Biological activities of *Sorbus aucuparia* L. leaves extract

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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 μg/ml) was the most active. Extract applied in a concentration of 200 μg/ml showed higher percent of DPPH inhibition compared with both concentrations of standard substances BHA and BHT (50 and 100 μg/ml). In the ABTS test, the activity of extract applied in 500 μg/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 μg/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*

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1. INTRODUCTION

Genus *Sorbus*, Rosaceae is organized in four subgenera: *Aria*, *Sorbus*, *Cormus* 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 diverse 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, 980 m in July 2017. Extraction with 70 % EtOH (1:10, w/v) was performed using maceration with con-

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

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

[a]Values 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.

tinuous 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±3.47 mg GAE/g (Singleton and Rossi, 1965) while the amount of total flavonoids was 20.78±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 µg/mL) and 900 µL of methanolic solution of DPPH (40 µg/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 µg/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 ± 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 ± 0.020 at 734 nm. The activity of three different concentrations (100, 200 and 500 µg/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 µg/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 ± 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 $FeCl_3$ x $6H_2O$ 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 µg/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 µg/mL) were used as standards. Standard solution of $FeSO_4$ x $7H_2O$ (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 µmol $FeSO_4$ x $7H_2O$/g dry extract.
All experimental measurements were carried out in triplicate and the results are expressed as the average of three measurements ± 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 (µg/mL)	AChE inhibitory activity[a] % of inhibition	TYR inhibitory activity % of inhibition
SLE dry extract	100	40.78±0.19b	42.57±1.37b
	200	43.19±1.20b	39.18±2.20b
	500	42.94±0.77b	31.21±1.55a
Galanthamine	100	57.11±1.68a	-
Kojic acid	100	-	51.81±2.55a

[a]Values are means ± 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 °C, and 200 mL of distilled water was added with vigorous shaking in order to prepare an emulsion. The extract (100, 200 and 500 µg/mL) and positive controls BHA, BHT and ascorbic acid (50 and 100 µg/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:

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

, 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 ± standard deviation.

2.3. Evaluation of antineurodegenerative activities

2.3.1. Acetylacholinesterase (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 tiocholine 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 µg/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 °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 ± 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 µg/mL) and kojic acid (100 µg/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 °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 ± standard deviation.

2.4. Statistical analysis

Results are presented as the mean value ± 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 µg/ml) were tested. Moreover, standard substances BHA, BHT, and ascorbic acid were tested in concentrations of 50 and 100 µg/ml. Concentration-dependent activity was noticed for all tested extract in assays and the most active was the highest concentration (500 µg/ml). SLE applied in a concentration of 200 µg/ml showed better activity in DPPH test compared with standard substances BHA and BHT applied to the concentration of 50 and 100 µg/ml (Table1) while the activity in ABTS was similar as was for BHA 100 µg/ml (Table 1). In FRAP assay, the concentration of SLE of 500 µg/ml was more active (840.18 µmol 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 µg/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 µg/ml reference drugs galanthamine 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 ACH test, there were no statistical differences among all applied concentrations while in TYR test most active was the lowest applied concentration of 100 µg/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. umbelleta* collected in two different localities had high inhibition potential against AChE i.e. 56.20±5.0 and 58.18±3.77 %, respectively. They pointed out marked variation 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.

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