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# Evaluation of biological active compounds found in sea buckthorn fruits

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**Abstract:** Sea buckthorn (*Hippophae rhamnoides* L.) is a shrub that can grow up to 5-6 meters high. The branches have many long, very sharp stems, and the leaves narrowed and colored in green-silver. The flowers, yellow-brown, are small and appear in March - April. The main parts used in food production are fruits. Fruits are rich in vitamin, as well as other vitamins E, K, F, P, B (B2, B6, B9, choline, inositol), carotenoids (beta carotene, xanthophylls), minerals, polyunsaturated fatty acids, complex oils, serotonin, volatile oils, flavonoids, pectins, tannins, amino acids, enzymes, phytosterols etc.

## • Introduction

Fruit extracts present antioxidant, emollient, cytoprotective, immunostimulatory, vitaminizing, depurative, anti-inflammatory effect. The aim of the present study was to extract the compounds with biological activity and to characterize them in terms of carotenoids, flavonoids and total polyphenols content and antioxidant activity.

## • Materials and methods

Sea buckthorn berries (*Hippophae rhamnoides* L.) were purchased at a commercial maturity from a local supermarket and directly refrigerated at 4°C, and subsequently frozen and stored at -20°C. Frozen sea buckthorn were lyophilized before processing. The reagents was used purchased from Sigma (Sigma-Altrich Co, St. Louis, USA).

The ultrasonic extraction method was used as one of the fastest and with a fairly high yield. For the extraction of hydrophilic and lipophilic compounds was used a mixture of polar (ethanol, acetone and ethyl acetate) and non-polar solvents.

Three methods were used for biological active compounds extraction: conventional solvent extraction, ultrasound-assisted extraction and microwave-assisted extraction. The combination of polar and non-polar solvents enhances the solubilization of non-polar carotenoids (lycopene and  $\beta$ -carotene), while polar solvents (ethanol, acetone and ethyl acetate) solubilize polar compounds.

All extracts were characterized from the point of view of the global phytochemical profile namely: carotenoid content, total polyphenol content, total flavonoid content and antioxidant activity

Step 1	Step 2	Step 3	Step 4	Step 5
				
Sea buckthorn berries ( <i>Hippophae rhamnoides</i> L.) were directly refrigerated at 4°C, and subsequently frozen and stored at -20°C, were lyophilized before processing.	•Ultrasound assisted extraction/ microwave assisted extraction and maceration.	Collected supernatant	Vacuum concentration	Redissolved extract Spectro-photometric analysis

## • Results and discussions

**Table 1** Content of total carotenoids and  $\beta$ -carotene in the samples analyzed by different extraction methods

Nr	Sample	Parameter	Total carotenoids, $\mu\text{g/g DW}$	$\beta$ -carotene, $\mu\text{g/g DW}$	Lycopene, $\mu\text{g/g DW}$
Ultrasound assisted extraction					
1	Ethanol- 96%		4,569 $\pm$ 0,009	3,835 $\pm$ 0,006	1,147 $\pm$ 0,003
2	Ethanol : Acetone(4:3)	45 min	25,082 $\pm$ 0,026	21,007 $\pm$ 0,013	5,972 $\pm$ 0,022
3	Ethanol : Hexane : Acetone(4:3:1)	40KHz	18,138 $\pm$ 0,032	15,123 $\pm$ 0,043	4,166 $\pm$ 0,010
4	Ethyl acetate: Hexane(2:1)	40-50°C	42,43 $\pm$ 0,17	35,35 $\pm$ 0,058	9,815 $\pm$ 0,029
5	Ethyl acetate: Hexane(1:2)		20,891 $\pm$ ,087	17,326 $\pm$ 0,078	4,315 $\pm$ 0,063
6	Hexane : Acetone(2:1)		25,198 $\pm$ 0,077	21,167 $\pm$ 0,285	5,518 $\pm$ 0,054
7	Acetone(80%):Ethanol (70%) (1:1)		0,909 $\pm$ 0,012	0,712 $\pm$ 0,015	0,351 $\pm$ 0,013
8	Ethanol (70%)		1,092 $\pm$ 0,011	0,87 $\pm$ 0,006	0,43 $\pm$ 0,004
9	Sunflower oil	15 min	0,232 $\pm$ 0,018	0,198 $\pm$ 0,018	0,051 $\pm$ 0,003
		40KHz			
		40-50°C			
		30 min	0,237 $\pm$ 0,031	0,201 $\pm$ 0,026	0,052 $\pm$ 0,007
		40KHz			
		40-50°C			
		45 min	0,243 $\pm$ 0,013	0,208 $\pm$ 0,01	0,058 $\pm$ 0,0016
		40KHz			
		40-50°C			
Microwave-assisted extraction					
1.	Ethanol (70%)	t=47 $\pm$ 61°C 15 sec, P=420W,	0,97 $\pm$ 0,015	0,768 $\pm$ 0,015	0,38 $\pm$ 0,008
2.	Sunflower oil	T=70,6°C 30sec P=1050W	0,265 $\pm$ 0,023	0,226 $\pm$ 0,019	0,067 $\pm$ 0,005
		T=47,5°C 40sec P=735W	0,265 $\pm$ 0,012	0,226 $\pm$ 0,01	0,062 $\pm$ 0,002
		T=54,2°C 30sec P=525W	0,216 $\pm$ 0,004	0,184 $\pm$ 0,004	0,048 $\pm$ 0,001
Extraction with maceration					
1	Ethanol (70%)	t=48h, T=40°C	1,094 $\pm$ 0,021	0,844 $\pm$ 0,018	0,425 $\pm$ 0,011

## Conclusions

The higher yield of the carotenoids extraction was found using the solvent mixture (in combination with ultrasonic extraction) ethyl acetate:hexane (2:1) when the total carotenoids content was 42.43 $\pm$ 0.17 mg/100 g DW,  $\beta$ -caroten content had a value of 35.35 $\pm$ 0.063 mg/100g DW and lycopene - 9,815 $\pm$ 0,029 mg/100g DW.

The mixture of ethanol:acetone (4:3) revealed the highest antioxidant potential of 3.465 $\pm$ 0.028 mM Trolox (TE)/g (ABTS-method).

The lowest values for carotenoid content extraction were found for sunflower oil samples in combination with microwave assisted extraction. Higher values for the total content of polyphenols and flavonoids were recorded by ultrasonic assisted extraction combined with ethanol: acetone (4: 3) solvent mixtures, the values were 805.34  $\pm$  5.5mg GA / g DW. The lowest polyphenol content was found for extraction using the mixture of ethyl acetate: hexane (2: 1), with values of 61.57  $\pm$  1.76 mg GA / g DW. The ultrasonic assisted extraction seems to be more useful for biological active compounds extraction compared to the other ones used.

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