Analytical characterisation of *Hippophae rhamnoides* juice by HPLC

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Abstract

The natural extracts are used for the treatment of some diseases but also in cosmetic purposes. They consist of complex mixtures of different classes of organic and inorganic compounds. Analytical characterization of most natural products is a very important and difficult problem, being equally a challenge for researches around the word.

In this paper we present the preliminary results concerning the separation and determination of some compounds from Hippophae rhamnoides fresh juice using HPLC system with diode array detection.

We studied juice obtained from fresh plants of Hippophae rhamnoides. The orange juice with suspensions was processed in order to obtain four extracts in different solvents: ethyl ether, methanol, water and HCl. The analytical used device was a High Performance Liquid Chromatograph GBC with photo-diode array detector - wavelengths range 190-700 nm. The analytical column was Spherisorb ODS 2 (C18) 5 mm,ID 150 x 4.6 mm. We identified some flavonoid, tannins and betacarotenoids compounds.

Introduction

Seabuckthorn (Hippophae sp.) is a deciduous, thorny and nitrogen-fixing shrub or small tree of 2-4 m height. Recent surveys indicate that this plant grows widely on riversides and slopes in dry temperate regions. The fruit of seabuchthars is quite rich in vitamins and other bioactive substances, which have been utilized in Russia, China, Finland, Romania and some other countries for the industrial production of health protection food product, medicine and cosmetics. High contents of vitamin C, flavonoids, tannins, oils and oil soluble bioactive compounds as well as minerals are the characteristics of the berry [1-3].

The reported methods of fresh juice's complete analytical characterization are generally: GC-MS for sugar, fruit acids, fatty acids, volatile compounds (head space technique) [4], TLC – MS for carotenoids [5], HPLC [6] for vitamin C [7], flavonoids [8-

10], carotenoids [11], RMN for tannins and flavonoides [12]. In the **table 1** we present the reported values for some of components from seabuckthorn berries:

Table 1. *The concentrations range of some components from* Hippophae sp. *fruits*

Compound	*Concentration, u.m.*
Vitamin C	*4.2 - 13.2 g/L*
Glucose	*0.9 – 5.5 g / 100mL*
Fructose	*0.2 – 3.8 g / 100 mL*
Oil	*2 - 11%*
Malic acid	*0.7 – 4.8 g/ 100mL*
Quinic acid	*0.8 – 4.9 g/100 mL*
Potassium	*6 – 14 g/kg dry weight (d w)*
Magnesium	*0.47 – 1.4 g/kg d w*
Calcium	*0.21 – 1.65 /kg d w*
Copper	*3.8 – 13mg/kg d w*
Iron	*22 – 282mg/kg d w*
Manganese	*8.1 – 17mg/kg d w*
Zinc	*8.8 – 48mg/kg d w*

The goal of this work is to report some results obtained in the studies about analytical characterisation of Hippophae rhamnoides fresh juice by HPLC with diode array detection.

Materials and Methods

A known quantity of Hippophae rhamnoides fresh juice was extracted with diethyleter and the extract was filtered on a quantitative filter paper (A extract). The insoluble part of the juice was then refluxed 30 minutes with methanol and the B extract was obtained. The remained insoluble components were dried and extracted with boiling water for 15 minutes (C extract). A half of the B extract's volume was refluxed with 15 ml HCl 10% (D extract). All utilized reagents were of chromatographic purity.

Analytical process was realized using a HPLC system from GBC Scientific Equipment with the configuration: LC1150 Quaternary Gradient Pump with 0-9,99 mL/min. and pressure range 0 – 40 MPa, LC1445 System Organiser with manual injector

Rheodine 7725 and injection volume 20µL, LC 5000 photo diode array detector (190-700nm). The column was Spherisorb ODS 2 (C18) 5µm, 150x4.6 mm. The acquisition set up applied for all extracts separation was: run time 10 min., wavelength range190-500nm, solvent methanol 100%. For the identification of separated compounds we used a spectrum library realized with pure compounds: quercetol, morin, rutin, tannin, betacarotene, nicotinic acid, vitamins.

Results and discussions

In the **figure 1** the chromatograms obtained for the A,B,C and D extracts are presented.

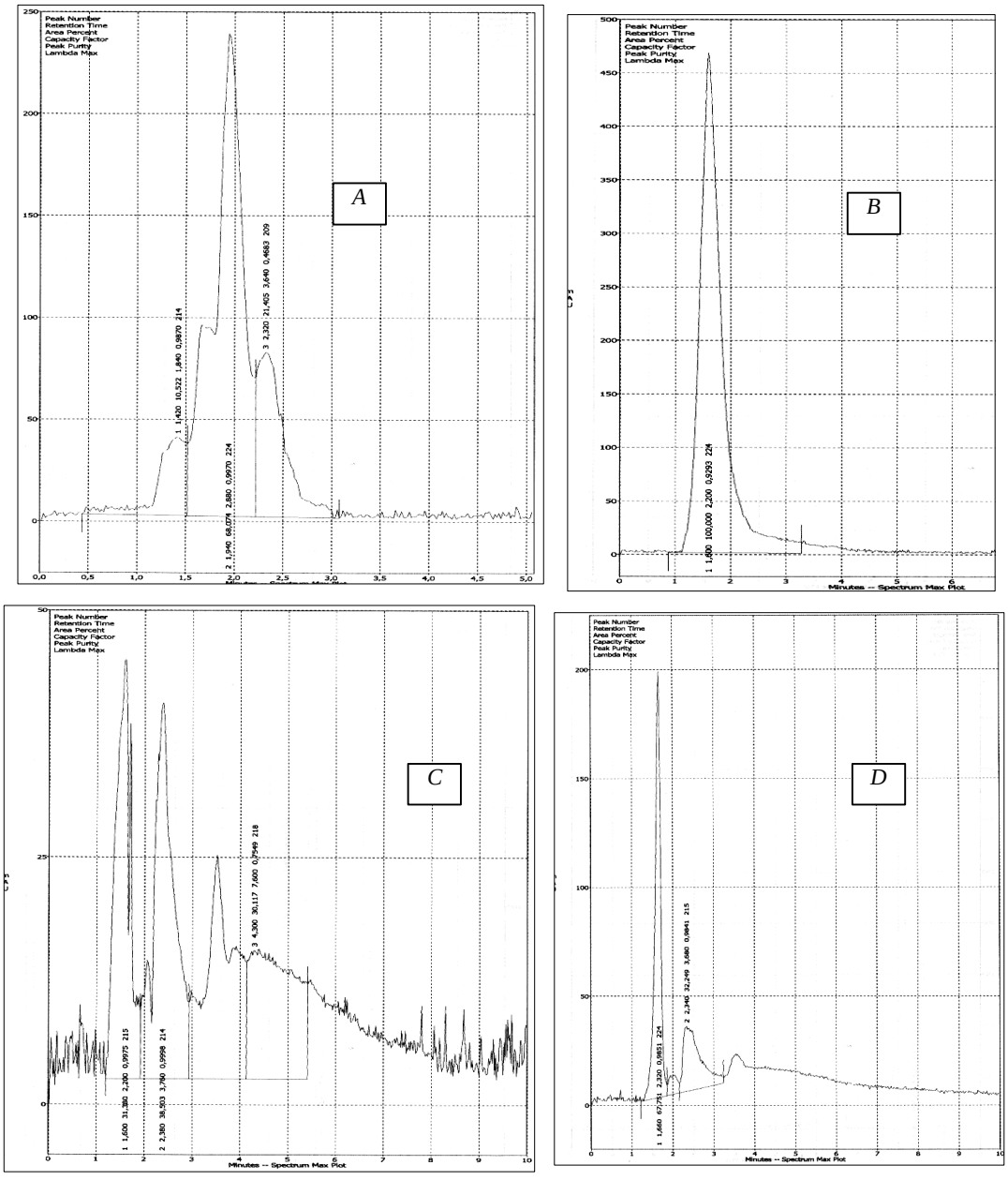

Fig.1. The four extracts chromatograms. A – extract in ethyl ether: B- extract in methanol; C- extract aqueous; D- extract in HCl

Studying these chromatograms we can summarize the obtained preliminary results in **table 2**.

Table 2. The description of A,B,C and D extracts chromatogram characteristics

Extract	Number of peaks	Peak 1		Peak 2		Peak 3	
		t_r, min	$?_{max}$, nm	t_r, min	$?_{max}$, nm	t_r, min	$?_{max}$, nm
A- ethyl ether	3	1.42	214	1.94	224	2.32	209
B- methanol	1	1.6	224	-	-	-	-
C – aqueous	3	1.6	215	2.38	214	4.3	218
D – HCl	2	1.66	224	2.34	215	-	-

By comparing the spectra of separated components with those from the library, we found that A extract contains betacarotene, B extract contains tannins, C and D extracts contain tannins and flavonoides.

Conclusions

The high pressure liquid chromatography is an useful tool for analytical characterization of various samples, including biological extracts with practical importance.

The fresh juice of Hippophae rhamnoides can be separated and analyzed by HPLC-DAD with accuracy.

References

1. *Wang S., Forest Research, 3(1), **1990**, 98*
2. *Brad I, Acta Phytoterapica Romanica, II (2), **1995**, 51*
3. *Yoshida T. et al, Phytochemistry, 30 (2), **1991**, 663*
4. *Yang F., et al, Journal of Chromatography A., 803 (1-2), **1998**, 298*
5. *Crapatureanu S. et al, Revue Roumaine de Biochimie, 33 (3-4), **1996**, 167*
6. *Klein E.J., Rivera S.L., Journal of Liquid Chromatography, **23** (14), **2000**, 2097*
7. *Lee H.S., Coates G.A., Journal of Liquid Chromatography, **22** (15), **1999**, 2367*
8. *Hakkinen S., Aurida S., Journal of Chromatography A, 829 (1-2), **1998**, 91*

9. *Zhang T.Y et al, Journal of Liquid Chromatography, 11 (1),* ***1988****, 233*

10. *Zhang T.Y. et al., Journal of Chromatography, 445 (1),* ***1998****, 199*

11. *Kurlich A.C., Juvik J.A., Journal of Liquid Chromatography,* ***22*** *(19),* ***1999****, 2925*

12. *Andrianaivoravelona J.O. et al, Phytochemistry, 52(6),* ***1999****, 1145*