The Influence of Solid-to-Solvent Ratio and Extraction Method on Total Phenolic Content, Flavonoid Content and Antioxidant Properties of Some Ethanolic Plant Extracts

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The purpose of this study was to determine to what extent plant material, solid-to-solvent ratio, and extraction method influence the content of total phenolics and flavonoids and the antioxidant activity of ethanolic extracts obtained from dried fruits of dog-rose, sea buckthorn and hawthorn. The extractions were performed by maceration, Soxhlet and ultrasound-assisted methods, with 60% ethanol, and the solid-to-solvent ratios used were 1/5 and 1/10 (w:v). For each extract it was determined the total phenolic content (TPC), the flavonoid content (FC), the ability to scavenge DPPH•, Fe^{3+} reducing power, and also the ability of chelating Fe^{2+}. The highest total phenolics and flavonoids contents were found in dog-rose fruits extracts. Maceration and ultrasound-assisted extraction methods led to the highest concentrations of phenolics and flavonoids, and solid-to-solvent ratio 1/10 (w:v) was the most effective. The extracts of dog-rose fruits showed the highest DPPH• scavenging activity and Fe^{3+} reducing power, and the hawthorn fruits extracts registered the highest capacity of chelating Fe^{2+}.

Key words: phenolics, flavonoids, solid-to-solvent ratio, extraction method

In the last decades, physicians and patients have looked for natural drugs, non toxic remedies, suitable to the organism [1]. The researchers suggested that compounds with antioxidant activity are able to remove the excess of free radicals in the body, and thus to prevent or to cure diseases caused by oxidative stress such as cancer, degenerative diseases and cardiovascular diseases. Due to the antioxidant properties and health benefits, the researchers turned their attention to plants rich in phenolics used in traditional medicine [2 - 5]. The researchers are interested in phenolic extracts ability to scavenge free radicals, and also on the influence of the methods and conditions of extraction on the antioxidant activity of the extracts [6, 7]. Phenolics can be extracted from fresh, frozen, or dried plant material with different solvents such as methanol, ethanol, acetone, ethyl acetate, and their combinations have been used for the extraction of phenolics from plant materials, often with different proportions of water [6].

The nature of the extraction solvent, the solid-to-solvent ratio and the extraction method determine the amount and type of extracted phenolics, and also the antioxidant activity of the extracts. The most used solvents for the phenolics extraction are methanol and methanol-water mixtures, but very good results can be obtained with ethanol and ethanol-water mixtures. Because the ethanol is not toxic, and the mixture polarity can be set by adjusting the ethanol/water ratio, some authors [8] recommend the use of this solvent for the extraction of phenolics.

Furthermore, the concentration of total phenolics and the antioxidant activity of the extracts depend on the solid-to-solvent ratio; the increase of solvent volume increases both the extraction efficiency and the price of the extract, and decreasing the solvent volume lowers the extraction efficiency due to saturation effects, but decreases the cost price.

In this study, we aimed to investigate in which way plant material, solid-to-solvent ratio, and extraction method influence the total phenolics and flavonoids content as well as the antioxidant activity of ethanolic extracts obtained from dried fruits of dog-rose *(Rosa canina),* sea buckthorn *(Hippophae rhamnoides)* and hawthorn *(Crataegus monogyna).*

Experimental part

Extractions

Dog-rose, sea buckthorn and hawthorn fruits were harvested from the geographical area of Slanic Prahova, Romania, dried in the dark and in airflow, and then grounded. Extraction of phenolics was performed with 60% ethanol at 50°C for 3 hours. The extractions were carried out by maceration, Soxhlet and ultrasound-assisted methods, for two solid to solvent ratios: 1/5, and 1/10 (w:v), respectively. The extraction by maceration was performed using a Thermolab-GFL 1092 shaking water bath. The extraction by Soxhlet method was carried out using a VELP Scientifica extractor. The ultrasound-assisted extraction method was performed with an Elmasonic S 80 H equipment, at 60 Hz.

Determination of total phenolic content (TPC)

For the determination of TPC, the method with Folin Ciocalteu reagent was used [9]. A volume of 500 μL of plant extract was pipetted into a 10 mL test tube which contained 7.0 mL distilled water. Then, 0.5 mL Folin Ciocalteu reagent were added and the reaction mixture was vortexed and left to stand for 2 min. In the end, 2.0 mL of 20 % (w/v) Na_2CO_3 solution were added. After 20 min, the absorbance was measured at 725 nm using V670 UV-VIS Jasco spectrophotometer. The results were expressed as mg gallic acid equivalent/g dry weight (mg GAE/g DW). All analyses were performed in triplicate.

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Determination of flavonoids content (FC)

For the determination of FC, the method with aluminium chloride was used [10]. The determination of total flavonoids in extract samples started by mixing 1 mL of sample solution with 0.3 mL of 5 % $NaNO_2$ in a 10 mL test tube. After 5 min, 0.3 mL of 10 % $AlCl_3$ were added to the solution by mixing in a vortex. After 6 min of reaction, the solution was neutralized with 2 mL of 1 M NaOH. The reaction mixtures were brought to volume with distilated water. These solutions were once more mixed in a vortex and transferred to a glass cuvette. The absorbance was measured using V670 UV-Vis Jasco spectrophotometer at 510 nm. The concentration of flavonoids was expressed as mg catechin equivalent/g dry weight (mg CE/g DW). All analyses were performed in triplicate.

Analysis of phenolics by thin-layer chromatography (TLC)

5.0 μL of the extracts and standard quercetin, rutin and kaempferol flavonoids, at a concentration of 0.1% in ethanol, were applied to 0.2 mm thick silica gel plates. The following TLC system was used: ethyl acetate:methyl ethyl ketone:formic acid:water (57:27:5:10, v/v/v/v); spraying with NP/PEG as follows: 5% (v/v) ethanol NP (diphenylboric acid 2-aminoethyl ester) followed by 5% (v/v) ethanol PEG 4000 (polyethylene glycol 4000); visualization under UV light at 366 nm [11].

Determination of the antioxidant activity of the extracts

Determination of DPPH radical scavenging activity of the extracts

To assess the ability of the extracts to scavenge 1,1-diphenyl-2-picrilhidrazil (DPPH•) synthetic radical, a *photocolorimetric* method was used [12]. Briefly, 0.3 mL of extract were mixed with ethanolic solution containing DPPH radical (0.004 g/100 mL, 2.7 mL). The mixture was vigorously shaken and left to stand for 30 min in the dark. The annihilation of DPPH radical was determined by measuring the absorbance of the mixture at 517 nm. The results were expressed as % Inhibition. All analyses were performed in triplicate.

Determination of the Fe^{3+} reducing power of the extracts

Fe^{3+} reducing power of the extracts was evaluated by a photocolorimetric method [13]. An aliquot of 0.5 ml plant extract was mixed with 1 mL phosphate buffer (0.2M, *p*H 6.6) and 1 mL 1% $K_3[Fe(CN)_6]$, shaken well and incubated at 50°C for 20 min. After incubation, 1 mL TCA (10%) was added to stop the reaction. The mixture was centrifuged at 3000 rpm for 10 min. 1.5 mL supernatant, 1.5 mL distillated water and 0.1 mL $FeCl_3$ (0.1%) were mixed and incubated for 10 min. The results were expressed as absorbance at 700 nm. All analyses were performed in triplicate.

Determination of the Fe^{2+} chelating activity of the extracts

The ability of plant phenolics to chelate Fe^{2+} ions was determined photocolorimetrically [14]. In this assay, the plant phenolics bind Fe^{2+} ion, using iron (II) sulphate as ion donor. 0.85 mL of the plant extract were mixed with 1.5 mL of Tris-HCl buffer (0.1M, *p*H 7.4), followed by the addition of 1.5 mL of 500μM iron (II) sulphate. The mixture was left to stand at room temperature of 5 min. 0.15 mL of 0.25% aqueous 1,10-phenanthroline were added. The absorbance of the solution was read at 510 nm against blank. The results were expressed as % Chelation. All analyses were performed in triplicate.

Results and discussions

Determination of TPC and FC

The comparative results on TPC and FC of investigated plants depending on solid/solvent ratio and extraction method are presented in Table 1. The TPC in plant materials was in range of 6.30-32.52 mg GAE/g DW and FC ranged between 2.10-23.82 mg CE/g DW.

The TPC and FC varied depending on the plant material, solid-solvent ratio and extraction method. The TPC found in rosehip was significantly ($p < 0.05$) higher compared to the levels found in sea buckthorn and hawthorn fruits; TPC mean value for rose-dog fruits was with 13.04 mg GAE/g DW higher than the one found in sea buckthorn fruits and with 13.55 mg GAE/g DW higher than the one found in hawthorn fruits. The FC found in rosehip was significantly ($p < 0.05$) higher than the ones found in sea buckthorn and hawthorn, FC mean values found in rosehip being with 12,01 mg CE/g DW higher than the ones found in the sea buckthorn fruits and with 9.07 mg CE/g DW higher than that found in hawthorn fruits (table 1).

Rosehip TPC values registered in this study are comparable to previ-ous findings which reported values between 78 mg GAE/100 g DW [15] and 9600 mg/100 g DW [16]. Some authors [17], reported, for different varieties of *Rosa canina* from Transilvania, a TPC that ranged between 326 and 575 mg GAE/100 g frozen pulp. The FC found in rosehip was higher compared to the level reported in some studies, 41 mg QE/100 g dry fruit [18]. These results show a high variability in the content of flavonoids in the dog-rose fruits. Other studies [17] also reported for different varieties of *Rosa canina* from Transilvania, FC values that ranged between 101.3 and 163.0 mg/100 QE g frozen pulp.

In a study on frozen pulp of sea buckthorn fruits, it was reported a TPC of 14.408 mg GAE/g and a FC of 6.794 mg RE/g [19]. In another study, TPC and FC in sea buckthorn fruits from 6 growers in Central Europe were determined and there were found values that ranged between 8.62 and 14.17 g GAE kg^{-1}FM, and between 4.18 and 7.97 g RE kg^{-1} FM, respectively [20].

Hawthorn fruits showed an important content of phenolics. The found values varied depending of extraction method and solid/solvent ratio. The TPC found in hawthorn fruits had values that ranged between 6.91 and 10.89 mg GAE/g DW. These values were lower compared to those reported by another study, 28.30 mg/g DM [21]. FC values ranged in hawthorn fruits from 5.23 to 7.78 mg CE/g DW, values close to those obtained for FC reported by the same study [21], 8.77 QE/g DW. In another study, phenolics and flavonoids from different parts of hawthorn fruits were extracted; TPC value in pulp was 122.6 mg GAE/100g DW, while in peel was 123.35 mg GAE/100 g DW, while reported FC values were 60.89 mg RE/100g DW, and 71.24 mg RE/100g DW, respectively [22].

Effect of solid/solvent ratio on phenolic compounds extraction

Compared to solid-to-solvent ratio of 1/5 (w:v), 1/10 (w:v) ratio was found to be favorable for phenolic compounds extraction (table 1). The use of 1/10 (w:v) solid-to-solvent ratio showed TPC mean values higher with 14.02 mg GAE/g DW for rosehips, with 3.81 mg GAE/g DW for sea buckthorn fruits, and with 2.92 mg GAE/g DW for hawthorn fruits, compared to the mean values registered in case of 1/5 (w:v) solid-to-solvent ratio.

When the 1/10 (w:v) solid-to-solvent ratio was used, the FC mean values were higher with 9.55 mg CE/g DW for rosehips, with 1.51 mg CE/g DW for sea buckthorn fruits, and with 2.22 mg CE/g DW for hawthorn fruits, compared to 1/5 (w:v) solid/solvent ratio. The TPC and FC found in all studied vegetal materials were significantly ($p < 0.05$) higher when the 1/10 (w:v) solid-to-solvent ratio was used,

Extraction method	Solid/solvent ratio (w:v)	TPC (mg GAE/g DW)	FC (mg CE/g DW)
Dog-rose fruits (rosehips)			
Maceration	1/5	17.52 ± 2.11	12.83 ± 1.28
Soxhlet	1/5	7.28 ± 0.66	5.32 ± 0.93
Ultrasound	1/5	20.66 ± 2.17	13.82 ± 1.88
Maceration	1/10	32.20 ± 2.33	21.40 ± 2.93
Soxhlet	1/10	22.78 ± 1.52	15.41 ± 1.33
Ultrasound	1/10	32.52 ± 3.68	23.82 ± 2.18
Sea buckthorn fruits			
Maceration	1/5	7.40 ± 0.66	2.78 ± 0.51
Soxhlet	1/5	6.30 ± 0.48	2.10 ± 0.54
Ultrasound	1/5	7.96 ± 0.85	3.10 ± 0.41
Maceration	1/10	13.08 ± 1.86	5.02 ± 0.52
Soxhlet	1/10	8.25 ± 0.94	3.10 ± 0.81
Ultrasound	1/10	11.75 ±1.19	4.39 ± 0.51
Hawthorn fruits			
Maceration	1/5	7.52 ± 0.68	5.26 ± 0.52
Soxhlet	1/5	6.91 ± 0.70	5.26 ± 0.45
Ultrasound	1/5	7.01 ± 0.63	5.23 ± 0.85
Maceration	1/10	10.32± 1.98	7.53 ± 0.63
Soxhlet	1/10	9.00 ± 0.87	7.10 ± 0.69
Ultrasound	1/10	10.89 ±1.07	7.78 ± 0.65

Table 1
TOTAL PHENOLIC CONTENT (TPC) AND FLAVONOIDS CONTENT (FC) OF PLANT MATERIALS, DEPENDING ON SOLID/ SOLVENT RATIO AND EXTRACTION METHOD

compared to 1/5 (w:v) solid-to-solvent ratio.

These results were consistent with mass transfer principles where the driving force for mass transfer is considered to be the concentration gradient between the solid and the solvent [23]. Higher solid-to-solvent ratio increases the concentration gradient, leading to an increased diffusion rate of the compounds from the extracted solid material into the solvent, but also determines the increasing of the necessary period of time to achieve equilibrium. Solid-to-solvent ratio could significantly affect the equilibrium constant and characterize the relationship between yield and solvent use as a steep exponential increase followed by a steady state to give the maximum yield [24]. Similar observations were reported also by other researchers. In a study on the effect of solid to solvent ratio on the extraction efficiency of phenolic compounds from *Aquilaria crassna* [25], it was fount that the TPC increased significantly when the solid-to-solvent ratio was increased from 1/10 to 1/20, and insignificantly when the solid-to-solvent ratio was increased to 1/60. In a study on extracted phenolic compounds from olive leaves [26], it was reported the increasing of extraction efficiency of total phenols until a solid-to-solvent ratio of 1/8, and constant extraction efficiency to a solid to solvent ratio of 1/10.

Effect of extraction method on phenolic compounds extraction

The TPC found in analyzed vegetal materials was significantly dependent ($p<0.05$) to the extraction method. The mean values obtained for the extractions carried out by maceration method were significantly ($p<0.05$) higher (with 4.59 mg GAE/g DW) compared to the mean values obtained after using the Soxhlet method (table 1). The TPC mean value found in vegetal materials extracted by ultrasound-assisted method was significantly higher (with 5.05 mg GAE/g DW) compared to Soxhlet method and insignificantly ($p>0.05$) higher compared to maceration method (with 0.46 mg GAE/g DW). FC found in vegetal materials extracted by maceration method and ultrasound-assisted method were significantly higher ($p<0.05$) compared to Soxhlet method, the mean values being higher with 2.76 mg CE/g DW, and 3.31 mg CE/g DW, respectively. The differences between the mean values registered for FC after ultrasound-assisted and maceration extraction methods were insignificant (0.55 mg CE/g DW; $p>0.05$).

Ýlbay Z. *et al.* (2013) extracted phenolics from dog-rose fruits by Soxhlet and ultrasound-assisted methods (with ethanol 50%), in different extraction conditions, and they reported TPC values that ranged from 20.23 to 31.37 mg GAE/g DW, and 41.52 mg GAE/g DW to 51.18 mg GAE/g DW, respectively [7]. In our study, the TPC found in dog-rose fruits by Soxhlet method was much lower than the one found in another study that reported a level of 62.79 mg GAE/g DM [27]. This difference could be explained by the different conditions of extraction used in that study (40°C for 24 h) [27].

Analysis of the phenolics by TLC

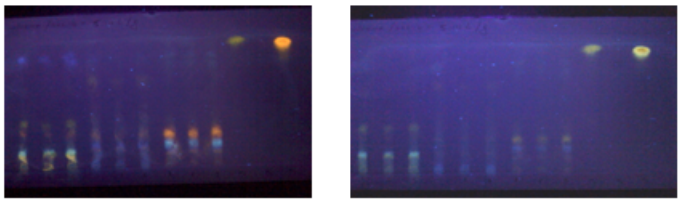
Figure1 shows the chromatographic profile of the ethanolic extracts obtained from dog-rose fruits, sea buckthorn fruits and hawthorn fruits by maceration, Soxhlet and ultrasound-assisted methods, for solid-to-solvent ratio of 1/5 and 1/10. The comparative analysis showed that the chromatographic profile of the extracts depended only on the vegetal material. The extracts obtained from the same vegetal material contained the same phenolics, regardless of the extraction method and solid-to-solvent ratio (table 2).

Antioxidant activity of the ethanolic extracts

Antioxidant activity of phenolic compounds derives from the ability of donating hydrogen atoms or electrons to reactive radicals [28] and to be effective as metal chelators [29]. Table 3 shows the antioxidant activity of ethanolic extracts depending on their TPC.

DPPH radical scavenging activity

The ability of the extracts to scavenge DPPH synthetic radical was significantly dependent ($p<0.05$) on the vegetal material and the solid-to-solvent ratio, and insignificantly dependent ($p>0.05$) on the extraction method. The highest DPPH radical scavenging activity was found for dog-rose fruits extracts, the mean values obtained being significantly ($p<0.05$) higher compared to the mean values obtained for sea buckthorn (by 10.21%) and hawthorn fruits (by 4.15%). In the case of ethanolic extracts obtained by 1/10 (w:v) solid-to-solvent ratio, it was found a DPPH radical scavenging activity significantly ($p<0.05$) lower compared to the one registered for 1/5 (w:v) solid-to-solvent ratio, excepting for rosehip extract obtained by maceration. The results obtained for all extracts are due to the lower concentration of phenolic compounds in the extracts obtained by 1/10 (w:v) solid-to-solvent ratio compared to those obtained by a 1/5 (w:v) solid-to-solvent ratio. In the case of 1/10 (w:v) solid-to-solvent ratio, dog-rose fruits extracts obtained by Soxhlet method registered a TPC concentration higher than the one obtained when 1/5 (w:v) solid-to-solvent ratio was used, but a lower DPPH radical scavenging activity was found. Some authors [30] suggested that dog-rose fruits phenolics have prooxidant activity at higher concentrations and that is why DPPH radical scavenging activity did not show an increasing trend at high concentrations.



1a 1b 1c 2a 2b 2c 3a 3b 3c 4 5 6
Solid/solvent = 1/5

1a 1b 1c 2a 2b 2c 3a 3b 3c 4 5 6
Solid/solvent = 1/10

Fig. 1. Comparative thin layer chromatography (TLC) for phenolics (λ excitation 366 nm) Vegetal material: 1 – dog-rose, 2 – sea buckthorn, 3 – hawthorn; The extraction method used: a – maceration, b – Soxhlet, c – ultrasounds

Fe^{3+} reducing power of the extracts

Fe^{3+} reducing power of the ethanolic extracts was significantly dependent ($p<0.05$) on the plant material and on the solid-to-solvent ratio; the extraction method insignificantly influenced ($p>0.05$) the reducing power of the extracts. Fe^{3+} reducing power of rosehip ethanolic extract was significantly ($p<0.05$) higher compared to the one of sea buckthorn and hawthorn fruits extracts, the mean values being higher with 0.14 and 0.18 (A700nm), respectively. The reducing power of the extracts registered for the 1/5 solid-to-solvent ratio was significantly higher

Table 2
RESULTS OF TLC ANALYSIS OF THE ETHANOLIC EXTRACTS OBTAINED FROM DOG-ROSE FRUITS, SEA BUCKTHORN FRUITS AND HAWTHORN FRUITS

Fluorescence (UV)	Maceration	Soxhlet	Ultrasound	Maceration	Soxhlet	Ultrasound
	Rf					
	Solid/Solvent 1:5 (w:v)			*Solid/Solvent 1:10 (w:v)*		
Dog-rose fruits						
light Blue	0.02	0.02	0.02	0.02	0.02	0.02
Yellow	0.09	0.09	0.09	0.09	0.09	0.09
light Blue	0.16	0.16	0.16	0.16	0.16	0.16
Orange	0.23	0.23	0.23	0.23	0.23	0.23
Orange	0.30	0.30	0.30	0.30	0.30	0.30
Yellow	0.37	0.37	0.37	0.37	0.37	0.37
Blue	0.87	0.87	0.87	0.87	0.87	0.87
Sea buckthorn fruits						
light Blue	0.02	0.02	0.02	0.02	0.02	0.02
Orange	0.13	0.13	0.13	0.13	0.13	0.13
light Blue	0.18	0.18	0.18	0.18	0.18	0.18
Orange	0.26	0.26	0.26	0.26	0.26	0.26
Yellow	0.38	0.38	0.38	0.38	0.38	0.38
Orange	0.68	0.68	0.68	0.68	0.68	0.68
light Blue	0.81	0.81	0.81	0.81	0.81	0.81
Hawthorn fruits						
Orange	0.05	0.05	0.05	0.05	0.05	0.05
Orange	0.11	0.11	0.11	0.11	0.11	0.11
light Blue	0.23	0.23	0.23	0.23	0.23	0.23
Orange	0.26	0.26	0.26	0.26	0.26	0.26
Blue	0.77	0.77	0.77	0.77	0.77	0.77
Blue	0.86	0.86	0.86	0.86	0.86	0.86

Extraction method	Solid/Solvent ratio (w:v)	TPC (mg GAE/mL extract)	DPPH (% Inhibition)	Fe^{3+} reducing power (A700nm)	Fe^{2+} chelating activity (% Chelation)
Dog-rose fruits					
Maceration	1/5	3.50 ± 0.42	93.22 ± 4.19	0.31 ± 0.01	7.79 ± 0.65
Soxhlet	1/5	1.46 ± 0.13	91.06 ± 4.27	0.32 ± 0.01	6.17 ± 0.51
Ultrasound	1/5	4.13 ± 0.43	93.11 ± 4.18	0.38 ± 0.02	28.73 ± 2.79
Maceration	1/10	3.22 ± 0.23	93.63 ± 4.21	0.24 ± 0.01	20.78 ± 1.96
Soxhlet	1/10	2.28 ± 0.15	89.83 ± 4.00	0.20 ± 0.00	27.60 ± 1.67
Ultrasound	1/10	3.25 ± 0.37	91.37 ± 4.08	0.23 ± 0.02	25.16 ± 2.22
Sea buckthorn fruits					
Maceration	1/5	1.48 ± 0.13	91.26 ± 4.08	0.19 ± 0.01	12.01 ± 1.05
Soxhlet	1/5	1.26 ± 0.10	87.40 ± 3.87	0.15 ± 0.01	11.20 ± 0.97
Ultrasound	1/5	1.59 ± 0.17	90.65 ± 4.04	0.16 ± 0.02	17.69 ± 1.63
Maceration	1/10	1.31 ± 0.19	75.03 ± 3.22	0.09 ± 0.00	49.19 ± 4.96
Soxhlet	1/10	0.83 ± 0.09	78.62 ± 3.40	0.11 ± 0.00	53.41 ± 4.40
Ultrasound	1/10	1.18 ± 0.12	68.04 ± 2.90	0.12 ± 0.01	25.65 ± 2.40
Hawthorn fruits					
Maceration	1/5	1.50 ± 0.14	90.75 ± 4.05	0.14 ± 0.00	32.31 ± 1.17
Soxhlet	1/5	1.38 ± 0.14	89.10 ± 3.96	0.13 ± 0.00	31.50 ± 2.08
Ultrasound	1/5	1.40 ± 0.13	90.44 ± 4.03	0.13 ± 0.01	44.81 ± 2.49
Maceration	1/10	1.03 ± 0.20	86.64 ± 3.82	0.08 ± 0.02	63.64 ± 3.49
Soxhlet	1/10	0.90 ± 0.09	84.50 ± 3.71	0.07 ± 0.02	62.10 ± 3.32
Ultrasound	1/10	1.09 ± 0.11	85.92 ± 3.79	0.07 ± 0.01	65.26 ± 2.66

Table 3
ANTIOXIDANT ACTIVITY OF PHENOLIC EXTRACTS DEPENDING ON TOTAL PHENOLIC CONTENT

($p<0.05$) compared to the one registered for 1/10 ratio, due to the higher concentration of phenolic compounds in the extracts.

Fe^{2+} chelating activity of the extracts

The ability of chelating Fe^{2+} ions by the tested ethanolic extracts was significantly dependent ($p<0.05$) on the plant material, the solid-to-solvent ratio, and the extraction method used. The hawthorn fruits ethanolic extracts showed a Fe^{2+} chelating activity significantly higher compared to those of dog-rose fruits (with 30.57%) and sea buckthorn fruits (with 21.75%). Generally, ethanolic extracts obtained for 1/10 solid-to-solvent ratio showed a Fe^{2+} chelating ability significantly ($p<0.05$) higher than the extracts obtained for the 1/5 ratio. For the 1/5 solid-to-solvent ratio, the ability to chelate Fe^{2+} of alcoholic extracts obtained by ultrasound-assisted method was significantly ($p<0.05$) higher than of the ones obtained by maceration and Soxhlet methods; the mean value found for the ultrasound-assisted method was with 14.12% higher compared to that registered for Soxhlet method, and with 13.04% higher than that found for maceration method.

Correlations

Phenolic compounds are widely studied for their antioxidant properties, although the term antioxidant has a broad range of meanings. Antioxidant activity refers to both the ability of phenolic compounds to prevent damage from reactive oxygen species (ROS) (such as through radical scavenging) or to prevent generation of these species (by binding iron) [29]. Radical scavenging activity of phenolic compounds is due to their ability to act as reducing agents, hydrogen or electrons donors and singlet oxygen quenchers. In this study, the correlation between TPC and FC, TPC/FC and the antioxidant activity were determined by using linear correlations. The correlations found between TPC and FC, for the extracts obtained from the three studied vegetal materials were strong positive: dog-rose fruits ($r = 0.973$), sea buckthorn fruits ($r = 0.979$) and hawthorn fruits ($r = 0.957$). DPPH radical scavenging activity was weakly positively correlated to TPC for the sea buckthorn fruits extracts ($r = 0.479$) and hawthorn fruits ($r = 0.466$); there was no correlation between TPC/FC and DPPH• scavenging activity for dog-rose fruits extract. These results indicate that DPPH radical scavenging activity of each extract could be related not only to the concentration of phenolic hydroxyl groups, but very important is the phenolic compounds structure. Phenolic compounds included tannins, flavonoids, phenolic acids and other compounds that have phenolic structure. Flavonoids are not always phenolic compounds; this is dependent on the position of OH radical into the flavonoid structure - only flavonoids that have OH radicals in A and/or B ring are characterized as phenolic compounds. Also, phenolic acids have a lower antioxidant activity than flavonoids [31]. In previous studies on the correlations of phenolic compounds and DPPH•, the scavenging activity showed that the phenolics were involved differently ($r = 0.792$; $r = -0.772$) or no correlation were found [32]. Other

authors [30] observed a negative correlation with the metal ion chelating activity and DPPH• % inhibition at higher concentration of phenolics from dog-rose fruits.

Also, they reported that in dog-rose fruits extracts, the DPPH radical scavenging activity did not show an increasing trend at higher concentrations, moreover, the scavenging activity decreased at higher concentration [30]. The ability of Fe^{2+} chelating activity and TPC was weakly positively correlated (r = 0.462) for the dog-rose fruits extracts, weakly negatively correlated (r = - 0.570) for the sea buckthorn fruits extract, and strongly negatively correlated (r = - 0.684) for the hawthorn fruits extract. These results demonstrate the molecular heterogeneity of the extracts, Fe^{2+} chelating activity being dependent of phenolic compounds structure. Metal chelating potency of phenolic compounds depends upon their unique phenolic structure and to the number and location of the hydroxyl groups [33]. In other studies, correlations between ferrous ion chelating ability and TPC were reported as being significantly positive (r = 0.978) [34], insignificantly negative (r = - 0.412) [35] or no correlations [36]. Between FC and Fe^{3+} reducing power of the extracts was found a strong positive correlation for the sea buckthorn fruits extracts (r = 0.654) and hawthorn fruits (r = 0.818), but there were no correlations for the dog-rose fruits extracts. In other studies on the flavonoids content and the Fe^{3+} reducing power, there were reported positive correlations (r = 0.974) for the ethanolic extracts, but there were not observed any correlations for the tamarillo aqueous extracts [37].

Between the solid-to-solvent ratio and the ability of chelating ferrous ions were found strong positive correlations for dog-rose fruits (r = 0.842) and hawthorn fruits (r = 0.980), and weak positive correlations for sea buckthorn fruits (r = 0.553). Between the ability of chelating ferrous ions and ferric ions reducing power were found strong negative correlations for sea buckthorn fruits (r = - 0.712) and hawthorn fruits (r = - 0.772), and no correlations were found for dog-rose fruits.

Conclusions

Dog-rose fruits had a TPC and a FC significantly higher than sea buckthorn and hawthorn fruits. By the maceration and ultrasound-assisted methods were found TPC and FC values significantly higher than those obtained by Soxhlet method. The reducing properties of the extracts significantly depended on the vegetal material and the solid-to-solvent ratio. The 1/10 (w:v) solid-to-solvent ratio was more favorable for phenolics and flavonoids extraction than 1/5 (w:v) ratio. Rosehips ethanolic extracts showed DPPH• scavenging activity and Fe^{3+} reducing power significantly higher than those of the sea buckthorn and hawthorn fruits extracts. Hawthorn fruits extracts had the highest ability of chelating Fe^{2+}, significantly higher compared to the ones registered for sea buckthorn and dog-rose fruits extracts.

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