OnLine Journal of Biological Sciences

Original Research Paper

Study of Chemical Composition of Extract from *Beta Vulgaris* Seeds and its Cytotoxic Activity

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Article history
Received: 24-03-2021
Revised: 15-04-2021
Accepted: 26-04-2021

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Abstract: Nowadays, a variety of plants is being investigated with the aim of developing new medicines with improved properties and expanding the range of efficient and safe preparations. Modern studies of representatives of the family *Amaranthaceae* (*Beta vulgaris*) have revealed medicinal properties in plants that were used previously only as food. Many medicinal properties are due to the presence of betalains and phenols in the roots and the stems of red beetroot (*Beta vulgaris L.* var. conditiva). Along with this, there is a lack of information on the chemical composition and biological activity of seeds of this species. The purpose of our research was to study the chemical composition of *Beta vulgaris* seeds and to assess the cytotoxic activity. The research objects: CO_2 extracts of beetroot seeds: Thick $POCO_2$ (1), liquid $POCO_2$ (2) and liquid alcohol extract POC_2H_5. Determination of the component composition of the extract was performed on the gas chromatograph Clarus 580 (PerkinElmer) with the mass spectrometric detector Clarus-SQ. To determine the cytotoxic activity, the marine crustaceans *Artemia salina* were taken. Based on the results of the analysis of the chemical composition of the thick CO_2-extract, 25 compounds were identified and the liquid CO_2-extract-11 components were identified. The dominant compounds in both extracts are phenol and creosol. The results of determining the cytotoxic activity allow determining that liquid CO_2 extract of beetroot seeds POC_2H_5 at a concentration of 10 mg/mL exhibits cytotoxicity (68%) and does not exhibit activity at concentrations of 5 and 1 mg/mL; thick CO_2 beetroot seed extract $POCO_2$ (1) at all tested concentrations does not exhibit cytotoxicity; liquid alcohol extract of beetroot seeds at all tested concentrations does not exhibit cytotoxicity.

Keywords: Carbon Dioxide, Seeds Alcohol Extract, Cytotoxicity, *Beta vulgaris*, Component Composition, Biological Activity

Introduction

Since ancient times, plants have been used by folk medicine for the treatment of various ailments (Kukovyakina *et al*., 2019). With the development of technology, people have learned to identify various active substances to develop new medicines with improved properties and expand the range of efficient and safe preparations for the treatment and prevention of a number of diseases in humans and animals.

To combat the negative influence of the environment, plants use a multicomponent defence system, including a hypersensitivity reaction, strengthening of protective barriers with the use of cell wall components and activation of the production of protective proteins and antimicrobial peptides (Goyal and Mattoo, 2014). Plants of the family *Amaranthaceae* (*Chenopodiaceae*), which have long been used both as a food product and as medicinal plants, are no exception (Clifford *et al*., 2015; Asgary *et al*., 2016).

Modern studies of representatives of this family have made it possible to discover medicinal properties in plants that were used previously only as food. Thus, in the leaves of *Beta vulgaris* L, the protein Beetin 27 (BE27), which inactivates the Ribosome (RIP), was found. Beetin 27 is supposed to be an antiviral protein induced by viruses and

signalling compounds such as hydrogen peroxide and salicylic acid. Its role as a protective protein is explained by its activity of the RNA polynucleotide: Adenosine glycosidase (Iglesias *et al*., 2005; 2015).

In vitro experiments have shown the inhibitory effect of *Beta vulgaris* root extract on the induction of the early antigen of the Epstein-Barr Virus (EBV-EA). Evaluation of the antitumor stimulating activity in vivo against bio samples of skin and lungs of mice also revealed a significant inhibitory effect of root extract of beetroot (Kapadia *et al*., 1996).

Aqueous extracts from *Beta vulgaris* root vegetables have shown antioxidant and antimicrobial activity in the experiments in vitro (Čanadanović-Brunet *et al*., 2011). Many medicinal properties are due to betalains and phenols found in the roots and the stems of red beetroot (*Beta vulgaris* L. var. conditiva) (Clements *et al*., 2014; Ben Haj Koubaier *et al*., 2014; Barlow *et al*., 2018). Despite the fact that the composition and properties of leaves and underground organs of *Beta vulgaris* are actively studied, there is a lack of information on the chemical composition and biological activity of seeds of this species.

We carried out preliminary studies with the seeds of *Beta vulgaris*, which showed the protective effect of aqueous extracts in the experiment in vivo (Konkabayeva *et al*., 2020).

In connection with the above, the purpose of our research was to study the chemical composition of *Beta vulgaris* seeds and to assess the cytotoxic activity.

Materials and Methods

Research Objects

CO_2 extracts of beetroot seeds: Thick $POCO_2$ (1), liquid $POCO_2$ (2) and liquid alcohol extract POC_2H_5.

CO_2-extracts were prepared on a CO_2-extractor from air-dry seeds at a pressure of 69-76 atm and a temperature of 18-21°C (Sisengalieva *et al*., 2015).

Determination by Gas Chromatography-Mass Spectrometry Method

The determination was carried out by gas chromatography-mass spectrometry on the gas chromatograph Clarus 580 (PerkinElmer). The detector was a mass spectrometric detector Clarus-SQ 8 with a NIST base of 300,000 compounds (the table shows the Kovats indices on a semi-polar column).

Method of Determining the Component Composition

Determination of the component composition of the extract was carried out on a gas chromatograph Clarus 580 (PerkinElmer) with a mass spectrometric detector Clarus-SQ 8. The sensitivity of the device is determined by the peak signal-to-noise ratio and is 800:1 (in reality, 2000:1).

Chromatographic conditions: capillary column Restek®-5Sil MS 0.25 mm ×30 m ×0.25 μm, sample volume: 1.0 μL, carrier gas: He; flow rate of carrier gas: 1 mL/min, flow division 1:25, t of the column: 40°C, temperature rising of 2°C/min up to 280°C, t of evaporator -280°C, mass spectrometric detector: t-240°C, EI+ = 70 eB, scanning time: from 4 to 120 min, ion scanning mode: 39-500 m/z. The percentage of components was calculated automatically based on the peak areas of the total ion chromatogram. The components were identified by mass spectra and retention times using the NIST library and comparing with the retention indices of normal alkanes.

Study of Cytotoxic Activity

The study of the cytotoxic activity of CO_2-extracts of beetroot seeds (thick $POCO_2$ (1) and liquid $POCO_2$ (2), liquid alcohol extract POC_2H_5) was conducted in the laboratory of the Institute of Applied Chemistry at L.N. Gumilyov Eurasian National University, according to the standard method (Terekhova, 2011).

To determine the cytotoxic activity, the marine crustaceans *Artemia salina* were taken. The technique is based on establishing the difference between the number of dead Artemia larvae in the analyzed sample (experiment) and water that does not contain toxic substances (control). The criterion for acute lethal toxicity of a solution of a substance is the death of 50% of the larvae and more in the experiment as compared with the control.

Dilution was made at the rate of 1 mg of substance per 1 mL of solvent. Each sample was tested in three parallel runs. It was carried out at a temperature of 20±2°C, at natural light period. The salinity of the control artificial water was 8.0-8.5 (pH). During the biotesting, Artemia larvae were up to 1-day old. The stocking density of larvae is 20-40 specimens per test tube.

Results

Based on the results of the analysis of the chemical composition of the thick CO_2-extract, 25 compounds were identified and the liquid extract -11 components were identified (Table 1, 2 and Fig. 1, 2).

The dominant components in both extracts are phenol and creosol. The first sample contains phenol -0.7%, cresol-0.5% of all volatile substances. The second sample contains phenol -0.4%, cresol -0.3% of all volatile substances.

Cytotoxic Activity

We studied the cytotoxic activity by the method of survival rate of the marine crustacean *Artemia salina*. The test flask was filled with artificial seawater and eggs of *Artemia salina* were added. *Artemia salina*. They were kept under a soft air supply for 3 days until the crustaceans hatched from their eggs.

Table 1: Component composition of thick CO_2-extract of beetroot seeds (sample 1)

	RI lit	RII	Component	Area %
	859±6	851	2-Furanmethanol	0.3
	918±3	910	2(5H)-Furanone	0.3
	980±4	980	Phenol	0.7
	1013 iu	999	2-Hydroxy-gamma-butyrolactone	1.1
	1043±0	1034	1,2-Cyclopentanedione, 3-methyl-	1.8
	1090±3	1078	Phenol, 2-methoxy-	1.8
			Unknown 1	1.0
			Unknown 2	6.3
	1110±6	1118	Maltol	0.4
	1091±17	1123	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	0.4
	1169±3	1164	Phenol, 4-ethyl-	0.4
	1193±3	1178	Creosol	0.5
	1129 iu	1192	5-Oxotetrahydrofuran-2-carboxylic acid, ethyl ester	1.0
	1205±5	1201	Catechol	1.8
	1207±8	1209	1,4:3,6-Dianhydro-à-d-glucopyranose	1.9
		1218	Unknown 3	0.8
	1268±4	1247	Pyrocatechol, 3-methoxy-	0.3
	1282±4	1264	Phenol, 4-ethyl-2-methoxy-	0.2
	1241±N/A	1288	Hydroquinone	0.5
	1355±5	1342	Phenol, 2,6-dimethoxy-	1.4
	1404±7	1389	Vanillin	0.4
			Unknown 4	1.0
	1534±5	1508	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	4.1
			Unknown 5	10.7
	1968±7	1970	n-Hexadecanoic acid	1.4
Total				40.5

Table 2: Component composition of liquid CO_2-extract of beetroot seeds (sample 2)

RI lit	RII	Component	Area %
980±4	982	Phenol	0.4
1090±3	1080	Phenol, 2-methoxy-	1.0
1029 iu	1095	1H-Pyrrole, 2,5-dihydro-1-nitroso-	0.4
		Unknown 1	0.6
1169±3	1165	Phenol, 4-ethyl-	0.6
1193±3	1179	Creosol	0.3
1207±8	1209	1,4:3,6-Dianhydro-α-d-glucopyranose	0.2
1282±4	1265	Phenol, 4-ethyl-2-methoxy-	0.4
1355±5	1343	Phenol, 2,6-dimethoxy-	0.3
		Unknown 2	0.3
		Unknown 3	1.0

Table 3: CO_2 extract of beetroot seeds POC_2H_5, (liquid), 10 mg/mL

	Number of larvae in the control		Number of larvae in the sample			% of surviving larvae in	% of surviving larvae in	Mortality	Presence of
Parallel	Survivor	Dead	Survivor	Dead	Par.	the control	the sample	rate, A, %	neurotoxicity, %
1	25	2	6	14	0	96	28	68	0
2	24	0	5	18	0				
3	23	0	7	16	0				
Average	24	1	6	16	0				

Actinomycin D or staurosporine was used as a referential preparation. Samples were tested at concentrations of 10, 5 and 1 mg/mL. The results of studies of cytotoxic activity for the liquid extract are presented in Table 3-5.

Discussion

Based on the performed experiment, it can be assumed that liquid CO_2 extract of beetroot seeds POC_2H_5 at a concentration of 10 mg/mL exhibits

cytotoxicity (68%) and does not exhibit activity at concentrations of 5 and 1 mg/mL.

The results of the study of the cytotoxic activity of the thick CO_2 extract of beetroot seeds $POCO_2$ (1) are shown in Tables 6-8.

Based on the experiment performed, it can be assumed that the thick CO_2 extract of beetroot seeds $POCO_2$ (1) does not exhibit cytotoxicity at all tested concentrations.

The results of the study of the cytotoxic activity of the liquid alcohol extract of beet seeds are presented in Tables 9-11.

Table 4: CO_2 extract of beetroot seeds POC_2H_5, (liquid), 5 mg/mL

	Number of larvae in the control		Number of larvae in the sample			% of surviving larvae in	% of surviving larvae in	Mortality	Presence of
Parallel	Survivor	Dead	Survivor	Dead	Par.	the control	the sample	rate, A, %	neurotoxicity, %
1	25	2	10	10	0	96	48	48	0
2	24	0	9	14	0				
3	23	0	10	10	0				
Average	24	1	10	11	0				

Table 5: CO_2 extract of beetroot seeds POC_2H_5, (liquid), 1 mg/mL

	Number of larvae in the control		Number of larvae in the sample			% of surviving larvae in	% of surviving larvae in	Mortality	Presence of
Parallel	Survivor	Dead	Survivor	Dead	Par.	the control	the sample	rate, A, %	neurotoxicity, %
1	25	2	23	0	0	96	96	0	0
2	24	0	22	0	0				
3	23	0	22	0	0				
Average	24	1	22	0	0				

Table 6: CO_2 extract of beetroot seeds $POCO_2$ (1), (thick), 10 mg/mL

	Number of larvae in the control		Number of larvae in the sample			% of surviving larvae in	% of surviving larvae in	Mortality	Presence of
Parallel	Survivor	Dead	Survivor	Dead	Par.	the control	the sample	rate, A, %	neurotoxicity, %
1	25	2	17	3	0	96	86	10	0
2	24	0	18	3	0				
3	23	0	18	4	0				
Average	24	1	18	3	0				

Table 7: CO_2 extract of beetroot seeds $POCO_2$ (1), (thick), 5 mg/mL

	Number of larvae in the control		Number of larvae in the sample			% of surviving larvae in	% of surviving larvae in	Mortality	Presence of
Parallel	Survivor	Dead	Survivor	Dead	Par.	the control	the sample	rate, A, %	neurotoxicity, %
1	25	2	20	3	0	96	88	8	0
2	24	0	21	3	0				
3	23	0	24	2	0				
Average	24	1	22	3	0				

Table 8: CO_2 extract of beetroot seeds $POCO_2$ (1), (thick), 1 mg/mL

	Number of larvae in the control		Number of larvae in the sample			% of surviving larvae in	% of surviving larvae in	Mortality	Presence of
Parallel	Survivor	Dead	Survivor	Dead	Par.	the control	the sample	rate, A, %	neurotoxicity, %
1	25	2	19	2	0	96	96	0	0
2	24	0	20	1	0				
3	23	0	24	1	0				
Average	24	1	21	1	0				

Table 9: Liquid alcohol beetroot seed extract, 10 mg/mL

	Number of larvae in the control		Number of larvae in the sample			% of surviving larvae in	% of surviving larvae in	Mortality	Presence of
Parallel	Survivor	Dead	Survivor	Dead	Par.	the control	the sample	rate, A, %	neurotoxicity, %
1	25	2	22	7	0	96	75	21	0
2	24	0	17	5	0				
3	23	0	15	7	0				
Average	24	1	18	6	0				

Table 10: Liquid alcohol beetroot seed extract, 5 mg/mL

	Number of larvae in the control		Number of larvae in the sample			% of surviving larvae in	% of surviving larvae in	Mortality	Presence of
Parallel	Survivor	Dead	Survivor	Dead	Par.	the control	the sample	rate, A, %	neurotoxicity, %
1	25	2	20	2	0	96	87	9	0
2	24	0	18	4	0				
3	23	0	18	4	0				
Average	24	1	19	3	0				

Table 11: Liquid alcohol beetroot seed extract, 1 mg/mL

	Number of larvae in the control		Number of larvae in the sample			% of surviving larvae in	% of surviving larvae in	Mortality	Presence of
Parallel	Survivor	Dead	Survivor	Dead	Par.	the control	the sample	rate, A, %	neurotoxicity, %
1	25	2	24	1	0	96	92	4	0
2	24	0	23	3	0				
3	23	0	24	2	0				
Average	24	1	24	2	0				

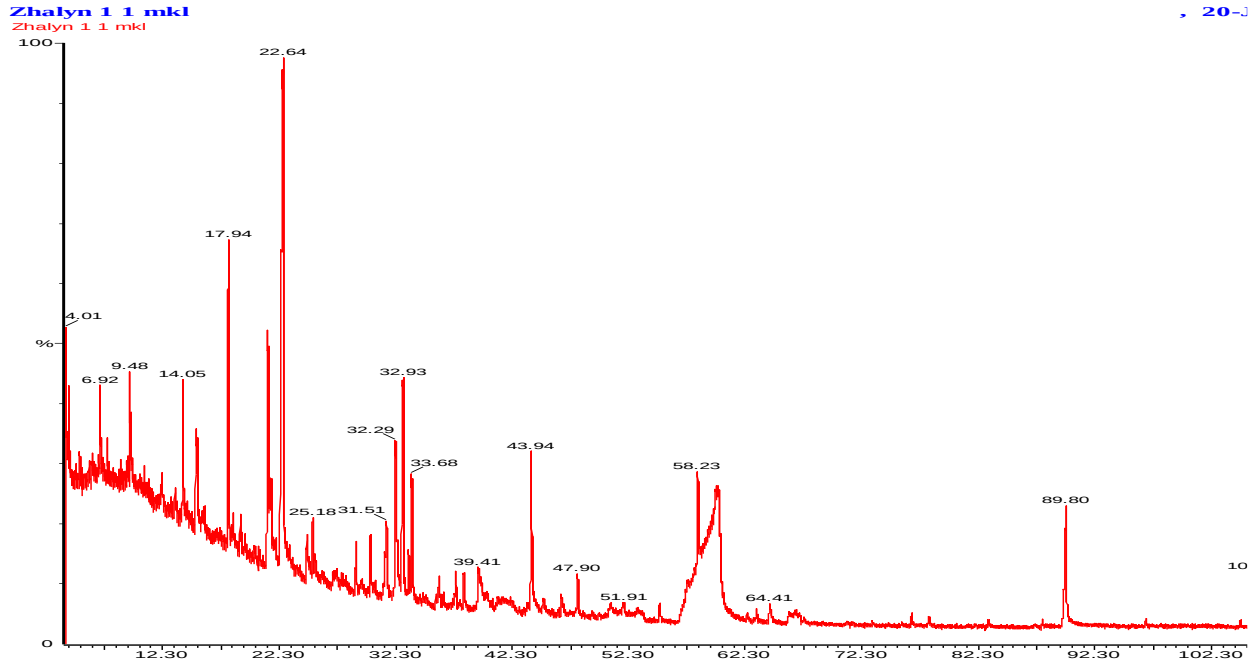

Fig. 1: Chromatogram of thick CO_2-extract of beetroot seeds (sample 1)

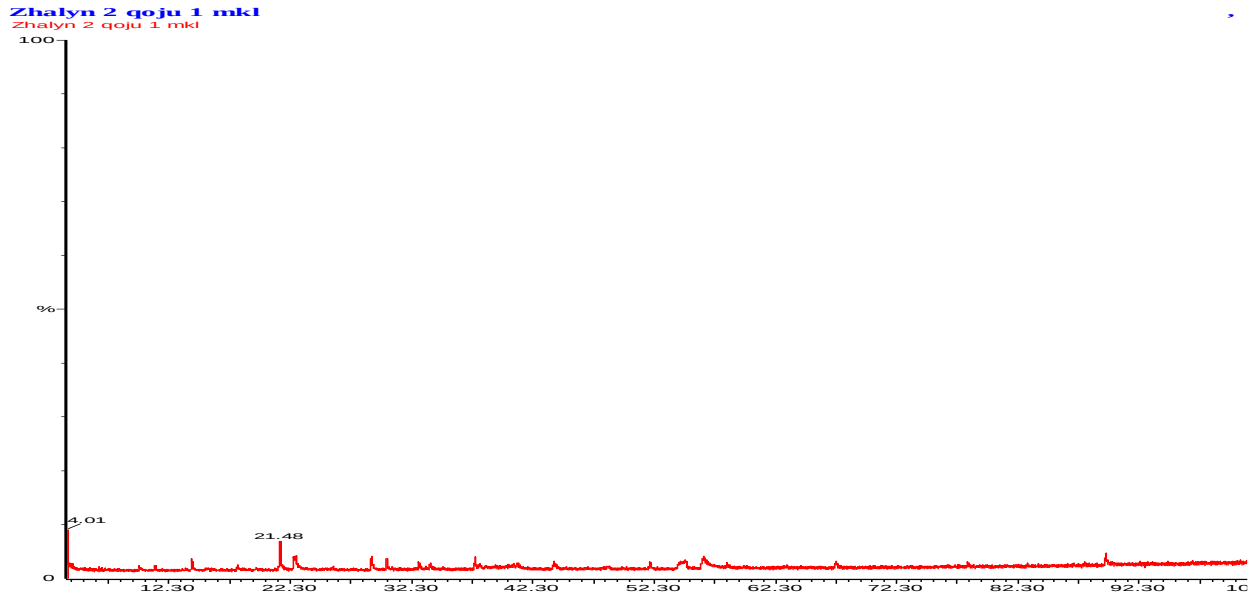

Fig. 2: Chromatogram of liquid CO_2-extract of beetroot seeds (sample 2)

Based on the experiment performed, it can be assumed that the liquid alcohol extract of beetroot seeds at all tested concentrations does not exhibit cytotoxicity.

Conclusion

Thus, a study of the component composition of CO_2-extracts from common beetroot seeds was carried out, according to the results of which 25 (for the thick extract) and 11 (for the liquid extract) components were identified. The dominant components are phenol and cresol.

The results of determining the cytotoxic activity allow determining that liquid CO_2 extract of beetroot seeds POC_2H_5 at a concentration of 10 mg/mL exhibits cytotoxicity (68%) and does not exhibit activity at concentrations of 5 and 1 mg/mL; thick CO_2 beetroot seed extract $POCO_2$ (1) does not exhibit cytotoxicity at all tested concentrations; liquid alcohol extract of beetroot seeds at all tested concentrations does not exhibit cytotoxicity.

Acknowledgement

The work was carried out within the framework of an internal grant from E.A. Buketov Karaganda University No. 219-B-19 “Study and evaluation of the medicinal properties of seeds of the plant *Beta vulgaris* of the family Amaranthaceae in the experiment”.

Author’s Contributions

Aiman Konkabayeva: Concept, manuscript writing, critical revision of manuscript, final approval.

Yerlan Suleimen: Concept, manuscript writing, data collection, analysis, final approval.

Margarita Ishmuratova: Analysis, manuscript writing, data interpretation, final approval.

Gulmira Tykezhanova: Data interpretation, manuscript writing, critical revision of manuscript, final approval.

Aidana Yerubay: Analysis, data collection, data interpretation, final approval.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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