

Antioxidative activity of extracts from *Rosa rugosa* harvested in 2 years

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ABSTRACT

Introduction: *Rosa rugosa* Thunb., commonly known as rugosa rose, has favorable properties. Due to the content of valuable compounds such as polyphenols and vitamins, it shows antioxidant activity, which can protect the organism against oxidative stress. The aim of this study was to evaluate the antioxidant potential of alcoholic extracts of *R. rugosa* leaves collected in 3 ripening periods and fruits at full ripening, harvested in 2 years.

Materials and methods: The plant extracts were prepared by ultrasound-assisted extraction, using 40%, 70%, 96% (v/v) ethanol and 99.8% methanol as solvents. The antioxidant activity was evaluated using the DPPH method.

Results: All the tested samples obtained from different parts of *R. rugosa* showed antioxidant potential. The antioxidant activity

of leaf extracts was significantly higher than that of fruit extracts. The highest antioxidant activity of 90% radical scavenging activity (RSA) was observed in extracts in 70% (v/v) ethanol (extraction time: 30 min) and in methanol (extraction time: 15 min) from dried leaves harvested during fruiting.

Conclusions: *R. rugosa*, especially its leaves, is a valuable source of antioxidants and could be used in the cosmetic, pharmaceutical and food industries. The extraction conditions, i.e. time, solvent and its concentration, affect the antioxidant activity of the obtained extracts.

Keywords: *Rosa rugosa* Thunb.; antioxidant activity; DPPH; ultrasound-assisted extraction; vegetation stages; ethanolic extracts.

INTRODUCTION

Natural antioxidants delivered to the organism are important factors against oxidative stress, which is one of the causes of many diseases, such as mental, metabolic, neurodegenerative, and cardiovascular system disorders [1, 2, 3, 4]. Plant products seem to be valuable substances due to their free radical scavenging ability, which is mainly due to their high content of polyphenols.

Rugosa rose (*Rosa rugosa*), also known as beach rose, Japanese rose or Ramanas rose, belongs to the family of *Rosaceae*. It is widespread in Europe and East Asia, from the Sea of Okhotsk to Korea, Japan, and China [5, 6, 7].

Major raw materials of this plant are usually fruits, containing pericarps and petals. Moreover, other parts, such as leaves and seeds, are used in the food, pharmaceutical, and cosmetic industries. The major source of biologically active compounds with pro-health properties are the fruits. They contain a high amount of vitamin C, and similarly to the *Rosa canina* fruit, the content of this vitamin is the highest among all raw materials sourced from plants in our climate zone. Moreover, *R. rugosa* fruit is rich in carotenoids, tocopherols, micro- and macrolelements, tannins, pectins, and flavonoids [7]. It is frequently used in herbal medicine to treat liver and gallbladder diseases, hyperacidity, and peptic ulcer disease, as well as the common cold [7, 8].

Due to its valuable components, mainly polyphenol content, *R. rugosa* reveals high antioxidant activity [7, 9, 10]. According to the literature, antioxidant activity of *R. rugosa* applies to its fruit [7, 11, 12, 13, 14], whereas fewer reports can be found about the antioxidant potential of leaves [15].

The aim of the study was to assess the antioxidant activity of alcoholic extracts of *R. rugosa* fruits and leaves, harvested in 2 years, at 3 ripening stages in the case of leaves and at full ripening in the case of fruits.

MATERIALS AND METHODS

2,2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) were obtained from Sigma Aldrich, USA; ethanol and methanol (all of analytical grade) were purchased from Chempur, Piekary Śląskie (Poland).

The raw material consisted of leaves (fresh and dried) and fresh fruits of *R. rugosa*. Leaves were harvested in 3 vegetation stages: before, during, and after fruiting, whereas fruits were only obtained at full ripening. The plant material was harvested from a natural state on the edge of a forest from June to October, in the area of forestry management Czarnobór in the West Pomeranian Voivodeship, Poland (N 53°42'28", E 16°41'39").

To prepare extracts using ultrasound-assisted extraction, 2 short-chain alcohols at different concentrations were applied: undiluted 99.8% (v/v) methanol and 40% (v/v), 70% (v/v) and 96% (v/v) ethanol. Extraction time was 15, 30 and 60 min.

The antioxidant activity was evaluated using the DPPH method [16, 17, 18]. All measurements of each extract were done in triplicate. Trolox was applied as a reference substance. The results were expressed as the percentage of free radical scavenging activity (% RSA) and as trolox equivalents in mg trolox/g of raw material. The meteorological conditions in the years 2016 and 2018, against the background of long-term averages in the area of plant harvesting, are given in Table 1.

The percentage of RSA was calculated by the formula:

$$\% \text{ RSA} = \frac{A_0 - A_s}{A_0} \times 100\%$$

where: % RSA – radical scavenging activity (%), A_0 – absorbance at 517 nm of the ethanolic solution of DPPH (control), A_s – absorbance at 517 nm of the test sample.

The results are presented as arithmetic means \pm standard deviation (mean \pm SD). The significance of differences between individual years as well as between individual parts of the plant were estimated using the Mann-Whitney test ($\alpha = 0.05$). The calculations were made using Microsoft Excel 2010 and Statistica 12PL (Statsoft) software.

RESULTS

Table 1 shows meteorological data in 2016 and 2018 compared to a multi-year period. The average air temperature from June to October, both in 2016 and in 2018, was slightly higher than the average in the years 1971–2000; in 2016, there was a difference of 0.7°C, and in 2018, it was 1.9°C. These differences were reflected in the average number of sunshine hours in that period – 1,035 h

in 2016, and 1,260 h in 2018 (mean value of the multi-year period – 960 h). The sum of atmospheric precipitation of 400 mm in 2016 was higher than the multi-year period and 2018 (only 230 mm). The highest average month air temperature in the 1st year of study was 18.5°C in July, but the highest number of sunshine hours in the same year was observed in July (290 h). In the 2nd year of research, the warmest months were July and August with an average temperature of 19.5°C. The highest sum of precipitation was observed in the 1st and 2nd year in July, reaching 150 and 100 mm, respectively. In turn, the month with a shortage of rainfall in 2016 was September, while in 2018 was August and September (25 mm).

Table 2 presents antioxidant activity of leaf extracts obtained at 3 ripening periods, determined by the DPPH method, and expressed as trolox equivalent. Both fresh and dried leaf extracts showed high antioxidant activity, mainly depending on the type of solvent and the extraction time. The plant vegetation stage, as well as the harvest year characterized by different climatic conditions, had an impact on the obtained values.

According to our findings, in the majority of cases, the leaves harvested in the 2nd year of the study had a higher ability to scavenge free radicals (Tab. 2). The highest antiradical activity was found in fresh leaf extracts, harvested during the flowering period – 3.71 \pm 0.08 mg trolox/g of raw material in the 1st harvest year and 3.35 \pm 0.06 mg trolox/g of raw material in the 2nd. In 2016, the highest antioxidant potential in this vegetation stage, reaching 3.99 \pm 0.04 mg trolox/g of raw material, was found in fresh leaf extracts in 96% (v/v) ethanol (extraction time: 15 min). However, in 2018, during flowering, the highest value was observed for leaves extracted in 70% (v/v) ethanol for 30 min – 4.29 \pm 0.09 mg trolox/g of raw material.

Dried leaf extracts, especially harvested in 2016 and prepared in concentrated ethanol, showed markedly lower antioxidant activity during the flowering period: only 0.05 \pm 0.02 mg trolox/g of raw material (extraction time: 60 min), and 0.97

TABLE 1. Meteorological data in the area of plant harvesting and its vicinity in 2016 and 2018 against the background of long-term averages

Years	Months					
	VI	VII	VIII	IX	X	VI–X
	Average air temperature (°C)					
2016	17.5	18.5	16.5	15.5	7.5	15.1
2018	17.5	19.5	19.5	14.5	10.5	16.3
1971–2000	15.5	17.5	17.5	12.5	9.0	14.4
	Total rainfall (mm)					
2016	65	150	85	25	75	400/80
2018	35	100	25	25	45	230/46
1971–2000	75	75	65	55	45	315/63
	Sunshine duration (h)					
2016	290	230	210	250	55	1035/207
2018	290	310	270	210	180	1260/252
1971–2000	230	250	230	145	105	960/192

Data comes from the Institute of Meteorology and Water Management – National Research Institute.

TABLE 2. Mean (\pm standard deviation – SD) antioxidant activity evaluated with the DPPH method and expressed as trolox equivalents (mg trolox/g raw material) of extracts of fresh and dried leaves harvested in 2 years in 3 vegetation stages

Solvent	Extraction time	Flowering		Fruiting		After vegetation
		fresh	dried	fresh	dried	dried
Trolox equivalent (mg trolox/g raw material)						
Year 2016						
96% (v/v) ethanol	15	3.99 \pm 0.04	0.97 \pm 0.12	3.72 \pm 0.02	3.44 \pm 0.06	3.37 \pm 0.08
	30	3.97 \pm 0.04	0.98 \pm 0.13	3.73 \pm 0.00	2.81 \pm 0.05	3.59 \pm 0.04
	60	3.85 \pm 0.03	0.05 \pm 0.02	3.64 \pm 0.05	2.95 \pm 0.09	2.81 \pm 0.06
70% (v/v) ethanol	15	3.79 \pm 0.06	3.20 \pm 0.04	3.76 \pm 0.03	3.85 \pm 0.07	3.81 \pm 0.01
	30	3.75 \pm 0.23	2.41 \pm 0.20	3.84 \pm 0.02	4.04 \pm 0.02	3.89 \pm 0.01
	60	3.86 \pm 0.04	2.99 \pm 0.04	3.67 \pm 0.01	3.97 \pm 0.02	3.85 \pm 0.01
40% (v/v) ethanol	15	3.43 \pm 0.23	3.07 \pm 0.08	3.85 \pm 0.01	3.98 \pm 0.01	3.59 \pm 0.01
	30	2.38 \pm 0.09	2.02 \pm 0.07	3.83 \pm 0.00	3.73 \pm 0.09	3.59 \pm 0.04
	60	3.96 \pm 0.05	2.47 \pm 0.20	3.90 \pm 0.02	3.82 \pm 0.03	3.31 \pm 0.02
99.8% (v/v) methanol	15	3.82 \pm 0.05	2.84 \pm 0.14	3.53 \pm 0.03	4.04 \pm 0.06	3.88 \pm 0.02
	30	3.84 \pm 0.03	2.89 \pm 0.16	3.63 \pm 0.03	3.31 \pm 0.13	3.84 \pm 0.01
	60	3.79 \pm 0.01	2.61 \pm 0.03	3.57 \pm 0.05	3.84 \pm 0.03	3.89 \pm 0.01
Year 2018						
96% (v/v) ethanol	15	2.30 \pm 0.12	1.68 \pm 0.09	1.13 \pm 0.63	1.76 \pm 0.05	1.93 \pm 0.06
	30	2.87 \pm 0.07	2.76 \pm 0.01	4.08 \pm 0.01	3.39 \pm 0.16	3.57 \pm 0.18
	60	4.19 \pm 0.01	3.54 \pm 0.02	3.18 \pm 0.11	3.99 \pm 0.03	1.26 \pm 0.05
70% (v/v) ethanol	15	1.30 \pm 0.08	3.87 \pm 0.01	1.11 \pm 0.14	4.47 \pm 0.01	3.88 \pm 0.03
	30	4.29 \pm 0.09	3.80 \pm 0.00	4.52 \pm 0.00	4.47 \pm 0.04	3.79 \pm 0.01
	60	4.15 \pm 0.06	3.75 \pm 0.01	4.37 \pm 0.03	4.40 \pm 0.03	3.80 \pm 0.02
40% (v/v) ethanol	15	1.32 \pm 0.15	3.87 \pm 0.02	2.32 \pm 0.12	4.40 \pm 0.03	3.36 \pm 0.03
	30	3.45 \pm 0.03	3.49 \pm 0.10	4.43 \pm 0.01	4.53 \pm 0.03	3.87 \pm 0.03
	60	4.20 \pm 0.04	3.46 \pm 0.02	4.25 \pm 0.04	4.48 \pm 0.02	3.91 \pm 0.02
99.8% (v/v) methanol	15	4.12 \pm 0.05	4.03 \pm 0.08	4.15 \pm 0.09	4.04 \pm 0.27	3.93 \pm 0.08
	30	4.07 \pm 0.02	4.11 \pm 0.01	4.36 \pm 0.08	4.52 \pm 0.01	4.11 \pm 0.00
	60	4.00 \pm 0.03	4.05 \pm 0.01	4.42 \pm 0.02	4.52 \pm 0.03	4.10 \pm 0.02

\pm 0.12 and 0.98 \pm 0.13 mg trolox/g of raw material (in case of extraction 15 min and 30 min). These results were the lowest taking into account all growing seasons of this plant (Tab. 2).

However, in 2018, the antioxidant capacity of some extracts harvested during fruiting was even above 4.50 mg trolox/g of raw material and was observed for fresh leaves extracted in 70% ethanol (extraction time: 30 min) and 4.52 mg trolox/g of raw material for dried leaves, prepared in concentrated methanol (extraction time: 30 and 60 min), whereas the highest activity of 4.53 \pm 0.03 mg trolox/g of raw material was found for dried leaves extracted for 30 min in 40% ethanol (Tab. 2).

Leaves harvested after fruiting were characterized by high antioxidant activity, 3.62 \pm 0.02 mg trolox/g of raw material (2016) and 3.46 \pm 0.04 mg trolox/g of raw material (2018) on average. The highest antioxidant activity in this stage of vegetation

was observed for leaves harvested in the 2nd year, extracted in methanol for 30 and 60 min, 4.11 \pm 0.00 to 4.10 \pm 0.02 mg trolox/g of raw material, respectively (Tab. 2).

Extracts of fruits harvested at full ripening in the 1st year showed higher antioxidant potential compared to the 2nd year. In 2016, this parameter ranged from 1.45 \pm 0.04 mg trolox/g of raw material for extracts prepared in undiluted ethanol (extraction time: 30 min) to 3.81 \pm 0.09 mg trolox/g of raw material for the samples extracted in methanol for 60 min.

However, in 2018, the trolox equivalent varied from 0.58 \pm 0.02 mg trolox/g of raw material for extracts prepared in concentrated ethanol for 15 min to 3.67 \pm 0.05 mg trolox/g of raw material, also for samples prepared with concentrated ethanol, but within 60 min (Tab. 3).

TABLE 3. Mean (\pm standard deviation – SD) antioxidant activity evaluated with the DPPH method, expressed as trolox equivalents (mg trolox/g raw material) of *R. rugosa* ripe fruit extracts harvested in 2 years

Solvent	Extraction time	Year 2016	Year 2018
		trolox equivalent (mg trolox/g raw material)	
96% (v/v) ethanol	15	2.18 \pm 0.10	0.58 \pm 0.02
	30	1.45 \pm 0.04	0.88 \pm 0.02
	60	2.42 \pm 0.13	3.67 \pm 0.05
70% (v/v) ethanol	15	3.10 \pm 0.16	1.12 \pm 0.06
	30	2.68 \pm 0.12	0.99 \pm 0.18
	60	3.33 \pm 0.05	3.36 \pm 0.01
40% (v/v) ethanol	15	2.57 \pm 0.06	0.70 \pm 0.07
	30	2.21 \pm 0.15	0.74 \pm 0.03
	60	2.82 \pm 0.01	2.23 \pm 0.05
99.8% (v/v) methanol	15	3.20 \pm 0.11	1.27 \pm 0.02
	30	2.90 \pm 0.18	1.89 \pm 0.03
	60	3.81 \pm 0.09	2.37 \pm 0.17

An analysis of statistical significance of the differences of the antioxidant activity between harvesting years and between fruit and leaf extracts, evaluated with the Mann–Whitney test, are summarized in Tables 4 and 5, respectively.

TABLE 4. Statistical differences of antioxidant activity between the analysed years (2016 and 2018) evaluated by the Mann–Whitney test

Vegetation phase	p	Z
Fresh leaf		
Flowering	0.4095	-0.8371
Fruiting	0.1781	-1.3567
Dried leaf		
Flowering	0.0004	-3.3197*
Fruiting	0.0055	-2.6876*
After vegetation	0.4095	-0.8371
Fruit		
Ripe	0.0141	-2.4537*

* significant differences ($p < 0.0500$)

TABLE 5. Statistical differences of antioxidant activity between individual parts of the plant, determined by the Mann–Whitney test

Raw material	p	Z
Year 2016		
Fruit/Leaf	0.0000	-3.6662*
Year 2018		
Fruit/Leaf	0.0011	-3.0888*

* significant differences ($p < 0.0500$)

Taking into account the year of harvesting, significant differences were found for dried leaves during flowering ($p = 0.0004$) and fruiting ($p = 0.0055$) as well as for ripe fruit ($p = 0.0141$) – Table 4.

If the individual parts of the plant are considered, significant differences were shown between the leaves and the fruits ($p = 0.0000$ in 2016 year and $p = 0.0011$ in 2018 year) – Table 5.

DISCUSSION

Our study revealed that extracts of all studied parts of the plant showed antiradical activity. However, the DPPH radical scavenging capacity of the leaf extracts was higher than the fruits, which were characterized by rather moderate activity, especially in the 2nd year of the study. In the case of dried leaves harvested during the fruiting period, RSA was up to 90%, both for extracts prepared in 70% ethanol (extraction time: 30 min) and extracts prepared in methanol (extraction time: 15 min). For the other extracts prepared from this raw material, it was demonstrated that the high radical scavenging ability was usually more than 80% (Fig. 1f).

Similar results were obtained by Leja et al., who observed a high antioxidant activity for the rosehip fruit, commonly known as the dog rose (*Rosa canina* L.). The samples were prepared in 80% methanol, and RSA measured by the DPPH method was 95% [13].

The results obtained by Leja et al. were comparable with the antiradical activities of our study, where similar values were demonstrated for the ripe fruit methanol extracts (86% RSA). The slightly higher result showed by Leja et al. could partly be due to another variety of rose species used for analysis and different storing conditions of the raw material. The raw material was frozen before the analysis (-20°C) which could contribute to better penetration of the plant material by the solvent. Freezing might cause some damage to the cell wall, which increased the penetration of active substances to the solvent and could result in higher antioxidant activity [13].

Rutkowska et al. demonstrated the ability of the ethanol extracts obtained from the dog rose fruit (*Rosa canina* L.) harvested at full ripening stage to neutralize free radical DPPH; antioxidant activity was 72% and 49% depending on the method of preparing the raw material. Higher results were observed by the authors for the dried product obtained by lyophilisation as compared to fruit dried conventionally [7].

In our study, the antioxidant activity of extracts of fully ripening fruit varied between 1.45–3.81 mg trolox/g of raw material in the 1st year of the study, which corresponded to the ability to scavenge free radicals of about 42% and 85% RSA. However, in the 2nd year, this capacity varied from 0.58 to 3.67 mg of trolox/g of raw material on average, which corresponds to 25% and 83% of RSA (Fig. 1f, Tab. 3).

The fruit of *R. rugosa*, as well as the petals, are often used to prepare various types of food products. Preserves, such as wine prepared from fruit or jams made of rose petals, are characterized by high antioxidant activity [11, 19].

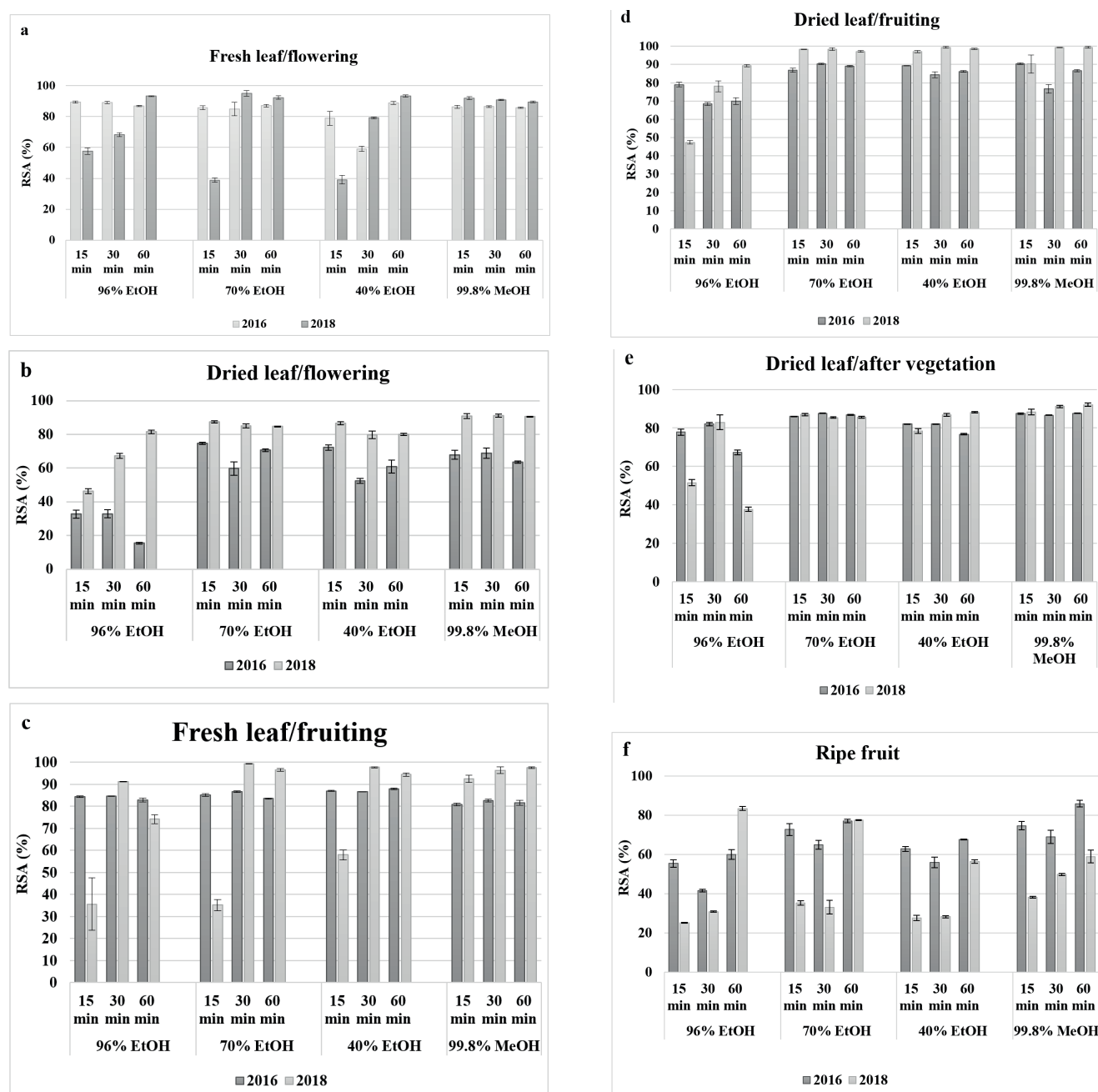


FIGURE 1. Mean antioxidant potential evaluated with DPPH method (radical scavenging activity – % RSA) of extracts of *Rosa rugosa* ripe fruit, and fresh and dried leaves harvested in 2 years in 3 vegetation stages. The vertical lines represent standard deviation

Rosa rugosa contains a lot of valuable substances, such as vitamins C, E, A, carotenoids, flavonoids, some micro-, macroelements, organic acids [11, 20, 21], and polyphenols, the major group of active substances showing antioxidant activity in plants [22].

Rugosa rose is considered to be one of the most valuable varieties of these species. Its fruits contain more antioxidants than the popular fruits of other wild species, such as hawthorn (*Crataegus monogyna* L.), sea buckthorn (*Hippophae rhamnoides* L.), black mulberry (*Morus nigra* L.), dogwood (*Prunus spinosa* L.), or rowanberry (*Sorbus aucuparia* L.) [13].

The meteorological conditions play a crucial role in plant ripening as well as in the content of biologically active substances, among other antioxidants [23, 24]. In our research, the climatic conditions in both years of the study were different

and could probably result in different antioxidant activity in particular years.

In the summer months, intensive fruit growth and its ripening, as well as the production and accumulation of active substances, are observed. In our study, the drought which occurred in August 2018 and high air temperature probably contributed to the lower antioxidant activity of fruits.

During preparation of plant extracts, the applied extractant seems to be an important factor affecting the antioxidant properties of the plant material. In our experiment, concentrated and diluted ethanol, as well as concentrated methanol, were used for the extraction of plant material.

When analysing the effect of the solvent on the tested extracts, the antioxidant potential was divergent, and the

lowest activity was observed for extracts in concentrated ethanol, especially concerning the fruit and dried leaves harvested during the flowering period (Fig 1).

Selecting the appropriate concentration of the solvent to obtain extracts with the highest antioxidant potential is also of great importance.

Liu et al. report on the hypoglycemic properties of *R. rugosa* extracts prepared with the use of 50% (v/v) ethanol. In *in vitro* tests, the authors assessed the inhibition of alpha-glucosidase activity and oxidative stress in rats with type 2 diabetes. In their study, the diet of the animals was enriched with the tested extract. As a final effect, after 4 weeks of observation, alpha-glucosidase inhibition in the liver of rats had been demonstrated. Extracts from *R. rugosa*, showing antioxidant activity, considerably reduced blood glucose levels, and improved the lipid profile in animals [8].

In the case of the rose, the major parts showing the ability to scavenge free radicals are leaves [15, 25], flowers [19, 26, 27, 28] and fruit [7, 11, 12, 13, 14]. In our study, extracts of both parts of the plant (fruits and leaves) were characterized by antioxidant activity. However, in most cases, the highest antioxidant activity has been found for the leaf extract (Fig. 1).

Antioxidant activity for individual raw materials delivered from 1 plant can vary depending mainly on the structure and chemical composition of the plant part. Leaves are characterized by a considerable content of polyphenols responsible for their antioxidant activity.

Baydar and Baydar also indicated a higher antioxidant activity (determined by the FRAP and DPPH method) in damask rose leaves (*Rosa damascene* Mill.) as compared to the flowers of this plant [15].

All plants during vegetation are characterized by variable chemical composition, which could affect their antioxidant abilities [29]. In our study, the antioxidant activity of leaves has been analyzed in 3 different ripening stages: flowering, fruiting, and after vegetation.

Raw material harvested after vegetation showed high antioxidant activity. In another study, high antiradical properties, measured by the DPPH method, were also found for *Ginkgo biloba* leaf extracts in 40% and 70% (v/v) ethanol harvested after vegetation [16]. Comparably, in the present study, rose leaves after the fruiting period, extracted in similar solvents, showed much higher antioxidant activity as compared to extracts in undiluted ethanol (Fig. 1e).

Plant leaves harvested after vegetation seem to be a valuable raw material to be applied in the cosmetic and pharmaceutical industries. The structure of polyphenols in leaves is subject to changes during yellowing, which can increase antioxidant potential [30]. *Rosa rugosa* seems to be a valuable plant with high antioxidant potential, which can be a crucial factor in daily health prophylaxis.

The right selection of raw material, paying attention to the stage of plant vegetation to choose the most valuable part of the plant, and the method of preparing the extracts, will allow to get the most beneficial material with high antioxidant potential. Products containing *R. rugosa* can provide efficient

antioxidant protection and can be useful both in cosmetic and pharmaceutical formulations.

CONCLUSIONS

1. All the examined extracts from leaves and fruits of *R. rugosa* showed antioxidant potential, evaluated by the DPPH method.
2. Leaves were the richer source of antioxidants as compared to fruits, while fruits had a moderate ability to scavenge free radicals.
3. The antioxidant activity of leaf extracts was usually high in all vegetation stages, but the most valuable properties were found in the case of extracts prepared from dried leaves in 70% ethanol (extraction time: 30 min) and in methanol (extraction time: 15 min) harvested during the fruiting, showing the scavenging of free radicals at a level of 90%.
4. The extraction conditions, i.e., the type of solvent used and extraction time, affects the antioxidant activity. Usually, the evaluated parameters were higher when samples were prepared in diluted ethanol.
5. *Rosa rugosa*, as a valuable source of antioxidants, could be applied in cosmetic formulations, pharmaceuticals, and in the food industry.

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