

Antioxidant properties of selected parts of *Syringa vulgaris* L.*

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ABSTRACT

Introduction: Antioxidants, in addition to their general positive effect on health, inhibit the aging of the skin. Many plant extracts are a valuable source of natural antioxidants that can be used in the production of cosmetics and cosmetology. *Syringa vulgaris* L. is a plant that contains flavonoids, one of the most important groups of natural compounds that eliminate free radicals.

The aim of this study was to determine and compare the antioxidant activity of the extracts prepared from selected *S. vulgaris* L. parts. We analyzed the influence of the solvent and the extraction time on the antioxidant potential of extracts.

Materials and methods: Alcoholic extracts from selected parts of *S. vulgaris* L. were prepared by ultrasound-assisted extraction, for 15, 30, and 60 min, and their antioxidant activity was evaluated. The analysis of the antioxidant potential was performed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) – ABTS methods using a spectrophotometer. The raw material used to prepare extracts comprised dried leaves and flowers harvested during flowering and dried fruits harvested during fruiting. The solvents applied were methanol, ethanol, iso-propanol, and n-propanol at concentrations of 40%, 70%, and 100%.

Results: The highest mean antioxidant activity evaluated using the ABTS method was 96.4% radical scavenging activity (RSA) for the leaf extract of *S. vulgaris* L., prepared in 70% methanol for 15 min. The highest antioxidant potential of the leaf extract determined using the DPPH method was 87.9% for concentrated ethanol applied for the same time. The lowest antioxidant activity was 1.36% RSA in a fruit extract extracted for 30 min, prepared in 99% n-propanol, determined by the ABTS method, and 6.3% RSA as evaluated using the DPPH method. The antioxidant potential was markedly lower for the fruit extracts.

Conclusions: The best antioxidant properties were demonstrated by the extracts made from the leaves of *S. vulgaris* evaluated using both methods. The lowest results were obtained for fruit extracts. The type of extractant used and the duration of the ultrasonically assisted extraction affected the ability of the obtained extracts to neutralize free radicals. The results show that *S. vulgaris* is a valuable source of antioxidants, especially its leaves and flowers.

Keywords: *Syringa vulgaris* L.; antioxidants; ultrasound-assisted extraction; antioxidant activity; DPPH; ABTS.

INTRODUCTION

Skin aging is a natural, irreversible, and inevitable physiological process that is influenced by many factors. Human skin is exposed every day to unfavorable factors such as wind, frost, ultraviolet radiation (UV), and all kinds of pollution. Years of exposure result in noticeable changes in the appearance and functioning of the epidermis, dermis, and subcutaneous tissue [1]. Aging skin is characterized, first of all, by a slowdown in regenerative processes and a decrease in the biological activity of cells. The atrophic processes begin to outweigh the cell growth. As a consequence, the skin loses its elasticity, collagen, and elastin fibers are degraded, and the activity of fibroblasts decreases. Structural changes become visible, mimic wrinkles appear, the eyelids become flaccid, “crow’s feet” appear around the eye sockets, and the face oval is distorted [1, 2, 3].

Reactive oxygen species (ROS) are formed during several cellular processes. Free radicals take part in many processes important for the body; however, at the same time they can

cause a number of harmful effects. To protect the body against the toxic activity of free radicals, antioxidants are applied. Under the homeostatic conditions, i.e. the balance of the organism, the antioxidant systems work without any interference from outside. If the balance between free radical reactions inducing the production of ROS and antioxidative reactions is disturbed, the oxidative stress occurs. When the antioxidant system cannot neutralize the excess ROS, it should receive exogenous antioxidants to protect the organism against increased oxidative stress. The basic activity of antioxidants is based on inhibiting the formation of free radicals, slowing down and breaking chain reactions that contribute to their formation, counteracting the oxidation of metals such as lead, mercury, cadmium, and copper, and repairing dysfunctions resulting from the action of ROS that cannot be repaired by the organism itself, stimulation of endogenous antioxidant systems for the synthesis of antioxidant enzymes [4], formation of a barrier to prevent the formation of ROS and their penetration

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into biological structures, absorption of energy, and binding electrons from ROS [5, 6].

Exogenous antioxidants are obtained from natural sources, most of all from plants. They consist of polyphenols, carotenoids, vitamins, and minerals such as zinc, selenium, and manganese. This group of substances prevents cell damage and inhibits unfavorable processes such as lipid, protein, and nucleotide peroxidation [7, 8, 9, 10].

Syringa vulgaris L., also known as common lilac, is an ornamental plant derived from the Oleaceae family. It grows in Asia and Europe and is most often found in fields, stony grounds, parks, and gardens. It is a plant that does not require special care; it likes drought, sunlight, and poor soils [11, 12, 13]. There are about 28 species of *Syringa*, but in Poland the most common is *S. vulgaris* L. [11]. It is a shrub that blooms profusely and produces large white to purple inflorescences, depending on the species. Small, 4-lobed flowers are gathered in large panicles, and the heart-shaped or ovate leaves grow opposite each other and form so-called opposite foliage. Solar radiation is one of the main factors determining leaf development [11, 13, 14]. The flowering period of lilacs falls between May and the beginning of June [11]. In autumn, the flower buds go dormant, the oblong fruits appear in bags (infructescences). In spring, the plant wakes up again from winter dormancy and produces buds [15]. The development of lilac flower buds lasts for 2 growing seasons [16]. Lilacs are frequently harvested for ornamental purposes, but in addition to decorating the interior, they have an intense aroma; essential oil from lilac is used in the production of perfumes. In addition, this plant has anti-inflammatory properties used to treat diseases such as rheumatoid arthritis, gout, rheumatism, and diabetes. In China, *S. vulgaris* is found in herbal remedies that reduce cough and diarrhea, and is used as well to heal bronchitis, conjunctivitis, hepatitis, and cardiovascular problems [13, 17, 18, 19, 20, 21]. In folk medicine, common lilac, especially its bark, was used commonly in the form of alcohol infusions and tinctures for colds and in the case of malaise. It also served as an analgesic, antimalarial, expectorant, and diaphoretic agent. Ointments and essential oils with flowers were used for rheumatic ailments. The bark and leaf extracts have been applied as a remedy for inflammation in the oral cavity [11, 13, 22]. In the flowers (*Flos Syringae*) and leaves (*Folium Syringae*) of the common lilac, flavonoids, iridoids, and cinnamic acid derivatives have been identified. These compounds are responsible for inhibition of lipid oxidation, trapping of free radicals, and reducing the negative effects of oxidative stress [17, 18, 23, 24]. Moreover, phenolic compounds, anthocyanins and rutin, can be also found [11]. However, the concentrations of these compounds in fruit extracts are low and only trace amounts could be isolated. Secondary metabolites in fruit are oleuropein, which is a glycosylated seco-iridoid, and nuzhenide, an antioxidant [17, 18]. Lilac fruits should be dried in a dry and ventilated place so that they remain viable for up to 2 years [25]. Leaves, fruit, and bark contain syringin, syringopicrin, farnesol, and monoterpene alkaloids [11]. The flavonoids present in the flowers and leaves have anti-inflammatory and antioxidant properties, and are

responsible for the aroma and taste of the plant. Moreover, they are also repellants that scare away predators as well as protect the plant from infections. Anthocyanins, which belong to the flavonoids, act as purple pigments of lilac and help attract pollinating insects [26]. Due to antioxidative properties, they have a health-promoting effect on the human body. Antiproliferative activity in neoplastic cells, positive effects on eyesight, and improved blood circulation have been also observed [27]. Both flavonoids and anthocyanins, due to their antioxidant properties, protect plants against the harmful effects of UV radiation and trap free radicals [26]. High concentrations of iridoids, mainly B and C syringopicrosides, i.e. natural compounds with anti-tumor, anti-inflammatory, anti-oxidant, and anti-fungal activity, were found in *S. vulgaris* leaves. These substances are produced for defense against microorganisms and predators [19]. The leaves are a rich source of secoiridoids, and also contain benzyl alcohol, syringin, lignans, coumarins, tannins, and flavonoids such as rutin and quercetin. Tannins protect the plant against bacteria and other pathogens, and have anti-inflammatory, soothing, and astringent properties on the skin. Another compound, coumarin, shows analgesic, anti-edema, sedative, as well as diastolic, antiallergic, and antioxidant activity [18, 28, 29]. Flavonoids and iridoids have been identified in the flowers and leaves of the common lilac, that inhibit lipid oxidation, the formation of free radicals, and the negative effects of oxidative stress [17, 18, 23, 24]. Leaves, fruit, and bark contain syringin, syringopicrin, farnesol, and monoterpene alkaloids [11].

As previously mentioned, antioxidants, in addition to their positive effect on health, inhibit skin aging. Many plant extracts are a valuable source of antioxidants used in the production of cosmetics and used in cosmetology treatments. *Syringa vulgaris* L. is a plant that contains flavonoids, one of the most important groups of natural compounds that eliminate free radicals, so in this study we evaluated the antioxidant activity of alcohol extracts of this plant using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) – ABTS methods. The purpose of the work was to determine the antioxidant activity of extracts prepared from various parts of *S. vulgaris* L. plant using the DPPH and ABTS methods, to evaluate the effect of the ultrasound-assisted extraction time and the solvent used during extraction on the antioxidant activity of the extracts from leaves, flowers, and fruits of *S. vulgaris* L. and to analyze which parts of the plant (leaves, flowers, fruits) have the highest antioxidant potential.

MATERIALS AND METHODS

2,2-diphenyl-1-picrylhydrazyl and ABTS diammonium salt were obtained from Sigma-Aldrich (USA), ethanol was from Linegal Chemicals, Warsaw, methanol, propan-1-ol, propan-2-ol, ascorbic acid, acetone, cholesterol, urea, bee wax, and sodium persulfate, all of analytical grade quality, were purchased from Chempur, Piekary Śląskie (Poland), whereas white petrolatum and eucerin – from COEL, Cracow (Poland).

The plant material applied in the study consisted of leaves, flowers, and fruits of the common lilac (*S. vulgaris* L.) growing in the field in the town of Gryfino, NW Poland. The leaves and flowers were harvested from a natural state in mid-May, and the fruits in early September at full ripening. All raw materials were dried at room temperature in a dark, ventilated place for 1 week.

Preparation of extracts from *S. vulgaris* L. To obtain the extracts 4 short-chain alcohols, i.e. methanol, ethanol, propan-1-ol and propan-2-ol at 3 concentrations, i.e. 40%, 70% and 100% were applied. For this purpose, 0,5 g of raw material and 10 cm³ of solvent were placed in test tubes. All the samples were subjected to ultrasound-assisted extraction for 15, 30, and 60 min. After extraction, the raw material was separated from the solution and the extracts were poured into plastic tubes sealed with a stopper and stored at room temperature in a dark place until the antioxidant activity was evaluated.

To estimate the antioxidant potential of obtained extracts, 2 methods, i.e. DPPH and ABTS, were applied as described previously [30, 31].

Shortly, the procedure to evaluate the antioxidant activity using the DPPH method is based on the reaction between the obtained alcoholic extracts of the tested raw material with an ethanolic DPPH radical solution. To prepare a stock solution of DPPH, 0.012 g of this radical was weighed and dissolved in 100 mL of concentrated ethanol using a magnetic stirrer. The absorbance of the applied DPPH working solution in 1 cm cuvettes must be in the range of 0.980–1.020 at a wavelength of 517 nm. For this purpose, it was necessary to dilute the obtained stock solution with 70% ethanol.

To determine the antioxidant activity by the DPPH method 1 cm cuvettes were filled with 2500 µL of DPPH working solution. Then, 132 µL of each of the plant extracts were added to each of the cuvettes. Three samples were made for each extract. The incubation time was 10 min and the absorbance was measured at 517 nm. The antioxidant activity was expressed in the form of the % radical scavenging activity (RSA) using the formula:

$$\%RSA = (1 - A_p/A_o) 100\%$$

where: %RSA – radical scavenging activity (%); A_p – absorbance of the test sample; A_o – absorbance of the control sample

Moreover, for this method calibration curve using ascorbic acid as the standard was prepared. A linear relationship between the antioxidant activity and concentration was observed (Fig. 1).

The second method applied to evaluate the antioxidant activity was the ABTS method based on the reaction between the obtained alcoholic extracts of the tested raw material with an ABTS radical solution. To obtain the proper reagent solutions, 100 cm³ of the 2.45 mM aqueous potassium persulfate solution was prepared. For this purpose, 0.066 g of potassium persulfate was dissolved in 100 cm³ of water. To form ABTS•+ cation radical, 0.038 g of the ammonium salt of ABTS was dissolved in 10 cm³ of the aforementioned solution. The prepared solution was placed in a dark glass bottle and had

to stand overnight in a dark place at room temperature. The time of reaction is at least 16 h. After 24 h, this solution had to be diluted with 50% (v/v) methanol to obtain a working solution with an absorbance range of 0.980–1.020 at a wavelength of 734 nm.

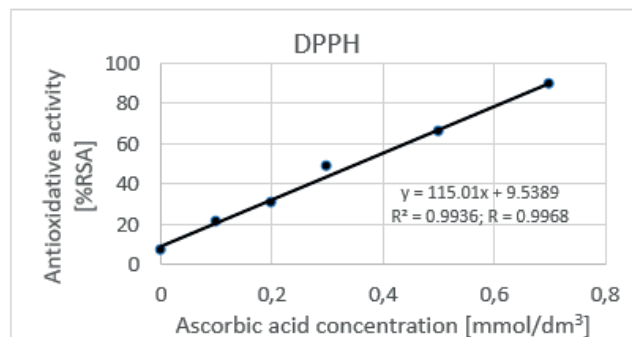


FIGURE 1. Calibration curve of the antioxidative activity vs. ascorbic acid concentration obtained using the DPPH method

To evaluate antioxidant potential of the extracts, 2500 µL of the working ABTS solution was placed in each cuvette, and then 25 µL of the plant extract was added. Three samples were made for each extract. The incubation time was 6 min and the absorbance measurements were made at a wavelength of 734 nm. As in the previous method, the antioxidant activity was expressed in the form of the %RSA using the formula:

$$\%RSA = (1 - A_p/A_o) 100\%$$

where: %RSA – radical scavenging activity (%); A_p – absorbance of the test sample; A_o – absorbance of the control sample

Also for the ABTS method, the calibration curve was prepared using ascorbic acid as the standard. Similarly to the DPPH method, a linear relationship between the antioxidant activity and concentration was observed (Fig. 2).

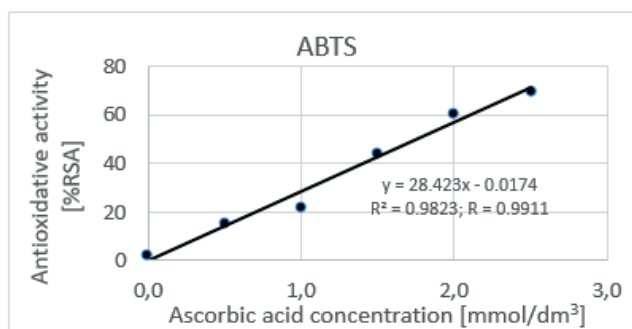


FIGURE 2. Calibration curve of the antioxidative activity vs. ascorbic acid concentration obtained using the ABTS method

Statistical analysis was performed using the Excel program for Windows (Microsoft Office). Arithmetic means and standard deviations (SD) were calculated for antioxidant activity. To establish the calibration curve of the relationship of antioxidative activity vs. the ascorbic acid concentration for the DPPH and ABTS methods, linear regressions and the correlation coefficients were determined.

RESULTS

Mean (\pm SD) antioxidative activities of extracts obtained from different parts of *S. vulgaris* using different short-chain alcohols and determined using the DPPH method are summarized in Table 1. Higher antioxidant potential was found for leaf and flower extracts as compared to the fruits.

Table 2 shows the mean (\pm SD) antioxidative activities of the same extracts, but evaluated using the ABTS method. As with

the results obtained using the DPPH method, lower activity was observed for fruit extracts than for leaf and flower extracts.

The effect of the applied solvent on the antioxidant potential of the obtained extracts of *S. vulgaris* leaves evaluated using the DPPH method is presented in Figure 3. The optimum extraction time to prepare leaf extracts of this plant, taking into account their antioxidant potential, seems to be 15 min, i.e. the shortest one. The highest activity was found for extracts prepared in

TABLE 1. Mean (\pm standard deviations) antioxidative activity of extracts of different parts of *Syringa vulgaris* evaluated using the DPPH method

Antioxidative activity [%RSA] determined using the DPPH method					
plant part analyzed	extractant		time of ultrasound-assisted extraction		
	solvent	concentration [%]	15 min	30 min	60 min
Leaf	methanol	40	74.76 \pm 0.65	57.42 \pm 0.90	59.37 \pm 0.68
		70	71.18 \pm 0.94	54.72 \pm 0.06	56.50 \pm 0.88
		99	86.15 \pm 2.38	73.50 \pm 0.98	73.44 \pm 0.15
	ethanol	40	76.32 \pm 0.41	60.06 \pm 1.46	61.50 \pm 0.37
		70	81.11 \pm 1.77	70.08 \pm 0.20	68.28 \pm 0.69
		96	87.90 \pm 2.54	75.59 \pm 0.52	82.34 \pm 0.69
	propan-1-ol	40	80.25 \pm 0.63	64.25 \pm 0.12	61.96 \pm 1.10
		70	82.37 \pm 1.58	64.55 \pm 2.71	68.13 \pm 1.05
		99	79.07 \pm 7.17	56.03 \pm 6.50	67.44 \pm 0.69
	propan-2-ol	40	80.44 \pm 2.74	67.36 \pm 0.55	64.04 \pm 3.92
		70	82.83 \pm 2.94	70.61 \pm 0.40	71.37 \pm 0.55
		99	74.01 \pm 2.65	72.90 \pm 2.26	73.74 \pm 0.61
Flower	methanol	40	71.88 \pm 0.33	70.02 \pm 0.83	79.78 \pm 3.23
		70	72.50 \pm 0.24	70.50 \pm 0.31	77.69 \pm 2.67
		99	83.90 \pm 0.32	78.62 \pm 1.94	83.92 \pm 4.34
	ethanol	40	74.62 \pm 0.00	67.41 \pm 5.15	67.93 \pm 2.80
		70	79.37 \pm 0.20	76.49 \pm 1.66	81.75 \pm 0.78
		96	63.17 \pm 0.76	79.94 \pm 2.43	90.11 \pm 3.76
	propan-1-ol	40	77.46 \pm 0.61	71.89 \pm 1.18	75.14 \pm 0.83
		70	80.38 \pm 0.34	75.07 \pm 1.73	78.57 \pm 0.51
		99	37.36 \pm 0.06	42.80 \pm 7.84	67.68 \pm 4.22
	propan-2-ol	40	76.53 \pm 0.49	75.20 \pm 2.18	76.09 \pm 0.46
		70	81.88 \pm 0.10	78.41 \pm 2.78	80.38 \pm 0.05
		99	30.59 \pm 0.34	36.24 \pm 1.42	58.71 \pm 2.00
Fruit	methanol	40	15.10 \pm 0.47	15.16 \pm 2.14	19.57 \pm 1.27
		70	16.82 \pm 0.87	13.31 \pm 0.43	38.10 \pm 0.55
		99	13.78 \pm 0.69	8.90 \pm 3.08	23.36 \pm 1.84
	ethanol	40	25.24 \pm 0.41	20.82 \pm 0.65	35.50 \pm 1.08
		70	17.01 \pm 0.35	10.43 \pm 0.50	40.77 \pm 3.13
		96	8.32 \pm 0.79	9.17 \pm 3.02	13.42 \pm 1.29
	propan-1-ol	40	16.09 \pm 2.66	26.59 \pm 2.21	58.51 \pm 0.47
		70	17.51 \pm 1.89	17.41 \pm 1.28	53.77 \pm 1.24
		99	14.54 \pm 0.15	13.19 \pm 3.38	11.29 \pm 4.33
	propan-2-ol	40	11.36 \pm 2.40	18.34 \pm 2.25	31.33 \pm 0.60
		70	16.12 \pm 1.48	16.12 \pm 0.81	43.83 \pm 0.40
		99	6.94 \pm 1.72	11.66 \pm 0.75	10.79 \pm 3.21

TABLE 2. Mean (\pm standard deviations) antioxidative activity of extracts of different parts of *Syringa vulgaris* evaluated using the ABTS method

Plant part analyzed	Antioxidative activity (%RSA) determined using the ABTS method				
	extractant		time of ultrasound-assisted extraction		
	solvent	concentration [%]	15 min	30 min	60 min
Leaf	methanol	40	23.53 \pm 4.10	92.92 \pm 4.37	30.72 \pm 2.07
		70	96.43 \pm 2.96	87.56 \pm 9.94	67.65 \pm 0.83
		99	36.51 \pm 2.24	91.82 \pm 3.39	55.88 \pm 4.63
	ethanol	40	83.66 \pm 2.61	73.02 \pm 1.55	29.84 \pm 4.03
		70	35.84 \pm 0.98	95.35 \pm 3.19	61.32 \pm 2.73
		96	32.17 \pm 3.18	26.20 \pm 2.29	76.44 \pm 6.91
	propan-1-ol	40	79.35 \pm 5.94	84.15 \pm 8.66	44.97 \pm 2.64
		70	68.81 \pm 2.97	91.29 \pm 1.51	53.08 \pm 8.98
		99	26.09 \pm 3.29	21.10 \pm 2.27	22.30 \pm 2.38
	propan-2-ol	40	29.17 \pm 3.30	95.97 \pm 2.06	66.35 \pm 2.36
		70	39.16 \pm 3.74	95.81 \pm 1.41	45.29 \pm 1.74
		99	19.05 \pm 0.57	27.42 \pm 2.37	29.18 \pm 0.40
Flower	methanol	40	31.78 \pm 1.90	50.35 \pm 1.50	57.99 \pm 1.30
		70	21.87 \pm 2.31	42.32 \pm 5.16	52.93 \pm 5.51
		99	57.72 \pm 7.35	43.31 \pm 6.16	56.90 \pm 4.70
	ethanol	40	93.43 \pm 5.80	43.74 \pm 1.59	71.66 \pm 2.19
		70	39.68 \pm 2.35	63.30 \pm 1.24	75.97 \pm 2.54
		96	22.12 \pm 1.17	30.51 \pm 9.88	47.90 \pm 11.94
	propan-1-ol	40	39.31 \pm 1.90	46.25 \pm 1.66	62.13 \pm 2.65
		70	48.99 \pm 3.25	49.78 \pm 5.78	57.23 \pm 1.68
		99	10.56 \pm 2.06	21.75 \pm 3.40	29.35 \pm 6.88
	propan-2-ol	40	50.54 \pm 2.17	56.62 \pm 8.23	70.32 \pm 0.89
		70	35.25 \pm 14.45	73.21 \pm 5.46	67.82 \pm 2.36
		99	10.01 \pm 4.21	15.15 \pm 1.29	21.81 \pm 3.76
Fruit	methanol	40	8.12 \pm 0.75	5.71 \pm 0.63	6.95 \pm 1.55
		70	9.34 \pm 0.46	4.91 \pm 0.65	11.58 \pm 0.92
		99	7.09 \pm 1.19	2.52 \pm 0.73	7.72 \pm 1.85
	ethanol	40	12.09 \pm 0.50	7.40 \pm 0.35	11.38 \pm 1.27
		70	8.65 \pm 1.01	4.68 \pm 1.74	15.97 \pm 5.27
		96	4.74 \pm 1.45	1.96 \pm 0.75	3.99 \pm 2.23
	propan-1-ol	40	5.58 \pm 0.61	7.64 \pm 0.76	33.60 \pm 4.20
		70	5.88 \pm 1.41	4.48 \pm 1.47	27.51 \pm 7.48
		99	4.98 \pm 1.05	1.36 \pm 0.86	6.69 \pm 5.62
	propan-2-ol	40	8.22 \pm 0.40	6.91 \pm 0.83	10.21 \pm 2.85
		70	9.94 \pm 0.72	2.79 \pm 0.82	16.10 \pm 5.45
		99	3.78 \pm 2.33	2.32 \pm 0.40	4.99 \pm 1.75

undiluted ethanol (87.90 \pm 2.54% RSA) and undiluted methanol (86.15 \pm 2.38% RSA).

Figure 4 presents the effect of the applied extractant on the antioxidant potential of *S. vulgaris* flower extracts determined using the DPPH method. In this case, the highest activity was observed for extracts prepared for 60 min. Similarly to the leaf extracts, the highest potential was found for extracts in undiluted ethanol (90.11 \pm 3.76% RSA) and concentrated methanol (83.92 \pm 4.34% RSA).

The effect of the applied solvent on antioxidant potential of the obtained extracts of *S. vulgaris* fruit also evaluated using the ABTS method is shown in Figure 5. The antioxidant activity of all the fruit extracts was markedly lower than that of leaf or

flower extracts. The highest antioxidative potential was found for extracts prepared for 60 min in 40% and 70% propan-1-ol – 58.51 \pm 0.47% RSA and 53.77 \pm 1.24% RSA, respectively.

Figure 6 presents the effect of the applied extractant on the antioxidant potential of *S. vulgaris* leaf extracts determined using the ABTS method. In this case, the highest activity was observed for extracts prepared mainly for 30 min. The highest potential was found for extracts in most of diluted alcohols.

The effect of the applied solvent on the antioxidant potential of the obtained extracts of *S. vulgaris* flowers evaluated using the ABTS method is presented in Figure 7. The most optimal extraction time to prepare flower extracts of this plant, taking into account their antioxidant potential, seems to be 60 min,

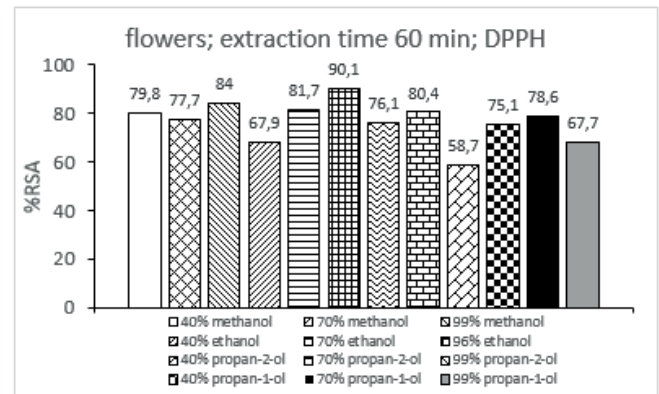
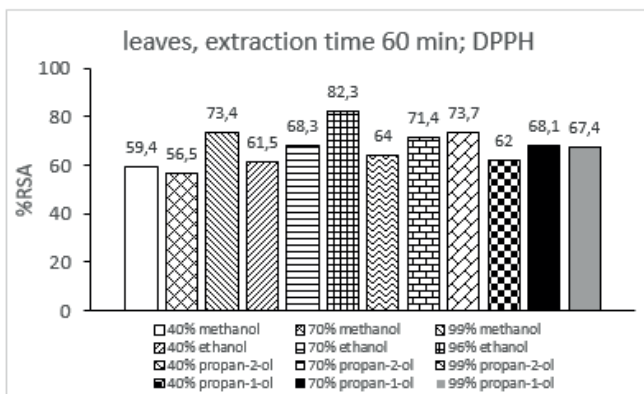
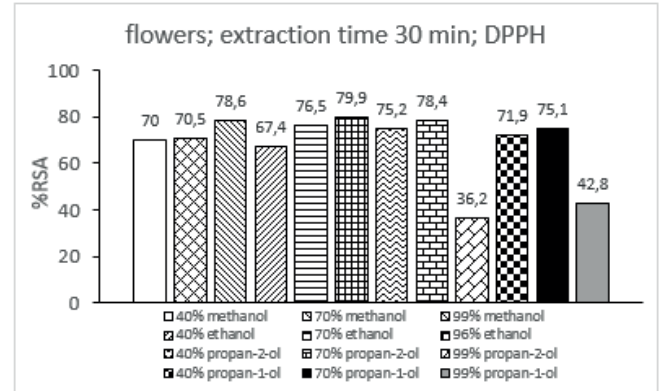
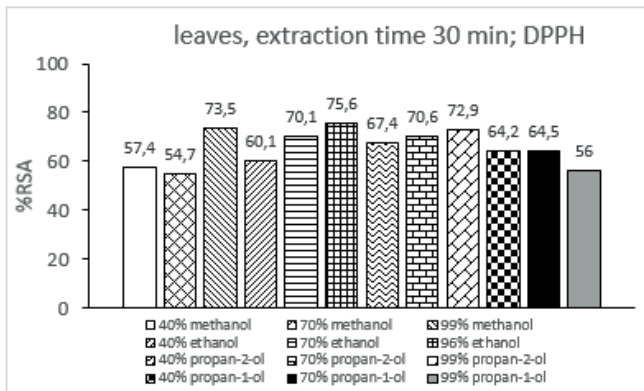
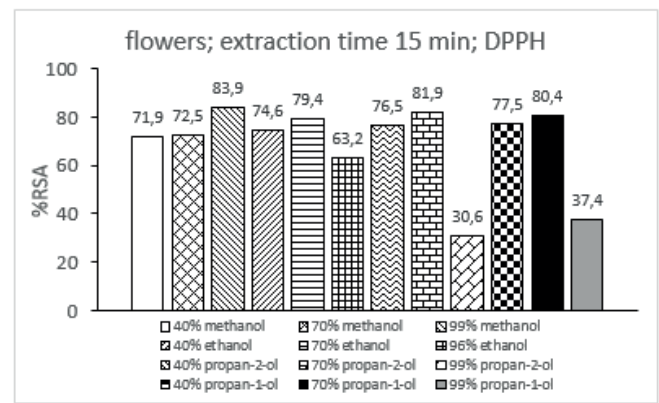
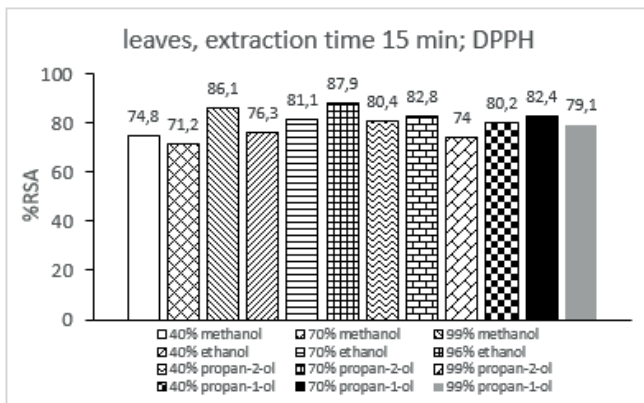


FIGURE 3. The effect of the applied extractant on the mean antioxidative activity (%RSA) of alcoholic extracts of dried *Syringa vulgaris* leaves obtained by 15, 30, and 60 min ultrasound-assisted extraction and evaluated using the DPPH method

FIGURE 4. The effect of the applied extractant on the mean antioxidative activity (%RSA) of alcoholic extracts of dried *Syringa vulgaris* flowers obtained by 15, 30, and 60 min ultrasound-assisted extraction and evaluated using the DPPH method

i.e. the longest one. The highest activity was found for extracts prepared in 70% and 40% ethanol ($75.97 \pm 2.54\%$ RSA and $71.66 \pm 2.19\%$ RSA, respectively).

The effect of the extractant on the antioxidant potential of the obtained extracts of *S. vulgaris* fruits evaluated using the ABTS method is shown in Figure 8. Similarly, the results obtained using the ABTS method showed that the antioxidant activity of all the fruit extracts was markedly lower than that of leaf or flower extracts. As for the DPPH method, the highest antioxidative potential was found for extracts prepared for 60 min in 40% and 70% propan-1-ol – $33.60 \pm 4.20\%$ RSA and $27.51 \pm 7.48\%$ RSA, respectively.

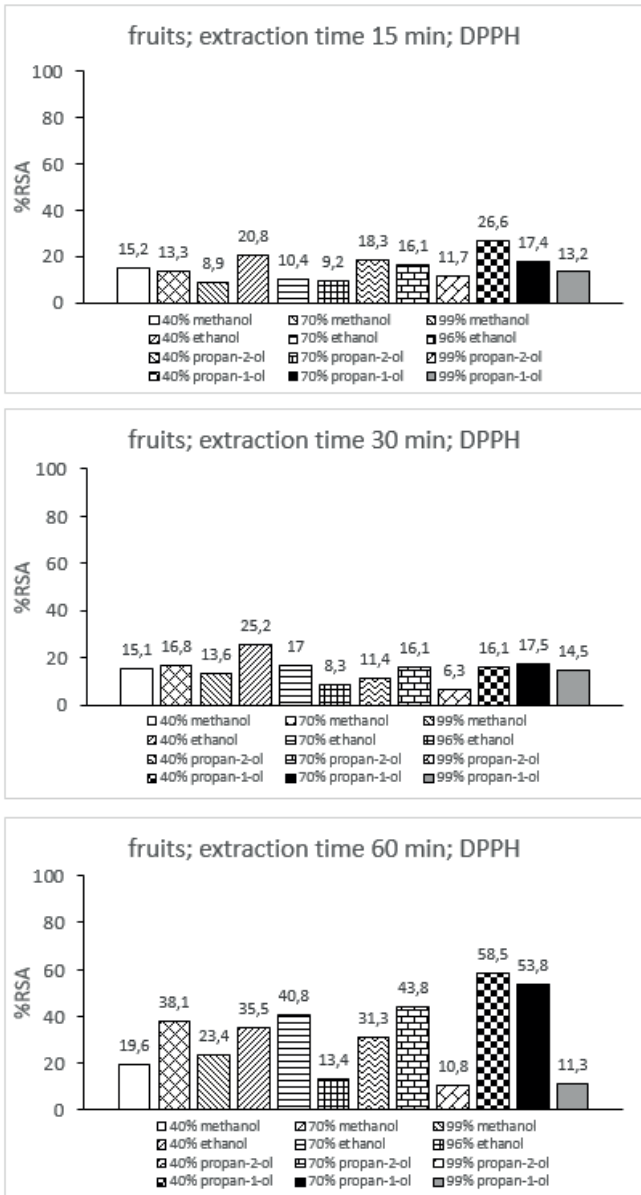


FIGURE 5. The effect of the applied extractant on the mean antioxidative activity (% RSA) of alcoholic extracts of dried *Syringa vulgaris* fruits obtained by 15, 30, and 60 min ultrasound-assisted extraction and evaluated using the DPPH method

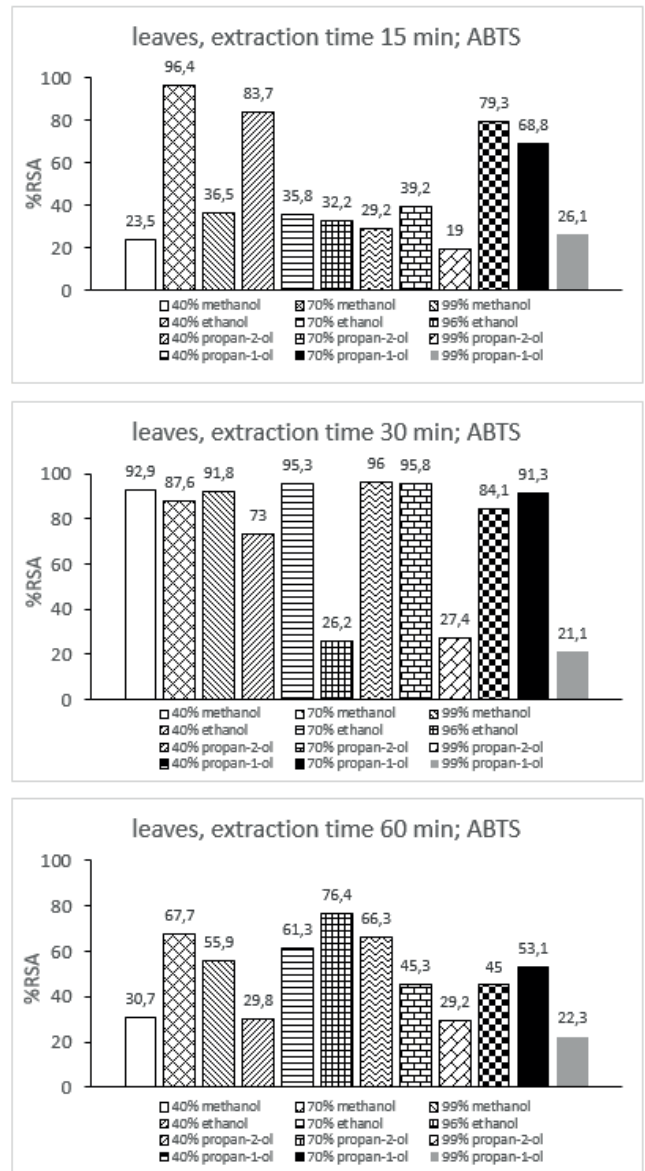


FIGURE 6. The effect of the applied extractant on the mean antioxidative activity (%RSA) of alcoholic extracts of dried *Syringa vulgaris* leaves obtained by 15, 30, and 60 min ultrasound-assisted extraction and evaluated using the ABTS method

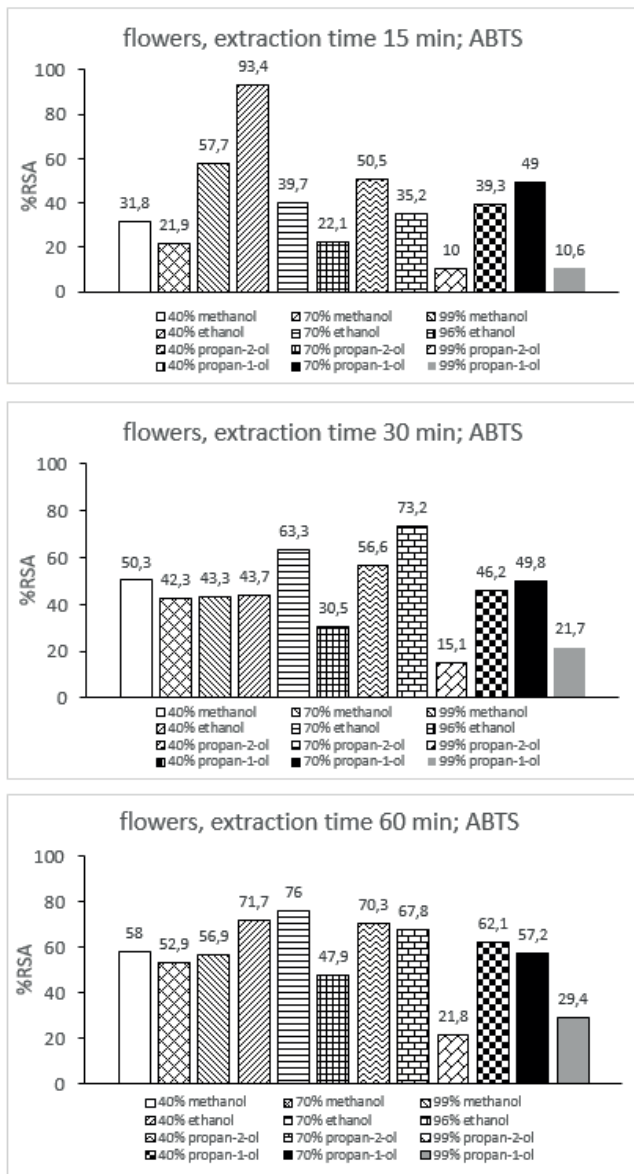


FIGURE 7. The effect of the applied extractant on the mean antioxidative activity (%RSA) of alcoholic extracts of dried *Syringa vulgaris* flowers obtained by 15, 30, and 60 min ultrasound-assisted extraction and evaluated using the ABTS method

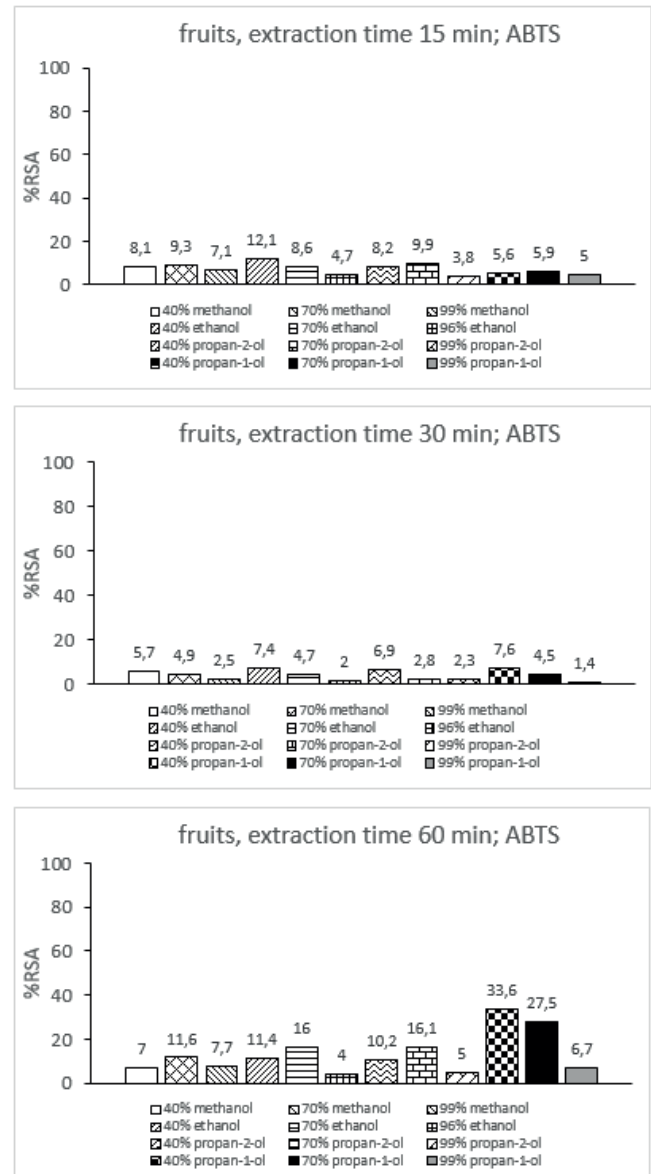


FIGURE 8. The effect of the applied extractant on the mean antioxidative activity (%RSA) of alcoholic extracts of dried *Syringa vulgaris* fruits obtained by 15, 30 or 60 min ultrasound-assisted extraction and evaluated using the ABTS method

DISCUSSION

Antioxidants are compounds that protect organisms against oxidative stress and inhibit and delay degenerative processes. As previously mentioned the main source of exogenous antioxidants are fruits and vegetables, especially those with specific colors, e.g. red and purple, which are characterized by a high content of anthocyanins. Their regular consumption protects the body against the adverse effects of free radicals and ROS. Thus, they contribute to the reduction of the occurrence of civilization diseases, e.g. cardiovascular and neoplastic diseases. Recently, there has been a great deal of interest in the therapeutic effects of antioxidative medicinal plants. Herbs owe their antioxidant potential to the content of biologically active substances, i.e. organic acids, mucilages, tannins,

phenolic compounds, and essential oils. Thus, they have the ability to protect the body from oxidative reactions. In order to stay healthy, fruit and vegetables are increasingly introduced into human diets [32, 33, 34, 35]. The ability of fruits and vegetables to neutralize ROS determines their total antioxidant potential [36].

Various parts of the *S. vulgaris* L. plant are used for medicinal purposes. Leaves, flowers, and bark are excellent anti-inflammatory agents. Talib and Mahasneh found that aqueous and ethanolic extracts of common lilac showed a high antiradical activity. Moreover, they extracted from the leaves and flowers minerals and amino acids, as well as micro- and macrolelements, and a particularly high concentration of silicon was noted. Among the amino acids, alanine, leucine, aspartic acid, and glutamic acid were found. The authors also showed that

the leaves and flowers were characterized by a high potassium content; however, the concentration of this element was higher in the leaves [37].

The main purpose of this study was to assess the antioxidant activity of individual parts of the *S. vulgaris* L. plant extracts. The study presents the research carried out using the DPPH and ABTS methods to determine the antioxidant potential. These methods were used to analyze the ability of plant extracts extracted from common lilac to scavenge free radicals. In the DPPH method, the color change of the sample from purple to yellow was evident to evaluate the concentration of antioxidants, while in the case of the ABTS method the content of antioxidants was confirmed by a decrease in the color intensity of the sample. The conducted study showed the high antioxidant capacity of *S. vulgaris* L. extracts, especially that of leaves and flowers. However, the results varied depending on the extraction time, the type of plant material and solvent used, as well as the spectrophotometric method applied for determination. Spectrophotometric tests using the DPPH method showed that the greatest amount of antioxidants was found in the extracts of *S. vulgaris* L. leaves, extracted in concentrated ethanol and methanol for 15 min 87.9 and 86.1% RSA, respectively. The flowers, extracted with the same solvents, showed the highest potential after 60 min ultrasound-assisted extraction – 90.1 and 84% RSA for 96% ethanol and, 99% methanol applied as extractants. Markedly lower activity was found for fruit extracts, where the highest activity was found for extracts in 70% alcohols after 60 min extraction. However, the highest potential was found for extract in 40% propan-2-ol – 58.5% RSA. After the application of the ABTS method to evaluate antioxidant capacity the highest activity in most cases of leaf extracts was found for the samples obtained after 30 min extraction, whereas for flowers the highest potential was found mainly for extracts obtained after 60 min extraction; however, the highest activity was found for the 15 min extraction in 40% ethanol. As concerns fruit extracts, their activity was markedly lower than that of leaf and flower extracts. The highest value of antioxidant activity was 33.6% RSA for 40% n-propanol extracts, extracted for 60 min, similar to the potential evaluated using the DPPH method. Similar observations regarding extraction conditions and the application of ultrasounds to aid extraction have been made with regard to different plants. For example, Flieger and Flieger, in their research on the goutweed (*Aegopodium podagraria* L.), proved that the use of dried plants and the reduction of the extraction time with the use of ultrasound helps obtain extracts with the highest antioxidant potential [7].

Abu-Darwish et al. evaluated the extracts from *Syringa* leaves harvested during the flowering period in mid-May; 40% ethanol was used as an extractant. The ratio of raw material to solvent was 1:10. Extraction was performed at 25°C. The biologically active compounds contained in the extract of *Syringa* leaves were identified using spectrophotometric and titration techniques. The presence of phenolic alcohols, flavonoids (rutin and quercetin), iridoids, and tannins has been found. On the basis of the obtained results, it was found that *Syringa* leaves

have anti-inflammatory and immunostimulating properties, therefore they can be used as a component of preparations and dietary supplements [38].

The properties of the bark and leaves of *S. vulgaris* L. were investigated by Varga et al. [39]. Phenols, flavonoids, and lignans were identified by means of the HPLC-DAD-ESI-TOF and HPLC-DAD-ESI-MS/MS methods. Analysis showed that syringin and rutin are the main phenolic compounds in leaves and bark. The *in vitro* DPPH and ABTS tests showed antioxidant activity of the leaves and bark of *S. vulgaris* L. [33]. In the studies of Tóth et al. on the identification of phenolic antioxidants using the HPLC-DAD-ESI-MS method in extracts from flowers and fruits of *S. vulgaris* L., phenols, flavonoids, and secooids were also identified. The ability to remove free radicals was tested using the DPPH and ABTS methods. Both flowers and fruits showed a high ability to scavenge free radicals, therefore they could be natural sources of phenolic compounds to be used in the cosmetics and pharmaceutical industries [17, 18]. Su et al. showed in their work that various extracts of the *Syringa* plant contained isolated compounds with anticancer, blood pressure lowering, anti-inflammatory, antifungal, and antioxidant properties. The research on the bark of *S. reticulata* (Japanese lilac) using the DPPH method showed that the extract in 70% EtOH has a high ability to scavenge superoxide anions [19].

In the present study, it has been found that the antioxidant potential of *S. vulgaris* leaf and flower extracts was higher than that of fruit extracts. Also, the results of many studies on other plants performed by others suggest that the antioxidant activity of leaf extracts is generally higher than that of fruit extracts. Muzykiewicz et al. evaluated the antioxidant activity of sea buckthorn leaves and fruit extracts. The extracts were prepared using the ultrasound-assisted extraction method. Next, the antioxidant potential of the resulting extracts was investigated using the DPPH, ABTS, and Folin-Ciocalteu methods. The leaves extracts showed a higher antioxidant activity than the fruit extracts. The best solvent was 70% methanol for extracts extracted for 60 min [33]. Another study assessed the antioxidant potential of extracts from the leaves and fruits of ripe rowan and quince. The raw materials were extracted in a Soxhlet apparatus or subjected to shaking. The extractants were 70% and 96% (v/v) ethanol, 99.85% (v/v) methanol and acetone; 5% extracts were obtained and were tested by various methods such as DPPH and ABTS, and the trolox served as the reference substance. It was found that the leaf extracts assessed using both DPPH and ABTS methods showed higher antioxidant activity than the fruit extracts. Both alcohols turned out to be more effective solvents [40]. Ahmed et al. conducted research on *Melia azedar* leaf extracts, which showed that their ethanolic extract contains the highest amount of phenolic compounds and at the same time shows the highest antioxidant activity [41]. The solutions were determined using the Folin-Ciocalteu and DPPH methods. The leaves were pulverized and then extracted into ethanol and petroleum ether using a Soxhlet apparatus at 55°C for 18 h. The antioxidant activity of the leaves was determined using the DPPH method; 3 replications were performed. The incubation time was 30 min,

the experiment was run at room temperature, and the absorbance measurements were made at 517 nm. Ascorbic acid was used as a reference. The study showed that *Melia azedaris* in ethanol exhibited the highest free radical scavenging ability compared to water and petroleum ether extracts. Methanol and ethanol were effective solvents for the extraction of phenolic compounds. However, ethanol proved to be more effective in reducing toxicity. The study showed that the higher the content of polyphenols in the plant raw material, the greater its antioxidant activity. Moreover, active ingredients such as flavonoids, tannins, saponins, phenols, glycosides, steroids, terpenoids, and alkaloids were isolated from the leaves of *Melia azedarach* [40, 41].

The results of the studies presented in this paper, as well as the studies cited above, show that *S. vulgaris* L. is a plant with a high antioxidant potential. The leaf extract in particular was characterized by high antioxidant properties. On the other hand, the fruit showed the lowest ability to scavenge free radicals. Based on the results of this research and those carried out by the other authors presented above, it can be concluded that the leaves and flowers of the common lilac are a valuable source of many active substances. Most of the research carried out concerned the leaves of *S. vulgaris* L. The researches carried out by Tóth et al. showed a high ability to scavenge free radicals by the flowers and fruits of the common lilac [17, 18]. Differences in the antioxidant activity of the fruit may be due to the use of a different solvent or the use of a different extraction method. The results were influenced by factors such as the type of plant raw material used, the type of solvent, extraction time, and the method used to measure the antioxidant potential. Taking into account the obtained results concerning the antioxidant potential of *S. vulgaris*, the plant may be recommended as a source of natural compounds in anti-aging cosmetics.

CONCLUSIONS

1. *Syringa vulgaris* L. showed varying antioxidant activity depending on the type of plant material.
2. Alcoholic extracts from the leaves and flowers of *S. vulgaris* L. showed higher antioxidant properties as compared to the fruit extracts of this plant.
3. The extraction time and the solvent used affected the antioxidant activity of the obtained extracts.

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