

ЕҢБЕК ҚЫЗЫЛ ТУ ОРДЕНДІ
«Ә. Б. БЕКТҰРОВ АТЫНДАҒЫ
ХИМИЯ ҒЫЛЫМДАРЫ ИНСТИТУТЫ»
АКЦИОНЕРЛІК ҚОҒАМЫ

ҚАЗАҚСТАННЫҢ ХИМИЯ ЖУРНАЛЫ

ХИМИЧЕСКИЙ ЖУРНАЛ КАЗАХСТАНА

CHEMICAL JOURNAL of KAZAKHSTAN

АКЦИОНЕРНОЕ ОБЩЕСТВО
ОРДЕНА ТРУДОВОГО КРАСНОГО ЗНАМЕНИ
«ИНСТИТУТ ХИМИЧЕСКИХ НАУК
им. А. Б. БЕКТУРОВА»

1 (61)

ЯНВАРЬ – МАРТ 2018 г.
ИЗДАЕТСЯ С ОКТЯБРЯ 2003 ГОДА
ВЫХОДИТ 4 РАЗА В ГОД

АЛМАТЫ
2018

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CHEMICAL COMPOSITION AND GROWTH REGULATING ACTIVITY OF *Tamarix hispida* SUBSTANCE

Abstract. This article considers the growth of regulating activity of 50 and 70% of the extract obtained from the aerial weight of the *Tamarix hispida* plant of the Tamaricaceae family harvested in Almaty in September 2016.

While testing the obtained extracts on the Lenma minor strain of the Araceae family found that 70% shows much greater activity when the concentration of the extract in the test medium is lower by 2 times. The obtained data prompted us to study in more detail the chemical composition of 70% alcohol extract by the method of high-performance liquid chromatography with a mass-selective detector. As a result, 70% of the extract identified a significant number of compounds structurally similar and potentially possessing a growth regulating ability. Namely chlorine azoorganic compounds of the type 7-chloro-4-(dimethylamino) 1,4,4a, 5,5a, 6,11,12a- 3,6,10,12,12a-pentahydroxy-1,11-dioxo-2-naphthacene-carboxamide, 3,5-dichloro-6-nitrocholestane, Phenol, 4,4'- (3H-2,1-benzoxathiol-3-ylidene) bis-2-bromo-3-methyl-6-(1-methylethyl)-, S, S-dioxide, and their share in the extract reaches more than 40% of all the identified substances.

Key words: *Tamarix hispida*, Tamaricaceae, growth-regulating activity, Lenma minor, HPLC-MS

Introduction. Among the many classes of biologically active substances isolated from the plant and obtained by the method of synthesis, substances with a growing regulation ability, that is capable of exerting a certain influence on the development of individual organs or plants as a whole, or stimulating a set of biomass [1].

Regulators of development are physiologically active substances that show their effect in extremely small amounts (usually the dosage is measured in microliters), they allow to influence the intensity and direction of physiological processes in agricultural crops, increasing their yield, speeding up the collection of green mass or vice versa suppressing the growth of weeds [2].

Growth regulators primarily include phytohormones are divided into 5 main groups, auxins, gibberlins, cytokinins, abscisins, and ethylene. The first 3 classes have the ability to induce growth, an example of such compounds is indole acetic acid and 2,4-dichloro-phenoxyacetic acid [3].

Abscisins and ethylene are natural inhibitors, that is, substances that inhibit growth. Natural inhibitors often accumulate in resting organs, in particular in the kidneys, seeds and tubers.

In our article, as an object, we chose the aboveground mass of the plant *Tamarix hispida* of the family *Tamaricaceae* harvested in September 2016 in the Almaty region.

Plants of this genus are basically perennial evergreen plants distributed in the arid zones of southern Europe, Iran, Pakistan, India, the South Caucasus, North America and Central Asia [4, 5].

Extracts obtained from representatives of the *tamarix* family were previously considered as medicinal phytocomplexes and capable of metabolites isolated from *tamarix* to regulate plant growth have not previously been studied.

Antioxidant, anti-bacterial, anti-cancer activity of various *tamarix* species, including hypsids, was previously considered in the study of alcohol extracts of the aerial part, many compounds of various classes were isolated, such as ursolic acid -1,2 methyl 3 β -al-D-firidoolean-14-ene-28-one acid, 3- α - [3'', 4''-dihydroxy-transcamolamoxy] -D-firidoolean-14-ene-28-tartaric acid (isotamarixene) -3,3- α -hydroxy -D-Freodoolean-14-ene-28-tartaric acid-4, 3- α - [4''-hydroxy-transcamolamoxy] -D-firidoolean-14-ene-28-ol Lota-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-dimethylbenzoic acid, 7,3', 4'-trigoroxy-5-methoxyflavone, 3,7,4'-trihydroxy-5-methoxyflavone, 3,5,7-trihydroxy-3', 4' dimethoxy flavone, Kampferid-3-O- β -glucopyranoside [4-6].

We studied the growth-regulating activity of extracts obtained from the above-ground mass of *Tamarix hispida* family *Tamaricaceae* using a standard test sample in the form of the *Lenma minor* family of *Araceae*, as well as the chemical composition of 70% alcohol-water extract by high-performance liquid chromatography with a mass-selective detector [7,8].

Methods

Preparation of 70% ethanol extract from the above-ground part of *Tamarix hispida* family *Tamaricaceae*. To determine the optimum from the point of view of the grow regulating ability of *Tamarix* extract, we prepared and sample, 50% and 70% alcohol extracts from the aerial part of the studied plant.

Extracts were obtained according to the following procedure:

The aerial part of a plant of the species *Tamarix hispida* harvested and dried in accordance with the requirements of the state Pharmacopoeia of the Republic of Kazakhstan [3-4] was crushed and the extractant in a ratio of 1 to 10. After them plant was insisted for 72 hours, then the extract was separated from the grass by filtration and concentrated extraction on a rotary evaporator until the solvent is completely removed at a temperature of 40-50 °C

Determination of grow regulation activity. Then, the determination of the growth regulating activity is carried out according to the following procedure:

The E-medium for plant development is obtained by mixing various components in 1000 ml of distilled water and adjusting the pH from 6.0 to 7.0 by adding KOH in the mother liquor.

Then the stock solution is dissolved to the working concentration by mixing 100 ml of the stock solution and 900 ml of water is distilled. Then prepare the test sample where 30 mg for the crude extract / compound is dissolved in 1.5 ml of solvent (methanol / ethanol, etc.) serving as the starting solution. Subsequently, 10, 100 and 1000 μl of the solution were inoculated from the mother liquor in 10, 100 and 1000 $\mu\text{g} / \text{ml}$ in three flasks. Then let the solvent evaporate overnight. After add 20 ml of working E. medium, and then plant *Lemma minor*, each of which contains a rosette of two or three leaves, in each flask. (a total of 20).

Other flasks prepare control experiments using plant growth inhibitors and promoters acting as a negative and positive control, respectively.

After all the cooking, place the flasks in the incubator cabinet for seven days. Plants should be inspected daily during incubation. The results are analyzed both in the regulation of growth of % age, calculated with reference to negative control.

The standard preparation was 1,1'-dimethyl-4,4'-bipyridinium hydrate hydrate

The results are shown in table 1.

Table 1 – Grow regulating ability of 50 and 70% ethanol extract from aboveground masses *Tamarix hispida* Wild

70% Ethanol extract					
Control plant	Concentration of sample	Types of standards		Degree of Growth	Concentration of standard drug
		Organisms that have not changed after the drug is administered	Control		
<i>Lemma minor</i>	10 μL	20	40	50 %	0,015
	100 μL	18		55 %	
	1000 μL	16		69 %	
50% Ethanol extract					
<i>Lemma minor</i>	10 μL	51	51	0 %	0,015
	100 μL	51		0 %	
	1000 μL	29		43.13 %	

Further, the extract exhibiting the highest activity values is analyzed by high performance liquid chromatography with a mass selective detector of Aligent Technologies 6400 Series Triple Quadrupole LC/MS. For analyze we use Poroshell 120 EC-C18 column (50 mm long, 3 mm in diameter, sorbent particle size 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 $^{\circ}\text{C}$.

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

From the data presented in table 1, we see that 70% ethanol extract from the aerial part of *Tamarix hispida* exhibits significantly greater growth regulating activity at a much lower concentration, that is, 50% extract exhibits significant activity (43.13%). Only at a maximum concentration of 100 µl, then time as 70% shows activity of 50% already at 10 µl. But when the concentration is increased, the changes are not so large and make up 55% at 100 µl and 69 at 1000 µl, which implies that a change in concentration of 10 does not lead to a significant increase in of activity, which confirms the statement of the feasibility of using growth regulating substances. Only in low concentrations, as the increase in concentration only leads to senseless consumption of the drug.

The data obtained clearly demonstrate that the growth regulating effect achieved in 50% extract at a concentration of 1000 µl per 1000 ml is 43.13% significantly less than in 70% at a concentration of 10 µl (50%) that is 70% extract shows more than 100 times greater efficiency in laboratory tests. This fact convinced us to consider 70% extract as the main one in determining growth regulating activity and further study its chemical composition in more detail.

From the data presented in table 2, we can say that the dominant compound in the 70% extract is 7-chloro-4-(dimethylamino) 1,4,4a, 5,5a, 6,11,12a-3,6,10, 12 , 12a-pentahydroxy-1,11-dioxo-2-naphthacencarboxamide (12.78%), 7-Azadabenz [a, e] azulene-12-one, 5,6,7,7a, 12,12a-hexahydro-7-methyl -8,9-bis (methoxy) -12a-hydroxy-2,3-methylenedioxy (13.46%), 5a, 7-dihydroxy-9a, 11a-dimethyl-1-(2-oxo-2H-pyran-5-yl) (14.88%), Phenol, 4,4' - (3H-2,1-benzoxathiol-3-ylidene)) bis-2-bromo-3-methyl-6- (1-methylethyl) -, S, S-dioxide (20.42%) and 4- (2-methyl-1-cyclohexenyl) -trans-3-butene ene-2-one 2,4-dinitrophenyl-hydrazone (10.74%). The proportion of beta carotene derivatives of 3,3'-dihydroxy-beta, beta-carotene-4,4'-dione is also significant 2.5%, the total number of organohalogen compounds is estimated at 35.9%, the quantity of nitrogen-organic substances in 48.89%, sulfur compounds in 24.88% and oxygen-containing organic substances 98.14%, ie the bulk. Of particular interest are natural halogen-organic compounds exhibiting, as a rule, high growth stimulating and potentially anti-cancer activity [9-18].

Conclusion. Thus, we studied the growth-regulating activity of 50 and 70 percent extracts of the aerial masses of the *Tamarix hispida* family *Tamaricaceae*.

On the test plant of the *Lenma minor* family *Araceae* with the comparative preparation being 1,1'-dimethyl-4,4'-bipyridinium hydrate hydrate, we found that 70% the extract shows 100 times more activity. That is, it shows a 50% result at 10 µl, but with an increase in the concentration of 70% the extract of multiple growth does not occur, but only 5% increase at 10 concentration increase and a result of 69%. The further study of the chemical composition revealed a large amount of chlorine and organo-organic compounds in a 70% extract of 35.9% and 48.89%, respectively, with some of the identified compounds. For example, 7-chloro-4- (dimethylamino) 1,4,4a, 5, 5a, 6,11,12a-3,6,10,12,12a - pentahydroxy-

Table 2 – Results of determination of the chemical composition of 70% alcohol-water extract obtained from the aerial part of the plant *Tamarix hispida* Wild

№	Name of compound	Formula	Molecular weight	Retention time	Content, %
1	7-chloro-4- (dimethylamino) 1,4,4a, 5,5a, 6,11,12a-3,6,10,12,12a-pentahydroxy-1,11-dioxo-2-naphthacencarboxamide	C ₂₁ H ₂₁ ClN ₂ O ₈	464	0,44	12,78
2	7-Azadabenz [a, e] azulen-12-one, 5,6,7,7a, 12,12a-hexahydro-7-methyl-8,9-bis (methoxy) -12a-hydroxy-2,3-methylenedioxy	C ₂₁ H ₂₁ NO ₆	383	0,56	13,46
3	5a, 7-dihydroxy-9a, 11a-dimethyl-1- (2-oxo-2H-pyran-5-yl) -hexadecahydronaphtho [1', 2': 6,7] indeno [1,7a-b] oxirene-2 -acetate	C ₂₆ H ₃₄ O ₇	458	1,401	14,88
4	3,3'-dihydroxy-beta, beta-carotene-4,4'-dione	C ₄₀ H ₅₂ O ₄	596	2,31	2,5
5	3,5-dichloro-6-nitro cholestane	C ₂₇ H ₄₅ Cl ₂ NO ₂	485	3,46	2,7
6	Carda-4.20 (22) -dienolide, 3 - [(6-deoxy-3-O-methyl-α-D-allopyranosyl) oxy] -1,14-dihydroxy-, (1β, 3β) -	C ₃₀ H ₄₄ O ₉	548	3,82	8,15
7	6-oxo-1-phenyl-1,6-dihydropyridazin-3-yloxy) acetic acid, ethyl ester	C ₁₄ H ₁₄ N ₂ O ₄	274	4,96	4,91
8	Phenol, 4,4' - (3H-2,1-benzoxathiol-3-ylidene) bis-2-bromo-3-methyl-6- (1-methylethyl) -, S, S-dioxide	C ₂₇ H ₂₈ Br ₂ O ₅ S	622	5,20	20,42
9	4,4', 4' - methylidennis (N, N-dimethylaniline)	C ₂₅ H ₃₁ N ₃	373	5,37	1,61
10	4- (2-methyl-1-cyclohexenyl) -trans-3-buten-2-one 2,4-dinitrophenylhydrazone	C ₁₇ H ₂₀ N ₄ O ₄	344	5,66	10,74
11	Benzo (g) indolo (2,3-a) quinolizine-1-carboxylic acid, 1,2,3,4,4a, 5,7,8,13,13b, 14,14a-dodecahydro-3-hydroxy-2 methoxy, methyl ester, 3,4,5-trimethoxybenzoate	C ₃₂ H ₃₈ N ₂ O ₈	578	6,01	3,14
12	2-thiophenecarboxaldehyde, 5-ethynyl-, (2,4-dinitrophenyl) hydrazone	C ₁₃ H ₈ N ₄ O ₄ S	316	6,55	4,46

1,11-dioxo-2-naphthacencarboxamide, 3,5-dichloro-6-nitrocholestane, are halogenated steroids exhibiting growth stimulating activity.

It should be noted that the growth-stimulating activity of the aboveground part of the Kazakhstan tamarix species was studied for the first time. As well as 7-chloro-4- (dimethylamino) 1,4,4a, 5,5a, 6,11,12a-3,6,10,12,12a- pentahydroxy-1,11-dioxo-2-naphthacencarboxamide, Phenol, 4,4' - (3H-2,1-benzoxathiol-3-ylidene) bis-2-bromo-3-methyl-6- (1-methylethyl) -, S, S-dioxide, 2-thiophenecarboxaldehyde, 5-ethynyl-, (2,4-dinitrophenyl) hydrazone were first found in the plant *Tamarix hispida*.

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Резюме

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**TAMARIX HISPIDA ХИМИЯЛЫҚ ҚҰРАМ ЖӘНЕ
ӨСУ НОРМАТИВІН РЕТТЕУ СУБСТАНЦИЯЛАРЫ**

Мақалада 2016 жылы қыркүйекте Алматыда жиналған *Tamarix* отбасының *Tamarix hispida* зауытында жер үстінен алынған 50-70% өсімдік сығындыларының өсуді реттеуші қызметі қарастырылған, *Agaceae* отбасының кішігірім *Lenina* өсімдігінің 70% экстракт кезінде аздаған активтілік екінші қатарда, тәжірибелік ортада

концентрациясы төмен. Алынған деретер бізді масс-селективті детектормен жоғары сапалы сұйық хроматография әдісімен 70% спирт сығындысының химиялық құрамын егжей-текжейлі зерделеуге мәжбүр етті.

Нәтижесінде 70% сығындыда құрылымдық, ұқсас және ықтимал өсу нормативін реттеу қабілеті бар атап айтқанда, 7-хлор-4-(диметиламин) 1,4,4а,5,5а,6,11,12а-3,6,10,12,12а-пентагидрокси-1,11-диоксо-2-нафтаценкарбоксамид, 3,5-дихлор-6-нитрохолестан, Фенол, 4,4'- (3Н-2,1-бензоксатиол-3-илиден) бис-2-бром-3-метил-6-(1-метилэтил)-S,S-диоксид, сияқты хлордың қосылыстары анықталды, олардың сығындылары 40%-дан асады.

Түйін сөздер: *Tamarix hispida*, *Tamaricaceae*, *регулярлық қызмет*, *Lenmaminor*, HPLC-MS.

Резюме

Е. С. Ихсанов, Н. А. Султанова, Ж. А. Абилов, М. И. Чоудхари

ХИМИЧЕСКИЙ СОСТАВ И РОСТРЕГУЛИРУЮЩАЯ АКТИВНОСТЬ СУБСТАНЦИИ *TAMARIXHISPIDA*

В статье рассматривается рострегулирующая активность 50 и 70% экстракта, полученного из наземной массы растения *Tamarix hispida* семейства *Tamaricaceae*, заготовленного в сентябре 2016 г в Алматинской области. В ходе испытаний полученных экстрактов на тестовом растении *Lenmaminor* семейства *Agaceae* было выяснено, что при более низкой концентрации экстракта в испытательной среде (на 2 порядка), 70%-ный экстракт проявляет намного большую активность. Полученные данные побудили нас более подробно изучить химический состав 70%-ного спиртового экстракта с помощью метода высокоэффективной жидкостной хроматографии с масс-селективным детектором. В итоге в 70%-ном экстракте было идентифицировано значительное количество соединений, структурно схожих и потенциально обладающих рострегулирующей способностью, а именно, хлор- и азоорганические соединения типа 7-хлор-4- (диметиламино) 1,4,4а, 5,5а, 6,11,12а-3,6,10,12,12а-пентагидрокси-1,11-диоксо-2-нафтаценкарбоксамид, 3,5-дихлор-6-нитрохолестан, Фенол, 4,4'- (3Н-2,1-бензоксатиол-3-илиден) бис-2-бром-3-метил-6- (1-метилэтил) -, S, S-диоксид, при этом их доля в экстракте достигает более 40% из всех идентифицированных веществ.

Ключевые слова: *Tamarix hispida*, *Tamaricaceae*, *рострегулирующая активность*, *Lenmaminor*, HPLC-MS.