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STUDIES ON THE ANTIOXIDANT POTENTIAL OF *ARONIA MELANOCARPA* EXTRACTS

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Abstract
Two hydro-alcoholic extracts of Aronia melanocarpa berries were obtained by different extraction techniques: classical solvent extraction by maceration with solvent, under stirring, for 10 hours and ultrasonic assisted extraction at 20 kHz for 5 minutes, respectively. In both cases the ratio dried vegetal material: hydro-alcoholic solvent mixture (3:1, v: v) was 1: 10 (g: g). The resulting extracts were analysed to evaluate their antioxidant capacity. An important antioxidant activity expressed by the amount of total polyphenols content and antiradical power it was shown for both extracts. The method using ultrasounds was more efficient leading to extracts richer in polyphenols (5376.947 mg GAE/10. 0g D.W.) and with higher antiradical power meaning higher percentage of inhibition of DPPH and lower EC_{50}. Using this powerful extract, the synthesis of some silver nanoparticles was performed, and the results were confirmed by molecular absorption spectrometry (MAS) and scanning electron microscopy (SEM). The characteristic peak for green silver nanoparticles has been found, in the region 450 nm of the absorption spectrum. Therewith, SEM images showed spherical nanoparticles with dimensions between 18 -105 nm.

Keywords: antioxidant activity, Aronia melanocarpa, silver nanoparticles

1. INTRODUCTION

Aronia melanocarpa called “black aronia” or “black chokeberry” (meaning „bitter berry”) is a species in *Rosaceae* family, *Maloideae* subfamily and it is a potential therapeutic food. It plays an important role in prevention of life-related common diseases due to the high levels of antioxidants compounds.

A lot of scientific researches have shown numerous beneficial actions of this species on the human body. These include: anti-inflammatory activity (Zapolska-Downar et al., 2012), decrease the risk of developing cardiovascular or other diseases associated with oxidative stress (Malinowska et al., 2013; Varela et al.,2016), neuroprotective effect (Lee et al., 2017), improves peripheral body temperature and blood flow (Sonoda et al., 2013), breast cancer having a beneficial effect on thiols concentration in plasma (Olas et al., 2010; Kędzierska et al.2013), prevention of metabolic diseases associated with obesity (Sikora et al., 2012).

Different parts of the plant have been analysed for antioxidants content, from flowers (Krivoruchko and Kovalev, 2011) to leaves and fruits (Szopa et al., 2017) or processed juice (Oszmiański et al., 2005). It is appreciated that the fruits have the highest contents, with a great variety of compounds, the most important being polyphenols, especially anthocyanins, procyanidins, flavonoids and flavonic acids (Kähkönen et al., 1999).

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Antioxidant properties depend not only on the amount of antioxidant compounds but also on the type and structure of them. Polymeric procyanidins composed of (-) epicatechin (flavan-3-ol) units represents 66% of total polyphenols (Kokotkiewicz et al., 2010). Anthocyanins come in the second place with 25% total polyphenols and are represented in particular by glucoside, arabinoside, galactoside and xyloside (Denev et al., 2012). Cyanidin-3-O-galactoside may be involved in the neuroprotective effect of *A. melanocarpa*, as exerted an antioxidant effect and a cognitive effect on spatial memory and regulates hippocampal ERK expression in senescence-accelerated mice. (Lee et al., 2017). Phenolic acids present in berries are hydroxylated derivates of benzoic and cinnamic acids as: chlorogenic acid, neoclorogenic acid, rosmarinic acid, 3,4-dihidroxyphenylacetic acid, protocatchuic acid (Szopa et al., 2017).
The contents of polyphenols are significantly influenced by the conditions in which the fruits of aronia are stored and processed. The extraction procedures using different solvents, particle size, solid–solvent ratio, pH and extraction time could have an impact in polyphenols levels (Ćujić et al., 2016). Different drying methods, such as freeze-drying, vacuum or microwave has also influence. It seems that the highest content of bioactive compounds is recording in freeze-dried samples (Samoticha et al., 2016).
Considering these aspects, the purpose of this work is to investigate the antioxidant activity of aronia berry extracts obtained by two extraction techniques and the possibility of obtaining silver nanoparticles using their reducing properties.

2. MATERIALS AND METHODS

Extraction

Aronia fruits were subjected to analysis. These were purchased from local producers and come from plants grown in the climate and soil conditions specific to the Arges region, Romania. The berries were separated, washed with water and rinsed with distilled water, then dried for 48 hours in a thermostat oven at 50^{o}C. They were subsequently ground and extracted with a mixture of H_2O: ethanol in a ratio of 3: 1 (V: V) by two methods, as shown in Table 1. The extraction ratio, aronia: hydroalcoholic mixture, was 1:10 in both methods.

Table 1. The extracted samples for analysis

Sample name	Extraction ratio Aronia: Solvent mixture (V:V)	Extraction parameters
Aronia 1	1:10	*Ultrasonic assisted extraction* 20 g of dried and minced vegetable material with 200 g of hydro-alcoholic (3:1) mixture were subjected to an ultrasound assisted extraction technique at 20 kHz, using a HIELSCHER UIP1000hDT ultrasonic device, for only 5 minutes. The average net power of ultrasound was about 180 W, and the temperature of the mixture was kept in the 16^{0}-31^{0}C range.
Aronia 2	1:10	*Solvent extraction* 10 g of dried and minced vegetable material with 100 g of hydro-alcoholic 3: 1 mixture were extracted under magnetic stirring, at 40^{o}C and 1500 rpm, for 10 hours. The extracts were filtered using filters with a pore size 2.5μm each (Whatman, grade 5, Qualitative filter paper), then subjected to analysis.

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The antioxidant activity

The antioxidant effects of chokeberry extracts were evaluated by two different in vitro assays.

a) Radical-scavenging activity by the DPPH test with 2,2-diphenyl-1-picrylhydrazyl

The antiradical ability or efficiency represents the property of a compound to remove from a system a free radical as a result of electrons, protons or both transfer reactions. The antiradical ability of the different plant extracts was evaluated by comparison with a stable organic radical, having a long life in aqueous solution, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH). The method is based on the theory that a donor of H atoms is an antioxidant so that it measures the ability of the compounds to capture radicals. 2,2-Diphenyl-picrylhydrazyl (DPPH) is one of the most stable organic radicals. It accepts an $H^{\cdot}$ radical from an antioxidant and the antioxidant effect of the extract is proportional to the disappearance of the DPPH radical in the sample. DPPH radical has a strong absorption maximum at 517 nm (purple). The colour turns to yellow as a result of DPPH H formation by fixing the $H^{\cdot}$ radical from an antioxidant. The antioxidant effect can be evaluated by following the decrease of the absorbance at 517 nm. (Brand-Williams et al., 1995; Kedare et al., 2011).

The extracts were reduced to remove the solvent. Four ethanolic dilution for each of the two samples, with the concentration between 0.02 - 0.2 mg extract / ml ethanol (20-200 ppm) were prepared. An ethanolic solution of DPPH with absorbance below 2 was also prepared. The reaction mixture consisted of adding 4 ml of DPPH solution in ethanol to 1.0 mL of each sample (Aronia 1-Aronia 2) and each concentration (C_1 -C_4). The changes in colour (from deep violet to light yellow) were read at 517 nm after 30 min of reaction. The control solution was prepared by mixing ethanol (1 mL) and DPPH radical solution (4.0 mL).

Percent inhibition (I%) was calculated, as has been shown previously (Topala and Tataru, 2019), from the decrease in absorbance with the relation:

$$I\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

where: A_{blank} is the absorbance for the blank (reference solution of ethanol-$DPPH^{\cdot}$ ethanolic solution) and A_{sample} is the absorbance for the samples mixed with DPPH reference solution.

Recording the percentage of inhibition for each concentration we obtain EC_{50} i.e. concentration required to obtain a 50% antioxidant effect. EC_{50} is a typically employed parameter to express the antioxidant capacity and to compare the activity of different compounds (Chen et al., 2013).

b) Total polyphenol content by Folin-Ciocalteu reagent

The total polyphenols content (TPC) can be expressed as Folin Ciocalteu index. That involved the reduction of the F-C reagent by the phenolic compounds in aronia extracts with the formation of a blue complex which absorb at λ_{max} = 760nm. The results were expressed in gallic acid equivalent (GAE) on a calibration curve obtained with a series of 12.5 – 150 μg/ml gallic acid solution and describe by a linear regression equation.

The results in absorbance were obtained as an average of three consecutive determinations and read at an UV–Vis Jasco 730 spectrometer.

Green synthesis of silver nanoparticles

2 ml of aronia extract 7.5 mg/ml was added slowly, under gentle stirring (500rpm) and at room temperature (25^{o}C), to 18 ml of $AgNO_3$ 10^{-3} mol/l solution. The pH was adjusted to 9. Absorption spectra were acquired after 10 min, 30 min, 1 hour and 24 hours of dark incubation, to observe the characteristic absorption peaks that indicate aronia antioxidants – AgNp formation.

Scanning electron microscopy (SEM) was also a tool for confirming nanostructures. The instrument was a Scanning Electron Microscope SU5000.

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3. RESULTS AND DISCUSSIONS

The results obtained by analysing the antioxidant activity for the two aronia extracts are presented in Table 2. As can be seen, both extracts have high polyphenolic content and a strong antiradical activity. The values obtained for our extracts are within the limits of 3000-8000 mg GAE / 100 g D.W. obtained by different researchers and cited in literature (Denev et al., 2012).
The total amount of polyphenols extracted from 100 g of dried berries is significantly influenced by the extraction method. Ultrasonically assisted extraction is much more efficient because it extracts, in a shorter time, an amount of polyphenols 39% higher than the classical maceration method.

Table 2. Antioxidant capacity for the dried berries of aronia extracts

Sample	TPC mg GAE/100 g D.W. ± SD*	DPPH test EC_{50} (µg/ml)
Aronia 1	5376.947 ± 0.6853	51.82 ± 0.0264
Aronia 2	3863.042 ± 0.2620	90.92 ± 0.0639

*Data are presented as the mean as ± SD of three individual measurements

The same tendency can be observed regarding the anti-radical activity. The diluted solutions of the extract, with the concentration between 0.02 - 0.2 mg extract/ml ethanol, lead to 30-90% percentage of inhibition of DPPH, after 30 minutes from the moment of radical reaction initiation.
For a better evaluation of the antiradical power and to eliminate accidental errors, the results were expressed as EC_{50} using a regression model. The values of I% obtained for every concentration were plotted to obtain EC_{50} values i.e. concentration in which the 50% of the free radical DPPH is reduced after 30 minutes. The lower this concentration, as in the case of Aronia 1 sample, the higher the antiradical power of the extract obtained.
Differences in analytical procedures applied to obtain the extracts influences their quality. Ultrasonically assisted method has higher efficiency and les extraction time, and it is safe for heat labile compounds.

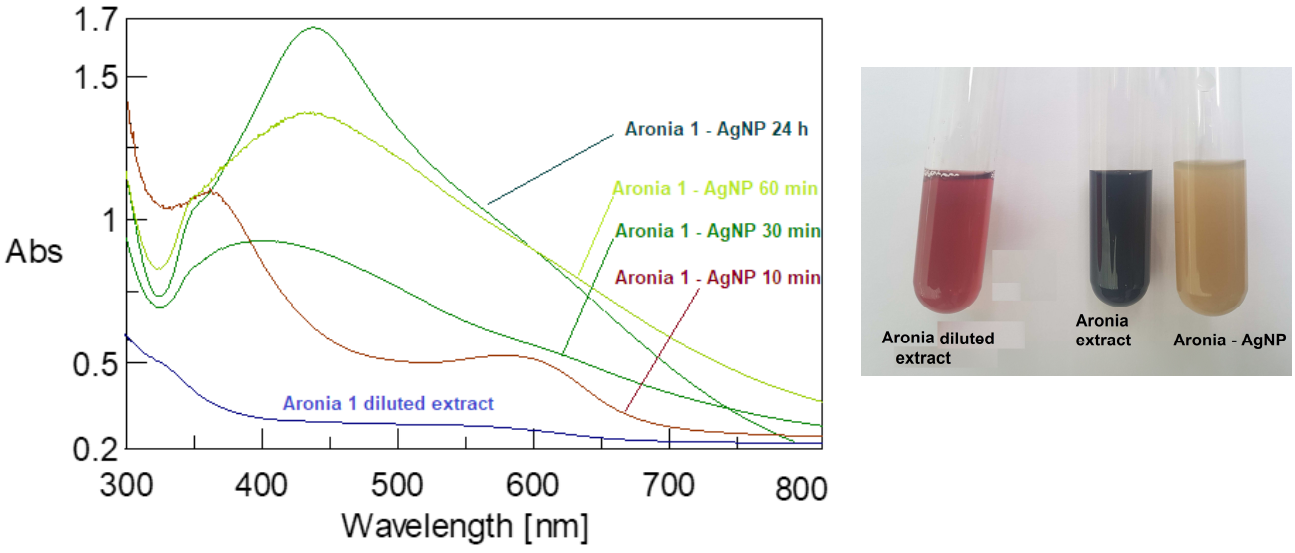

Figure 1. Aronia 1 – Ag NP – spectral and visual characteristics

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The Aronia 1 sample was used in order to obtain Ag nanoparticles by green synthesis. After 10 minutes starting with the moment when the redox reaction was initiated, the reducing effect of the extract was observed in the solution, respectively its discoloration and the appearance of a brown opalescent colour characteristic to the colloidal Ag (Figure 1).

The recording of molecular absorption spectra revealed a wide peak in the area of 450 nm that represents the formation of silver nanoparticles. By increasing the contact time, the particle is further improves so that the width of the absorption band decreases and the intensity of the peak increases.

The Aronia - Ag NP were confirmed by scanning electron microscopy (SEM). The SEM image of synthesized silver nanoparticles shows that the particles are nanoscale. The shape of the nanoparticles is spherical and the size ranges of 18 to 105 nm (Figure 2).

Nanoparticles tend to clump together, which is why it is necessary to use a dispersing agent for better preservation.

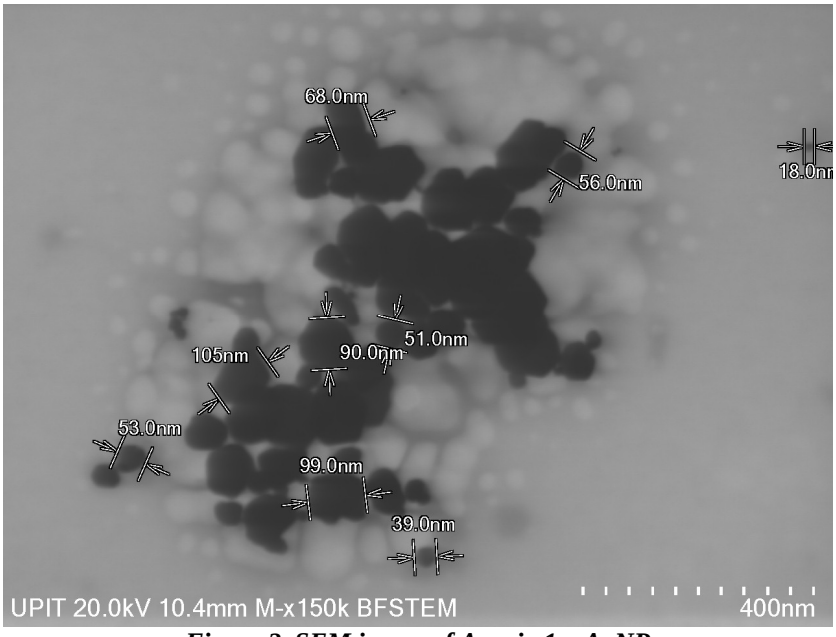

Figure 2. SEM image of Aronia 1 – AgNP

4. CONCLUSIONS

In this study hydro-alcoholic the extracts of *Aronia melanocarpa* berries were applied to green synthesis of silver nanoparticles and their antioxidant activity was tasted.

The antioxidant capacity of aronia dried berries depends of extraction technology. Our results show that the ultrasonically assisted method leads to extracts with better antioxidant activity, both in terms of total polyphenol content and in terms of antiradical power.

Green synthesis of of silver nanoparticles using aqueous extract of chokeberries is a clean, inexpensive and safe method as long as it does not use other chemical reagents or solvents. The obtained nanoparticles, concentrate the antioxidant activity and have germicidal and bactericidal action representing potential forms for the replacement of chemical drugs.

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