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ANALYSIS OF SOME PRIMARY AND SECONDARY METABOLITES OF THE AERIAL PART OF *TAMARIX HISPIDA*

Abstract. The article provides an analysis of the quantitative and qualitative content of some groups of primary and secondary metabolites contained in the aerial part of the plant Tamarixhispida harvested during flowering in 2016 in the territory of the Almaty region of the Republic of Kazakhstan. We carried out a qualitative determination of the presence of various groups of BASs in the aerial part of Tamarixhispida. Then the quantitative content of BAS groups interested in us was determined, in particular, the quantitative content of the sum of alkaloids, polysaccharides, carotenoids and riboflavin was reduced, and finally we studied the ethilacetate extract from the aboveground masses of Tamarixhispida by high-performance liquid chromatography with a mass-selective detector.

As a result, in the aboveground part of the plant Tamarixhispida we detected the presence of tannins, alkaloids, polysaccharides, flavonoids, coumarins, anthraquinones, carotenoids, organic acids, carbohydrates, amino acids and riboflavin. We also determined the quantitative content of the sum of alkaloids, polysaccharides, carotenoids and riboflavin.

Finally, by the method of high performance liquid chromatography with a mass-selective detector. WE was 15 compounds in the ethyl acetate extract. The dominant of which was dicyclohexylphthalic acid ester with a content of more than 50%, a significant amount of morphine derivatives (Morphinan-4,6-diol, N- formyl-6-acetate), some halogen and sulfur-containing compounds (Milbemycin B, 6.28-anhydro-15-chloro-25-isopropyl-13-dehydro-5-O-demethyl-4-methyl, 2-methoxy-N methyl-4- (methylsulfanyl) -N-phenyl-benzamide, 5-(2-Chloro -6-fluorobenzyl) -6-methyl-2- (2-propinilsulfanil) -4-pyrimidinol, oryzalin, Alkometazon).

Key words: *Tamarixhispida*, BAS, *Tamaricaceae*, metabolites, HPLC-MS.

Introduction. Organs of higher plants contain many primary and secondary metabolites belonging to different classes of organic substances; many of them exhibit different types of biological activity and have different effects on the human body. In particular, compounds of the alkaloid class exhibit, anesthetic or stimulating action, flavonoids and tannins substances have an antioxidant and astringent effect; some types of sulfur-containing compounds affect the growth of pathogenic organisms and exhibit actericidal properties. In this connection, knowledge of the qualitative and quantitative composition of various plants is of obvious practical interest [1].

We studied the qualitative and quantitative composition of the aerial mass of *Tamarixhispida* family *Tamaricaceae* harvested in 2016 in the Almaty region during the flowering period.

From the work on the aerial mass of the *Tamarixhispida* of the family Tamaricaceae. Itwas found out that in the aerial part of *Tamarixhispida* a significant amount of flavonoids, tannins and terpenoid.

Was found, and the structure of a number of new compounds, such as (octane, genicosan, docosane, tricano, heptadecane, nonadecane, hexacosane, pentacosane) terpenoids (α-copene, g-element, g-cadien, isophytol, phytol, β-cubenene, 3-α- [3", 4" -dihydroxy-trans-cinamoyl] -oxy-D -fridolean-14-ol-28-xylitol, β-systenrol, α-cadinol, d-cadinol, α-bisabolol) and their derivatives, polar acid esters (hexanoic acid, heptanoic acid, 2-ethylhexanoic acid, methyl palminat, hexadecanoic acid, octanoic, dodecanoic), aromatic compounds (vanillin, benzyl, benzoate, benzyl tsinamat) and alcohols (tridecanol, eugenol) [2].

Of the aerial mass directly *Tamarixhispida*, compounds of various classes were isolated, such as: ursolic acid-1, 2 methyl ester of 3β-al-D-freeoolean-14-ene-28-one acid, 3-α- [3 " 4"-dihydroxy-transcinamoloxy] -D-firdoolean-14-ene-28-tartaric acid (isotamarixene) -3,3-α-hydroxy-D-fridooleane-14-ene-28-tartaric acid 4-, 3- - [4"-hydroxy-transcamolamoxy] -D-firdoolean-14-ene-28-olal kilo-5. isoramnetin, 3,5-dihydroxy-4 ', 7-dimethoxyflavone, rhamnocetri, afzelin, 5,3'-dihydroxy-7,4'-dimethoxyflavone 3-O-β-D-glucopyranoside, 4-hydroxy-3,5-O-dimethyl benzo 7'-tetrahydrofuran, 3,7,4'-trihydroxy-5-methoxyflavone, 3,5,7-trihydroxy-3 ', 4'-dimethoxyflavone, Kampferid-3- O-β-glucopyranoside [3].

It was also proved that the extract from the aboveground mass *Tamarixhispida* has antibacterial and antioxidant activity [4].

Methods. After the study, previously published by *Tamarixhispida*, we decided to determine the quantitative composition of the least studied *tamarix* metabolites, namely alkaloids, polysaccharides, carotenoids and riboflavin

For which the following methods were used.

Determination of the quantitative content of alkaloids. About 10 grams (accurately weighed) of the crushed material are placed in a 250 ml flask, 100 ml of chloroform or ethyl acetate are added, 5 ml of concentrated ammonia solution, covered with a stopper and shaken on a vibrating apparatus for 2 hours or left at room temperature for 15 hours, after which they shake another 30 minutes. Chloroform extraction is filtered through cotton wool. 50 ml of the filtrate is transferred to a 100 ml flask and the chloroform is distilled to a volume of 1-2 ml. The remaining chloroform is removed by blowing air. To the remainder, add 2 ml of sodium hydroxide solution (0.1 mol/l) with a pipette and rub with a stick until the lumps disappear completely, then add 8 ml of water and mix 2-3 minutes. 10 ml of a solution of hydrochloric acid (0.1 mol/l) are added to the contents, pipetched gently and left for 8-10 minutes, then shaken on a vibratory shaker for 8-10 minutes and filtered through a triple paper filter, 7 cm in diameter. 10 ml The filtrate is transferred to a 50 ml flask, 10 ml of water, 2 drops of methyl red solution are added and the excess acid is titrated with a solution of sodium hydroxide

(0.01 mol/l) until a yellow color appears. At the same time, they conduct a control experiment. Add 1 ml of sodium hydroxide solution (0.1 mol/l) to a 50 ml flask, add 4 ml of water and 5 ml of hydrochloric acid (0.2 mol/l), mix, add 2 drops of methyl red solution and titrate excess acid sodium hydroxide solution (0.1 mol/l) until yellow coloration appeared. The content of the sum of alkaloids in terms of thermopsin and absolutely dry feedstock (X) in percent is calculated by the formula:

$$X = \frac{(V1 - V2) \times 0.0244 \times 4 \times 100 \times 100}{m \times (100 - W)},$$

where 0.0244 – the number of alkaloids in terms of thermopsin, corresponding to 1 ml of a solution of hydrochloric acid (0.1 mol/l), g; V1 is the volume of a solution of sodium hydroxide (0.1 mol/l), which has gone for titration of the control experiment, ml; V2 is the volume of a solution of sodium hydroxide (0.1 mol/l), which has gone for titration of the test solution, ml; m is the mass of the raw material, g; W is the loss in mass when the raw material is dried,%.

Determination of the quantitative content of polysaccharides. Approximately 5 grams (accurately weighed) of the crushed raw material are placed in a 100 ml flask, 50 ml of purified water are added, the flask is attached to a reflux condenser and boiled while stirring in a water bath for 1 hour, cooled. Extraction with water is repeated twice for 30 minutes under the same conditions. Water extracts combine and filter into a volumetric flask with a capacity of 250 ml ml through 3 layers of gauze. The filter is rinsed with purified water and the volume of the solution is adjusted with water purified to the mark. 25 ml of the resulting solution are placed in a centrifuge tube, 75 ml of ethyl alcohol 95% is added, mixed, heated in a water bath at a temperature of 60 ° C for 5 minutes. After 30 minutes, the contents are centrifuged at a rotation speed of 5000 rpm for 30 minutes. The supernatant is filtered under vacuum through a glass filter POR 16 dried to a constant weight. The precipitate is then quantitatively transferred to the same filter and washed with 15 ml of ethyl alcohol 95%. The filter with the precipitate is dried at a temperature of 100-105 ° C to constant weight. The content of polysaccharides in terms of absolutely dry raw materials in percent (X) is calculated by the formula:

$$X = \frac{(m2-m1)\times 250\times 100\times 100}{m\times 25\times (100-W)},$$

where m1 - filter weight, g; m2 - filter weight with precipitate, g; 32 m - weight of the sample of raw materials, g; W is the moisture content in the mass when the raw material is dried, %.

Determination of the quantitative content of carotenoids. Approximately 5 grams (accurately weighed) of the crushed plant material are placed in a conical flask with ground glass stopper of 100 ml capacity, 50 ml of a mixture of hexane-alcohol ethyl 96% (1: 1) are poured, kept for 2 hours with constant stirring, filtered. 15 ml of the filtrate is placed in a 25 ml volumetric flask and the volume

is adjusted to a label with a mixture of hexane-ethyl alcohol 96% (1: 1). [Approximately 1 g (exact sample) of the preparation is dissolved in a mixture of these solvents in a 50 ml volumetric flask, the volume of the solution is brought to the mark with the same mixture and mixed.] * The optical density of the solution is measured at a wavelength of 450 nm in a cuvette with a layer thickness of 10 mm , using a mixture of hexane-ethyl alcohol 96% (1: 1) as the reference solution. In parallel, the optical density of the potassium dichloride CO solution is measured. The content of carotenoids in terms of β-carotene in mg% (X) is calculated by the formula:

$$X = \frac{D1 \times 0.00208 \times 25 \times 50 \times 100 \times 100}{D0 \times m \times 15 \times (100 - W)}$$

where D1 - optical density of the solution under study at a wavelength of 450 nm; D0 is the optical density of the solution of potassium bichromate CO at a wavelength of 450 nm; 0,00208 - the amount of β-carotene in milligrams, in a solution corresponding to the color of the solution of potassium bichromate CO; m is the weight of the sample of raw materials, g; W - loss in mass during drying of raw materials,%

Preparation of potassium bichromate (CO) solution: 0.0036 g (exact sample) of potassium bichromate potassium salt is dissolved in water, purified in a volumetric flask with a capacity of 1 liter, the volume of the solution is adjusted with water purified to a mark, and mixed. The color of the solution corresponds to a solution containing 0.00208 mg of β-carotene in 1 ml.

Determination of the quantitative content of riboflavin. 0.06 g of the plant material is placed in a 1000 ml flask, 20 ml of glacial sulfuric acid are added, 500 ml of purified water, heated in a water bath, then cooled to room temperature, filtered and the volume of the solution is adjusted to the mark with water. 10 ml of filtrate are taken from the resulting solution, placed in a 100 ml volumetric flask, 3.5 ml of 0.1 mol/l sodium acetate solution is added and the volume of the solution is adjusted to the mark with water. 34 The optical density of the resulting solution is measured at a wavelength of 270 nm in a cuvette with a layer thickness of 10 mm. The content of riboflavin in percent (X) is calculated by the formula

$$X = \frac{D \times 10000}{a \times 850}$$

Where D is the optical density of the test solution at a wavelength of 270 nm; 850 - specific absorption index of riboflavin at a wavelength of 270 nm; a - the mass of the sample of raw materials, g.

The quantitative content of alkaloids, polysaccharides, carotenoids and riboflavin is given in table 1.

Next, we studied the ethylacetate extract obtained from the aerial mass of Tamarixhispida family Tamaricaceae according to the following procedure.

Vegetable raw materials are harvested in the southern region of the Republic of Kazakhstan (Almaty region). The ground air dry powder (500 g) was extracted with ethyl acetate into the feed / reagent ratio (1:10) in a Soxhlet apparatus. The resulting extract was concentrated under mild conditions (water bath temperature 40-450 °C).

The extract was examined by a high-performance liquid chromatograph with a mass selective detector of Aligent Technologies 6400 Series Triple Quadrupole LC/MS.

We was use: a Poroshell 120 EC-C18 column (50 mm in length, 3 mm in diameter, 4.0, 2, 7 and 1.9 μm) with 10% methanol with an aqueous solution of methanol as the starting solvent and 90% methanol as the final solvent at a pressure of 11.5 mPa and a temperature of 40 °C.

The components were identified by mass spectra and retention times, using the NIST library and Wiley LC / MS.

The results are shown in table 2.

Results and discussion

Chemical analysis of the aerial mass of *Tamarixhispida* family *Tamaricaceae* showed that in the investigated object there are alkaloids in the amount of 0.32%, polysaccharides (1.22%), carotenoids, 10.32% and riboflavin (2.09%), the data prompted us to further study the aboveground masses of Tamarixhispida already using the methods of mass spectrometry and high-performance liquid chromatography.

Table 1 – Content of alkaloids, polysaccharides, carotenoids and riboflavin in the aerial part of *Tamarixhispida* family *Tamaricaceae*

№	BAS Group	Content, (%)
1	Alkaloids	0.32
2	Polysaccharides	1.22
3	Carotenoids	10.32
4	Riboflavin	2.09

As a result of studying the hexane extract, 96.93% of the substances were identified. It was found that in the above-ground part of the *Tamarix hispida* family *Tamaricaceae* is dominated by dicyclohexyl ester of phthalic acid contained in an amount of 54.58%, of the compounds of the alkaloid class is dominated by Morphinan-4,6-diol, N-formyl-6-acetate (6.49%), total fraction of nitrogen-preserving compound is 14.17%, in addition to the derivatives of morphine, we classified benzenamine, 4-methyl-N, N-bis (4-methylphenyl) (1.94%), 6H-benzo [b] naphtho [2,3-H] carbazole (3 , 46%), 44-t-butyl-2- (4-methoxyphenyl) -6-p-tolyl-pyridine (1.27%), pyrrole, 2- (2-naphthyl) -3,5-diphenyl (1.01 %).

In addition to the nitrogen-containing compounds, we have identified some compounds related to terpenoids, namely (6E, 8E, 10E, 12E, 14E, 16E, 18E, 20E, 22E, 24E, 26E, 28E) -31-methoxy-2,6,10, 14,19,23,27,31-octamethyldotriaconta-6, 8,10,12,14,16,18,20,22,24,26,28-dodecaen-2-ol (3.68%), and the produced heterocycles 3 , 5-progesterol acetate (2.93%).

Table 2 – Component composition of the ethyl acetate extract of the aboveground mass *Tamarixhispida* Willd.

№	ConnectionName	Formula	Molecular weight	RT	Content, %
1	Milbemycin B, 6.28-anhydro-15-chloro-25-isopropyl-13-dehydro-5-O-demethyl-4-methyl	C33H47ClO7	590	0,48	3.06
2	Morphinan-4,6-diol, N-formyl-6-acetate	C19H23NO4	329	5,85	6.49
3	(6E, 8E, 10E, 12E, 14E, 16E, 18E, 20E, 22E, 24E, 26E, 28E) -31-methoxy-2,6,10,14,19,23,27,31-octamethyldotriaconta-6, 8,10,12, 14,16,18,20,22,24,26,28-dodecen-2-ol	C41H60O2	584	6,97	3.68
4	3,5-progesterol acetate	C23H32O3	356	7,48	2.93
5	2-methoxy-N-methyl-4-(methylsulfanyl) -N-phenylbenzamide	C16H17NO2S	287	8,07	8.57
6	Benzenamine, 4-methyl-N, N-bis (4-methylphenyl)	C21H21N	287	8,40	1.94
7	5- (2-chloro-6-fluorobenzyl) -6-methyl-2- (2-propynylsulfanyl) -4-pyrimidinol	C15H12ClFN2OS	322	8,78	0.65
8	6H-benzo [b] naphtho [2,3-H] carbazole	C24H15N	317	8,92	3.46
9	4- (dipropylamino) -3,5-dinitrobenzenesulfonamide	C12H18N4O6S	346	9,37	4.51
10	Alclomethasone	C28H37ClO7	520	9,73	0.65
11	4-t-Butyl-2- (4-methoxy-phenyl) -6-p-tolyl-pyridine	C23H25NO	331	10,03	1.27
12	Pyrrole, 2- (2-naphthyl) -3,5-diphenyl	C26H19N	345	10,76	1.01
13	2-methyl-4- (1,1,3,3-tetramethylbutyl) phenol	C15H24O	220	11,64	3.36
14	2,4,6-decathrienoic acid, 1a, 2,5,5a, 6,9,10,10a-octahydro-5,5a-dihydroxy-4- (hydroxymethyl) -1,1,7,9-tetramethyl-11 -oxo-1H-2,8a-methanocyclopenta [a] cyclopropa [e-cyclodecene-6-yl ester, [1aR-(1aα, 2α, 5β, 5aβ, 6β, 8aα, 9α, 10aα)]	C30H40O6	496	12,81	0.77
15	dicyclohexyl ester of phthalic acid	C20H26O4	330	13,88	54.58

A significant amount of organo-halogen compounds has been identified with a total content of 4.36% and includes the following compounds: Milbemycin B, 6.28-anhydro-15-chloro-25-isopropyl-13-dehydro-5-O-demethyl-4-methyl (3.06 %), 5- (2-chloro-6-fluorobenzyl) -6-methyl-2- (2-propynylsulfanyl) -4-pyrimidinol (0.65%), alclometasone (0.65%).

In addition, we identified a number of sulfur-containing compounds of various classes with a total fraction of 13.73% in the ethylacetnane extract, the following compounds were dentified: 22-methoxy-N-methyl-4- (methylsulfanyl) -N-phenylbenzamide (8.57%) and 4- (dipropylamino) -3,5-dinitrobenzenesulfonamide (4.51%).

Some of the identified compounds were not previously found in representatives of the tamarix family, in particular halogen-containing organic compounds have been detected for a while for the metabolites of a plant in the family of Tamaricaceae [5-9].

Conclusion. Thus, we determined the quantitative content of 4 groups of metabolites in the aerial part of *Tamarixhispida*, namely, alkaloids, carotenoids, polysaccharides and riboflavin, and the presence of alkaloids was confirmed by HPLC mass spectrometry.

In addition to the groups of substances by the method of high-performance liquid chromatography with a mass-selective detector. In the ethylacetate extract from the aboveground weight of *Tamarixhispida*, we found 15 compounds of various classes. 2 of them ether, 1 phenolic compound, pyrolysis derivative, pyridine derivative, pyromadyl derivative and morphine derivatives, also identified 3 sulfur-containing compounds including the class of sulfonamides; in addition, 3 halogen-containing compounds, namely Milbemycin B, 6.28-anhydro-15-chloro-25-isopropyl-13-de 5-O-demethyl-4-methyl, 5- (2-chloro-6-fluorobenzyl) -6-methyl-2- (2-propynylsulfanyl) -4-pyrimidinol, which had not previously been identified in plants of the tamarix genus [11].

The data obtained are in general consistent with the results of the literature review, since the identified BAS groups have anti-inflammatory and bactericidal activity

In particular, the sulfamide class compounds are known as highly effective antibiotics, and in a number of literature it is claimed that the *tamarix* aerial extract has a tonic and anesthetic effect, which is confirmed by the presence in the composition of alkaloids-derivatives of morphine, known for their high therapeutic efficacy w [12-15].

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Резюме

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TAMARIXHISPIDA БӨЛІГІНІҢ КЕЙБІР БАСТАПҚЫ ЖӘНЕ ЕКІНШІ МЕТАБОЛИТТЕРІН ТАЛДАУ

Мақалада бастапқы және қайталама метоболиттердің кейбір топтарының сандық және сапалық мазмұнын талдау. 2016 жылы Алматы облысының аумағында гүлдену кезінде жиналған *Tamarix hispida* жер асты бөлігінде орналасқан. Біз *Tamarix hispida*жоғары бөлігінде әртүрлі топтардың болуын сапалы анықтауды жүзеге асырдық. Және бізді қызықтыратын БАВ топтарының сандық ортасы анықталды. Атап айтқанда, соманың сандық мазмұны алкалоидтар,полисахаридтер, каротиноидтар және рибофлавина.Қорытындысында біз жер асты массасынан алынған Tamarix hispida этилацетаттың экстрактісін, селективті-масса детектормен жоғары сапалы сұйық хромотография әдісімен зерттедік.

Нәтижесінде жерасты *Tamarix hispida* өсімдігі дубилді заттардың қатысында анықталды, алкалоидтық, полисахаодтік, флаваноидтық, кумариндық, антрахинондық, каротиноидтық, органикалық қышқылдық, көміртектік аминқышқылдық және рибофлавиндік. Және де алкалоидтардың, полисахаридтердің, каротиноидтардың және рибофлавиндердің

Түйін сөздер: *Tamarix hispida, БАВ, Tamaricaceae,* метаболитырын, *HPLC-MS.*

Резюме

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АНАЛИЗ НЕКОТОРЫХ ПЕРВИЧНЫХ И ВТОРИЧНЫХ МЕТАБОЛИТОВ НАДЗЕМНОЙ ЧАСТИ *TAMARIXHISPIDA*

Анализ количественного и качественного содержания некоторых групп первичных и вторичных метаболитов, содержащихся в надземной части растения *Tamarixhispida*, заготовленного в период цветения в 2016 г. на территории Алматинской области Республики Казахстан. Нами было проведено качественное определение присутствия различных групп БАВ в надземной части *Tamarixhispida*, затем было определено количественное содержание заинтересовавших нас групп БАВ. В частности, было поределено количественное содержание суммы алкалоидов, полисахаридов, каротиноидов и рибофлавина и в заключение мы изучили этиацетатный экстракт из надземной масс *Tamarixhispida* методом высокоэффективной жидкостной хроматографии с масс-селективным детектором.

В результате в надземной части растения *Tamarixhispida* нами было обнаружено присутствие дубильных веществ, алкалоидов, полисахаридов, флаваноидов, кумаринов, антрахинонов, каротиноидов, органических кислот, углеводов, аминокислот и рибофлавина. Также нами было определено количественное содержание суммы алкалоидов, полисахаридов, каротиноидов и рибофлавина.

В завершении методом высокоэффективной жидкостной хроматографии с масс-селективным детектором в этилацетатном экстракте было идентифицировано 15 соединений, доминирующим из которых являлся дициклогексиловый эфир фталевой кислоты с содержанием более 50%, также обнаружено значительное количество производных морфина (Морфинан-4,6-диол, N-формил-6-ацетат), некоторое количество галоген и серосодержащих соединений (Милбемицин B, 6,28-ангидро-15-хлор-25-изопропил-13-дегидро-5-O-деметил-4-метил, 2-метокси-N-метил-4- (метилсульфанил) -N-фенилбензамид, 5-(2-Хлор-6-фторбензил) -6-метил-2- (2-пропинилсульфанил) -4-пиримидинол, Оризалин, Алкометазон).

Ключевые слова: *Tamarixhispida, БАВ, Tamaricaceae,* метаболиты, *HPLC-MS.*