

Biological activities of *Sorbus aucuparia* L. leaves extract

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Received: October 29, 2018

Accepted: November 16, 2018

Published on-line: December 20, 2018

Published: December 25, 2018

Biological activities of *Sorbus aucuparia* L. leaves extract were tested in our study. Antineurodegenerative activity was evaluated by acetylcholinesterase (AChE) and tyrosinase (TYR) inhibitory activity assays while antioxidant activity was tested using four different assays (DPPH, ABTS, FRAP, and β -carotene test). In all antioxidant tests, concentration-dependent activity of leaves extract was noticed and the highest applied concentration (500 $\mu\text{g/ml}$) was the most active. Extract applied in a concentration of 200 $\mu\text{g/ml}$ showed higher percent of DPPH inhibition compared with both concentrations of standard substances BHA and BHT (50 and 100 $\mu\text{g/ml}$). In the ABTS test, the activity of extract applied in 500 $\mu\text{g/ml}$ was comparable or higher than BHA and BHT/Vitamin C, respectively. Moreover, all concentrations of tested extract were significantly more active in the β -carotene test than vitamin C. The lowest concentration of extract (100 $\mu\text{g/ml}$) was the most active in TYR assay reaching 42.57% of the inhibition while in AChE assay there was not statistically significant differences among all applied concentrations.

Key words: *Sorbus aucuparia*, dry extract, antioxidant activity, antineurodegenerative activity

<http://dx.doi.org/10.5937/leksi1838022S>

1. INTRODUCTION

Genus *Sorbus*, Rosaceae is organized in four subgenera: *Aria*, *Sorbus*, *Cornus* and *Torminaria* that comprises about 250 species commonly distributed in the northern hemisphere with a colder climate. The most familiar species in Europe are *S. aucuparia* L. (rowanberries), *S. aria* (L.) Crantz, *S. torminalis* (L.) Crantz and *S. domestica* L. and their fruits have been traditionally used as anti-inflammatory, vasoprotective and diuretic agents. Fruits represent rich source of vitamins and antioxidative compounds having a significant role in nutrition and well-being (Hallmann et al., 2011; Kylli et al., 2010; Olszewska and Michel, 2009). Although, berries are well known as a valuable source of bioactive compounds, berry leaves recently are becoming more interesting material for research as a new source of polyphenols (Matczak et al., 2018). *Sorbus aucuparia* is a tree widely distributed in the mountain regions of Europe and it is also popular as an ornamental plant (Kylli et al., 2010). Different polyphenolic compounds such as phenolic acids, flavonols and their glycosides, flavan-3-ols etc. have been found in *S. aucuparia* (Kylli et al., 2010; Olszewska et al., 2012). Šavikin et al. (2017a) have also reported, carotenoids, tocopherols, and chlorophylls in berries from *S. aucuparia* collected in Serbia and Montenegro. Due to valuable chemical composition di-

verse biological activities have been reported for *S. aucuparia* such as antioxidant and antimicrobial (Hukkanen et al., 2006; Kylli et al., 2010; Šavikin et al., 2017a). Moreover, leaves of different *Sorbus* species including *S. aucuparia* were found to be biologically active due to its chemical composition (Raudone et al., 2015). Also, according to traditional Polish and East-European medicine rowanberry inflorescences has been used as a diuretic and anti-inflammatory agent (Olszewska and Michel, 2009).

According to literature, very limited reports (Denev et al., 2014; Šavikin et al., 2017a) could be found regarding the chemical composition and biological activity of *Sorbus* species from Balkan Peninsula. Moreover, such reports for the leaves of *S. aucuparia* lack. Taking into account these facts, the aim of our work was to study antioxidant and antineurodegenerative activities of leaves of *S. aucuparia* collected on Stara Planina Mountain, Serbia.

2. MATERIALS AND METHODS

2.1. Extract preparation

Leaves of *S. aucuparia* were collected on natural locality, Stara Planina Mountain at 980 m.a.s.l. in July 2017. Extraction with 70% EtOH (1:10, w/v) was performed using maceration with

Table 1. Antioxidant activity of *Sorbus aucuparia* leaves (SLE) dry extract

Sample	Concentration ($\mu\text{g/mL}$)	DPPH radical scavenging activity ^a	ABTS radical scavenging activity	FRAP activity	β -carotene bleaching activity
		% of inhibition	% of inhibition	$\mu\text{mol Fe(II)/g}$	% of inhibition
SLE dry extract	100	34.27 \pm 0.87 f	18.23 \pm 0.97 f	180.16 \pm 5.18 f	35.53 \pm 1.44 c
	200	51.21 \pm 0.67 d	31.12 \pm 0.66 e	582.81 \pm 7.47 b	38.99 \pm 0.98 c
	500	86.52 \pm 0.67 b	63.21 \pm 0.52 a	840.18 \pm 5.89 a	48.11 \pm 1.70 b
BHA	50	28.21 \pm 0.60 g	56.18 \pm 0.80 b	231.22 \pm 4.71 e	50.79 \pm 2.76 b
	100	43.33 \pm 0.87 e	64.95 \pm 0.63 a	572.85 \pm 5.71 b	57.70 \pm 1.91 a
BHT	50	20.86 \pm 0.81 h	37.47 \pm 0.67 c	364.05 \pm 5.62 d	48.74 \pm 1.79 b
	100	34.31 \pm 0.43 f	55.11 \pm 0.44 b	413.03 \pm 3.13 c	56.29 \pm 1.44 a
Vit C	50	58.99 \pm 1.01 c	35.14 \pm 0.58 d	427.56 \pm 5.18 c	1.73 \pm 1.19 d
	100	91.65 \pm 0.21 a	55.70 \pm 0.69 b	576.17 \pm 7.61 b	2.99 \pm 2.13 d

^aValues with the same letter (a-h) in each column showed no statistically significant difference ($P < 0.05$); Statistical analysis was based on a one-way ANOVA and Bonferroni tests.

continuous stirring at 100 rpm during 24 h at room temperature. Extraction was repeated till the solvent became colorless. Further, extracts were combined, filtered and evaporated under reduced pressure till complete dryness (Buchi rotavapor R-114). Crude extracts were stored at +4 °C. The amount of total phenolics was 138.17 \pm 3.47 mg GAE/g (Singleton and Rossi, 1965) while the amount of total flavonoids was 20.78 \pm 1.52 mg QE/g (Park et al., 1997).

2.2. Evaluation of antioxidant activity

2.2.1. DPPH assay

Radical scavenging activity of the extract was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Blois, 1958). One hundred μL of the extract solution in methanol (100, 200 and 500 $\mu\text{g/mL}$) and 900 μL of methanolic solution of DPPH (40 $\mu\text{g/mL}$) were mixed. The absorbance of the reaction mixture was measured after incubation for 30 minutes in the dark at room temperature at 517 nm. BHA, BHT and ascorbic acid in concentrations of 50 and 100 $\mu\text{g/mL}$ were used as positive controls. The percentage of neutralization of DPPH radical was calculated using the equation:

$$\text{Inhibition of DPPH radical (\%)} = \frac{AC - AS}{AC} \times 100\%$$

where AC is the absorbance of control (without test sample) and AS is the absorbance of the test sample at different concentrations.

All experimental measurements were carried out in triplicate and the results are expressed as the average of three measurements \pm standard deviation.

2.2.2. ABTS assay

The scavenging activity of the extract was evaluated by ABTS assay (Miller et al., 1993), with some modifications. The stock solution of ABTS⁺ (7 mM) was prepared 12-16 hours before the experiment in 2.46 mM potassium-persulfate and stored in the dark at room temperature. Prior analysis it was diluted by distilled water to obtain an absorbance of working solution 0.700 \pm 0.020 at 734 nm. The activity of three different concentrations (100, 200 and 500 $\mu\text{g/mL}$) of the extract was tested by

mixing 25 μL with 1 mL of working ABTS⁺ solution and incubated for 30 min at 30 °C. The same procedure was applied for positive controls BHA, BHT and ascorbic acid in concentrations of 50 and 100 $\mu\text{g/mL}$. Absorbance was recorded at 734 nm. The decrease of ABTS radical absorption at 734 nm was calculated using the equation:

$$\text{Inhibition of ABTS radical (\%)} = \frac{AC - AS}{AC} \times 100\%$$

where AC is the absorbance of control (without test sample) and AS is the absorbance of the test sample at different concentrations. All experimental measurements were carried out in triplicate and the results are expressed as the average of three measurements \pm standard deviation.

2.2.3. Ferric-reducing ability of plasma (FRAP) assay

Ferric-reducing ability of plasma (FRAP) assay evaluates the total antioxidant capacity of the extract by measuring its ability to reduce ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) at low pH. The FRAP assay was performed according to Benzie and Strain (1996) procedure with slight modifications. FRAP reagent was prepared freshly and it contained sodium acetate buffer (300 mmol/L, pH 3.6), 10 mmol/L TPTZ in 40 mmol/L HCl and FeCl₃ \times 6H₂O solution (20 mmol/L), in proportion 10:1:1 (v/v/v), respectively. Extract solutions in methanol (40 μL) in concentrations of 100, 200 and 500 $\mu\text{g/mL}$ were added to 1200 μL of FRAP reagent and absorbance was recorded at 593 nm after 4 minutes. BHA, BHT and ascorbic acid (in concentrations of 50 and 100 $\mu\text{g/mL}$) were used as standards. Standard solution of FeSO₄ \times 7H₂O (0.2-1.6 mmol/L) was used for constructing the calibration curve. FRAP values of samples were calculated from the standard curve equation and expressed as $\mu\text{mol FeSO}_4 \times 7\text{H}_2\text{O/g}$ dry extract. All experimental measurements were carried out in triplicate and the results are expressed as the average of three measurements \pm standard deviation.

2.2.4. β -carotene bleaching (β -CB) assay

β -carotene bleaching assay, designed to evaluate the capacity of the antioxidants to reduce degradation of β -carotene in a β -carotene linoleic acid emulsion, was performed according

Table 2. Antineurodegenerative activity of *Sorbus aucuparia* leaves (SLE) dry extract

	Concentration ($\mu\text{g/mL}$)	AChE inhibitory activity ^a	TYR inhibitory activity
		% of inhibition	% of inhibition
SLE dry extract	100	40.78 \pm 0.19b	42.57 \pm 1.37b
	200	43.19 \pm 1.20b	39.18 \pm 2.20b
	500	42.94 \pm 0.77b	31.21 \pm 1.55a
Gаланthamine	100	57.11 \pm 1.68a	-
Kojic acid	100	-	51.81 \pm 2.55a

^aValues are means \pm standard deviation. n = 3; Mean values within a column with different letters are significantly different at P<0.05; Statistical analysis was based on a one-way ANOVA and Bonferroni tests.

to the slightly modified procedure of Dapkevicius et al. (1998). β -carotene (1 mg), linoleic acid (50 μL) and Tween 40 (400 mg) were dissolved in 2 mL of chloroform. Chloroform was removed using a rotary evaporator at 40 $^{\circ}\text{C}$, and 200 mL of distilled water was added with vigorous shaking in order to prepare an emulsion. The extract (100, 200 and 500 $\mu\text{g/mL}$) and positive controls BHA, BHT and ascorbic acid (50 and 100 $\mu\text{g/mL}$) were prepared in methanol. Aliquots of 1000 μL of the emulsion and 140 μL of samples (extract/standard) were mixed. The absorbances were measured immediately (t=0 min) and after 2 h incubation (t=120 min) at 490 nm. The antioxidant activity of the sample was evaluated using the following equation:

$$\% \text{ Inhibition} = \frac{A_{120} - C_{120}}{C_0 - C_{120}} \times 100 \%$$

where A_{120} and C_{120} are the absorbances measured in t=120 minutes for sample and control, respectively, while C_0 is absorbance of control in t=0 min.

All experimental measurements were carried out in triplicate and the results are expressed as average of three measurements \pm standard deviation.

2.3. Evaluation of antineurodegenerative activities

2.3.1. Acetylcholinesterase (AChE) inhibitory activity assay

AChE inhibitory activity assay was performed according to the spectrophotometric method described before (Ellman et al., 1961), with slight modifications (Šavikin et al., 2017b). The AChE activity was measured by monitoring of increase of yellow color produced from tiochrome when it reacts with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) ion. The test reaction mixture (S) was prepared by adding 140 μL of sodium phosphate buffer (0.1 M, pH 7.0), 20 μL of DTNB, 20 μL of extract-buffer solution containing 5% DMSO (concentration of 100, 200 and 500 $\mu\text{g/mL}$) and 20 μL of AChE solution (5 units/mL). The mixture without extract was used as the control (C), while blank (B) did not contain AChE solution. The commercial anticholinesterase alkaloid-type of drug galanthamine was used as a reference. After incubation (15 min, 25 $^{\circ}\text{C}$), the reaction was initiated with the addition of 10 μL of acetylthiocholine iodide and absorbance was measured at wavelength of 412 nm using Tecan Sunrise SN microplate reader equipped by XFluor4 software. Percentage of inhibition of AChE by sample was determined using the formula:

$$\text{Inhibition of AChE (\%)} = \frac{C - (S - B)}{C} \times 100\%$$

All experimental measurements were carried out in triplicate and the results are expressed as average of three measurements \pm standard deviation.

2.3.2. Tyrosinase inhibitory activity assay

Tyrosinase inhibitory activity assay was performed according to a slightly modified spectrophotometric method of (Masuda et al., 2005). The extract prepared in different concentrations (100, 200 and 500 $\mu\text{g/mL}$) and kojic acid (100 $\mu\text{g/mL}$) used as reference compound was dissolved in sodium phosphate buffer (0.1 M, pH 7.0) containing 5% DMSO and phosphate buffer. The wells were designed as: A (containing 120 μL of sodium buffer and 40 μL of tyrosinase in the same buffer (46 units/L)), B (containing only buffer), C (containing 80 μL of buffer, 40 μL of tyrosinase and 40 μL of sample) and D (containing 120 μL of buffer and 40 μL of sample). After the addition of 40 μL of L-DOPA and incubation (30 min, 25 $^{\circ}\text{C}$), absorbance was measured at 475 nm using Tecan Sunrise SN microplate reader equipped by XFluor4 software. Percentage of inhibition of tyrosinase was determined using the formula:

$$\text{Inhibition of tyrosinase (\%)} = \frac{(A - B) - (C - D)}{(A - B)} \times 100\%$$

All experimental measurements were carried out in triplicate and the results are expressed as average of three measurements \pm standard deviation.

2.4. Statistical analysis

Results are presented as the mean value \pm standard deviation of three independent experiments (n = 3). Statistical analysis was based on a one-way ANOVA test. Statistically significant effects were further analyzed and means were compared using Bonferroni test. A level of P<0.05 was taken as statistically significant.

3. RESULTS AND DISCUSSION

3.1. Evaluation of antioxidant activity

Antioxidant activity of *S. aucuparia* leaves extract (SLE) was evaluated using four in vitro tests. Results are presented in Table 1. Three concentrations of extract (100, 200 and 500 $\mu\text{g/mL}$) were tested. Moreover, standard substances BHA, BHT, and ascorbic acid were tested in concentrations of 50 and 100 $\mu\text{g/mL}$. Concentration-dependent activity was noticed for all tested extract in assays and the most active was the highest concentration (500 $\mu\text{g/mL}$). SLE applied in a concentration of 200 $\mu\text{g/mL}$ showed better activity in DPPH test compared with standard substances BHA and BHT applied to the concentration of 50 and 100 $\mu\text{g/mL}$ (Table 1) while the activity in ABTS was similar as was for BHA 100 $\mu\text{g/mL}$ (Table 1). In FRAP assay, the concentration of SLE of 500 $\mu\text{g/mL}$ was more active (840.18 $\mu\text{mol Fe(II)/g}$) compared with all standard substances applied in both tested concentrations (Table

1). On the other hand, activity of SLE in the β -carotene test was lower compared with BHA and BHT but significantly higher than of vitamin C all applied in both concentrations (Table 1). This significant antioxidant activity could be at least partially explained by high content of polyphenols and flavonoids in tested extract (138.17 ± 3.47 mg GAE/g and 20.78 ± 1.52 mg QE/g, respectively). Olszewska et al. (2010) have shown that antioxidant activity of tested inflorescences and leaves of some *Sorbus* species is in correlation with high values of total phenolic content as well as with the content of specific groups of polyphenols proanthocyanidins, chlorogenic acids isomers, and flavonoids. Olszewska and Michel (2009) have investigated antioxidant activity of different parts (inflorescences, leaves, fruits) of three well known traditionally used *Sorbus* species (*S. aucuparia*, *S. aria*, *S. domestica*) where rowanberry extracts were the most active in DPPH, ABTS and FRAP assay. Among them, rowanberry inflorescences and leaves extract showed more pronounced effect compared to the activity of the fruits. To the best of our knowledge, this is the first report on the antioxidant activity of rowanberry leaves extract tested for antioxidant activity in the β -carotene assay. Although, rowanberry leaves have been previously tested in AAPH [2,2-azobis-(2-amidinopropane) dihydrochloride]-induced linoleic acid (LA) peroxidation test, where the similar mechanism of the antioxidant mechanism is involved (Olszewska et al., 2010).

3.1.1. Evaluation of antineurodegenerative activities

Enzyme inhibition activity of SLE was tested against acetylcholinesterase (AChE) as well as tyrosinase (TYR). Those enzymes are connected with the development of some neurodegenerative diseases. Reduced level of neurotransmitter acetylcholine is connected with the loss of memory that occurs in Alzheimer's disease (AD) so cholinesterase inhibitors are found to be effective in patients that suffer from mild to moderately level of AD (Ahmed et al., 2013). Tyrosinase is connected with Parkinson's disease (PD) as it is involved in the formation of neuromelanin which hyperproduction could be a trigger for PD (Greggio et al., 2005). As the standard therapy for neurodegenerative diseases is often accompanied by side effects, natural products which could prevent, slow down or cure neurodegenerative diseases are of great interest (Morzelle et al., 2016). Three concentrations (100, 200 and 500 μ g/ml) of SLE were used in AChE and TYR inhibition assays for testing the anti-neurodegenerative potential. The activity was compared with those of 100 μ g/ml reference drugs galantamine and kojic acid, respectively (Table 2). Galantamine achieved an inhibition of 57.11% while kojic acid achieved 51.81%. SLE reached 43.18 and 42.57% of inhibition in AChE and TYR assays, respectively. In AChE test, there were no statistical differences among all applied concentrations while in TYR test most active was the lowest applied concentration of 100 μ g/ml of extract. Although the activity of SLE was weaker comparing to standards, it could be caused by type of solvent used for the extraction and, consequently, with the type and the amount of extracted compounds (Ćujić, 2017). Hasbal et al. (2015) showed that water extract of fruits of *S. torminalis* possessed dose-dependent AChE inhibitory activity but, it was not as potent as standard galantamine. Similar activity was evaluated for SLE. They concluded that beneficial effect can be explained by the antioxidant activity of the extract connected with the presence of polyphenols. Moreover, Ekin et al. (2016) used 75% ethanolic extract to test acetylcholinesterase inhibitory activity of thirty-four Rosaceae samples among them of 7 *Sorbus* species collected on different localities in Turkey. Extracts of *S. umbellata* collected in two different localities had high inhibition potential against AChE i.e. 56.20 ± 5.0 and $58.18 \pm 3.77\%$, respectively. They pointed out marked varia-

tion in chemical composition and bioactivity not only between different species of the same genera but, also, among the same plant species growing in different localities.

CONCLUSION

In our study, we screened antioxidant and antineurodegenerative activities of *Sorbus aucuparia* leaves dry extract. The highest antioxidant activity has been achieved in DPPH and β -carotene test where the activity of the extract was more potent than of standard substances BHA and BHT and, vitamin C, respectively. On the other hand, antineurodegenerative activity of *S. aucuparia* extract was determined as moderate. Future study will be focused on the optimization of extraction procedure and extract fractionation aimed to increase the amount of biologically active compounds in the extract.

ACKNOWLEDGMENTS

Acknowledgment. This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, project number 46013, and 173029.

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