International Journal of Environmental Engineering– IJEE
Volume 2 : Issue 1 [ISSN : 2374-1724]
Publication Date: 30 April, 2015

Optimal process of obtaining of biologically active substance from Euphorbia soongarica Boiss.

[Sluken Rakhmadiyeva, Dina Kassenova, Darya Kushnarevich]

***Abstract*— CO_2 – extract for removal of poisonous milk sap and resin was used in order to optimize the process of obtaining of the number of biologically active substance (BAS) from the herb *Euphorbia soongarica Boiss.*, growing on the territory of Kazakhstan and having a wide range of biologic activeness. Component composition of CO_2 – extract was identified with the method of HGLC (high gas-liquid chromatography). Hygrophilous components obtained from defatted product are represented by polyphenols, amino acids and carbohydrates. Their quantitative content was identified with the method of complexometry, spectrometry and amino acid analysis. Mineral composition was identified in ash residue.**

***Keywords*— *Euphorbia soongarica Boiss*., BAS, technology, CO2-extract, methods.**

I. Introduction

Herbs of genus Euphorbia L. is very interesting as a source of biologically active substance and renewable feedstock, this herb is used for treatment of different diseases in Chinese, Korean, Indian and Tibetan folk medicine for a long time [1]. For herbs of *Euphorbia L*. genus antitumor, proteoclastic [2], antimicrobial and bactericidal activity [3] are identified. Number of BAS of the herb *Euphorbia soongarica Boiss*. possess antitumoral, anti-inflammatory, bactericidal, anti-hypoxic, antioxidant, immunity modelling activity. Resins and milk sap are used in folk medicine for removal of pigment spots and carbuncles [2]. The process of defatting of herbal substances is very important during emission and segregation of substances of polyphenol nature into individual components and during development of methods of BAS production. In the past such chemicals as benzole, chloroform, hexane and acetone were used for defatting. During extract of substance with benzole and hexane full extract of chlorophylls, carotenes occurs, but resins are poorly extracted and they remain in oil cake. Extraction with acetone leads to full extraction of not only hydrophobias but also hydrophilic components such as flavonoids. Full removal of lipophilic components is observed during extraction with chlorophorm. But because of their toxicity during optimization of technology of extract of BAS at the stage of de-resining resource-saving technology was applied with application of carbon dioxide extraction [4].

S. Rakhmadiyeva, D.Kassenova, D. Kushnarevich
L.N. Gumilyov Eurasian National University
Kazakhstan

II. Materials and methodology

A. Plant material and reagents

The plant materials were collected from Almaty region during flowering stage. The sample was collected in 2005-2008 Republic of Kazakhstan. Certified reference standard: tannin, quercetin. Carbon dioxide (99,99% purity), contained in a cylinder with an eductor tube. In work were use chemicals from Sigma-Aldrich Chem. (Germany).

B. Procedure for the preparation of biologically active substances

- Preparation of plant material. Breaking of dried grass. Standard unit FBU-2 (feed-breaking unit) with the capacity of 30 kat is used. Breaking is performed in batches right before extraction (for better opening of plant cell and extraction of its lipophilic part). Feedstock losses at this stage of feedstock processing are 5% of gross mass.
- Carbon dioxide extraction. Defatting is performed with liquid carbon dioxide. Broken mass is filled into two extractors and sealed. After that flushing with liquid carbon acid is started at the temperature of 25C and pressure of 65 bars. The duration of extraction is 5 hours. After first extraction the pressure is dropped down to 0 bars in the unit and feedstock is filled into bags and then it is broken for the second time and then extracted with addition of some amount of fresh broken herbs (considering losses during breaking and refilling). After second extraction defatted, fine and sterilized herbs (oil cake) is put into bags by weight and packed. Feedstock losses at the second stage are 0,2 – 0,4 %.
- GS and GS/MS analyses. GS analyses were performed using a Hewlett-Packard 5890 series II gas chromotograph (Folsom, CA, USA) equipped with mass-selective detector HP-5972. Experimental conditions: capillary column HP-5 length 32 m (diameter 0.25 mm, carrier gas -helium. Total flow rate 29 ml/min, flow split 1:20). Temperature gradient is from 50 to 280C^0 within 10 minutes. The sample was injected with concentration of 1% in methanol. Identification of individual compounds was performed automatically by analogy with mass spectrum of samples, entered in computer data bank of the device. Only samples that had degree of confidence above 90% of the analog were taken into account [5].

SEEK DIGITAL LIBRARY

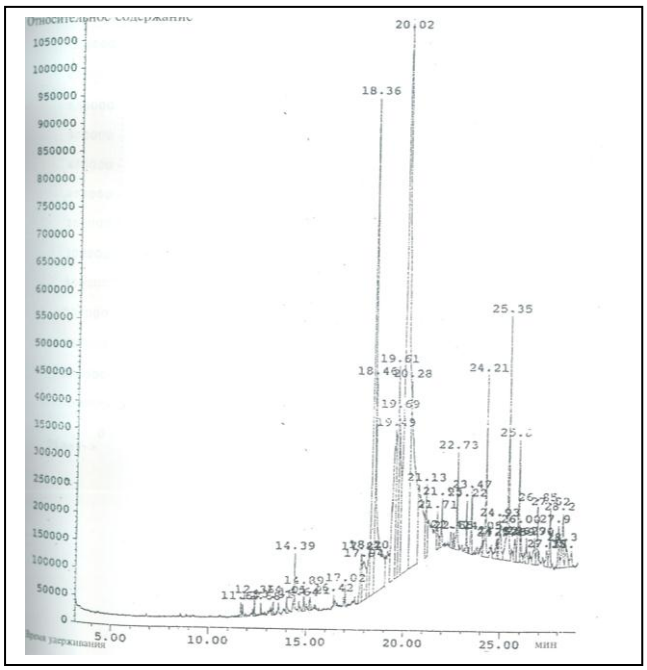

Figure 1. HGLC analysis of CO_2–extract of *Euphorbia soongarica Boiss*

- Extraction of defatted feedstock with organic solvent. 0,5 kg *of Euphorbia soongarica* defatted herb is placed in flat-bottomed flask, fill with 1,85l of water acetone (one-to-one) and leave for 5 hours. The extract is poured out and oil cake is filled with fresh portion of water acetone and then extract for 5 hours again. This procedure is repeated two times. Water-acetone extraction is united in one vessel with volume of 2-2,5 l. The feedstock is taken out and dried out under the air stream. Under production conditions it is recommended to perform extraction at room temperature during stirring. Water-acetone extract is concentrated at temperature 50-55C^0, at under-pressure of 0,6 Atm. up to 1/3 of the volume, water residue is concentrated at temperature of 55-60C^0 at under-pressure of 0,95-1 Atm. until it dries out and turns into powder – end product. The extract is amorphous powder of brownish color with inclusions of darker particles with slight specific smell, water-absorbing. It is soluble in water-alcohol mixtures, insoluble in benzole, chloroform and ester.
- Stripping of the extract. Stripping of water-acetone extract is performed up to 1/3 of the volume at the temperature of 50-55%C^0 and at under-pressure of 0,6 Atm. After that water residue is concentrated at temperature of 55-60C^0 and at under-pressure of 0,95 – 1 Atm., until dry powder is produced – end product.
- Standardization of CO_2 – extract and total BAS. In order to standardize CO_2 – extract of *Euphorbia soongarica* as per methods accepted in State Pharmacopoeia of the USSR XI refraction index, humidity, acid number and solubility were identified.

TABLE I. CHEMICAL COMPOSITION OF CARBON DIOXIDE EXTRACT OF *EUPHORBIA SOONGARICA BOISS*..(HGLC, time 5-25 min, t - 50 - 280C^0)

№	Name	Structural formula	Split vent time	Persentage, %
1. Fatty acids (63,54%)				
1	myristic acid	$CH_3(CH_2)_{14}COOH$	18.37	10.73
2	ethyl ester of palmitic acid	$CH_3(CH_2)_{14}COOC_2H_5$	18.46	7.93
3	methyl ester of stearic acid	$CH_3(CH_2)_{16}COOCH_3$	19.69	0.12
4	oleic acid	$CH_3(CH_2)_7$-CH=CH-$(CH_2)_7$-COOH	19.49	6.07
5	linoleic acid	$CH_3(CH2)_3$-CH=CH-CH_2-CH=CH$(CH_2)_7$COOH	19.99	28.16
6	methyl ester of linoleic acid	$CH_3(CH_2)_3$-CH=CH-CH_2-CH=CH$(CH_2)_7$COOCH_3	19.61	4.34
7	aldehyde of linoleic acid	$CH_3(CH_2)_3$-CH=CH-CH_2-CH=CH$(CH_2)_7$COH	20.28	10.65
2. Fatty hydrocarbons (11.15%)				
8	octadecane	C_8H_{38}	21.13	1.47
9	eicosane	$C_{20}H_{42}$	24.26	5.79
10	dotrikontan	$C_{32}H_{66}$	24.21	2.05
11	tetratikontan	$C_{34}H_{70}$	25.88	1.81
3.Terpenes and oxygenated derivatives				
12	squalene	$C_{30}H_{50}$	25.35	3.46
4. Oxygen compounds of hydrocarbons (28.35%)				
13	R(-)-14 methyl-8-hexadecin-1-ol		20.28	10.65
14	cyclopropyl naphthalene 2-(4-en)-on		20.37	9.76
15	tetrahydroxycyclopentadienone-3		21.71	0.56
16	bis (2-ethylhexyl) phthalate		23.14	4.53
17	α-ethyl-1-naftaleneprop anol		25.20	2.92

For the purpose of standardization of total BAS following indicators such as composition, description solubility, authenticity, quantitative estimation, content of heavy metals, residual quantity of acetone wereestablished.For quantitative estimation of tannins complexonometric and permanganatometric methods were applied. The Complexonometric method is based on precipitation of tannins from water-alcohol mixture of 1% solution of zinc salt in ammonium

SEEK DIGITAL LIBRARY

TABLE II. DATA ANALYSES OF *EUPHORBIA SOONGARICA BOISS.* BEFORE AND AFTER LIQUEFIED CARBON DIOXIDE PROCESSING

№	Index, %	Before CO2 processing, %	After CO2 processing, %
1	Loss on drying	9,74	8,9
2	Ash	8,93	12,86
3	Extractive substances	29,65	27,88
4	Hydrolysable tannin	6,4	6,68

buffer pH = 9-10 in the form of insoluble residue with further decomposition of complex in acid and complexonometric titration of zinc ions with solution of trilon B with xylenol orange indicator. Tannin is used as a standard [6].Quantitative estimation of flavonoids was performed with the method of spectophotometery using SP-46 device, and quercetine was used as a standard [7].Content of phenol acid was identified with a method of neutralization 0,1 n NAOH with the use of methylorange indicator [8].Content of amine acids was identified with the method of photometry [9], mineral compounds were identified with spectrographic method using DFS-13 equipment.

III. Results and discussion

The purpose of this work is to develop optimal method of obtaining of total of biologically active substances from the herb *Euphorbia soongarica Boiss*, which possesses hepatoprotective, immunity-modelling and antioxidant activity.

Phytochemical study of above-ground parts of *Euphorbia soongarica* indicated presence of polyphenol compounds: hydrolyzed tanning agents, phenol acids, flavonoids; also presence of free carbohydrates, amine-acids, micro-elements and minors. Milk sap of the herb *Euphorbia* is poisonous and it consists of lipophilic components: resins, chlorophylls A and B, carotenoids. Milk sap is contained mainly in new-gathered feedstock where the content of lipophillic components is insignificant and stays within the range of 0,5-0,8%.

One of the perspective approaches of processing of herbal raw material is extraction with light solvents. This method allows for high intensification of the process and there is a possibility of selective extraction of substances. An important stage of obtaining of hygrophilous biologically active components from Euphorbia is a stage of defatting (resin extraction), because herbal Euphorbia contains milk sap and resins both in the aboveground and underground part that prevent from splitting and purification of hygrophilous components. During defatting of herbal raw materials with benzole and hexane full extraction of chlorophylls and carotenoids was observed and resins were not fully extracted.

TABLE III. DATA ANALYSES OF THE PREPARATION OBTAINED BY DIFFERENT EXTRACTIONS

№	Preparations	Humidity, %	Ash content ,%	Flavonoids, %	Amino acids, %	Phenolic acids, %	Tannins	
							Permanganatometry, %	Complexometry, %
1	Preparation 1 (water.alcohol)	13,2	14,1	2,87	7,7	10,29	40,7	19,3
2	Preparation 2 (water.acetone)	11,2	10,3	5,07	14,7	20,76	67,8	40,3

During extraction with acetone not only lipophillic components were extracted but also flavonoids. Only extractions with chloroform lead to full extraction of lipophillic components. In order to exclude and minimize spending of fire hazardous and toxic chemicals and their emissions to environment these chemicals were substituted with environmentally friendly chemicals. For this reason environmentally clean, non-energy intensive and economic technology was applied with the use of liquid carbon acid that helps to keep thermal and oxy instable in the extract at the stage of resin extraction. Liquefied carbon acid has a number of positive properties as a solvent: it is chemically indifferent in relation to extracted substances and fully evaporates from them, it is inflammable, not explosive and during treatment with this agent it sterilizes raw material and it is permitted for use in production of food products [10]. Treatment of raw materials is performed in low temperature mode in inert gas ambience. Unlike extraction with organic solvents CO_2-extraction provides full extraction of lipophillic components from herbal stock and increases production of end products – total of polyphenol substances by 15-20%, reduces consumption of organic solvents at further stages of extraction of hygrophilous components, reduces the process duration because it excludes the process of drying of raw material and sterilizes it, which is important for storage of a large volume of feedstock after defatting.

CO_2 – extract of *Euphorbia soongarica Boiss*. was examined for content of fatty acids and volatile substances with a method of highly effective gas-liquid chromatography. It was identified that resin components in examined CO_2 – extract are fatty, unsaturated acids: linolic acid (28,6%), its aldehyde (10,65%) and oleic acid (6,07%). As for fatty acids prevail palmic acid (7,93%), myristic acid (10,73%), hydrocarbons are represented by eicosane (1,11%), squalen (3,46%), tetratriacontane (2,05%), dotriacontane (0,57%), octadecane (1,47%). (*Fig.1*, Table I).

An analysis of raw material before treatment and after treatment with carbon dioxide was performed in order to assess an impact of resin extraction on the quality of raw materials and content of hydrolyzed tanning substances in it. Following process parameters were applied: humidity, ash-content, extractive substances and tanning substances (Table II).

SEEK DIGITAL LIBRARY

TABLE IV. CONTENT OF MINERAL COMPOUNDS IN *EUPHORBIA SOONGARICA BOISS.*

Element	**barium**	**arsenic**	**cobalt**	**boron**	**phosphorus**	**manganese**	**lead**	**copper**	**chrome**	**gallium**	**vanadium**
Percentage, %	0	0	6	20	15	5	2	4	2,5	3	0,6
Element	**nickel**	**beryllium**	**titanium**	**tin**	**molybdenum**	**zirconium**	**zinc**	**scandium**	**silver**	**strontium**	**yttrium**
Percentage, %	0	2	0,3	2	15	10	6	8	5	8	1,5

Qualitative test of defatted raw material showed that extraction with liquefied carbon dioxide does not have any impact on the main components – hydrolyzed tanning substances, does not destruct them, oxidize them and does not change their quantitative content.

Total of polyphenol compounds in examined herb was obtained by extraction of defatted raw material for 3-5 days with water acetone or water alcohol of different concentrations, and further stripping of solvent until obtaining of the end product – total of BAS.

For selective extraction, in particular hydrolyzed tanning substances a study was performed for application of different extracting agents for treatment of the same herbal material. Optimal extraction conditions were defined during the experiment: type of raw material (above-ground part at the phase of blossoming), fineness factor (3-4mm), exposure to humidity (air-dry), extraction agent (water acetone 50-70%), method of extraction (maceration), time (3-5 days at room temperature), raw material – extraction agent ratio (1:8). For further improvement of extraction method and in order to exclude of fire hazardous solvents a possibility of substitution of extraction of defatted herbal raw material with extraction with water alcohol. The results of experimental studies indicated that extraction capacity of water acetone is higher than extraction capacity of water alcohol and this reflected in the quantity of acting substances (hydrolyzed tanning substances) (Table III). Content of tanning substances during extraction with water acetone is twice higher. Application of water acetone increases solubility of natural compounds by generation of poly-component system where molecules of the solvent, substance and water are connected with each other by hydrogen bonds. This allows for reduction of unit consumption norms in the process flowsheet.

Hydrolyzed tanning substances are leading group of active substances of polyphenol complex of the herb *Euphorbia soongarica* because this group of substances, according to information contained in literature, has high antineoplastic activity after examination of different extracts of herbs. For this reason standardization of raw material was performed based on the content of hydrolyzed tanning substances. In order to confirm nature of hydrolyzed tanning substances we used two qualitative reactions: reaction on pyrogallic group with salts of ferric iron and on ellagotannins with sodium nitrite in acid ambience. Hydrolyzed tanning substances consist of monomers and dimmers of ellagitannins, zoongarine A, B, C and geraniine. For analysis of the raw material for tanning substances an official method of permanganatometric titration was used as express method. Content of hydrolyzed tanning substances in dried raw material is within the range from 4,71 to 5%, identified by the method of complexometry; and from 9 to 11,2% - by the method of permanganatometry. Out of these two method of complexometric titration is more accurate. Both methods were used with calculation of tanning.

Phenol acids are represented by gallic, ellagic, dehydro-degallic and protocatechuic acids; flavonoids are represented by quercetine and its mono-glycosides: hyperene and quercitrine. Content of phenol acids did not exceed 20%, total of flavonoids was not less than 3%. Complexes of microelements with polyphenols in herbs considerably exceed their biologic activity due to better absorption by organism. The presence of glucose, rhamnose, arabinose, fructose, 20 amino acids was established, in which cysteine, alanine, tyrosine, glutamine are on large amounts. [11]. The content of free carbohydrates is not more than 15%, the content of amino acids is not more than 20%, and for elements is not more than 0.29%. The mineral composition of the ash amount of BAS was determined and identified 19 micro-and macroelements. In which phosphorus, boron, manganese and titanium are in large amounts (Table IV). The content of microelements corresponds to the MAC.

IV. Conclusion

An optimal technology of obtaining of polyphenol substances from the herb *Euphorbia soongarica Boiss* was established. Polyphenol substances are extracted by the method of defatting of raw material with liquefied carbon deoxide that has a number of advantages against toxic and fire hazardous chemicals: high level os extraction of lipophillic components is achieved, nativeness of components remains and there is a possibility of a complex application of the raw material.

Acknowledgment

This publication was made as part of the Sub-project “Development of regulatory technical documentation for the original medical product made of local herbal raw material that possesses hepatoprotective activity”, financed as part of the grant program Project “commercialization of technologies” of the component 2: “Development of bonds between science

SEEK DIGITAL LIBRARY

and production" of "Center of commercialization of technologies".

References

[1] Ибрагимов Ф. И., Ибрагимова Б.С. Основные лекарственные средства китайской народной медицины. М.: Медгиз, 1960, 412 с.

[2] Chopra R.N., Nayar S.L., Chopra I.S. Glossary of Indian medicianal plants. – New – Delhi, 1956, 330 p.

[3] Zhbe Txcon. Лекарственные растения. М.: Медицина, 1987, 405 с.

[4] Государственная фармакопея Республики Каахстан. Том II, Астана, 2009, с 821-822.

[5] Knipe, C.R., Miles, W.S., Rowland, F. and Randall, L.G., Designing a Sample Preparation Method that Employs Supercritical Fluid Extraction. Hewlett-Packard Company, USA, part no. 07680-90400, 1993.

[6] Государственная фармакопея СССР: вып. 2 Общие методы анализа. Лекарственное растительное сырье.. 11 изд. Медицина, Москва, 1991, 400 с.

[7] Беликов В.В., Точкова В.В., Калесник Н.Т. Унифицированная методика определения флавоноидов для стандартизации фитохимических перпаратов // Всесоюз. конф. «Новые лекарственные препараты из растений Сибири»: Тезисы докл.- Томск, 1989, 21 с.

[8] Пашинина Л.Т. Методические указания к практикуму по качественному и количественному анализу природных полифенолов и углеводов. КазГУ, Алма-Ата, 1997, 43с.

[9] Плешков В.П. Практикум по биохимии растений. Колос, Москва, 1968, 88 с.

[10] Mostafa Khajen, Yadollah Yamini, Shahad Shariati, "Comparison of essential oils compositions of Nepeta persica obtained by supercritical carbon dioxide extraction and steam distillation methods", Food and bioproducts processing, vol. 88, pp. 227-232, 2010.

[11] Губаненко Г.А., Рубачевская Л.П., Репях С.М. Экстракция растительного сырья сжиженными газами. // Материалы Междунар. совещания, посвящ. памяти В.Г. Минаевой «Физиолого-биохимические аспекты изучения лекарственных растений». Новосибирск, с.22, 1998.

[12] К.Н. Разаренова, Е.В. Жохова, "Сравнительная оценка содержания дубильных веществ в некторых видах рода Geranium L. флоры северо-запада," Химия растительного сырья , №4, сс. 187-192, 2011.

[Sluken B. Rakhmadiyeva graduated from Faculty of Chemistry of the Kazakh State University in Almaty in 1973, received shis PhD in Chemistry studies at the Kazakh State University (1974-1977). She carried out postdoctoral studies at the Kazakh State University (1997-2000). Doctor of Chemistry Sciences, the Doctoral thesis "The Hydrolysable tannins and related substances purslane plants and their biological activity". She was appointed Lecturer in 1978, and in 2003 he became full Professor of Chemistry at L.N. Gumilyov Eurasian National University (ENU). Shis research is represented byaround 250 papers and patents covering several topics: bioactive natural products, medicinal chemistry, organic chemistry. She is now interested on polymeric nanostructures forbiomedical applications. She has authored three textbooks for students and served the ENU as professor of Chemistry Department and Director of Research Institute of Bioorganic chemistry, Dean of the Faculty.]

[Dina Kassenova. I graduated East-Kazakhstan State University named after S.Amanzholov as bachelor of natural science in June 2013. Now I study master of Natural science in L.N. Gumilyov Eurasian National University. In 2011 work laboratory assistant for research on «Extraction and analysis of essential oils from plant material». Also in 2011 I finished Course of «Modern physical and chemical methods of the analysis. High resolution of nuclear magnetic resonance spectroscopy in chemical and biological researches».
Interests: Chemistry of Natural Compounds.]

[Darya Kushnarevich. I graduated L.N. Gumilyov Eurasian National University as bachelor of natural science in June 2014. I was studying chemical engineering in Politechnical University of Gliwice (Poland) as Erasmus student from October 2102 to February 2013. In present time I study master of Natural science in L.N. Gumilyov Eurasian National University and continue to do my bachelor work "Development of new methods for the determination of biologically active substaces in *Calligonum aphyllum* and extracts of this plant".]

SEEK DIGITAL LIBRARY