



PHYTOCHEMICAL STUDY OF *TRIBULUS TERRESTRIS* L.

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Tribulus terrestris L., an annual herb belonging to the *Zygophyllaceae* family and growing in moderate and tropical climates, has a rich chemical composition of biologically active substances and chemical elements.

The aim of the work is a phytochemical study of *Tribulus terrestris* L. growing in different geographical zones.

Materials and methods. The objects of study were herb specimens of *Tribulus terrestris* L. collected in different habitats. The samples of the raw materials were shade-dried. The determination of saponins in the raw materials, was carried out by high performance liquid chromatography with a mass spectrometric detection (HPLC-MS / MS). The study of the qualitative and quantitative composition of the elements was carried out on an X-ray fluorescence spectrometer.

Results. The saponins had been studied by HPLC-MS/MS, according to which in all the studied samples, dioscin and protodioscin were found. Their retention times coincided with the retention times of dioscin and protodioscin standards. It has been established that among the macroelements of *Tribulus terrestris* L., potassium and calcium are mostly accumulated. They account for about 90% of the total content of the elements in the plant. It has been revealed that the distribution of macro- and microelements in the plant, varies significantly depending on their place and growing conditions.

Conclusion. The maximum dioscin content was observed in the samples harvested in Moldova, and the minimum – in the samples from the nursery garden of the All-Russian Scientific Research Institute of medicinal and aromatic plants. The largest amount of protodioscin was found out in the samples from the Crimea, and the minimum – in the samples from Moldova. The carried out study of the elements content of *Tribulus terrestris* L. showed that the habitats (geographical zones) in which the studied samples of raw materials had been were collected, affect the accumulation of the elements by the plant. Based on the data obtained, biological absorption series have been compiled for the samples from each habitat.

Keywords: *Tribulus terrestris* L., high performance liquid chromatography with a mass spectrometric detection, HPLC-MS/MS, elements content, phytochemistry

ФИТОХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ ТРАВЫ ЯКОРЦЕВ СТЕЛЮЩИХСЯ

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Якорцы стелющиеся, или якорцы наземные – *Tribulus terrestris* L., семейство парнолистниковые – *Zygophyllaceae*, однолетнее травянистое растение, произрастающее в умеренном и тропическом климате, имеет богатый химический состав биологически активных веществ и химических элементов.

Цель работы – фитохимическое исследование якорцев стелющихся, произрастающих в разных географических зонах.

Материалы и методы. Объектами исследования являлись образцы травы якорцев стелющихся, собранные в различных условиях

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обитания. Образцы сырья были высушены воздушно-теневым способом. Определение сапонинов в сырье проводили методом высокоэффективной жидкостной хроматографии с масс-спектрометрическим детектированием (ВЭЖХ-МС/МС). Изучение качественного и количественного состава элементов проводили на рентгенофлуоресцентном спектрометре.

Результаты. Проведено исследование сапонинов методом ВЭЖХ-МС/МС, по результатам которого, во всех исследуемых образцах обнаружены диосцин и протодиосцин, времена удерживания которых совпадают с временами удерживания стандартов диосцина и протодиосцина. Установлено, что в образцах якорцев стелющихся травы среди макроэлементов в наибольшем количестве накапливаются калий и кальций, на их долю приходится около 90% от общего содержания элементов в растении. Выявлено, что распределение макро и микроэлементов в растении существенно различается в зависимости от места и условий произрастания.

Заключение. Максимальное содержание диосцина наблюдали в образцах, заготовленных в Молдове, а минимальное – в образцах из питомника ФГБНУ ВИЛАР. Наибольшее количество протодиосцина обнаружено в образцах из Крыма, а минимальное – в образцах из Молдовы. Проведенное исследование элементного состава якорцев стелющихся травы показало, что места обитания (географические зоны), в которых осуществлялся сбор исследованных образцов сырья, влияют на накопление элементов растением. С учетом полученных данных составлены ряды биологического поглощения для образцов из каждого места произрастания.

Ключевые слова: якорцы стелющиеся, *Tribulus terrestris*, ВЭЖХ-МС/МС, элементный состав, фитохимия

INTRODUCTION

Currently, a lot of attention is focused on complex preparations containing vitamins, amino acids and microelements. It has been established that manganese (Mn) and molybdenum (Mo) potentiate the effect of cardiac glycosides, Mn potentiates the effect of ascorbic acid and carotenoids in medicinal plants. In addition, the microelements found in plants, are better absorbed by the human body since they are found in plant materials in “biological” concentrations [1, 2].

Tribulus terrestris L. is an annual herb belonging to the *Zygophyllaceae* family.

The plant has a tap-root system with a framework of fibrous lateral roots. Its leaves are pinnately-paired compound. The flowers with a diameter of 1–1.2 cm, are located in the leaf angles. The calyx consists of 5 long-pointed, externally pressed hairy calyx lobes 4–5 mm long, 1–1.3 mm wide [3–5].

It grows in moderate and tropical climates in southern Europe, South Asia, Africa, Northern Australia.

In the Russian Federation, *Tribulus terrestris* L. grows in the European part, in the North Caucasus, in Western and Eastern Siberia and in the Crimea. It grows in dry, sandy and rocky steppes on clay-silty, saltiera, sandy loamy soils, moist meadows, along river valleys, like weeds in crops, along roads [6]. Raw materials base consist of wild plants.

One of the major groups of *Tribulus terrestris* L. biologically active substances are steroidal saponins, represented by Dioscin, Protodioscin, Tribestin, Prototribestin, Methylprototribestin, methyl protodioscin, pseudo-protodioscin, Tribulosin and other compounds [7–10]. In the raw materials there are flavonoids, mainly derivatives of quercetin, astragalin, 3-rutinoside, 3-genciobioside kaempferol, 3-genciobioside isorhamnetin, tribuloside, rutin, kaempferol, quercetin, 3-O-rhamnoside quercetin [7, 11–13]. In the roots, aerial parts, fruits, flowers there is hecogenin, in the roots, aerial parts there is neo-tigogenin, in roots and flowers there are such substances as β -sitosterol, stigmaterol, campesterol; in the aerial part and flowers there is ruskogenin.

Carotenoids: in leaves there is α carotene; in fruits there are phenolcarboxylic acids – ferulic and p-hydroxybenzoic; alkaloids: tribulusamide C, tribulusterine, trib-

ulusin, harmine. Harmine; other components include organic acids, amino acids; as for organic acids, there are benzoic, vanilla, ferulic and succinic acids. The main amino acids are alanine and threonine, in addition, there are the following substances there: coumarin, emodin and fission [6, 14–17].

Extracts from *Tribulus terrestris* L., have hypocholesteremic, anti-inflammatory, hepatoprotective, antihypertensive, immunomodulating and antioxidant effects, they enhance intestinal motility, stimulate the function of the sex glands, and have the properties of an aphrodisiac [18].

Tribulus terrestris L. is widely used in folk medicine in many countries [19]. In folk medicine, it is used to treat impotence [19]. In folk medicine of Iran – as a diuretic, laxative, for poultices, in the treatment of syphilis [20]. In Nepal it is used for urogenital infections [21], in the Indian medicine – for radiculitis, inflammation of the pelvic and sacral organs, dry cough and respiratory disorders [22]. In the traditional Chinese medicine, it is used for the treatment of eyes, swelling, abdominal bloating, pathological pains and sexual dysfunctions. In the Shern-Nong Pharmacopoeia, *Tribulus terrestris* L. is presented as a very valuable drug [19].

In the study conducted in Egypt in 2015 in an elder age group of men suffering from age-related androgen deficiency, the use of a *Tribulus terrestris* L. extract showed a statistically significant difference in the increase in testosterone levels [23].

The study of 2016 carried out in Iran on human spermatozooids, showed improvements in various parameters of male sperm [24]. Another study on 65 males in Brazil, 2016, showed a significant increase in its quality [25]. A randomized, double-blind, placebo-controlled study confirmed a high effectiveness *Tribulus terrestris* L. in the treatment of female sexual dysfunction [26].

In 2016, scientists from Iran showed that ethanol extract of *Tribulus terrestris* L. effectively reduced glucose levels compared with placebo in women with type 2 diabetes [27]. The mechanism of hypoglycemic action, most likely, is associated with inhibition of the activity of α -glucosidase in the small intestine [28, 29]. A mild α -amylase inhibitory effect was also established [28].

Steroid saponins of *Tribulus terrestris* L. have an antifungal effect against fluconazole-resistant fungi of the *Candida* genus [30]. Other *in vitro* studies have also shown both the antifungal and antibacterial properties of this plant [31].

In vitro studies of heart cells and whole animals' hearts showed that *Tribulus terrestris* L. substances have a protective effect on the heart tissue [32], as well as the neuroprotective effect of steroidal saponins [33]. On the basis of *Furostanol-Type Steroidal Saponins*, Bulgarian scientists have created Tribestan, the drug which has hypolipidemic and hypocholesteremic effects [34].

A neogalenical drug Tribusponin (the sum of steroid saponins), has been obtained by Georgian scientists on the basis *Tribulus terrestris* L.; it reduces a blood cholesterol level and increases a lecithin/cholesteric coefficient [35].

The drug "Tribusponin" has been registered in the Russian Federation in the dosage form of tablets (0.1 g) with *Tribulus terrestris* L. extract as an antisclerotic agent; the extract is a part of the complex preparations "Fitovit", "Speman", "Speman forte". ZAO "Evalar" produces dietary supplements "Effex tribulus" for the treatment of erectile dysfunction and to increase potency.

Because of a widespread currency and use of the plant in scientific and folk medicine, it is of great interest in studying the chemical composition of the raw materials of *Tribulus terrestris* L. collected in different habitats.

THE AIM of the work is a study of *Tribulus terrestris* L. harvested in different geographical zones.

MATERIALS AND METHODS

Object of the study

The object of the study is *Tribulus terrestris* L. collected in different geographical zones: Syrian Arab Republic, the vicinity of Damascus; Republic of Moldova, the vicinity of Chisinau (Kishinev); the Botanical Garden of VILAR; the peninsula of the Crimea, the peak of the Mount Kush-Kaya (Table 1). The samples were collected during flowering and fruiting periods and represented the entire aerial part of the plant with the root. The primary processing of the raw materials was to remove the root, brown parts of the plant. After the shade drying, the raw materials met the requirements of All-Russian Federal Assembly No. 42-827-79 "Herba tribuli terrestris" in terms of "authenticity" and "good quality".

Methods of determining of saponins

Quantitative determination of saponins in *Tribulus terrestris* L. was performed by HPLC-MS/MS using a liquid triple quadrupole chromatomass spectrometer LCMS-8050 (Shimadzu) under the following conditions:

– chromatographic column: Luna 5u C18(2) (2.1×150 mm 3.5-Micron);

– column thermostat temperature: 40°C;

– eluent: 0.1% formic acid solution – acetonitrile;

– elution mode: gradient;

– mobile phase flow rate: 0.4 ml/min.

– The conditions of mass spectrometric detection are presented in Table. 2.

Sample preparation

About 0.5 g of the crushed raw materials (accurately weighed quantity) was placed in a conical flask with a capacity of 100 ml, 25 ml of methanol (c.p.) was added. Next, the contents of the flask were extracted in an ultrasonic bath for 30 minutes at the temperature of 30±1°C. 1 ml of the resulting extraction was transferred to a centrifuge tube and centrifuged for 5 minutes at 10.000 rpm.

50 µl of the supernatant was placed in a 2 ml vial, 950 µl of methanol was added and mixed up. The volume of the analyzed sample was 5 µl.

The identification and quantitative determination of saponins in the extracts from the plant raw materials was carried out using standard samples of dioscin and protodioscin (Sigma-Aldrich, Germany). Accurately weighed quantities (about 1 mg) were dissolved in 1 ml of methanol. The resulting solutions were used to prepare methanol solutions with the concentration of 1000 ng/ml of each standard.

The detection of the generated ions was carried out in the MRM mode (monitoring of multiple reactions). Ionic transitions for dioscin were 869.50 → 415.00, for protodioscin 1031.5 → 415.0 [36].

The determination was carried out in three replications.

Methods of determining elements content

The elements content was studied using an X-ray fluorescence method recommended for the study of the elemental composition of medicinal raw materials (State Pharmacopoeia XIV) [37].

Sample preparation

About 10 g of dry *Tribulus terrestris* L. was ground to a powdery state, placed in a crucible and burned on a stove in an exhaust fume hood until smoking stopped. The crucible was placed in a muffle furnace at a temperature of 500±1° C, kept there for about 2 hours, until it was completely salted and there was no black coal mass. After complete cooling in the fume hood, 50% nitric acid was added to the crucible and evaporated on a tile with a shut down spiral, avoiding splashing. Then it was placed in a muffle furnace at the temperature of 500±1° C for 2 hours [38].

After cooling the crucible, the qualitative and quantitative composition of the elements was determined in the ash residue on a Thermo Scientific QUANT'X X-ray fluorescence spectrometer [39]. The determination was carried out in three replications.

Statistical processing of results

Processing of chromatographic information was carried out using the software "LabSolutions" (Shimadzu).

Table 1 – Zones for harvesting the samples of *Tribulus terrestris* L. raw materials

Habitat	Date of collecting
Syrian Arab Republic, vicinity of Damascus	June 2018
The peak of the Mount Kush-Kaya, Laspi Bay, Republic of the Crimea, the Russian Federation	July 2017
The vicinity of Chisinau (Kishinev), Republic of Moldova	June 2017
The Botanical Garden of "All-Russian Scientific Research Institute of medicinal and aromatic plants" (VILAR)	July 2016

Table 2 – Conditions of mass spectrometric analysis

Interface	DUIS (electrospray + chemical ionization)
Spray Gas Flow	3 L/min
Heating gas flow	10 L/min
Drying gas flow	10 L/min
Interface temperature	300°C
DL temperature	200°C
Heat Block Temperature	400°C
Drying gas Consumption	10 L/min
Capillary voltage	4000 V
Ionization source polarity	Positive
Impact Cell Energy	30 eV

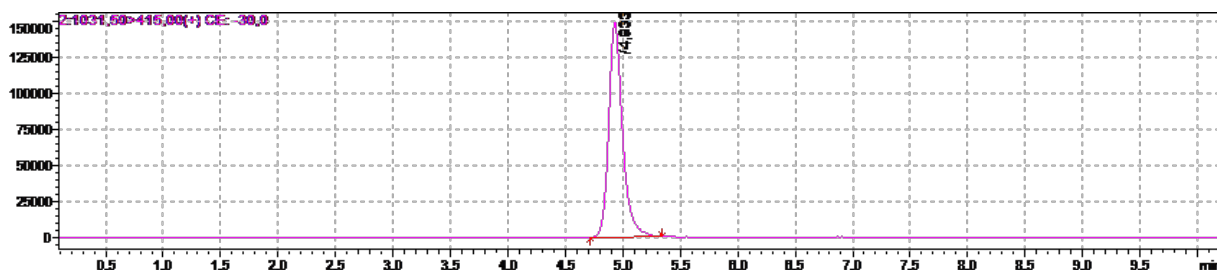


Figure 1 – Chromatogram of a protodioscin standard sample solution (concentration 1333 ng/ml)

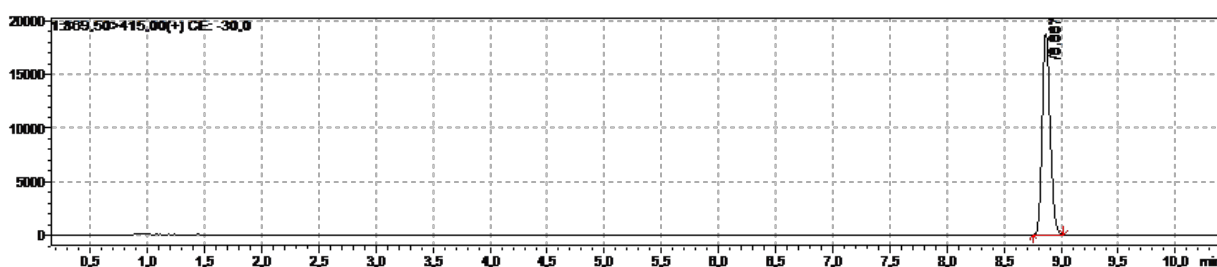


Figure 2 – Chromatogram of a dioscin standard sample solution (concentration 900 ng/ml)

For the statistical analysis of the results obtained, the statistical programming language R CRAN was used. The results were processed using Microsoft Excel. To compare the analyses of the results, Student's test was used with an assessment of the reliability of differences ($p < 0.05$).

The results were visualized using non-metric multidimensional scaling. To make up the scaling, the Bray distance (also known as the *Sørensen* test) was applied [40, 41]. To minimize the stress function, centering and Procrustean rotation were performed

RESULTS

Fig. 1–2 show chromatograms of solutions of dioscin and protodioscin standard samples in methanol.

The retention times of protodioscin and dioscin were 4.93 ± 0.03 and 8.86 ± 0.03 min, respectively.

Chromatograms of the extracts from the studied samples of plant materials are presented in Fig. 3 (A–D).

The identification of dioscin and protodioscin on the chromatograms was carried out by retention times in comparison with standard samples. Additional peaks observed on the chromatograms of the extracts corre-

sponding to the coextractive compounds, are well separated and do not interfere with the determination of the target analytes. To quantify saponins in plant materials, the external standard method was used.

The results of the studies showed that dioscin and protodioscin are found in all the studied samples. The maximum dioscin content is observed in the samples of the raw materials collected in the Crimea – 1.90 ± 0.02 ($p < 0.05$), and the minimum – in the samples from the nursery garden of the nursery garden of the Federal State Budgetary Scientific Institution “All-Russian Scientific Research Institute of medicinal and aromatic plants” (VILAR). No statistically significant differences were found out between the dioscin content in the samples collected in the Crimea and Syria. The largest amount of proto was also found in the samples from the Crimea – 15.59 ± 0.28 ($p < 0.05$), and the minimum – in the samples from Moldova (tab. 3).

The next stage of the work was the determination of the key elements in the elements content of *Tribulus terrestris* L. collected in different habitats, because these key elements are necessary for plant life and affect the processes occurring in the human body (tab. 4).

The data of statistical processing of the analyses results are presented in table. 5.

Fig. 7 shows a mutual disposition of the samples in the reduced attribute space and the contribution of each of the elements studied to the main coordinates.

Fig. 7 shows that the samples characterizing each geographical zone, are localized densely, especially the samples from the VILAR nursery garden. The least similar are the samples from Syria and Russian VILAR, whereas the samples from the Crimea and Moldova, by contrast, are relatively close.

As a result of the elements analysis of *Tribulus terrestris* L. samples, it has been found out that among the macroelements in all the studied samples, potassium and calcium are accumulated most of all, they account for about 90% of the total contents of elements in the plant. Among others, *Tribulus terrestris* L. contains essential elements, such as iron, copper, zinc, manganese, chromium and molybdenum.

The distribution of macro- and microelements in the plants differs significantly depending on the place and conditions of their growth. So, for example, the content of titanium in the samples of the raw materials collected in the VILAR nursery garden, is 5–6 times higher than that in other samples (tab.4). A high content of titanium in these samples is associated with the growing conditions of the plant (cultivated in the VILAR nursery garden). The minimal accumulation of titanium is found out in the samples from Syria. There was more Zinc in the samples collected on the Crimean Peninsula, and its minimum content was in the samples from the VILAR nursery garden. There was most of all of Manganese in the samples from the VILAR nursery garden; and in the samples from Syria, its minimum content was established.

Manganese is involved in metabolism, it improves

physiological processes in the body. It takes part in oxidative processes, in the regenerative process of nitrates during photosynthesis, as well as in the antagonism between manganese and other chemical elements [42]. This leads to the conclusion that the intensity of metabolic processes is higher in the plants cultivated in the VILAR nursery garden. A high intensity of metabolic processes indicates favorable conditions for the plant.

The data provided by other researchers [42], showed a certain relationship between the contents of iron and manganese. With a decrease in the manganese content in the plant, an excess amount of active nitrous iron is accumulated [42]. A similar interdependence is found out in some of the studied samples.

The organic compounds containing ferrum, are necessary for plants to ensure the biochemical processes that occur during respiration and photosynthesis. Compounds with ferrum in the structure, act as electron carriers in biochemical processes, since they are components of the enzymes dihydrogenases and cytochromes. Ferrum takes part in the process of chlorophyll biosynthesis, so with a limited supply of it, severe plant diseases, in particular chlorosis, may occur, [42, 43].

The following pattern of ferrum accumulation is presented in the studied samples. Its content was higher in the samples collected in Moldova. There is less iron in the samples collected in Syria.

The maximum amount of silicon was found out in the samples from the VILAR nursery garden, while its minimum amount was found in the samples from Syria.

In the VILAR samples there was twice as much Aluminium as in the other samples. In the human body, aluminum is involved in the construction of epithelial and connective tissues, bone regeneration, mineral metabolism, etc. [1, 44]. It is also worth noting that a high content of aluminum can have a negative effect on the human body, since excessive intake may have an impact on the central nervous system, the progression of Alzheimer's disease and depression [1].

According to GPM 1.5.3.0009.15. “Determination of the content of heavy metals and arsenic in medicinal plant materials and herbal medicines”, the maximum permissible concentration (MPC) of lead is 6.0 mg/kg. No lead has been detected in the samples from Syria, the Crimea and Moldova. The lead content in the samples collected in the VILAR nursery garden, did not exceed the maximum permissible concentration.

Based on the results obtained, a series of biological uptake of chemical elements has been compiled for *Tribulus terrestris* L.

The samples harvested in Syria: $K > Ca > Na > S > P > Al > Mg > Si > Fe > Zn > Ti > Mn > Cu > Ni$.

The samples harvested in the Crimean Peninsula: $K > Ca > Na > S > Fe > P > Si > Al > Mg > Ti > Zn > Mn > Cu$.

The samples harvested in Moldova: $K > Ca > Na > S > Fe > Si > P > Al > Mg > Ti > Zn > Mn > Cu > Cr$.

The samples harvested in the VILAR nursery garden: $Ca > K > Na > Al > S > Si > Ti > Fe > P > Mg > Mn > Zn > Cu > Cr > Pb > Cu$.

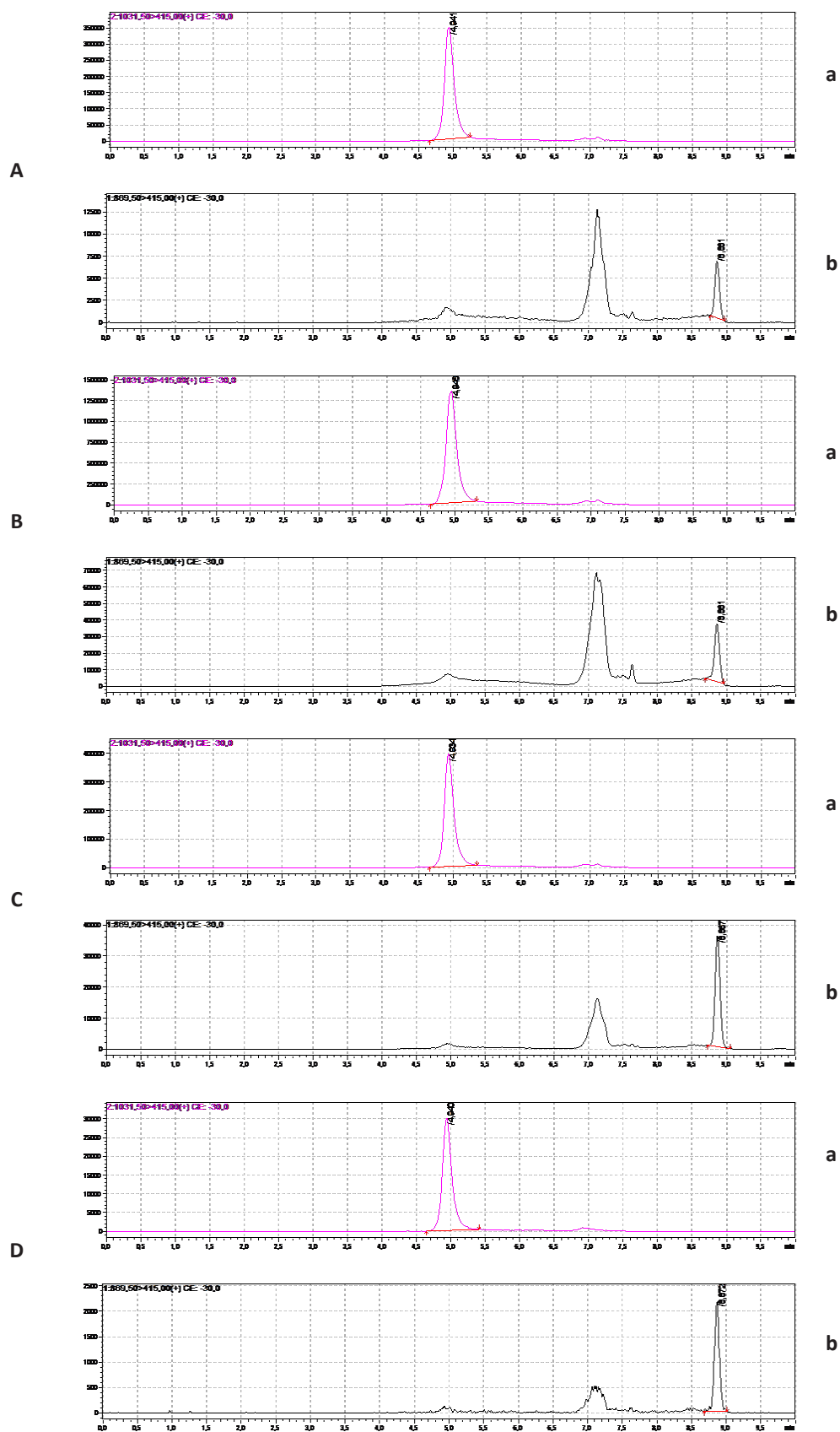


Figure 3 – Chromatograms of extracts from the samples of raw materials collected in Moldova (A), Crimea (B), Syria (C) and VILAR nursery (D)

Note: a – detection of protodioscin at ionic transitions (1031.5 → 415.0), b – dioscin (869.50 → 415.00)

Table 3 – The results of dioscin and protodioscin quantitative determination in the samples of plant materials by method of HPLC-MS/MS

Samples	Content of dioscin, mg (in 1 g of raw materials) X cf ± SD	Content of protodioscin, mg (in 1 g of raw materials) X cf ± SD
Moldova (2017)	0.265±0.011*	3.17±0.13*
Crimea (2017)	1.90±0.02	15.59±0.28*
Syria (2018)	1.67±0.04*	3.80±0.11*
VILAR (2016)	0.097±0.02*	1.435±0.38*

Note: * – significance of differences p <0.05 significance of differences

Table 4 – Elements content of *Tribulus terrestris* L. samples

Sl.No.	Element	% of the total content of elements in the samples, in terms of dried raw materials			
		Syria (2018)	Crimea (2017)	Moldova (2017)	VILAR (2016)
Macroelements					
1	Na	7.70±0.15	3.20±0.26	3.5±0.6	4.30±0.211
2	K	50.71±0.56	64.65±0.39	63.97±0.30	36.27±0.42
3	Mg	0.47±0.09	0.498±0.056	0.58±0.042	0.31±0.01
4	P	0.69±0.10	0.878±0.098	0.88±0.08	0.35±0.04
5	S	1.43±0.13	1.099±0.124	1.08±0.20	1.02±0.11
6	Ca	37.83±0.54	27.22±0.23	26.98±0.21	46.59±0.35
Microelements					
7	Cu	0.0323±0.001	0.0163±0.004	0.018±0.001	0.018±0.002
8	Zn	0.0685±0.008	0.0959±0.007	0.074±0.003	0.054±0.011
9	Al	0.600±0.079	0.628±0.048	0.736±0.045	1.555±0.124
10	Si	0.458±0.058	0.656±0.072	0.957±0.043	0.908±0.062
11	Ti	0.056±0.001	0.134±0.007	0.143±0.008	0.630±0.075
12	Cr	0	0	0.008±0.002	0.016±0.001
13	Mn	0.046±0.010	0.065±0.004	0.067±0.008	0.215±0.008
14	Fe	0.356±0.088	0.880±0.07	0.962±0.054	0.474±0.045
15	Co	0	0	0	0
16	Ni	0.004±0.002	0	0	0.011±0.001
17	Pb	0	0	0	0.011±0.003
18	Mo	0	0	0	0
19	Sn	0	0	0	0
20	Ba	0	0	0	0

Table 5 – Statistical processing of the results of the elements content analysis

	Df	Sums of Sqs	Mean Sqs	FM model	R ²	Pr (>F)
Group	3	0.158631	0.052877	951.25	0.9972	0.001
Residuals	8	0.000445	0.000056	0.0028		
Total	11	0.159075	1			

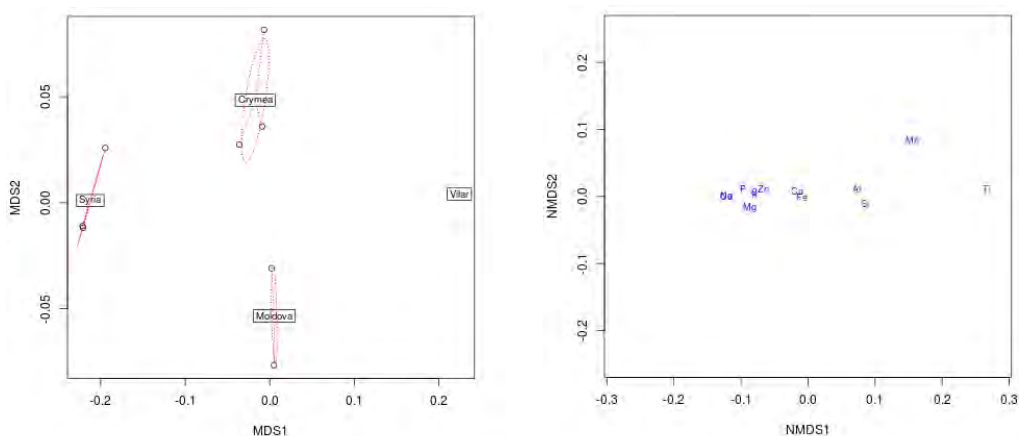


Figure 7 – A mutual disposition of the samples and the contribution of each of the elements studied to the main coordinates

DISCUSSION

The plants of *Tribulus terrestris* L. from different geographical regions, are known to contain saponins different in composition [45, 46]. The accessible literature contains information about the saponins isolated from *Tribulus terrestris* L. growing in China, India, New Zealand, South Africa, Bulgaria, Georgia and Moldova [46].

According to Szakiel et al. [47], the structure of saponins and their quantitative contents in this plant depend on several factors: climate, light exposure, moisture and soil composition, as well as some other local regional characteristics. In addition, in the majority of previously performed studies, the stage (phase) of plant growth or when and how the raw material was harvested, has not been reported.

It is also clearly stated that the method of steroidal saponins extraction, obviously, affects the results of their determination in the *Tribulus terrestris* L. raw materials [45]. Therefore, the data on the absence, for example, of dioscin in some samples analyzed by other research groups [48] suggest that the phase of *Tribulus terrestris* L. development and the specific methods of the extraction of steroid saponins have a significant effect on the result.

The studies performed by our group, allow us to conclude that in the flowering and fruiting phases, all the samples, regardless of the sample preparation zone, contain dioscin and protodioscin. Moreover, in some samples, the content of protodioscin is several times higher than the content of dioscin.

The scientific literature devoted to the study of *Tribulus terrestris* L., provides information on various methods used to assess the contents of steroidal saponins, including dioscin and protodioscin, in this medicinal plant material [46, 48].

According to A.N. Stavrianidi et al. [45], "the best detection method (by informative value, selectivity and sensitivity) for determining saponins is considered to be electrospray ionization in combination with tandem mass spectrometry".

Our methods we based on using HPLC-MS/MS, allows us to reliably assess the quality of *Tribulus terrestris* L. raw materials by the content of dioscin and protodioscin in it using the external standard method.

According to some researchers, a characteristic set of macro- and microelements in this plant is considered an important component of its chemical composition [49–51].

R. Selvaraju et al. register *Tribulus terrestris* L. as a good source of Na, K, Ca, Mg, and Fe. However, a significant concentration of some heavy metals can accumulate in the flowers [49]. They also showed that the contents of

minerals in *Tribulus terrestris* L. varies depending on the composition of the soil on which the plants grow. Our results also allow us to conclude that Na, K, and Ca are the predominant elements in *Tribulus terrestris* L.

A study by A. Ghani et al. has established that *Tribulus terrestris* L. accumulates Cu^{2+} up to 0.044% (dry weight), Ni^{3+} – 0.239%, Zn^{2+} – 0.434%, Co^{2+} – 0.161%, Cr^{3+} – 0.241%, Cd^{2+} – 0.384%, Fe^{2+} – 0.349%, Mn^{3+} – 0.527%, Pb^{2+} – 0.494%, Mg^{2+} – 0.541% [50]. The ability of this plant to accumulate cadmium and lead, depending on the composition of the soil, allows us to consider that control over the content of heavy metals in the *Tribulus terrestris* L. raw materials, is necessary.

Daur et al. [51] have found out that the accumulation of mineral elements in *Tribulus terrestris* L. is associated both with stressful conditions in the places where the plants grow and with their content and availability in the soil.

The series of biological uptake of macro- and microelements, which can be compiled on the basis of the results obtained for *Tribulus terrestris* L. samples collected by these researchers in Saudi Arabia and Pakistan – $\text{K} > \text{N} > \text{Mg} > \text{Ca} > \text{P} > \text{Na}$; $\text{Mn} > \text{Fe} > \text{Zn} > \text{Ni}$ – allow, in comparison with the data obtained by us, to speak about the influence of the place (zone) of growth on the mineral composition of the raw materials of this plant.

CONCLUSION

As a result of the studies by HPLC-MS/MS methods, dioscin and protodioscin have been found in all the studied samples. The maximum dioscin content was established in the samples of *Tribulus terrestris* L. raw materials harvested in Moldova, and the minimum was in the samples from the VILAR nursery garden. The largest amount of protodioscin was established in the samples of *Tribulus terrestris* L. raw materials collected in the Crimea, and the minimum – in the samples from Moldova. The obtained data and the methodology for assessing the quality of raw materials have been used in the preparation of the draft pharmacopeia article "*Tribulus terrestris* L.", sent to the "Scientific center for expert evaluation of medical products of the Ministry of Health of the Russian Federation".

The carried out study of the *Tribulus terrestris* L. elements content showed that the habitats (geographical zones) in which the studied samples of raw materials were collected, affect the accumulation of elements by the plant. Based on the obtained data, biological uptake series have been compiled for the samples from each habitat. All the tested samples of raw materials met the requirements of GPM.1.5.3.0009.15 SP on the content of heavy metals.

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AUTHORS' CONTRIBUTION

All authors equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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