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ANALYSIS OF CYTOTOXIC ACTIVITY AND QUANTITY OF BIOLOGICALLY ACTIVE METALS OF THE AERIAL MASS OF *TAMARIX HISPIDA*

Abstract. This article considers the quantitative content of bioactive metals, and the cytotoxic activity of the aboveground mass of the plant Tamarixhispida of the family Tamaricaceae harvested in September 2016 in the Almaty region. Determination of the number of metals was carried out by atomic absorption spectroscopy, and cytotoxic activity using the larvae of Artemiasalina shrimps in concentrations of 10, 100 and 1000 μl in aqueous medium by the LD 50 method, that is, determination of the concentration level of the substance in which more than 50 subjects do not survive organisms.

As a result, we found out that the content of biometals in the above-ground mass of Tamarixhispida does not exceed the parameters specified in the State Pharmacopoeia as permissible, and the cytotoxic activity of 50% ethanol, 70% ethanol, ethyl acetate, hexane and dichloromethane extract is practically absent, while 90 % the extract showed a relatively high level of cytotoxic activity

Key words: *Tamarixhispida, Tamaricaceae, Artemiasalina,* cytotoxic activity, alcohol extracts.

Introduction. It is known that indicators of good quality and safety are an extremely important parameter of the good quality of medicinal plant raw materials and the possibility of its use in medicine.

The medicinal raw materials contain many compounds that are potentially capable of harming the human body, such as mineral components, for example, salts of heavy metals, and various organic substances, both natural metabolites of plants, and those that have fallen into the raw materials from the environment (pesticides used in agriculture or emissions from industrial enterprises).

As an object of research, we chose the aboveground mass of the plant *Tamarixhispida* of the family *Tamaricaceae* harvested in September 2016 in the Almaty region.

The genus *Tamarix* belongs to the family *Tamaricaceae*. Types of *Tamarix* are ornamental shrubs or trees, mostly related to evergreen plants.

Tamarix can grow on highly saline soils due to the ability to regulate the salt balance by extracting excess Salt through the deciduous glands and consuming large amounts of water from underground sources.With the help of a developed root system, which facilitates the accumulation of a large number of mineral components in the overground mass of the plant [1-3].

In folk medicine, decoctions of the aerial part are used for rheumatism, gastrointestinal diseases, treatment of inflammation of the oral cavity. In the review of Sharma there is information about the use of representatives of the family *Tamarix* as an antioxidant for cosmetic purposes [4, 5].

To determine the content of heavy metals, we selected the method of atomic emission spectroscopy [6, 7], the cytotoxic activity was determined using larvae of Artemiasalina. Eggs of *Artemiasalina* are easily accessible, as a food for fishes in pet shops. When placed in artificially obtained sea water, eggs are opened within 48 hours, which provides a large number of larvae. This is a fast, inexpensive, general Biological Analysis that was developed for screening, fractionation and monitoring of physiologically active natural products.

Methods

Determination of heavy metals. The initial medicinal plant material was analyzed for the presence of heavy metals by atomic absorption spectroscopy using the following procedure

About 3-5 g of crushed medicinal plant raw materials (exact weighed portion) are placed in a pre-calcined and precisely weighed porcelain crucible.

The crucible is then gently heated, giving the substance first to burn or to evaporate at the lowest possible temperature. With incomplete combustion of coal particles, the residue is cooled, wetted with water or a saturated solution of ammonium nitrate, evaporated in a water bath and the residue is calcined. If necessary, repeat this operation several times.

Calcination leads to about 500 ° C to constant mass, avoiding the fusion of ash, and sintering with the walls of the crucible. At the end of the calcination, the crucible is cooled in a desiccator and then, the resulting ash is burned again at 600 ° C until a uniform gray color is obtained.

Finally, the precipitate is dissolved in 5 ml of HNO_3. The resulting solution must be heated on the tile to wet salts. The result is dissolved in 10-15 ml of 1H HNO_3 and transferred to a 25 ml volumetric flask, bring the volume to the mark. In parallel, prepare a blank sample with 1N HNO_3 solution

The analysis was carried out on the Atomic Absorption Spectrophotometer Shimadzu 6200

With a wavelength range of 190-900 nm with a measurement step of 0.2 nm

The results of determination of the concentration of heavy metals are given in the table1

Determination of cytotoxic activity. Test specimen, *Artemiasalina*, sea salt (38 g / l D / W, pH 7.4), Honed tray with a perforated septum, lamps for attracting larvae in brine, micro pipettes (10, 100, 1000 μl) , a tray for bottles, 9 bottles with samples of organic solvents (methanol / ethanol / acetone / water), distilled water.

Getting the larvae. Tray for the reproduction of larvae (rectangular dish (22x32 cm)) is half filled with filtered saline solution then *Artemiasalina* (50 mg) eggs are introduced. Incubate at 37º C for 48 hours.

Sample preparation. Dissolve the test sample (20 mg) in 2 ml of the appropriate solvent at concentrations of 10, 100 and 1000 μg / ml, respectively, and leave under the traction for 24 hours to remove the solvent. After 2 days from hatching and maturation, how to select 10 larvae, using a Pasteur pipette. Then place in 5 ml with seawater. Incubate the selected group at 25-27 ° C for 24 hours, under constant illumination. Add samples to the medium with the test substance.

Parallel to the same procedure, prepare positive and negative samples.

Data analysis is performed using the Finney computer program to determine LD 50 values with 95% confidence intervals.

The results of the determination of cytotoxic activity are shown in table 2.

Results and discussion

As a result of studying the aboveground mass of *Tamarixhispida* family *Tamaricaceae*, we determined that the lead content is 1.11 mg/kg that does not exceed the maximum permissible standards in accordance with the requirements of the State Pharmacopoeia [8].

The content of other metals is also within safe limits.

Table 1 – The content of heavy metals medicinal plant raw materials (aboveground mass *Tamarixhispida* Wild.)

Metal	Cu	Fe	Ni	Pb	Mn	Zn	Cd
Number of metals in mg/kg	0.62	40.36	0.51	1.11	3.13	2.26	0.01

For the most complete determination of cytotoxic activity in order to further develop several technologies for obtaining the substance based on the aboveground masses of *Tamarixhispida*, we prepared several extracts, namely, ethanol extracts with an ethyl alcohol concentration of 70, 50 and 90 percent, dichloromethane, hexane and ethyl acetate extracts [9].

As a result of the comparison of this cytotoxic activity, we see that in all the samples, in addition to 90% of the ethanol extract, mortality does not exceed 20% of the total number of organisms. Which allows us to speak about a sufficiently low level of toxicity and as a result of safety of preparations based on *tamarix* overground weight, 90 % alcohol extract the concentration at which more than 50% of the test organisms die is more than 400 μl. Which, given the sensitivity of *Artemiasalina* larvae to toxic compounds, is not an absolute proof the danger of 90% of the extract for humans and requires additional research.

Conclusion. As a result, we found out that the above-ground mass of the *Tamarixhispida* family of *Tamaricaceae* and preparations based on it are sufficiently safe for humans.The content of metals such as cadmium, lead, manganese, nickel, iron, copper and zinc is in acceptable concentrations and meets the requirements State Pharmacopoeia, the cytotoxic activity of both polar (various alcohol extracts) and nonpolar fractions is relatively low and does not prevent the

Table 2 – Cytotoxic activity of various extracts obtained from the aerial mass of *Tamarixhispida* Wild.

Dose	Number of organisms (initial)	Number of surviving organisms	LD 50	Standard drug	LD 50
70% ethanol					
10	30	30	–	Etoposite	7.4625
100	30	30			
1000	30	29			
50% ethanol					
10	30	29	–	Etoposite	7.4625
100	30	29			
1000	30	26			
90% ethanol					
10	30	28	489.6056	Etoposite	1.4625
100	30	27			
1000	30	0,9			
Dichloromethane					
10	30	21	–	Etoposite	1.4625
100	30	26			
1000	30	26			
Hexane					
10	30	29	–	Etoposite	1.4625
100	30	29			
1000	30	28			
Ethylacetate					
10	30	30	–	Etoposite	1.4625
100	30	29			
1000	30	29			

use of *tamarix* preparations in medicine, even at high concentrations. The analysis of the cytotoxic activity of various extracts of *Tamarixhispida*was performed for the first time.

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Резюме

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АНАЛИЗ ЦИТОТОКСИЧЕСКОЙ АКТИВНОСТИ И КОЛИЧЕСТВА БИОЛОГИЧЕСКИ АКТИВНЫХ МЕТАЛЛОВ НАДЗЕМНОЙ МАССЫ *TAMARIXHISPIDA*

Рассмотрено количественное содержание биоактивных металлов и цитотоксическая активность надземной массы растения Tamarixhispida семейства Tamaricaceae, заготовленного в сентябре 2016 г. в Алматинской области. Определение количества металлов проводилось методом атомно-абсорбционной спектроскопии, а цитотоксической активности с применение личинок рачков вида Artemiasalina в концентрациях 10, 100 и 1000 мкл в водной среде по методу LD 50, то есть определения уровня концентрации вещества, при котором не выживает более 50 испытуемых организмов.

В результате выяснено, что содержание биометаллов в надземной массе Tamarixhispida не превышает параметры, указанные в Государственной Фармакопее, как допустимые, а цитотоксическая активность 50% этанольного, 70% этанольного, этилацетатного, гексанового и дихлорметанового экстракта практически отсутствует, в то время как 90% экстракт проявил сравнительно высокий уровень цитотоксической активности

Ключевые слова: *Tamarixhispida, Tamaricaceae, Artemiasalina,* цитотоксическая активность, спиртовые экстракты.

Резюме

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БЕЛСЕНДІ МЕТАЛЛ ЖЕР ҮСТІ МАССАСЫНЫҢ ЦИТОТОКСИКАЛЫҚ БЕЛСЕНДІЛІГІН ЖӘНЕ СОМАСЫН ТАЛДАУ

Мақалада қарастырамыз: сандық мазмұны бар биоактивті металдар және активті цитотоксикалық жер үсті массасының өсімдігі Tamarix hispida тұқымдасы Tamaricaceae; 2016 қыркүйек айында Алматы облысында дайындалған. Металдардың санын атомдық – адсорбциялық спекртоскопия әдісі арқылы жүргізілді, цитотоксикалық белсенділік Artemia salina құрттарын пайдаланып, олардың концертициялары 10,100 және 1000 мкл сулы ортада LD50 әдісімен жүргізу, яғни заттың концентрациясын анықтау,онда 50-ден астам организмдер өмір сүрмейді.

Нәтижесінде; биометалдар құрамын, жер үсті массасында Tamarix hispida, мемлекеттік Фармакопия ұсынған параметрлерден аспайды, ол цитоткисикалық белсенділік 50%-дық этанолдық, 70 %-дық этанолдық, этилацетаттың, гексанның және дихлорметанның экстракты іс-жүзінде жоқ, сол уакытта 90% экстракт салыстырмалы жоғары деңгейде цитотоксикалық белседілігін көрсетті.

Түйін сөздер: *Tamarix hispida, Tamaricaceae, Artemia salina*, цитотоксикалық белсенділігін, этанолдық экстракт.