Artsakh floras, and cultivated in the hydroponic conditions, and to provide a scientific basis for the further application in medical practice.

Material and methods

Herbal material

From the vicinities of Armenian villages of Arzakan, Hankavan, Voghjaberd, and from the vicinities of the villages of Surenavan, Nakhijevanik, Berdadzor of Artsakh from the natural populations, several samples (shrubs) of *Ziziphora clinopodioides Lam.* and the herbs cultivated in soil and hydroponics conditions were collected (from June to July, 2017) for scientific research and then identified by the registry for species identification (*Z.clinopodioides Lam.,* 1791, Tabl. Encycl. Meth. Bot., Illustr.1:63) according to Takhtajyan (1987) and Grossgeym (1949), (GACP, WHO 2003).

The voucher specimen (ERE N194583) of the plant was deposited in the Institute of Botany after A.L. Takhtajyan of NAS RA.

Method of cultivation

For the cultivation, nearly 50 plant of *Ziziphora* bushes collected in the area of the village of Voghjaberd and Hankavan, Kotayk region, in mid-April (15.04.17) were planted on ($5m^2$) hydroponic and soil areas. Black slag was used as nutrient filler in a 3–15 mm diameter, which was previously disinfected with 0.05% solution of $KMnO_4$ (Davtyan 1980; Chichoyan *et al.* 2015). In the process of vegetation, the plants were nutriated according to Davtyan's (1980) nutrient solution (pH 5.5-6.5), 1–2 times a day. The first collection of the raw materials was in early July, at the beginning of the flowering phase (GACP, WHO 2003).

Preparation of the extract

Extracts preparation were carried out by the extraction of the air-dried plant raw material of the *Ziziphora* in 50% ethanol during 30 minutes with further evaporation. The dry extracts were filtered and evaporated to dryness under the reduced pressure in a rotary evaporator (Quality control methods for herbal materials. WHO, 2011).

Then the total amount of flavonoids in extracts of the *Ziziphora* with respect of 7-methyl sudahitin was defined (Georgievskii *et al*. 1990; Chakchir and Alekseev, 2002; Ulikhanyan *et al*.2017).

DPPH (free radical-scavenging) assay:

In the research the methanol, a stable radical of DPPH ((2,2-diphenyl-1-picrylhydrazyl, $C_{18}H_{12}N_5O_6$, M = 394.33) (Sigma Aldrich GmbH)) and the dry alcohol extracts of the Z.*clinopodioides* were used.

Determination of the antioxidant activity was carried out using the spectrophotometric method, in which the natural antioxidant interacts with the stable chromogen radical DPPH [1]. The antioxidant activity (in the percentage) was determined after the interaction of the methanol solution of DPPH with the test solution and calculated using a calibration graph of the DPPH (the dependence of the optical density from the DPPH concentration). The absorbance of the samples was determined at the wavelength 515 nm.

Definition the optical density of the tested solutions was done by the device Helios Comp Thermoelectron (England). The measurements were carried out in five replicates. The optical density of the DPPH was recorded after 1; 5 and 20 minutes.

The scattering effect in percentage was defined according to this formula:

Antioxidant Activity (%) = $((C_c-C_{ext}) / Cc) * 100\%$,

C_c-control absorbance concentration; C_{ext}-corresponds to the absorbance in the presence of extracts (Mnatsakanyan *et al.* 2009; Ananikyan *et al.* 2007).

Statistical analysis

Statistical analysis was done by SPSS® for Windows (Version 19.0, Chicago, IL, USA). The results were presented as means ± standard error of mean (S.E.M) of at least five measurements; $P < 0.05$ was regarded as statistically significant. The data were assessed by one-way analysis of variance (ANOVA) followed by the Tukey's test.

Results and Discussion

Free Radical Scavenging Activity (% DPPH inhibition):

As the results of the study show, all samples of the raw material are manifested a positive antioxidant activity, which was determined by the rate of inhibition of the DPPH.

A correlation between the concentration of inhibition of the DPPH and the optical density of the specimens was determined. A correlation was found between the concentration of DPPH binding and the optical density of absorption of solutions. Figure 1 show the correlation graphs of the DPPH of the plants grown up in the hydroponic conditions (Z3), wild plant Z.*clinopodioides* in the floras of Armenia (Z1)and Artsakh (Berdadzor) (Z2), and figure 2 show the relationship between the optical density (D) and concentration (C) of the inhibition DPPH; Z1 (plants from Berdadzor), Z2 (plants from Voghjaberd) and Z3 (plants from the hydroponics) the results of which are given in the *Tables I, II, III, Figure 1.*

Table I

Correlation of the DPPH for the (plants from the hydroponics) Z3 and (plants from Berdadzor) Z1

Optical density for the Z1 and Z3	**Concentration (c)**
X	У
Y=33,962264*X-1,226415	
0,625	20
0,095	2

Table II

Correlation of the DPPH for the (plants from the hydroponics) Z3 and (plants from Voghjaberd) Z2

Optical density for the Z2	**Concentration (c)**
X	У
Y=35,087719*X-1,368421	
0,609	20
0,096	2

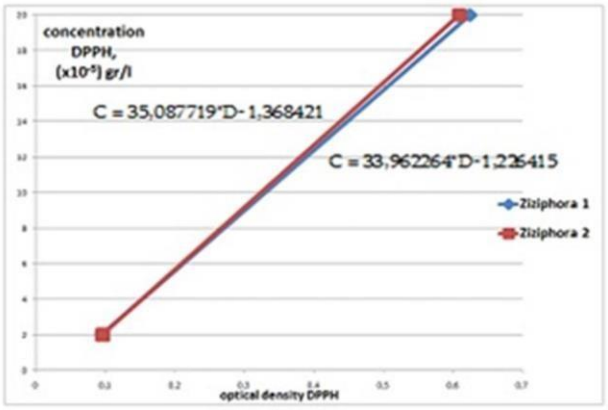

Figure1. *Correlation graph of the DPPH for Z1 ((plants from Berdadzor) and Z3 (plants from the hydroponics) (the line Ziziphora1), Z2 (plants from Voghjaberd) (the line Ziziphora 2).*

From the results of the studies presented in the *Tables VI, VII* and *Figure 2*, it was found that the extracts of the raw material from the hydroponics and wild plants show a pronounced antioxidant activity, differing from each other in the rate of manifestation of the reaction (rate of inhibition of the DPPH).

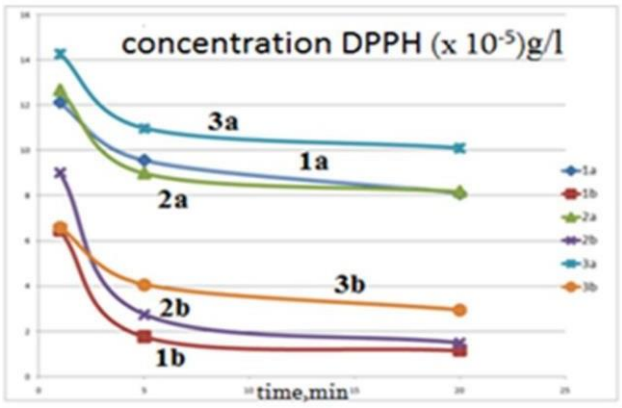

Figure 2. *The relationship between the optical density (D) and concentration (C) of the inhibition DPPH; Z1 ((plants from Berdadzor), Z2 (plants from Voghjaberd) and Z3 (plants from the hydroponics);a-6mg,b-12mg.*

Table III

The relationship between the optical density (D) and concentration (C) [(x 10^{-5}) g/l] of the inhibition DPPH (n=5, $\overline{x}$ – mean, E_s – standard error of the mean)

Analytical samples of the raw materials / The areas of the plants collected	Extract (mg)	The time of the inhibition DPPH (min) $\overline{x} \pm E_s$		
		1	5	20

Hydroponics (black slag)	*D-6	0,446±0,001	0,352±0,001	0,327±0,001
	D-12	0,277±0,001	0,155±0,002	0,123±0,002
Hydroponics (black slag)	**C-6	14,287±0,048	10,975±0,045	10,119±0,042
	C-12	8,358±0,05	4,084±0,04	2,947±0,049
Voghjaberd	*D-6	0,409±0,001	0,301±0,002	0,277±0,002
	D-12	0,302±0,001	0,117±0,001	0,08±0,002
Voghjaberd	**C-6	12,975±0,038	9,207±0,057	8,358 ±0,037
	C-12	9,228±0,04	2,751±0,042	1,453±0,043
Berdadzor	*D-6	0,393±0,002	0,318±0,001	0,275±0,001
	D-12	0,227±0,002	0,088±0,001	0,07±0,002
Berdadzor	**C-6	12,41±0,057	9,8±0,043	8,295±0,05
	C-12	6,61±0,06	1,733±0,038	1,091±0,053

***** *D-6, D-12 – the values of the optical densities of the extracts with concentrations of 6 mg and 12 mg, respectively*

****** *C-6, C-12 – the values of the concentrations of the DPPH of 6 mg and 12 mg, respectively*

Table IV

The antioxidant activity of the dry 50% ethanol extracts of *Ziziphora clinopodioides* Lam, (n=5, $\overline{x}$ – mean, E_s – standard error of the mean).

Analytical samples of the raw materials / The areas of the plants collected	Extract (mg)	The time ofthe inhibition DPPH, min and antioxidant activity(%) $\overline{x} \pm E_s$		
		1	5	20
Hydroponics (black slag)	6	28,56±0,238	45,12±0,225	49,4±0,212
	12	67,51±0,277	79,58±0,212	85,26±0,248
Voghjaberd	6	36,67±0,181	54,85±0,194	59,06±0,181
	12	54,95±0,276	86,3±0,21	92,47±0,21
Berdadzor	6	39,46±0,277	52,06±0,205	59,37±0,244
	12	67,52±0,278	91,12±0,183	94,28±0,265

The antioxidant activity of the extracts of *Z. clinopodioides* was carried out for the first time (Table IV), and as the results of the study showed, all the samples of the raw material exhibit a positive antioxidant activity, which was determined by the rate of the inhibition of the DPPH and the following conclusions were done; the extracts of the raw material *Z. clinopodioides* collected in the vicinities of the villages Voghjaberd and Berdadzor show a positive antioxidant activity, and the highest antioxidant activity exhibits extract of the raw material collected in the vicinity of the village Berdadzor, which, at the concentration of 12 mg, reached 94,28 ± 0,265% to the 20th minute.

At a concentration of 12 mg for the extract of the raw material cultured in the hydroponic conditions, at the 20th minute the rate of inhibition of the DPPH reached 85,26 ± 0,248%, for the extract from the raw material of a wild plant collected in the vicinity of the village Voghjaberd reached 92,47 ± 0,21%.

Conclusions

The results of the study showed that the extracts from the plants grown up in the hydroponic conditions and wild population show almost identical a pronounced antioxidant activity; it could be noted that the future prospects of the use of this raw material will be not only for wild-growing raw material of Z.*clinopodiodes*, but also for the raw material grown up in the hydroponic conditions, since stocks of the raw materials Z.*clinopodiodes*, in nature are limited.

The results of the research show that studying the flora of the Armenia and Artsakh, as well as the plants grown up in the hydroponic conditions, will create the backgrounds for identifying the promising plant sources of the flavonoids, and creating the effective herbal preparations with antioxidant activities on their basis.

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