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COMPARISON OF ANTIOXIDANT ACTIVITY BETWEEN *ROSA CANINA* L. AND *HIPPOPHAE RHAMNOIDES* L. HARVESTED FROM ROMANIA

Dejeu Ioana Lavinia*, Frenţ Olimpia Daniela*, Sacalîş Ileana, Zbârcea Claudia***, Marian Eleonora*, Bodea Anca Salomea******

*University of Oradea, Faculty of Medicine and Pharmacy, 29 N. Jiga St., 410028, Oradea, Romania
e-mail: ioana.dejeu@gmail.com
**Pharmacist
***Secondary School "Mrs. Oltea", Oradea, Romania
****County Clinic Emergency Hospital Oradea, Department of Dermatology, Oradea, Romania

Abstract

Rosa canina L. is a medicinal plant used in traditional folk medicine with good results in arthritis, rheumatism, gout, fever, gastrointestinal disorders. Rosa canina fruits and hips contain a large amount of Vitamin C. The rose hips are used for the production of jams, jellies or beverages.

Hippophae rhamnoides L. known as sea buckthorn contains antibacterial, anti-fungal, anti-sebum properties and also is a good source of Vitamin C. Sea buckthorn is a valuable plant because it contains flavonoids, carotenoids, minerals and Omega 3. The aim of the present study was to investigate the antioxidant activity of rosehip and sea buckthorn fruits harvested from two sources from Romania and we observed that the highest concentration of polyphenols and antioxidant activity (by FRAP, DPPH methods) is found in the ethanolic extract of rosehip fruits, regardless of the area from which they were harvested and the highest concentration of flavonoids is in the ethanolic extract from sea buckthorn fruits.

Key words: *Rosa canina* L., medicinal plant, Vitamin C, sea buckthorn, antioxidant

INTRODUCTION

Rosa canina L. is a medicinal plant with therapeutic activities against arthritis, rheumatism, gout, sciatica, fever, colds, gastrointestinal disorders, gastric ulcers, gallstones and it is used for its anti-inflammatory, laxative, astringent, antioxidant and diuretic effect (Orhan et al., 2009).

The vegetable product of Rosa Canina, rosehip, is a shrub up to 3 meters tall, thorny, very common near fences, meadows, in the forest, from the Black Sea area to the mountain regions at an altitude of 1700 meters. Rosa canina fruits are a valuable source of polyphenols and vitamin C (Fig. 1) (Fan et al., 2014).

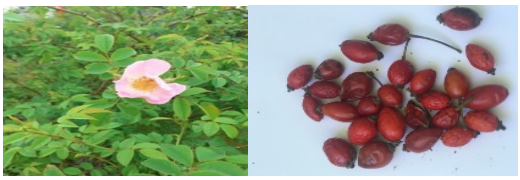

Fig. 1. Rosa Canina plant and fruits

It is also called field rose and wild rose and is widespread in Europe, West Asia and North Africa. In the spontaneous flora of our country we find 30 species of rosehip, of which 8 are also cultivated (Palade, 1998, Pârvu, 2002). Flowers, leaves and fruits can be used from rosehip. The flowers and leaves are harvested only in May and June, and the fruits are harvested from late July, August, early September, when they are still pleasant-looking red-orange and have the highest content of vitamin C.

Hippophae rhamnoides L. from the Elaeagnaceae Family is also called Sea buckthorn, blue sea buckthorn, spiny sea buckthorn, river-sea buckthorn, is a bushy, rustic shrub, found in clusters or extensive bushes, on the banks of rivers, stony shores, especially on the geological formations of the salt marshes, mountainous, in cold-temperature regions of Northern Europe, China, Mongolia and Russia (Fig. 2) (Tkacz et al., 2019). In the Alps it can be found even at an altitude of 2000 meters, and in the Himalayas it can be found even at an altitude of 5000 meters (Palade M, 1998, Tița, 2005).

In Romania we can find it in large areas in the Subcarpathians of Muntenia and Moldova, in the Bistrița Valley and the Siret Valley. Today sea buckthorn is considered one of the most valuable species because it contains flavonoids, carotenoids, minerals, Omega 3 and has antibacterial, anti-sebum, antifungal properties (Pundir et al., 2020).

Fig. 2. *Hippophae rhamnoides* L. plant and fruits

The fruit of *Hippophae rhamnoides* L. is used as a food with high nutritional and medicinal values and is a good source of vitamin C (500-900 mg/100g) (Fig. 2) (Bal et al., 2011, Ma et al., 2020, Makovics-Zsohar et al., 2014).

MATERIAL AND METHOD

Materials: in this study we compared the antioxidant activity of rosehip and sea buckthorn fruits from two sources: sea buckthorn fruits harvested from Bihor county, Oradea area and Maramureș county, Berbești area and rosehip fruits harvested from Bihor county, Oradea area and Maramureș county, Berbești area.

Methods: The sea buckthorn fruits were harvested by hand in October 2018, when their color was yellow-orange and the taste was sour, slightly astringent, and the rose hips in September 2018, when the fruits change from reddish to red-orange. Sea buckthorn and rosehip fruits were harvested from two areas of Romania, the northern area, Maramureș County and the northwestern area, Bihor County.

The fruits were harvested without any impurities (leaves) or unripe fruits. The washing was done in several waters, and for the rosehips the darker end was cleaned. Drying was performed in a drying chamber at a temperature of 30-35°C for 24 hours. The extraction was performed for 90 minutes, using a Soxhlet device, and methyl alcohol as solvent, purchased from Silver Chemicals, Romania.

The obtained extracts were concentrated with a rotary evaporator at a temperature of 40°C, at 80 rpm and a pressure of 200 atm, and then ethyl alcohol was added.

Determination of the total polyphenols, total flavonoids and antioxidant activity of the extracts was performed by spectrophotometric methods, using a UV-VIS PG Instruments T70 spectrophotometer.

RESULTS AND DISCUSSION

Determination of the total polyphenol content by the Folin-Ciocâlteu method: 1.7 mL deionized water, 0.2 mL Folin-Ciocâlteu reagent, 1 mL Na_2CO_3 solution of 20% concentration are added to 0.1 mL alcoholic extract, kept in the dark for 90 minutes and read the absorbance at 765 nm. Then we calculate the total polyphenol concentration using the regression equation; the concentration in total polyphenols of the extracts.

The results are expressed in milliequivalents of gallic acid GAE/100 g DW (dry sample) (Everette et al., 2010, Ikawa et al., 2003, Jurca et al., 2016).

The results obtained for total polyphenols by the Folin-Ciocâlteu method for the four samples are shown in Table 1.

From the analysis of the data provided by Table 1 it is observed that the highest concentration of total polyphenols is found in the ethanolic rosehip extract from Bihor (536.43 ± 5.19 mg GAE/100 g dry matter), followed by the ethanolic rosehip extract from Maramureș (523.18 ± 4.73 mg GAE/100 g dry matter), the differences not being significant. The results obtained are consistent with other determinations made by other researchers (541.12 mg GAE/100g dry matter), who placed in descending order the content of total polyphenols several species of Rosa: R. spinosissima> R. canina> R. rugosa> R. gallica (Koczka et al., 2018).

Table 1

Calculation of the average total polyphenol concentration of rosehip and sea buckthorn fruit extracts expressed in mg GAE/100 g dry product

Sample	Absorbance sample read at 765 nm	Sample concentration (mg GAE/100 g dried substance)	Average sample concentration (mg GAE/100 g dried substance)
Rosehip from Bihor	0.725	531.241	536.432±5.191
	0.742	543.472	
	0.730	534.583	
Rosehip from Maramureș	0.713	521.880	523.183±4.731
	0.721	527.915	
	0.710	519.764	
Sea buckthorn from Bihor	0.347	251.182	247.772±3.411
	0.339	244.953	
	0.342	247.171	
Sea buckthorn from Maramureș	0.349	252.645	256.653±4.161
	0.355	256.802	
	0.360	260.502	

Other data obtained for *Rosa canina* L. were: Roman et al. (Roman et al., 2013) found a TPC range of 326 mg to 575 mg GAE/100 g DW, Yoo et al. (Yoo et al., 2008) determined 818 mg GAE/100 g DW in extracts obtained with water extraction solvent, Fattahi et al. (Fattahi S. et al., 2012) found 180-225 mg GAE / 100 g DW, Yilmaz and Ercisli et al. (Yilmaz Ercisli, 2011) 102 mg GAE / 100 g DW and Barros et al. (Barros et al., 2011) 149.35 mg GAE / g extract in methanolic extracts.

The data in the literature are very varied, so the total polyphenol content depends on the extraction solvent and the geographical area from which the rosehip fruits were harvested. It was also noted that a higher polyphenol content and antioxidant activity were obtained for alcoholic, methanolic or ethanolic extracts from various plant products, compared to extracts obtained with water as a solvent. For these reasons, in recent scientific studies, alcohol extraction is more frequently used than water extraction to determine the antioxidant properties of various plant products (Koczka et al., 2018).

From the analysis of the data provided in Table 1 it is observed that the highest concentration of total polyphenols is found in the sea buckthorn ethanolic extract from Maramureș (256.65 ± 4.16), followed by the sea buckthorn ethanolic extract from Bihor (247.77 ± 3.41), the differences not being significant.

The determination of the total flavonoid content was done using a colorimetric method described in other studies (Jurca et al., 2016, Kim et al., 2003). The flavonoid concentration of the fruit extract samples was expressed in mg equivalents gram of quercetin / mL.

The results of the analyzes are shown in Table 2.

Table 2

Calculation of the average flavonoid concentration of ethanolic fruit extracts (rosehip / sea buckthorn) expressed in mg EQ / 100 g dry product

Sample	Absorbance sample read at 510 nm	Sample concentration in flavonoids (mg EQ/100 g dried substance)	Average sample concentration in flavonoids (mg EQ/100 g dried substance)
Rosehip from Bihor	0.320	39.085	39.407 ±0.888
	0.318	38.842	
	0.330	40.295	
Rosehip from Maramureș	0.334	40.779	40.537 ±0.727
	0.338	41.264	
	0.324	39.569	
Sea buckthorn from Bihor	1.201	145.756	141.841±3.915
	1.146	139.096	
	1.159	140.671	
Sea buckthorn from Maramureș	1.208	146.604	145.201±1.531
	1.198	145.393	
	1.184	143.670	

By observing the data in Table 2 it can be concluded that the highest concentration of flavonoids between the two ethanolic extracts of rosehip fruits is obtained for the sample from Maramureș, even if there are no significant differences. In fact, the concentration of flavonoids obtained for rosehip fruit is in line with other data provided by current research, 41 mg QE / 100 g dried fruit (Amadczak A. et al., 2012), 101.3-163.2 mg QE / 100 g frozen fruit pulp for different varieties of rose canine (Roman et al., 2013).

For ethanolic sea buckthorn extracts, the analysis of the data in Table 2 shows that for sea buckthorn from Maramureș has a higher amount of flavonoids than the sample from Bihor. There are no big differences between the sea buckthorn fruits from the two counties. However, comparing the two fruits analyzed, the highest amount of flavonoids is found in sea buckthorn fruits compared to rosehip fruits.

Determination of the antioxidant activity with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method is a fast, cheap and simple method. The method is widely used to measure the antioxidant activity of some

compounds in various foods against free radicals or compounds that can release hydrogen ions (Jurca et al., 2016).

The results obtained after performing the DPPH method are presented in Table 3.

Table 3

DPPH test results on rosehip / sea buckthorn ethanolic extracts

Sample	White absorbance	Sample absorbance	Percentage of inhibition %	Average percentage of inhibition %
Rosehip from Bihor	0.6959	0.1453	79.1201	81.0074±1.8873
		0.1224	82.4110	
		0.1288	81.4912	
Rosehip from Maramureș	0.6959	0.1205	82.6841	82.5933±0.4261
		0.1188	82.9286	
		0.1241	82.1672	
Sea buckthorn from Bihor	0.6959	0.3239	53.4604	52.0979±1.3625
		0.3402	51.1103	
		0.3359	51.7231	
Sea buckthorn from Maramureș	0.6959	0.3215 0.3316 0/3154	53.8010 52.3495 54.6774	53.6093 ±1.2598

The data provided in Table 3 show that the highest percentage of inhibition, so the best ability to neutralize free radicals, was found in the ethanolic extract of rosehip from Maramureș, followed by that of rosehip from Bihor. The highest percentage of inhibition among the ethanolic extracts of sea buckthorn fruits was the one from fruits in Maramureș. Comparing the extracts from the two fruits taken in the analysis, the rosehip has the highest antioxidant activity.

Determination of antioxidant activity by the FRAP method (ferric reducing antioxidant power), is a simple spectrophotometric method that tests the antioxidant capacity of some compounds and is based on the reduction of ferric tripyridyltriazine (Fe (III) -TPTZ) complex, freshly prepared in ferrous tripyridyltriazine complex ((Fe (II) -TPTZ) by a reducer. The reaction takes place at acid pH (Jurca et al., 2016).

Table 4 shows the results obtained for the antioxidant activity of ethanolic extracts from rosehip / sea buckthorn fruits by the FRAP method.

Table 4

The results of the FRAP method on ethanolic extracts from rosehip / sea buckthorn fruits

Sample	Absorbance read at 595 nm	Sample concentration (µmoli TE/100 g dried substance)	Average sample concentration (µmoli TE/100 g dried substance)
Rosehip from Bihor	0.2495	95.4706	94.0812±1.3894
	0.2449	92.7647	
	0.2470	94.0082	
Rosehip from Maramureș	0.2493	95.3510	94.6815±1.1168
	0.2462	93.5647	
	0.2489	95.1289	
Sea buckthorn from Bihor	0.1629	44.5312	44.1403±0.3909
	0.1616	43.7894	
	0.1622	44.1002	
Sea buckthorn from Maramureș	0.1639	45.1427	45.2437±0.6136
	0.1652	45.8573	
	0.1632	44.7311	

The data provided by Table 4 show that the highest antioxidant capacity has the ethanolic rosehip extract from Maramureș, followed by the ethanolic rosehip extract from Bihor (Jurca T. et al., 2016). It can be seen that the ethanolic extract of sea buckthorn fruits from Maramureș has the highest antioxidant activity. Comparing the extracts from the two fruits taken in the analysis, the rosehip fruits have a higher antioxidant activity, compared to the sea buckthorn fruits.

CONCLUSIONS

The results obtained in this study show that the antioxidant activity of rosehip and sea buckthorn fruits in different locations, grown in different environmental conditions, requires further investigation. The highest concentration of polyphenols and antioxidant activity (by FRAP, DPPH methods) is found in the ethanolic extract of rosehip fruits, regardless of the area from which they were harvested and the highest concentration of flavonoids is in the ethanolic extract from sea buckthorn fruits.

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