UDC 544:615.322 https://doi.org/10.26577/2218-7979-2015-8-1-47-51

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Phytochemical study of conditional phytopreparation and liposoluble contents of *Halostachys caspica* with immunostimulatory activity

The study presents the results of a biologically active complex (BAC) study obtained from the aerial part of Halostachys caspica from the Chenopodiaceae family. Studies using gas chromatography-mass spectrometry revealed a significant amount of biologically active substances (BAS) in the BAC obtained by hydroalcoholic extraction, followed by lyophilization of the extract obtained, compared to the raw material. By passing this, the result of this study is the detection of such esters of higher saturated and unsaturated carboxylic acids and fatty acids as ethyl esters of oleic, hexadecanoic and octadecanoic acids.

Key words: Halostachys caspica, Chenopodiaceae, phytochemical composition of biologically active substances (BAS), biologically active complex (BAC), herbal, gas chromatography-mass spectrometry.

Introduction

To date, with all the certainty we can say that one of the most promising areas of development of drugs, is the search of physiologically active compounds through research phytochemical composition of plant facilities. However, determining the precise phytochemical composition as vegetable and herbal remedies obtained therefrom is still a difficult and time consuming task. However, technological progress and the associated development of physico-chemical methods of analysis, as exemplified by the widespread use of hybrid methods of analysis, the most important in the chemistry of natural compounds, as most of the analyzed samples are mixtures. Even with the use of effective methods of sample preparation for the isolation of interesting compounds still have to analyze the mixture. The value of gas and liquid chromatography is their ability to separate multicomponent mixtures. The combination of gas chromatography and mass spectrometry provides a method by which all components of a complex mixture can be separated and identified, even if their content in a sample is extremely small [1-3].

Thus, the relevance and appropriateness of discharge from the aerial part of the *Halostachys caspica* polyphenol complex as well as a study of its phytochemical composition, based on the meager assortment inmunostimulatory herbal drugs in the global pharmaceutical practice, high immunostimulatory, antidiabetic and antioxidant actively and there is sufficient commercial reserves in Kazakhstan [4, 5].

GC-MS is a hybrid method of analysis for this reason should be viewed as a combination of chromatography (gas or liquid) and mass spectrometry. The processes of separation and analysis here occur entirely independently of each other [6].

Materials and methods

At the initial stage of raw material was dried, shredded and standardized in accordance with the procedures described in the USSR and GF RK [7-9].

In the next stage, 500 g of the vegetable raw material was obtained BAC by the following procedure:

500-1000 g of plant (from the aerial part *Halostachys caspica*) were poured in 2500-5000 ml of 50% ethanol-water at a ratio of raw materials – 1:5 extractant, and infused for 24 hours protected from light at a temperature of 24-28 °C. The flask contents were cooled, thoroughly stirred, and filtered through filter paper into a dry flask. The extraction process was repeated three more times as described above. Then extracts were combined, filtered through the same filter to the same flask and concentrated to dryness under water pump vacuum at a temperature of 35-40 °C to a volume of 100-120 ml. At last, the extraction was poured into special molds and placed in

 Printed in Kazakhstan

a freezer at -20 °C for 12 hours. Then frozen extract was sublimated in the freeze-drying Rime-4.

At the end of the process we obtained 150g of BAC, and the phytochemical composition of the plant and BAC were studied.

Also the standardized BAC was investigated by gas chromatography-mass spectrometry according to the following procedure [10] in order to determinate the liposoluble contents in the conditional phytopreparation.

The operating mode chromatography-mass spectrometer was:

Type column: DB35ms
Detector: mass spectrum Agilent 5975s
Chromatograph: Agilent 7890A
Pressure: 12,051psi
Flow rate: 18 mL / min
Time: 38min

5g BAC were poured in 50ml of chloroform and were stirred for 1 hour and filtered through filter paper. The resulting filtrate was concentrated on a rotary evaporator and dewatered. Then prepared samples are sent to the center of physical and chemical methods of research and analysis for studying the phytochemical composition by gas chromatography-mass spectrometry.

Results and discussion

Object of study is the aerial part of the *Halostachys caspica* from the *Chenopodiaceae* family, collected in the flowering phase in Ili district of Almaty region in 2013 and the BAC obtained form this plant.

These study results of the high quantitative indicators of raw materials were shown in Table 1.

Table 1 – High quantitative indicators from the aerial part of the *Halostachys caspica* and BAC of the plant

Parameter	Content %	
	Plant raw material	BAC
Humidity	4.14	8.48
Total ash	30.24	43.19
Ash insoluble in 10% hydrochloric acid	30.34	38.90
Sulphated ash	35.41	50.58
Extractives (water)	53.76	-
Extractives (50% propyl alcohol)	31.51	-
Extractives (50% ethanol)	41.98	40.05

As can be seen from the data presented in Table 1, the aerial part of the tested plant and its BAC, we can remark the high ash content, which means a significant amount of mineral components in the medicinal plant of raw materials and a high content of extractives on the ground, which may suggest a high content of biologically active substances. Based on the data obtained as the optimal solvents we selected 50% aqueous ethyl alcohol and water, as they are more than 40% recovered from vegetable material.

Also study of the mineral composition of the phytopreparation revealed high content of calcium and sodium compounds and other metals, which explains how the properties of medicinal plants belonging to class *Salicornia* and close proximity to the place of gathering Balkhash metallurgical combine. However, it should be noted that the metal content of the plant material does not exceed the maximum allowable concentrations.

According to generally accepted methods of GF USSR and the State Pharmacopoeia of Kazakhstan was quantified the amount of the main BAS groups. The results are presented in Table 2.

Table 2 shows that in the quantitative content from the aerial part of the *H. caspica* the highest percent of BAS are amino acids, riboflavin, saponins, flavonoids and carotenoids, and in the BAC from this plant, free organic acids, amino acids, carotenoids, alkaloids and flavanoids have the higher index. And we see that the amount of the main groups of biologically active substances increases in more than two times in most of the groups.

Table 2 – The contents of the main biological active substances groups from the aerial part of *Halostachys caspica* and BAC of the plant

Main BAS groups	Content %	
	Plant raw material	BAC
Free organic acids	4.95	13.09
Amino acids	10.56	12.29
Coumarins	0.31	0.60
Carbohydrates	0.21	0.36
Polysaccharides	0.25	0.37
Riboflavin	4.49	5.83
Carotenoids	4.21	9.52
Alkaloids	1.30	14.16
Flavonoids	2.63	18.03
Tannins	2.40	6.10
Saponins	4.23	7.03

In order to extend the study of the BAC we used gas chromatography-mass spectrometry method to determinate the substances in the liposoluble contents. The results are given in Figure 1 and Table 3.

Table 3 – Determined compounds of liposoluble contents of a 50% aqueous ethanol extract from *Halostachys caspica* by using the method of gas chromatography-mass spectrometry

N	RT	Area	Substance
1	9.067	0.13	2-butenal, 3-methyl- 2h pyran
2	9.214	0.25	1-pentene, 3,3-dimethyl- 4-trifluoroacetoxyoctane 1-pentyn-3-ol
3	9.750	0.39	1-butene, 4-methoxy
4	9.980	0.18	2-pentene
5	24.182	0.02	n-heptadecane
6	25.201	0.11	ethyl ester of tetradecanoic acid
7	25.289	0.12	Octadecane
8	25.761	0.16	2-pentadecanone, 6,10,14-trimethyl
9	26.049	0.1	phthalic acid, isobutyl 4-methylpent-2-yl ester
10	26.338	0.11	Nonadecane
11	26.592	0.12	1-pentadecene
12	26.980	0.08	methyl 9,12-heptadecadienoate
13	27.028	0.32	1,2-benzenedicarboxylic acid, buty
14	27.081	0.19	ethyl 9-hexadecenoate
15	27.193	0.01	isohexyl trans-hex-3-enyl ester
16	27.263	6.27	hexadecanoic acid,ethyl ester
17	27.340	0.14	Eicosane
18	27.434	0.13	5-ethyl-8-(trimethylsilylmethyl)dimethylsilyloxydecane
19	27.705	0.05	nonane, 2,5-dimethyl-
20	27.959	0.05	heptadecanoic acid, ethyl ester

Continuation of table 3

N	RT	Area	Substance
21	28.029	0.17	n-heneicosane
22	28.171	0.23	bicyclo(3.3.1)nonane-2,6-dione
23	28.224	0.08	heptadecanoic acid, ethyl ester
24	28.295	0.07	Heneicosane
25	28.336	0.16	9-octadecenoic acid, methyl ester,(e)-
26	28.943	6.33	ethyl oleate
27	29.008	75.67	ethyl oleate
28	29.208	6.62	octadecanoic acid, ethyl ester
29	30.333	0.27	Nonadecane
30	31.152	1.60	.alpha.-(p-chlorobenzoyl)-p-chloroacetophenone
31	31.424	0.65	heptadecanoic acid ethyl ester
32	32.166	0.01	(3.beta.,24r)-(24r)-5-ergosten-3.beta.-ol (24r)-5-ergoste n-3beta-ol
33	32.655	1.51	9-octadecenal, (z)-
34	33.480	0.27	bis(2-ethylhexyl) phthalate
35	34.358	0.13	docosanoic acid, ethyl ester
36	36.415	0.32	Heptacosane

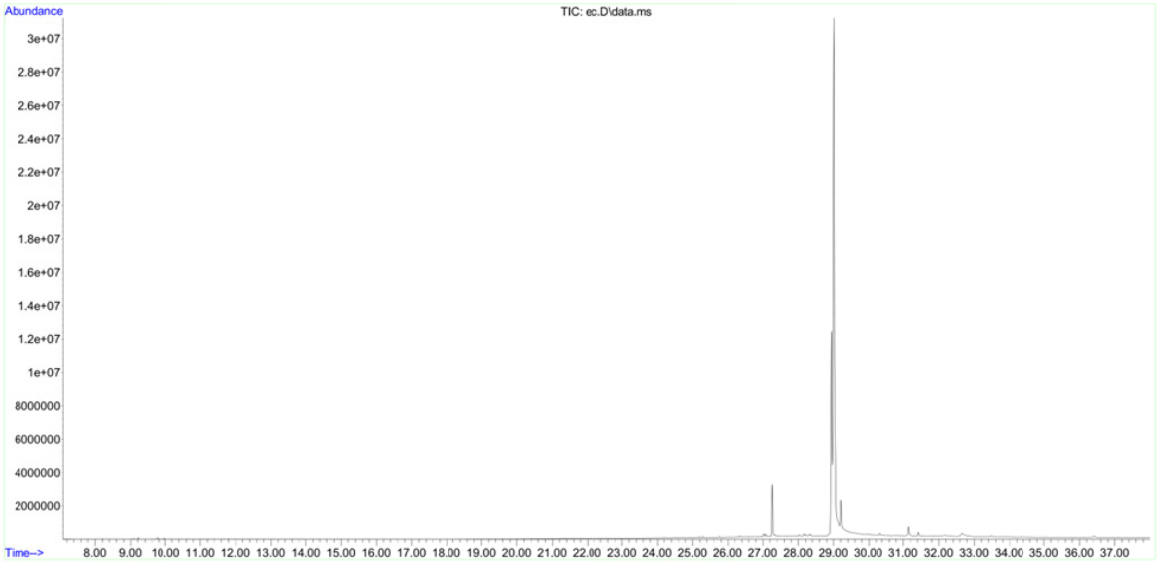

Figure 1 – Gas chromatography-mass spectrum of liposoluble contents of a 50% aqueous ethanol extract from *Halostachys caspica*

These data of the gas chromatography-mass spectrum adduces that most of the compounds in the liposoluble contents in the BAC are esters of higher carboxylic acids (oleic acid ethyl ester, hexadecanoic acid ethyl ester, octadecanoic acid ethyl ester).

Conclusions

We identified the high quantitative indicators and the phytochemical composition of the aerial part of the *Halostachys caspica* and BAC of the plant.

By using gas chromatography-mass spectrometry was done a study of the liposoluble fraction from the hydroalcoholic extract of *Halostachys caspica* by for the first time.

We still go on our studies of the chemical composition and the biological activity of *Halostachys caspica* and phytopreparations obatained from the aerial parts of the plant.

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