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Original Article

Hippophae salicifolia D. Don: A Miraculous Species Less Known in Europe

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Abstract

Hippophae salicifolia is an Euro-Asian species used in many industries, from medicine to cosmetics, nutrition, or soil sciences (enriching degraded fields, diminishing soil erosion, preventing and treating diseases). The purpose of this study was to analyse the culture of this species in Europe together with the chemical content of its fruits. In order to achieve this, *Hippophae salicifolia* seeds were sown in greenhouses, seedlings were planted in fields, while the fruits were harvested and analysed both fresh, as well as after preservation for 1 year and 5 months. The properties of *H. salicifolia* fruits were also compared with the fruits of *Hippophae rhamnoides*, both fresh and preserved for 7 months. The analysis have shown that fresh fruits contain an average quantity of 31; 811 and 231 mg/100 total carotenoid, polyphenol, and ascorbic acid, respectively. The vitamin C content was much higher than that observed for *H. rhamonides.* If kept in adequate conditions, *H. salicifolia* fruits lose only a small amount of vitamin C and exhibit a vitamin C content (224 mg/100g) superior to the fresh fruit of *H. rhamonides* (100-150 mg/100 g). By comparing the chemical characteristics of *H. salicifolia* and *H. rhamnoides* fruits preserved over a long period of time, a higher concentration of vitamin C was observed in *H. salicifolia*, while the differences between the other chemical characteristics were insignificant. The obtained results strongly suggest that *H. salicifolia* can be successfully cultured in Europe, while its exceptional fruit qualities can be capitalised on by a variety of industries.

Keywords: Vitamin C; sea buckthorn; seedlings; polyphenol

Introduction

Hippophae genus (*Elaeagnaceae* family) is considered to contain seven species, one of which is *Hippophae salicifolia* D. Don (Geetha and Asheesh, 2011; Pant *et al*., 2014). *H. salicifolia* is a resistant shrub with falling leaves from the mountain regions of China and Russia (Gupta and Ahmed, 2010), with a large but uneven distribution in Euro-Asia, namely between 27 and 69 °N latitude and 7 °W and 122 °E longitude (Pant *et al*., 2014).

H. salicifolia spreads naturally at altitudes between 2000-3600 m in Euro-Asia, especially in Central and East Asia, in the Himalayan region around Pakistan, Nepal, and East India (Uprety *et al*., 2010). This species grows naturally on sandy soils in areas with a cold climate and can resist temperatures between -43 °C and +40 °C. It is also highly resistant to drought (Yao and Tigerstedt, 1994). However, optimal conditions include average annual temperatures between 4.7 and 15.6 °C and with annual average precipitation of 250-800 mm (Basistha *et al*., 2009).

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H. salicifolia prefers to grow in conditions of low humidity, alluvial gravels, wet landslips, and riversides. However, it can also grow in arid to wet conditions (Yao and Tigerstedt, 1994). Reproduction occurs through suckers (Airi *et al*., 2009), while natural regeneration through seeds is scarce (Sankhyan *et al*., 2005). Seeds are viable for 2 years, while freshly harvested seeds enter a period of physiological dormancy (Singh *et al*., 2008; Gupta *et al*., 2011b). The leaves are alternate, narrow, and lanceolate with a greyish appearance (Synge, 1974). The male and female plants are similar in morphology. Differences are only observed after 3-4 years of growth and only during blooming (Gupta *et al*., 2012).

H. salicifolia has a number of important characteristics: controls soil erosion, creates a proper habitat for wild fauna, is resistant in severe meteorological conditions, and develops a rich rootlet system, even on scarce soils, by fixing nitrogen in the soil (Gupta and Ahmed, 2010). Furthermore, this species is thought to improve the soil's fertility and can regenerate degraded fields (Huxley, 1992; Airi *et al*., 2009). It can also be found on riverbanks, lakeshores, steep slopes, and other susceptible terrains (Basistha *et al*., 2009). The species has a considerable importance not only for increasing soil fertility in fields with high slopes where it prevents erosion and landslides, but also as firewood and as forage (Rongsen, 1992).

Some studies have suggested that this species is a potential source of bioactive antimicrobial agents and could be used as a natural preservative and for nutraceutical formulations (Gupta *et al*., 2011a). The leaves and seeds has a higher total phenolic content, comparing to other sea buckthorn species (Lu, 1992), as well leaves showed anti-inflammatory properties (Padwad *et al*., 2006). During the last year, extracts from this plant were used in the USA as an food supplement (Saikia and Handique, 2012). This species contains a series of secondary metabolites, including carotenoids, flavonoids, tocopherols, sterols, unsaturated lipids, vitamin C and tannins. These compounds are well documented to have antioxidant, antitumor (Matheus and MacLeod, 1994; Singh *et al*., 2010), hepato-protective as well as immunomodulatory and anti-stress properties (nutritive, therapeutic, pharmaceutical, and cosmetic properties (Kaushal *et al*., 2013). A previous study demonstrated that the seeds of *H. salicifolia* have a greater antioxidant capacity than the leaves (Saikia and Handique, 2012).

The fruits of *H. salicifolia* have higher quantities of vitamin C and flavonoids, while the seeds represent between 3.4 and 4.1% of the fruit's mass (Ranjith *et al*., 2006). The fruits are edible and represent a rich source of vitamins, which are used in the preparation of local drinks (Gaur, 1999). Due to the high degree of perishability and acidic taste, the fruits cannot be consumed fresh and thus require rapid processing (Kaushal *et al*., 2013). In addition to its medicinal use, the fruits can be processed as juice and marmalade or used for adding flavour to lactic products due to its distinct taste (Gao *et al*., 2000; Saikia and Handique, 2012). The fruits are commonly used for preparing drinks by mixing other fruits or with the addition of sugar (Kaushal *et al*., 2013).

H. salicifolia seeds, juice, and pulp contain over 190 compounds (Gupta and Ahmed, 2010). Amongst them are the liposoluble vitamins A, K, and E, 22 fatty acids, 42 lipids, organic acids, amino acids, glucides, vitamins C, B1, and B2, folic acid, tocopherol, flavonoids, phenols, terpene, tannins, and 20 mineral elements; most of which are known for their beneficial health effects. Due to the high level of unsaturated fatty acids, the seeds are adequate for decreasing heart disease; this risk is also reduced by the presence of antioxidants, which prevent the oxidation of cholesterol (Gupta and Ahmed, 2010). The linolenic acids are also useful role in the treatment of rheumatoid arthritis, multiple sclerosis, psoriasis, and lupus. In China and the former Soviet Republics, medicinal sea buckthorn was used to treat the harmful effects of radiation, mouth burns, inflammation, and gastric ulcers (Gupta and Ahmed, 2010). The oil from this species blocks ultraviolet rays and helps in regenerating tissues (Gupta and Ahmed, 2010). The sea buckthorn oil, leaves and bark are known for their medicinal properties and have been used in treating the symptoms of the high number of lipids in blood, gingivitis, eye, or skin diseases, as well as cardiovascular diseases (Yang *et al*., 2000; Saikia and Handique, 2012). In China and Russia, the fruits have been used for years as prime material in alimentation and medicine (Cheng *et al*., 2003; Saikia and Handique, 2012). In conclusion, *H. salicifolia* has a wide range of uses (Fig. 1).

Sea buckthorn (*Hippophaë rhamnoides* L.) is much more studied than *Hippophaë salicifolia*. It grows wildly in Euro-Asia and has been domesticated, being cultivated especially in the Carpathian regions, showing a good adaptability to various climate conditions and extensive genetic variability. Also, sea buckthorn is well known for its large scale utilization in industry, pharmacology, forest and land rehabilitation provided by its unique rich biochemical composition, variety of species, fast fruiting, high productivity and ecological adaptation (Bal *et al*., 2011; Pop, 2013). In addition to producing juice extremely rich in vitamin C (up to 20 g/L) and flavonoids, sea buckthorn berries are used for edible and cosmetic oil production, especially in China, Russia, and, more recently, in Europe. Relatively high contents of oil are found in both kernels and berry pulp. The kernel oil is a significant source of tocopherols, tocotrienols, plant sterols, and carotenoids; it contains essential fatty acids such as linoleic acid (35-45%) and α-linolenic acid (20-36%). Pulp oil is more saturated, as more than half of the fatty acids are palmitic and palmitoleic acids, esterified as triglycerides. The yellow or orange sea buckthorn berries are rich in bioactive compounds (i.e. carotenoid pigments with provitamin A activity, folate-derivatives and phylloquinone, phenolics, unsaturated lipids, tocopherols and phytosterols), as well volatiles which contribute to the increasing interest for sea buckthorn utilisation for human nutrition and health promotion (Singh, 2005; Bal *et al*., 2011; Giuffrida *et al*., 2011). Forty-one different carotenoids have been reported in various cultivars, with zeaxanthin, beta-cryptoxanthin, and beta-carotene as the predominant ones (Raffo *et al*., 2004; Andersson *et al*., 2009; Giuffrida *et al*., 2011), but also minor ones (lycopene and canthaxanthin) (Kallio *et al*., 2002; Yang and Kallio, 2005; Vescan *et al*., 2010). The

presence of carotenoid esters has been also reported in sea buckthorn berries (Pintea *et al*, 2005; Parlog *et al*., 2009), but the identification and quantification of the individual carotenoid esters is poorly described, especially in Romanian varieties (ssp. *carpatica*) (Socaciu, 2008). Significant quantities of fruits of sea buckthorn are harvested annually from this species (169 t in the year 2016; Vasile *et al*., 2016), sometimes with numerous medicinal uses (Vasile *et al*., 2015). In Romania, white sea buckthorn is used in the ecological reconstruction of degraded fields (Constandache *et al*., 2016), or for planting in waste heaps (Dincă *et al*., 2011).

As such, the plant's high adaptability, characteristics and potential are the main reason for enterprising this study that intends to increase awareness for its benefits, characteristics rendered in well-analyzed numbers and recommended usages.

Materials and Methods

Sea buckthorn seeds used

Hippophae salicifolia seeds, native to China, were sowed in the year 2010 in the greenhouse at I.N.C.D.S. (National Forest Research-Development Institute) Brașov, Romania. The 3-year-old seedlings were planted in fields (Bolotești nursery garden, Vidra Experimental Basis – I.N.C.D.S. Focsani, Romania). The first fructification occurred in the fifth year (2015). Based on the fruit's morphological (shape, measure) and organoleptic (taste, colour) characteristics, two main varieties were identified (noted as 1 and 2). Fruits were harvested from these varieties to determine their chemical properties:

1 = yellow-orange colour, medium size ellipsoidal seeds (5-6 mm/2-3 mm), flavoured;

2 = bright red colour, small round seeds (3-4/2-3 mm).

Material from other I.N.C.D.S. Brasov *H. salicifolia* plantations was used for comparison. Fruits from the year 2015 were harvested, as well as *H. rhamnoides* fruits preserved for 7 months at a constant temperature of –15 °C. The samples were analysed at the Forest Product Laboratory at Transylvania University, Brașov, Romania.

Sowing and cultivating the species in fields

The sowing can be performed in autumn, after harvesting the coarse fruits, or in spring with seeds extracted from the fruits and stratified. In the present case, sowing was performed in the spring, after stratification of seeds in the sand for 90 days at a temperature of 3 °C. The sowing depth was 2 cm, with a seed norm of 2 g per linear meter. Emergence occurred at approximately 20-30 days from dissemination. The optimal cultivation density was 30-40 seedlings per linear metric. Sowing was performed in plastic caskets of 60 × 40 × 15 cm.

The substratum in which sowing was performed was composed of a mixture of 1:1 peat and sand, and disposed in the caskets over a grovel layer 5 cm deep. Two types of peat were used: oligotrophic peat (35% organic matter, 0.4% total N and pH 3.4-4) and eutrophic peat (19% organic matter, 0.62% total N and pH 7.10). The mixture of blonde peat and sand had a pH of 4.14, while the mesotrophic and sand mixture had a pH of 6.91. Pricking out the obtained seedlings from the cuttings was performed in plastic pots, in a mixture of 2:1 common beech humus and sand, with a pH of 5.71 (Fig. 2). The adequate types of fertilisers were used.

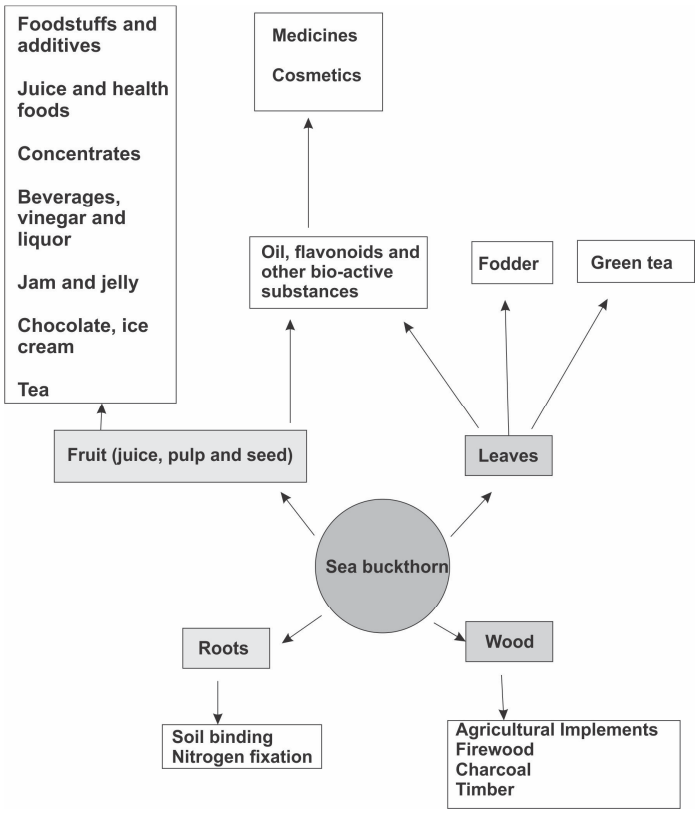

Fig. 1. Use of various parts of *Hippophae salicifolia* (Rajesh, 2009)

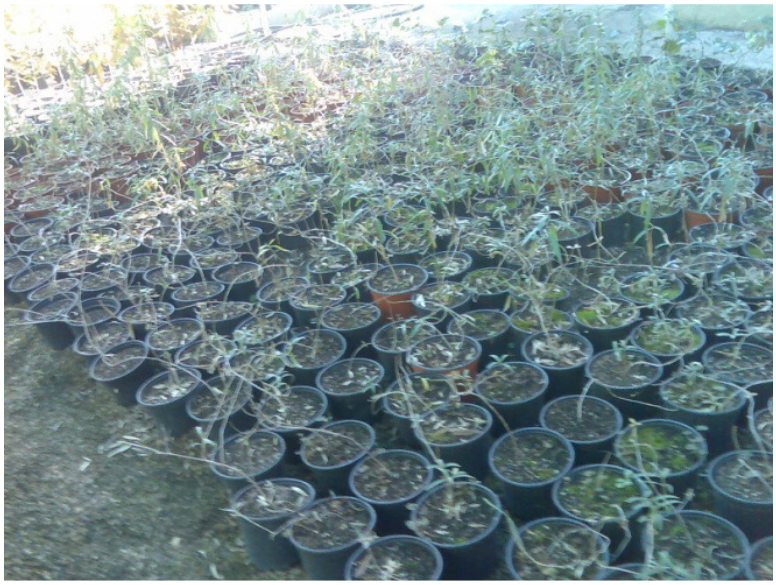

Fig. 2. One-year seedlings of *H. salicifolia* in the I.N.C.D.S. Braşov greenhouse

In terms of preventing disease and controlling harmful agents, the main problem that appeared in the installed cultures was provoked by *Aphide* (lice, flea) and the white greenhouse fly (*Trialeurodes vaporariorum*). To control these harmful agents, the insecticides were applied by alternative spraying.

Planting the seedlings in the field (Boloteşti nursery garden) was performed at the beginning of April 2013, before the start of vegetation. The field was flat and field preparation consisted of cleaning vegetal remains and stones, deep ploughing (25-30 cm), followed by two discing. The soil was alluvial, with a reduced humus content, loamy texture, and granular-poliedric structure, with approximately 20-30% coarse fragments.

Seedling plantation was performed in common graves of 30 × 30 × 30 cm in a 2.5 × 1.5 m schema, while the rows were oriented in the East-West direction. Seedlings with protected roots were planted in the grave from the recipient in which it was grown. Afterwards, maintenance was performed each year: soil mobilisation, which consisted of two hoeings per row and one furrow between the rows. The ratio of male to female specimens is 1:1 (which has determined the weak fructification). Normally, this ratio would be 1 male to 6-7 females.

Chemical analyses

After defrosting, total contents of water, soluble solids, lipids (including pro-vitamin A), vitamin C, invert sugar, and ash were determined for both sets of material. Humidity of the samples during quantified was 73%.

The vitamin C content (mg vitamin/100 g fruits) was determined with the iodometric method (Kallner, 1987; Daviers *et al*., 1991), with extraction in 2% HCl and potassium iodate N/1000 titration (Corlăţeanu *et al*., 1975). The acidity (g acid malic/100 g fruits) was determined with the titrimetric method (Sinclair *et al*., 1945), and with 0.1 N sodium hydroxide titration (***, 2011) for juice obtained from the fruits. The total content of soluble solids was determined refractometrically (OECD, 2009), with an Optech hand-held refractometer. Inverted sugar was determined with the Ofne method (McDonald and Turcotte, 1946). The lipids and vitamin A extracts were obtained in petroleum ether with the Soxhlet apparatus, after the defrosted samples naturally achieved a humidity of 10.9%. The ash was obtained through dry ashing in a muffle furnace at 550 °C (Schuck *et al*., 2012).

Extraction of HS berries for spectrometry and chromatographic analysis. Aliquots of 10 g were taken in duplicate from each variety (V11, V12 and V21, V22 respectively). The extraction of unpolar components was done in a volume of 100 ml mixture of chloroform: methanol (2:1, *v/v*). The samples were sonicated for 1 h and stirred for another 30 min using a magnetic stirrer in dark conditions. Further, all samples were centrifuged for 10 min at 2500 rpm and 4 °C. The pellet and supernatant were recovered. Afterwards, the pellet was re-extracted twice using the same procedure. The three supernatants were combined and then separated in a separation funnel using water to induce phase separation (lower lipophilic phase and upper hydrophilic phase). The lower chloroform phase was further concentrated under vacuum and kept in the freezer until analysis.

The UV-Vis spectral fingerprinting of HS extracts. UV-Vis spectra were recorded for each HS berty extract using a Jasco V 530 Spectrophotometer, in the range 300-550 nm. There were identified the maximum wavelengths specific for the carotenoids and there were compared the fingerprints of the four samples (V11, V12, V21, V22). For qualitative evaluation, it was used the general formula (Britton *et al*., 1995).

Carotenoids (expressed in mg/g berry) = (Absorbance $_{450\,nm}$ × extract volume × dilution × 10000)/(2500 × 100)

UHPLC-DAD analysis of the HS extracts. For chromatographic analysis it was used a UHPLC-QTOF-ESI$^+$-MS Bruker Maxis Impact device (Dionex HPLC coupled with Bruker mass Spectrometer). For separation, a column Acquity BEH C18, 1.7 µm, 2.1 × 75 mm was used, with Run-to-run time set at 20 min. Injection volume was 1µl, column temperature 32 °C, flow: 0.1 ml/min, with isocratic mobile phases: ACN/MeOH/IPA (45/50/5) +

0.1% FA. For diode array detection of carotenoids, there were chosen 280 nm, 340 nm, 445 nm and 475 nm, for a possible identification of phenolics (phenolic acids absorbing at 280 nm, flavonoids absorbing at 340 nm, and carotenoids absorbing at 445 and 475 nm, respectively).

For the adequate identification of carotenoids separated on the column, the optimal QTOF-ESI$^+$-MS parameters were chosen: capillary voltage 3500 V, drying gas flow 12 l/min, and drying temperature 300 °C. The m/z values were identified, in the range 250-1000 Da. The control of the whole instrument and the data processing were done using the specific software TofControl 3.2, HyStar 3.2 and Data Analysis 4.1 (Bruker, Daltonics).

Results

Cultivating the species in fields

Better results were obtained with the eutrophic substratum, compared with the oligotrophic one.

The attachment was 95% and 3 years after plantation (2016), the keeping was 75%. This decrease was caused by drought. Approximately 10% of the remaining specimens exhibited an abnormal vegetative state (dry sprouts, discolouration, and defoliation). The biometric characteristics of the culture (at 3 years) are the following: minimum height, 120 cm; maximum height, 260 cm; average height, 170 cm; and crown diameter, 85-90 cm minimum and 200 cm maximum.

In 2014 and 2015, forming cuttings were applied, namely choosing the ax and 3-5 lateral branches that are best situated on the axe. Both branches as well as the axe were shortened by one-half or one-third based on the robustness of growth by trimming the axe from 30 to 30 cm with skeleton branches up to a height of 1.5-1.7 m, considered suitable for the manual work of cutting and harvesting.

During the following spring (2016), fructification cutting was also performed, oriented mostly on the fruiting branches, so that the fruit was closer to the periphery of the crown. The sea buckthorn enters fructification in the second, third, or fourth year from plantation (Fig. 3). At the beginning, fructification is concentrated on the axe and at the base of skeleton branches. As the plants age, the fructification is directed towards the crown's periphery by cutting. Furthermore, the cuttings intend to evenly distribute the fructification branches, as well as their rejuvenation.

Afterwards, intervention of fructification formation is performed annually, so that they remain at a distance of 10-12 cm in less bright areas and 6-7 cm towards the crown's periphery. A part of the fructification formation is eliminated from the base, while another is cut in 1-1.5 cm snags. From the sprouting formed from the snags, the most vigorous and best-situated one is chosen to replace the fructification formation that fructified the previous year. This alternative replacement operation is repeated every year, based on the same principle. Fructification cutting is performed in the fall or spring, but can also be partially performed during the period of fruit maturation, when harvesting is performed by detaching the fructification branches.

Fig. 3. *H. salicifolia* fructification of 5-year-old seedlings

Chemical properties of H. salicifolia fruits

The chemical properties of fresh *H. salicifolia* fruits are shown in Table 1 and the chemical properties of *H. salicifolia* fruits preserved for 1 year and 5 months as well as *H. rhamnoides* preserved for 7 months are shown in Table 2.

From the data shown in Table 1, high variability regarding the quantity of total carotenoids (coefficient of variation, CV = 38.87), vitamin C concentration (CV = 24.01), and homogeneity with respect to the quantity of polyphenols (CV = 11.93) can be seen.

UV-Vis spectral fingerprinting and total carotenoid content

The comparative UV-VIS spectra are presented in Fig. 4, as well as the calculation of the carotenoid concentration in the extracts according to the formula generated by Britton *et al.* (1995).

Table 1. Chemical properties of fresh *Hippophae salicifolia* fruits

Sample name	Total carotenoids (mg/100 g sample)	mg polyphenols /100 g sample	Vitamin C concentration (mg ascorbic acid /100 g sample)
H. salicifolia, V 1.1.	35.18	715.629	197
H. salicifolia, V 1.2.	45.45	904.092	314
H. salicifolia, V1, average	40.32	809.86	255.50
H. salicifolia, V 2.1.	19.64	740.272	202.5
H. salicifolia, V 2.2.	22.51	883.801	211.25
H. salicifolia, V 2, average	21.08	812.04	206.88
H. salicifolia, average	30.70	810.95	231.19
Coefficient of variation	38.87	11.93	24.01

Table 2. Chemical properties of preserved sea buckthorn fruits

Characteristic	Values			Autochthon reference values (*H. rhamnoides*)
	Minimal	Maximum	Average	
Vitamin C content (mg/100 g fruits)				
-H. salicifolia *-H. rhamnoides*	221.27 33.89	227.64 36.49	223.56 35.19	-For fresh fruits: 100-200 (240) mg % (Brad *et al.*, 2002; Beldeanu, 2004); 66-142 mg% (Pascanut *et al.*, 2010b); -for raw juice: 71.85-214.00 mg% (Beldeanu, 1975; Brad et *et al.*, 2002); -for dry skim fruits: 72.26-235.13 mg % (Brad *et al.*, 2002).
Total acidity (g acid/100 g juice)				
-H. salicifolia *-H. rhamnoides*	2.68 3.22	3.54 3.40	3.04 3.28	- For fresh fruits: 2.33-2.55 g% (Brad *et al.*, 2002); - for the juice from fresh fruits: 2.58-3.50 g% (Brad *et al.*, 2002); 2.77-4.58 (Pascanut *et al.*, 2010a); - for dry unskimmed fruits: 6.30-15.32 g% (Brad *et al.*, 2002).
Content of dry soluble substance (%)				
-H. salicifolia *-H. rhamnoides*			18 9.8	-For fresh fruits: 17.7-21.4% (Beldeanu, 1975); -for raw juice: 11.0-13.31% (Beldeanu, 1975) and 7.0-10.5% (Pascanut *et al.*, 2010).
Inverted sugar content (% of juice)				
-H. salicifolia *-H. rhamnoides*	1.36 1.36	1.75 2.11	1.58 1.61	-For dry fruits: 0.47-0.53% (Brad *et al.*, 2002); -for juice: 0.42% (Brad *et al.*, 2002); 1.75-.9.74% (Pascanut *et al.*, 2010a).
Lipids and pro-vitamin A extract in petroleum ether (% dry substance)				
-H. salicifolia *-H. rhamnoides*			0.56 2.12	-For fresh fruits: 4-9% (Beldeanu, 1975); -for fruits after two refrigeration months: 0.14-0.72% (Dinulica, personal data); -for raw juice: 1-3% (Beldeanu, 1975).
Ash (% dry substance)				
-H. salicifolia *-H. rhamnoides*			0.205 0.138	-For fresh fruits: 0.65-0.70% (Brad *et al.*, 2002).

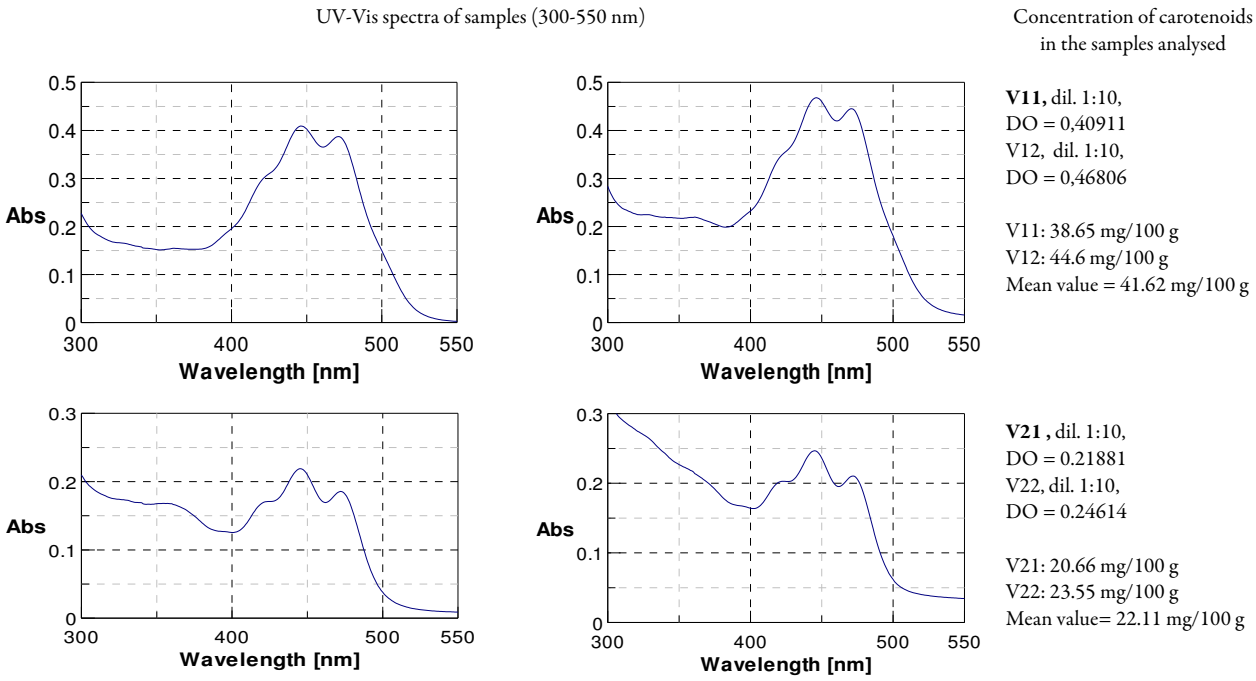

Fig. 4. Comparative UV-Vis spectral fingerprints of the HS extracts (V11, V12, V21, V22)

Chromatographic fingerprinting HPLC- DAD and UHPLC-TOF-ESI⁺-MS

The comparative HPLC-DAD chromatograms of samples V11 and V21 are presented in Fig. 5. The fingerprints are different, showing qualitative and quantitative differences between these samples. Generally, carotenoid molecules are separated at retention times (TR) between 15.4 and 20 min. The peaks are visible at 445 and 475 nm wavelengths, which are specific for xanthophylls (zeaxanthin, lutein), beta and alpha-carotenes and lycopene, respectively.

Table 4 includes the main carotenoids separated from these extracts, identified after a preliminary calibration with pure standards, as well their percentage, calculated from the peak areas. The qualitative and quantitative composition of V12 and V21 is slightly different, the first sample being more concentrated in beta and alpha carotene, as well xanthophylls (especially zeaxanthin), while the second one being relatively concentrated in lycopene. The total carotenoid concentration was also different, the first sample being around 2 times more concentrated in carotenoids pigments.

Table 3. Vitamin content of *H. salicifolia* fruits (Lu, 1992)

Comparison of the vitamin contents (mg/100 g) of *H. salicifolia* and others species	Vitamin A	Vitamin B1	Vitamin B2	Vitamin C
Hippophae salicifolia	11.00	0.04	0.56	300-1600
Cilicrosa roxburghii	4.83	0.05	0.03	1000-3000
Hawthorn	0.82	0.02	0.05	100-150
Orange	0.55	0.08	0.03	50.0
Tomato	0.31	0.03	0.02	11.8
Carrot	4.00	0.02	0.05	8.0

Table 4. Retention times (tR, min), carotenoids separated and identified by HPLC-DAD technique in samples V11 and V21

Sample V11			Sample V21		
t_R (min)	Carotenoid identified	%	t_R (min)	Carotenoid identified	%
15.5	Lutein	12	15.5	Lutein	13
15.8	Zeaxanthin	16	15.8	Zeaxanthin	13
16.8	Beta-cryptoxanthin	12	16.8	Beta-cryptoxanthin	10
18.0	Alpha-carotene	20	18.0	Alpha-carotene	12
18.4	Beta-carotene	22	18.4	Beta-carotene	14
18.9	Lycopene	18	18.9	Lycopene	38
20.0	NI	-	20.0	NI	-

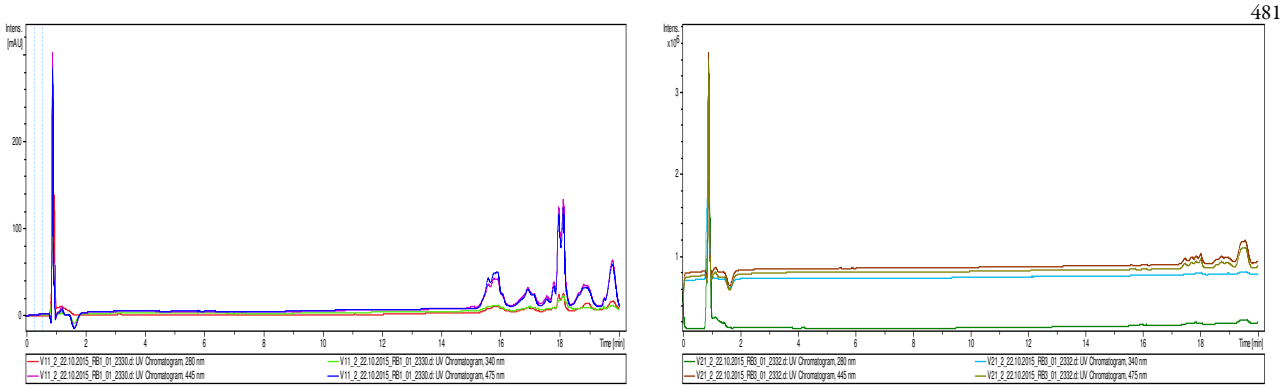

Fig. 5. Comparative HPLC-DAD chromatographic fingerprints of the HS extracts: V11 (left) and V21 (right)

Discussion

The vitamin C content of *H. salicifolia* fruits is much higher than that in *H. rhamnoides* fruits: 231 mg ascorbic acid/100 g sample in comparison with 100-200 (240) mg % (Brad *et al*., 2002; Beldeanu, 2004) and 66-142 mg % (Pascanut *et al*., 2010b), if we refer only to the analyses performed in Romania.

From the data shown in (Table 2), it can be observed that *H. salicifolia* fruits do not lose, except for a small amount, their vitamin C content if they are maintained at lower temperatures (i.e., in a freezer) even over long periods of time. Based on our data, the differences between fresh fruits and those preserved for 1 year and 5 months ranged from 231 to 224 mg vitamin C/100 g. Moreover, the fruits kept in the freezer for longer periods of time still have a superior vitamin C content (224 mg/100 g) in comparison with *H. rhamnoides* fresh fruit (100-200 mg/100 g).

There are significant differences in vitamin C content between *H. salicifolia* and *H. rhamnoides* preserved fruits: the *H. salicifolia* fruits maintained a relatively constant vitamin C content over long period of preservation, and this number is approximately seven times higher than *H. rhamnoides*. The other fruit characteristics do not register significant differences. Previous studies have also recorded a decrease in vitamin C content for White Sea Buckthorn (from 135 to 102 mg/kg, after preservation for 90 days after the fruits underwent a process of refrigeration between 0 °C and 5 °C for 1-2 days, and were then frozen at –15 °C; Păşcănuţ *et al*., 2010b).

As previously discussed in the literature, there is variability in the chemical composition of *H. salicifolia* fruits among different plant populations (Ahmad *et al*., 2005). Furthermore, the concentration of vitamin C in *H. rhamnoides* was highly variable among different populations (Karhu *et al*., 1999). The results obtained in this study regarding vitamin C concentration in *H. rhamnoides* fruits (231 and 224 mg/100 g for fresh and frozen fruits, respectively) are similar with those obtained in previous studies [196 mg/100 g (Ahmad *et al*., 2005)], but lower than those obtained by Kaushal *et al*. (2013) (490.5 mg/100 g), Rongsen (1992) (from 200-1500 mg/100 g), or Pant *et al*. (2014) (2984 mg/100 g).

H. salicifolia fruits have a much higher vitamin C content (2984 mg/100 g) than *H. tibetana*, *H. rhamnoides*, or even *Citrus sinensis* (879, 230, and 50 mg/100 g, respectively). As can be observed in the values in (Table 3), *H. salicifolia* is richer in vitamins C, B2, and A than hawthorn, orange, tomato, or carrot.

The carotenoid quantities determined in this study in the *H. salicifolia* fresh fruits (30.7 g/100 g) are slightly higher than the ones obtained by Kaushal *et al*. (2013) (18.3 mg/100 g). Major carotenoid pigments include δ and β-carotene, lycopene flavaxanthin, progestin, kryptoxanthin, violaxanthin, and neoxanthin (Ranjith *et al*., 2006; Beveridge *et al*., 1999; Xing, 2003).

The polyphenol quantity determined in this study in *H. salicifolia* fresh fruits (811 mg/100 g) is higher than that determined by Pant *et al*. (2014) for *H. salicifolia* (591 mg/100 g). The same authors indicated that there exists a higher concentration of polyphenols in *H. salicifolia* fruits than for *H. tibetana* and *H. rhamnoides* (572 and 521 mg/100 g, respectively). Higher phenolic content in *H. salicifolia* compared with *H. rhamnoides* has been confirmed in previous studies (Sharma *et al*., 2013; Ranjith *et al*., 2006).

Total acidity of the fruit determined in this study (3.04 g/100 g) was similar to values determined by Brad *et al*. (2002) and Kaushal *et al*. (2013) (2.33-2.55 and 2.7 g/100 g, respectively).

The sugar content for fresh fruits determined in our investigations (1.58%) is situated within the limits obtained by other researchers: 0.5% (Brad *et al*., 2002) and 6.29% (Dwivedi *et al*., 2006).

Conclusions

As a highly adaptable plant that grows in varied conditions of climate and soil, abundant in compounds, nutritive and bioactive substances, *Hippophae salicifolia* has numerous usages in industries as varied as medicine, cosmetics or soil sciences. The aim of this paper was to ascertain its numerous properties, focusing on its chemical propertis and to offer future directions.

As such, sowing, cultivating and managing *H. salicifolia* cultures do not pose considerable problems. Based on our analysis, the best cultures are realized during the spring, at a depth of 2 cm, with a seed norm of 2 g per linear meter, or a substratum composed of a mixture of peat and sand. Three years from planting, the plant's dimensions were as follows: minimum height, 120 cm; maximum height, 260 cm; average height, 170 cm; and crown diameter, minimum 85-

90 cm and maximum 200 cm. Another aspect revealed by our study is that forming cuttings in the crown is a necessary measure for obtaining maximal fructification. The comparison with *H. rhamonides* has helped us in emphasizing the properties and advantages of creating and sustaining *H. salicifolia* cultures.

The higher content of vitamin C (231 mg ascorbic acid/100 g sample) preserved and even increased in comparison with *H. rhamonides* (224 vs. 100-150 mg/100 g) if the species are kept in proper conditions, recommend the species for cultivation. Insignificant differences were obtained for other chemical characteristics, including total acidity, inverted sugar content, and concentration of dry substances. We observed also variability in the chemical composition of *H. salicifolia* fruits among different plant populations.

As such, the culture of *H. salicifolia* species in Europe is possible (on different field categories, especially degraded fields) and even recommended due to its ability to fix and enrich the soil, but especially because of the multiple uses of its fruits, leaves, or wood.

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