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Extracts from *Corylus avellana* as a source of antioxidants useful in cosmetic preparations*

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ABSTRACT

Introduction: Human skin is constantly exposed to harmful external factors that are often the cause of premature aging. Antioxidants (including vitamins and flavonoids) are an important group of compounds that are necessary to scavenge harmful free radicals. As a result of the universally functioning cult of beauty, cosmetics manufacturers increasingly often add antioxidants to slow the aging process of the skin. In beauty salons, these preparations are used during cosmetics treatments to facilitate the penetration of active ingredients into the skin, including mesotherapy, iontophoresis, or sonophoresis. Researchers, on the other hand, are looking for new rich sources of antioxidants and for more effective methods of their isolation. Raw materials from common hazel (*Corylus avellana* L.) are characterized by a very high antioxidant potential, but they are relatively rarely used in the production of any cosmetic preparations.

The aim of the study was to evaluate the impact of the hazel vegetation period as well as extraction conditions on the antioxidative activity of alcoholic extracts of selected parts of *Corylus avellana* L.

Materials and methods: Ultrasound-assisted extraction was applied to obtain alcoholic extracts from male inflorescences and common hazel leaves in different vegetation phases. Ethanol, methanol, isopropanol, and n-propanol at concentrations of 40%, 70%, or 96/99% were applied as extractants. The antioxidative potential was assessed using the DPPH and ABTS methods. Absorbance measurements were made using a spectrophotometer.

Results and conclusions: All the tested raw materials from *Corylus avellana* L. were characterized by a very high antioxidant activity. The highest scavenging capacity of free radicals was observed for dried leaves before and during fruiting determined using the DPPH and ABTS methods. The lowest activities were obtained in extracts in propan-1-ol and propan-2-ol from dried leaves during fruiting and male inflorescences. The results confirm the high content of antioxidants in the studied plant and suggest that raw materials derived from common hazel may be used in the production of cosmetics.

Keywords: common hazel (*Corylus avellana* L.); DPPH; ABTS; antioxidant activity; alcoholic extracts.

INTRODUCTION

The aging of an organism is a multifactorial process taking place not only inside the organism but also its skin. Most of the problems being a result of aging are mainly due to 4 factors – mechanical damage (caused by facial expressions), biological (genetically determined hormonal changes), environmental (the influence of UV radiation and active or passing smoking) as well as lifestyle (diet, skincare, stress, diseases, and the amount of sleep). These factors can be also divided into 2 groups: external or intrinsic [1]. The latter can be divided into physiological (aging as a result of disrupting the functioning of physiological systems), genetic (similarly aging individuals within one species), and non-genetic factors (accumulation of harmful substances in a living organism) [2].

With age, the ability of keratinocytes to proliferate within the epidermis decreases, and thus – its living layers become thinner. The keratinized layer becomes thicker as a result of an incorrect exfoliation process due to the disturbance of the proteinase activity responsible for the corneosome disintegration. The number of Langerhans cells gradually decrease, in older adults even down to 50%, which causes a decrease in the skin's immune resistance. Limiting the sebum secretion leads to dry skin and to changes in the composition of the hydrolipid mantle. Inadequately oiled skin may cause the enlargement of the sebaceous glands, causing their overgrowth. In the elderly, the pH of the skin also changes, and could also reduce the resistance to harmful environmental factors.

Another symptom of aging of the epidermis is a decrease in the number of melanocytes, which decreases the body's protection against the harmful effects of UV radiation [3]. An increased transepidermal water loss and a decrease of fatty acids, ceramides, and triglycerides concentration is another important factor. Moreover, skin aging results in enzyme degradation of hyaluronic acid, collagen, and elastin contained in the skin, causing loss of skin elasticity and firmness [4].

At the dermal-epidermal border the flattening of the epidermal icicles and dermal papilla occurs, and there is also

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a reduction in intercellular junctions, which affects the gradual reduction of the adherence area of the dermis and the epidermis. These changes lead to a reduction of nutrient penetration between the layers [5]. The number of fibroblasts in the skin decreases, and their size and activity decrease. A reduction in cellular adenosine triphosphate and oxygen consumption can also be observed, and these changes may lead to impaired protein synthesis with a simultaneous reduction in the ability to penetrate into the cell. The synthesis of collagen and elastic fibers is disturbed, and as a result of progressive disturbances in their physico-chemical properties, they become hard, unevenly distributed, and stiff. The number of macrophages is clearly reduced, contributing to decrease of collagenase and proteinases – enzymes responsible for the decomposition of cross-linked collagen. During the aging process of the skin, its ability to bind water decreases, because the composition of the proteoglycan gel changes, and thus the amount of hyaluronic acid is reduced. In addition, there are changes in the blood vessels, the walls of which may thicken or thin, while affecting the blood flow and skin nourishment [3, 5].

Intrinsic aging is an inevitable, continuous, and genetically controlled physiological process. This type of aging is also called chronological or natural because it is associated mainly with internal and not external factors. With age, the production of collagen and elastin gradually decreases and the number of newly formed cells decreases. Wrinkles appear on the face, the skin becomes thin, and the fatty tissue under the skin is reduced, causing the skin to sag and "sagging cheeks". In older people, dark circles become visible under the eyes, the lips are less convex, the face oval begins to drop, and excessive hair appears on the surface of the skin. Mimic wrinkles become permanent, the nasolabial furrow becomes expressive, and the skin is thin and transparent. All these changes increase significantly in the postmenopausal period when hormonal fluctuations occur [6].

Extrinsic skin aging is caused by factors from the environment in which we live. They contribute to the acceleration of aging or are the source of the premature skin aging. This group includes environmental pollution, nicotine, UV radiation, as well as improper nutrition. Cigarette smoke contains many harmful substances that have an adverse effect not only on active smokers but also on passive smokers. Prolonged exposure to tobacco smoke, causing biochemical changes, has negative effects on both general health and the skin. It manifests itself, inter alia, in premature aging, numerous skin discolorations, hypoxia, immune disorders of the skin, the appearance of characteristic smoker's wrinkles, and even the development of various diseases, including cancer. Ultraviolet radiation is the main, extrinsic factor that causes skin aging because doses of UV accumulate in the skin throughout life and could lead to the so-called photoaging and numerous neoplasms. Its harmful effects are associated with the action of free radicals [7, 8, 9, 10].

Free radicals are highly reactive and unstable molecules or atoms that contain unpaired electrons on the valence shell. They arise as a result of environmental pollution, UV radiation, and chemical reactions, as well as during respiration, enzymatic reactions, bacterial and viral infections, and phagocytosis [11]. The imbalance between the production of free radicals and their elimination from cells by antioxidant systems is called oxidative stress. This phenomenon very often contributes to the occurrence of diseases of the nervous system, cancer, atherosclerosis, and diabetes [12].

Free radicals are controlled by 2 antioxidant systems. They work by slowing down free radical chain reactions and protecting the cell against harmful effects or complete neutralization of free radicals. The enzyme system contains superoxide dismutase, glutathione peroxidase, and catalase – the so-called enzyme triad. The second system, the non-enzymatic one, consists of low-molecular-weight protective substances, so-called free radical scavengers, that transfer their electrons to free radicals and transform them into an oxidized form characterized by low reactivity. This group includes mainly vitamins, plasma proteins, glutathione, and melatonin [13, 14]. The antioxidant compounds found in plants include mainly vitamins A, C, and E and the group of polyphenols [15].

Hazelnut (*Corylus avellana* L.) belongs to the genus *Corylus*, the family Corylaceae, while formerly it was included in the Betulaceae family [16]. Several varieties can be distinguished, including *Corylus avellana* var. Pontica, *C. maxima* Mill., *C. tubulosa* Willd., *C. lamberdi* Lodd., *C. mashurica* Maxim., *C. colchica* Alb., *C. culurna* L., *C. arborescens* Munch. However, *C. avellana* L. is the most common. The aforementioned varieties differ in their sensitivity to low temperatures, diseases and pests, shape of the crown, structure of fruit and fruit cover, flowering and fruiting time, as well as the abundance of crops [17].

Corylus avellana is considered a shrub, but its size is very often the same as trees (about 6 m high). It was probably the first species of shrub trees to appear after the Ice Age. At that time, it dominated in the areas of today's North America and Europe, and its fruits constituted an important element of diet [17].

In the wild, *C. avellana* prefers the phytocoenosis of low-density forests – mainly mixed or leafy, rarely coniferous, where it forms a layer of undergrowth. It can also be found very often on the edges of mid-forest clearings, in ravines, on mountain slopes, at the shores of lakes and forests, as well as in thickets of various types of wastelands. *Corylus avellana* can be found in most temperate countries. In Poland, it grows in practically every forest, mostly in the south-eastern part of the country. It is characterized by extraordinary vitality, which is confirmed by the very high resistance to frost (even down to –30°C). It gives the most abdundant crops in fairly moist and fertile soils. Due to its taste and nutritional values, *C. avellana* is grown more and more often in orchards, plantations, allotments, and houses. It is sometimes used as a hedge and wind protection. The largest producers of hazelnuts on an industrial scale are Turkey, Italy, Spain, the USA, Azerbaijan, Georgia, Kazakhstan, as well as China and France. The cultivation method varies from country to country [17].

Hazel is distinguished from other plants by a very short dormancy period. Male inflorescences appear on the twigs as early as July, develop slowly in the following months, and at the turn of late December and early January, they start pollinating with

yellow pollen. Immediately after the emergence of male inflorescences, female flowers appear. Hazel is a wind-pollinated plant, which results in a low proportion of bees in the pollination process. However, due to the fact that hazel is one of the first plants to bloom in early spring, it provides bees with valuable pollen, which is the main nutrient medium for broods [17].

After pollination, the fruits (proper nuts) ripen through July and August protected by a serrated covering consisting of 3 sub-flowers equal to or shorter than the height of the nut. Its shape varies depending on the variety of hazel, it can be sleeve-shaped, skull-shaped, or bell-shaped. The unbellate, single-seeded and consisting of 2 cotyledons and a hidden embryo between the fruits, is covered with a leathery seed coat. The inside of the nut is protected by a woody pericarp (shell), which gradually turns brown and falls out of the dried cover (August–October). The nuts can be oblong or spherical, and medium or small in size (about 2.5 cm high) [17].

Hazelnuts contain many essential nutrients. They are very caloric – about 680 calories in 100 g, with the fat percentage reaching almost 60%. Taking into account the taste and nutritional value, hazelnut oil is one of the best vegetable oils. Hazelnuts are a valuable source of magnesium, iron, calcium, sodium, phosphorus, and potassium compounds, as well as B vitamins, and vitamins A, C, and E. Moreover, they contain proteins, carbohydrates, and unsaturated fatty acids (oleic acid). They are recommended by nutritionists for daily consumption especially for diabetics, children, heavy workers, and older adults, as well as for humans suffering from kidney diseases, anemia, stomach weakness, and ulceration. Hazelnuts support the body in the fight against obesity and fatigue, prevent sclerosis, and have a positive effect on the maintenance of beautiful skin and well-being. They have the ability to lower LDL-cholesterol, thus protecting the body against stroke and heart attack. They are used both in the food industry for chocolate products and confectionery, as well as in the cosmetics and pharmaceutical industries. The leaves and bark of hazel contain tannins, essential oils, glycosides, organic acids and sugars, which is why they are used to prepare therapeutic agents for bathing, washing difficult-to-heal wounds, and treating varicose veins. However, it should be remembered that hazelnuts can cause food allergies and life-threatening anaphylactic reactions [17, 18, 19].

Since the dawn of time, there has been a search for a substance that could help maintain a healthy and youthful appearance. The aging process of the skin consists of many factors of both endogenous (e.g. free radicals) and exogenous origin (e.g. UV radiation, improper skincare). Understanding the mechanisms of changes in the skin allows a more effective use of cosmetic methods and preparations to counteract this process. *Corylus avellana* L. is a valuable source of flavonoids and vitamins A, C, and E showing strong antioxidant properties, so the use of extracts from this raw material in the cosmetic industry may prove to be an effective anti-aging agent [2, 18].

The aim of the study was to compare the antioxidant properties of extracts from different morphological parts of *C. avellana* (fresh and dried leaves and inflorescences) determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), to determine of antioxidant properties of extracts from selected morphological parts of the studied plant depending on its vegetation phase, to evaluate the effect of the type of solvent used for extraction on the antioxidant properties of extracts from *C. avellana* and to determine the effect of ultrasound-assisted extraction time of raw materials on the antioxidant potential of the plant.

MATERIALS AND METHODS

DPPH and ABTS were purchased from Sigma-Aldrich (USA). Methanol, propan-1-ol, propan-2-ol, sodium persulphate and ascorbic acid, all of analytical grade, were from Chempur, Piekary Śląskie and ethanol was from Linegal Chemicals, Warsaw.

The following plant material was evaluated in this study: dry inflorescences harvested in March, fresh leaves harvested before fruiting (June), dried leaves harvested before fruiting (June), dried leaves harvested during the fruiting stage (July) and dried leaves collected after the end of vegetation (November). The raw material came from *C. avellana* L. growing in a home garden in Zieleniewo (near Stargard) in the West Pomeranian Voivodeship. It was dried at room temperature in a well-ventilated dark place.

Extracts were prepared in 4 short-chain aliphatic alcohols. The following alcohols, all at 3 concentrations, i.e. 40%, 70%, and undiluted, were used: methanol, ethanol, propan-1-ol, propan-2-ol. To obtain extracts 0.5 g of raw material and 10 cm3 of solvent were put into a stoppered test tube. All the tubes containing raw material and solvent were subjected to ultrasound-assisted extraction (Sonic 0.5, Polsonic, Poland) for 15, 30, or 60 min. This technique is generally considered a so--called green-extraction technique [20]. A total of 36 extracts were prepared from each raw material. After extraction, the extracts were separated from the remaining plant material by filtration. The pure extracts were transferred to plastic tubes sealed with a stopper and stored in a dark place at room temperature until analysis of antioxidant potential.

The antioxidant activity of the obtained extracts was evaluated using the DPPH and ABTS methods that have been described previously in literature [20, 21]. Briefly, to evaluate the antioxidant activity using the DPPH method, ethanolic DPPH radical solution was prepared by dissolving 0.012 g DPPH in 100 cm^3 96%(v/v) ethanol using a magnetic stirrer for 1 h. To obtain a working solution this concentrated solution was diluted with 70% ethanol until the absorbance in a 1 cm cuvette at 517 nm was 1.00 ±0.02. Next, 132 mm^3 of examined extracts were added to 2500 mm^3 of working solution in spectrophotometric cuvettes. After 10 min incubation at room temperature absorbance at 517 nm was measured (Spectroquant Pharo 300, Merck, Germany). Three samples were made for each extract. Antioxidant activity was expressed as ascorbic acid equivalents (mmol/dm^3) based on the prepared calibration curve ($y = -1.2771x + 0.9926$). A linear relationship between absorbance and concentration of ascorbic acid was found ($R^2 = 0.996$).

The second applied method to evaluate the antioxidant potential of the studied extracts was the ABTS technique based on the reaction of $ABTS^{+\bullet}$ cation radical with the tested solution. At the beginning, it is necessary to obtain the above-mentioned radical. For this purpose, 0.038 g of the ABTS ammonium salt was dissolved in 10 cm^3 2.45 mM aqueous potassium persulfate solution in a dark glass bottle. This solution was left overnight in a dark place at room temperature. After 24 h, this solution was diluted with 50% (v/v) methanol to obtain a working solution. The absorbance of this solution was 1.00 ±0.02 at a wavelength of 734 nm in a 1 cm cuvette. To evaluate the antioxidant potential to 2500 mm^3 of the working ABTS solution, 25 mm^3 plant extract was added, mixed and incubated in dark at room temperature for 6 min. The absorbance was measured at 734 nm. Three samples were made for each extract. Antioxidant activity was expressed as ascorbic acid equivalents (mmol/dm^3) based on the prepared calibration curve ($y = -0.5038x + 0.9785$). A linear relationship between absorbance and concentration of ascorbic acid was found ($R^2 = 0.992$).

Statistical analysis was performed using the Excel program for Windows (Microsoft Office). Arithmetic means and standard deviations (SD) were calculated. To prepare the calibration curve of the relationship of absorbance vs. ascorbic acid concentration for both applied methods, linear regressions and correlation coefficients were calculated.

RESULTS

Table 1 summarizes the mean (±SD) antioxidant activities of *C. avellana* extracts obtained from inflorescences and green leaves harvested before fruiting evaluated with the DPPH method and expressed as ascorbic acid equivalents. Antioxidant activities of inflorescence extracts were higher than those obtained from green leaves harvested before fruiting.

TABLE 1. Mean (±standard deviations) antioxidative activity expressed as ascorbic acid equivalents of extracts of inflorescences and green leaves harvested before fruiting of *Corylus avellana* evaluated using the DPPH method

Antioxidative activity (mmol/dm^3) determined using the DPPH method					
plant part	extractant		time of ultrasound-assisted extraction		
	solvent	concentration (%)	15 min	30 min	60 min
Inflorescences	methanol	40	0.634 ±0.002	0.585 ±0.004	0.695 ±0.003
		70	0.661 ±0.011	0.665 ±0.003	0.714 ±0.001
		99	0.509 ±0.025	0.550 ±0.014	0.684 ±0.007
	ethanol	40	0.626 ±0.009	0.640 ±0.005	0.690 ±0.019
		70	0.689 ±0.002	0.702 ±0.004	0.718 ±0.004
		96	0.173 ±0.053	0.223 ±0.005	0.265 ±0.019
	propan-1-ol	40	0.622 ±0.009	0.616 ±0.001	0.685 ±0.005
		70	0.668 ±0.002	0.668 ±0.012	0.721 ±0.001
		99	0.125 ±0.012	0.080 ±0.004	0.139 ±0.002
	propan-2-ol	40	0.632 ±0.003	0.614 ±0.003	0.678 ±0.019
		70	0.676 ±0.007	0.676 ±0.003	0.713 ±0.003
		99	0.077 ±0.041	0.082 ±0.005	0.121 ±0.006
Green leaves before fruiting	methanol	40	0.397 ±0.007	0.389 ±0.005	0.378 ±0.012
		70	0.465 ±0.006	0.498 ±0.019	0.602 ±0.005
		99	0.648 ±0.012	0.691 ±0.012	0.677 ±0.004
	ethanol	40	0.370 ±0.006	0.545 ±0.006	0.577 ±0.014
		70	0.317 ±0.012	0.668 ±0.012	0.649 ±0.013
		96	0.232 ±0.005	0.411 ±0.016	0.700 ±0.001
	propan-1-ol	40	0.238 ±0.054	0.585 ±0.031	0.636 ±0.010
		70	0.488 ±0.028	0.726 ±0.003	0.681 ±0.002
		99	0.098 ±0.010	0.569 ±0.003	0.496 ±0.012
	propan-2-ol	40	0.512 ±0.012	0.609 ±0.010	0.690 ±0.000
		70	0.480 ±0.013	0.313 ±0.016	0.641 ±0.005
		99	0.185 ±0.016	0.420 ±0.021	0.564 ±0.014

TABLE 2. Mean (±standard deviations) antioxidative activity expressed as ascorbic acid equivalents of extracts of dry leaves collected before, during, and after fruiting of *Corylus avellana* evaluated using the DPPH method

Antioxidative activity (mmol/dm^3) determined using the DPPH method					
plant part	extractant		time of ultrasound-assisted extraction		
	solvent	concentration (%)	15 min	30 min	60 min
Dry leaves before fruiting	methanol	40	0.730 ±0.009	0.683 ±0.005	0.681 ±0.010
		70	0.733 ±0.003	0.698 ±0.003	0.741 ±0.010
		99	0.640 ±0.015	0.690 ±0.011	0.758 ±0.012
	ethanol	40	0.714 ±0.001	0.684 ±0.004	0.680 ±0.010
		70	0.732 ±0.011	0.700 ±0.006	0.744 ±0.002
		96	0.290 ±0.011	0.225 ±0.004	0.635 ±0.032
	propan-1-ol	40	0.732 ±0.007	0.712 ±0.003	0.706 ±0.003
		70	0.722 ±0.001	0.720 ±0.005	0.713 ±0.004
		99	0.130 ±0.004	0.154 ±0.007	0.219 ±0.020
	propan-2-ol	40	0.729 ±0.004	0.700 ±0.009	0.694 ±0.005
		70	0.740 ±0.003	0.714 ±0.008	0.716 ±0.003
		99	0.197 ±0.013	0.155 ±0.006	0.120 ±0.020
Dry leaves during fruiting	methanol	40	0.523 ±0.030	0.528 ±0.063	0.586 ±0.010
		70	0.450 ±0.079	0.603 ±0.004	0.608 ±0.008
		99	0.519 ±0.103	0.658 ±0.027	0.634 ±0.007
	ethanol	40	0.498 ±0.047	0.571 ±0.004	0.652 ±0.046
		70	0.554 ±0.073	0.622 ±0.010	0.559 ±0.146
		96	0.548 ±0.070	0.583 ±0.011	0.663 ±0.007
	propan-1-ol	40	0.560 ±0.016	0.578 ±0.001	0.569 ±0.021
		70	0.581 ±0.033	0.617 ±0.010	0.618 ±0.011
		99	0.042 ±0.036	0.171 ±0.004	0.339 ±0.007
	propan-2-ol	40	0.502 ±0.049	0.565 ±0.005	0.554 ±0.039
		70	0.493 ±0.098	0.638 ±0.006	0.633 ±0.017
		99	0.058 ±0.003	0.167 ±0.015	0.212 ±0.026
Dry leaves after fruiting	methanol	40	0.681 ±0.018	0.697 ±0.031	0.735 ±0.003
		70	0.717 ±0.006	0.738 ±0.003	0.756 ±0.005
		99	0.662 ±0.012	0.733 ±0.009	0.763 ±0.004
	ethanol	40	0.671 ±0.014	0.722 ±0.007	0.732 ±0.002
		70	0.696 ±0.027	0.743 ±0.007	0.740 ±0.041
		96	0.531 ±0.011	0.720 ±0.008	0.706 ±0.043
	propan-1-ol	40	0.702 ±0.003	0.719 ±0.005	0.720 ±0.009
		70	0.722 ±0.015	0.738 ±0.006	0.738 ±0.007
		99	0.153 ±0.040	0.266 ±0.009	0.369 ±0.016
	propan-2-ol	40	0.704 ±0.029	0.721 ±0.008	0.729 ±0.011
		70	0.707 ±0.029	0.751 ±0.006	0.754 ±0.004
		99	0.208 ±0.009	0.377 ±0.012	0.557 ±0.008

Mean (±SD) antioxidant potential of *C. avellana* extracts obtained from dry leaves collected before, during, and after fruiting determined using the DPPH method is presented in Table 2. The antioxidant activity of extracts obtained from dry leaves harvested before fruiting was higher than the activities of extracts of leaves harvested after and during fruiting.

The mean (±SD) antioxidant activities of *C. avellana* extracts obtained from inflorescences and green leaves harvested before fruiting evaluated using the ABTS method and expressed as ascorbic acid equivalents are presented in Table 3. Similarly to the results obtained using the DPPH method, higher antioxidant potential of inflorescence extracts as compared to those obtained from green leaves harvested before fruiting was observed.

Table 4 contains mean (±SD) antioxidant activities of *C. avellana* extracts obtained from dry leaves harvested before, during, and after fruiting evaluated using the ABTS method and expressed as ascorbic acid equivalents. Antioxidant potential of extracts from dry leaves harvested before fruiting was in most cases higher than the activities of extracts of leaves harvested after and during fruiting.

The effect of the solvent concentration used to prepare the extracts of *C. avellana* and the time of ultrasound-assisted extraction on the antioxidant activity of the obtained extracts determined by applying the DPPH method is shown in Figures 1, 2, 3, 4 for methanol, ethanol, propan-1-ol, and propan-2-ol, respectively.

Figures 5, 6, 7, 8 present the effect of methanol, ethanol, propan-1-ol and propan-2-ol concentration used to prepare the extracts of *C. avellana* and the time of ultrasound-assisted extraction on the antioxidant potential of the obtained extracts evaluated by applying the ABTS method and expressed as ascorbic acid equivalents (mmol/dm^3).

TABLE 3. Mean (±standard deviations) antioxidative activity expressed as ascorbic acid equivalents of extracts of inflorescences and green leaves before fruiting of *Corylus avellana* evaluated using the ABTS method

Antioxidative activity (AAE [mmol/dm^3]) determined using the ABTS method					
plant part	extractant		time of ultrasound-assisted extraction		
	solvent	concentration (%)	15 min	30 min	60 min
Inflorescences	methanol	40	1.255 ±0.061	0.911 ±0.044	2.703 ±0.185
		70	1.694 ±0.032	1.562 ±0.126	2.932 ±0.352
		99	0.586 ±0.008	0.619 ±0.021	1.094 ±0.088
	ethanol	40	2.003 ±0.019	1.423 ±0.056	4.005 ±0.011
		70	1.608 ±0.025	1.605 ±0.059	3.641 ±0.141
		96	0.212 ±0.078	0.191 ±0.042	0.306 ±0.085
	propan-1-ol	40	3.222 ±0.029	2.910 ±0.084	4.030 ±0.007
		70	2.376 ±0.109	1.920 ±0.077	2.021 ±0.006
		99	0.135 ±0.068	0.115 ±0.054	0.014 ±0.004
	propan-2-ol	40	1.705 ±0.050	3.294 ±0.078	4.009 ±0.055
		70	1.281 ±0.181	1.186 ±0.126	2.020 ±0.004
		99	0.030 ±0.019	0.091 ±0.036	0.030 ±0.012
Green leaves before fruiting	methanol	40	0.451 ±0.076	0.514 ±0.088	0.459 ±0.014
		70	0.539 ±0.040	0.560 ±0.042	0.727 ±0.046
		99	0.565 ±0.064	0.780 ±0.162	1.000 ±0.016
	ethanol	40	0.406 ±0.042	0.684 ±0.049	0.691 ±0.036
		70	0.396 ±0.080	0.980 ±0.091	1.029 ±0.041
		96	0.365 ±0.064	0.500 ±0.004	0.916 ±0.018
	propan-1-ol	40	0.395 ±0.014	0.683 ±0.019	1.370 ±0.050
		70	0.492 ±0.044	1.001 ±0.025	1.999 ±0.001
		99	0.185 ±0.038	0.651 ±0.104	0.692 ±0.019
	propan-2-ol	40	0.548 ±0.022	0.661 ±0.030	0.897 ±0.061
		70	0.284 ±0.057	0.133 ±0.013	0.926 ±0.023
		99	0.537 ±0.046	0.458 ±0.061	0.726 ±0.083

TABLE 4. Mean (±standard deviations) antioxidative activity expressed as ascorbic acid equivalents of extracts of dry leaves collected before, during, and after fruiting of Corylus avellana evaluated using the ABTS method

Antioxidative activity (mmol/dm³) determined with ABTS method					
plant part	extractant		time of ultrasound-assisted extraction		
	solvent	concentration (%)	15 min	30 min	60 min
Dry leaves before fruiting	methanol	40	2.368 ±0.124	2.767 ±0.051	3.383 ±0.066
		70	1.650 ±0.017	1.884 ±0.050	3.206 ±0.479
		99	0.767 ±0.038	1.205 ±0.044	1.468 ±0.034
	ethanol	40	2.551 ±0.104	2.685 ±0.070	3.462 ±0.308
		70	1.995 ±0.018	1.587 ±0.168	3.222 ±0.209
		96	0.389 ±0.097	0.473 ±0.073	0.687 ±0.021
	propan-1-ol	40	2.591 ±0.112	3.455 ±1.083	6.217 ±0.106
		70	1.761 ±0.090	1.533 ±0.200	2.855 ±0.215
		99	0.152 ±0.012	0.209 ±0.045	0.226 ±0.025
	propan-2-ol	40	2.762 ±0.083	1.860 ±0.027	3.711 ±0.029
		70	1.393 ±0.189	1.557 ±0.106	2.274 ±0.002
		99	0.117 ±0.041	0.160 ±0.042	0.175 ±0.025
Dry leaves during fruiting	methanol	40	1.314 ±0.076	1.702 ±0.110	2.534 ±0.049
		70	1.130 ±0.198	1.508 ±0.059	2.589 ±0.121
		99	1.305 ±0.261	1.442 ±0.014	1.394 ±0.097
	ethanol	40	1.251 ±0.118	1.613 ±0.023	2.438 ±0.024
		70	1.394 ±0.185	1.798 ±0.034	2.977 ±0.149
		96	0.873 ±0.177	0.538 ±0.076	1.113 ±0.028
	propan-1-ol	40	1.407 ±0.041	0.968 ±0.028	3.468 ±0.118
		70	1.460 ±0.084	1.708 ±0.064	2.453 ±0.036
		99	0.047 ±0.008	0.013 ±0.011	0.493 ±0.157
	propan-2-ol	40	1.261 ±0.124	1.855 ±0.066	2.461 ±0.011
		70	1.238 ±0.248	1.781 ±0.043	2.183 ±0.097
		99	0.140 ±0.008	0.241 ±0.010	0.244 ±0.068
Dry leaves after fruiting	methanol	40	1.725 ±0.091	3.074 ±0.727	3.058 ±0.105
		70	1.563 ±0.036	2.765 ±0.057	3.384 ±0.051
		99	0.700 ±0.142	1.332 ±0.007	1.584 ±0.015
	ethanol	40	1.593 ±0.024	3.440 ±0.221	3.451 ±0.148
		70	1.017 ±0.054	1.787 ±0.118	2.315 ±0.273
		96	0.661 ±0.055	0.858 ±0.072	0.858 ±0.074
	propan-1-ol	40	1.790 ±0.118	3.360 ±0.255	5.253 ±0.163
		70	1.210 ±0.029	3.216 ±0.151	3.475 ±0.520
		99	0.183 ±0.092	0.265 ±0.052	0.373 ±0.053
	propan-2-ol	40	1.527 ±0.074	3.789 ±0.036	3.757 ±0.225
		70	1.134 ±0.083	1.959 ±0.040	2.443 ±0.495
		99	0.209 ±0.033	0.474 ±0.048	0.543 ±0.143

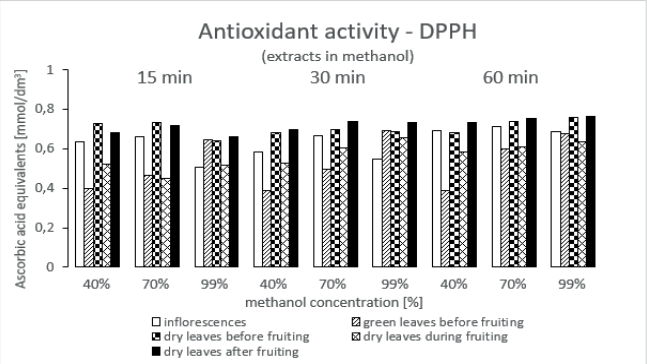

FIGURE 1. Antioxidant activity evaluated by the DPPH method and expressed as ascorbic acid equivalents (AAE; mmol/dm³) of methanol extracts of all evaluated parts of *Coryllus avellana* – the extracts were prepared in 40%, 70%, and 99% methanol by ultrasound-assisted extraction (15, 30, or 60 min)

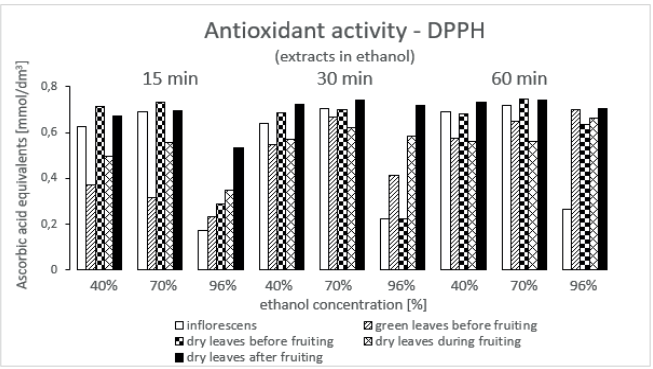

FIGURE 2. Antioxidant activity evaluated by the DPPH method and expressed as ascorbic acid equivalents (AAE; mmol/dm³) of ethanol extracts of all evaluated parts of *Coryllus avellana* – the extracts were prepared in 40%, 70%, and 96% ethanol by ultrasound-assisted extraction (15, 30, or 60 min)

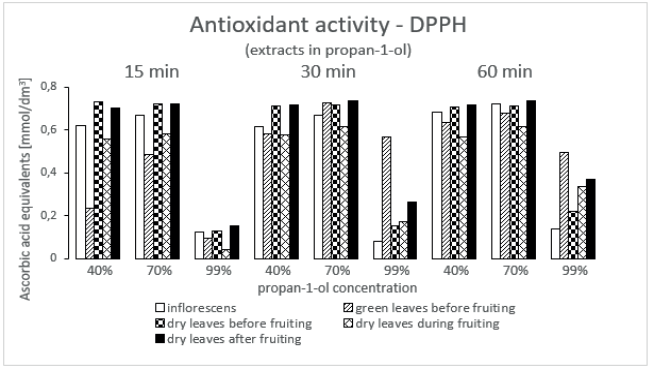

FIGURE 3. Antioxidant activity evaluated by DPPH method and expressed as ascorbic acid equivalents (AAE; mmol/dm³) of propan-1-ol extracts of all evaluated parts of *Coryllus avellana* – the extracts were prepared in 40%, 70%, and 99% propan-1-ol by ultrasound-assisted extraction (15, 30, or 60 min)

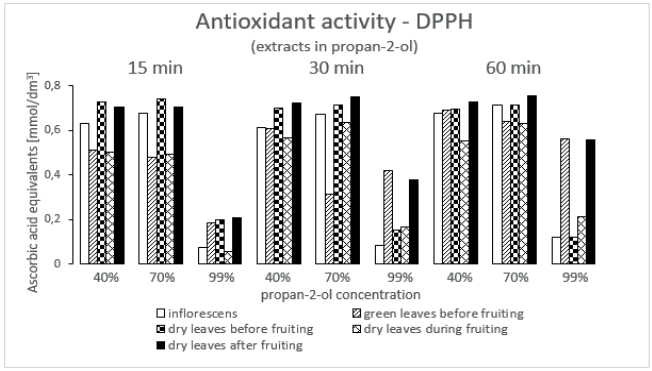

FIGURE 4. Antioxidant activity evaluated using the DPPH method and expressed as ascorbic acid equivalents (AAE; mmol/dm³) of propan-2-ol extracts of all evaluated parts of *Coryllus avellana* – the extracts were prepared in 40%, 70%, and 99% propan-2-ol by ultrasound-assisted extraction (15, 30, or 60 min)

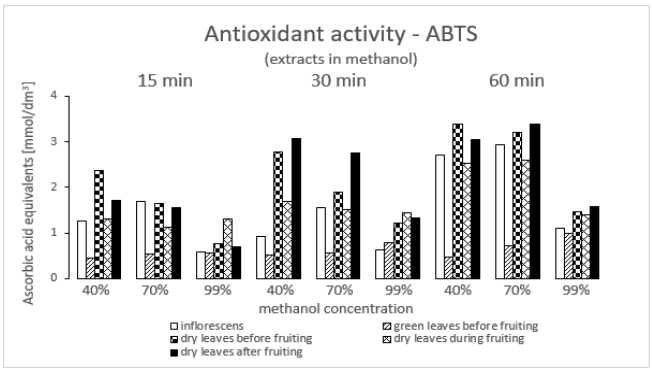

FIGURE 5. Antioxidant activity evaluated using the ABTS method and expressed as ascorbic acid equivalents (AAE; mmol/dm³) of methanol extracts of all evaluated parts of *Coryllus avellana* – the extracts were prepared in 40%, 70%, and 99% methanol by ultrasound-assisted extraction (15, 30, or 60 min)

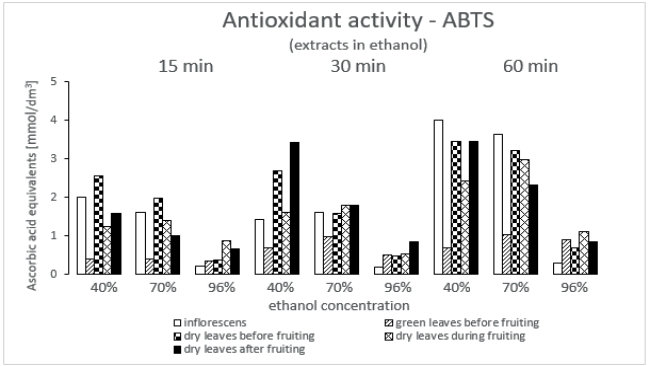

FIGURE 6. Antioxidant activity evaluated using the ABTS method and expressed as ascorbic acid equivalents (AAE; mmol/dm³) of ethanol extracts of all evaluated parts of *Coryllus avellana* – the extracts were prepared in 40%, 70%, and 96% ethanol by ultrasound-assisted extraction (15, 30, or 60 min)

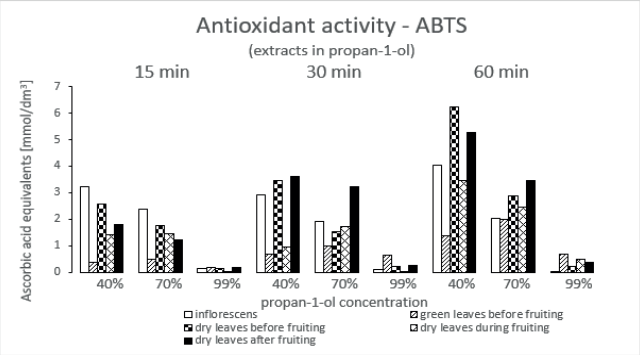

FIGURE 7. Antioxidant activity evaluated using the ABTS method and expressed as ascorbic acid equivalents (AAE; mmol/dm³) of propan-1-ol extracts of all evaluated parts of *Coryllus avellana* – the extracts were prepared in 40%, 70%, and 99% propan-1-ol by ultrasound-assisted extraction (15, 30, or 60 min)

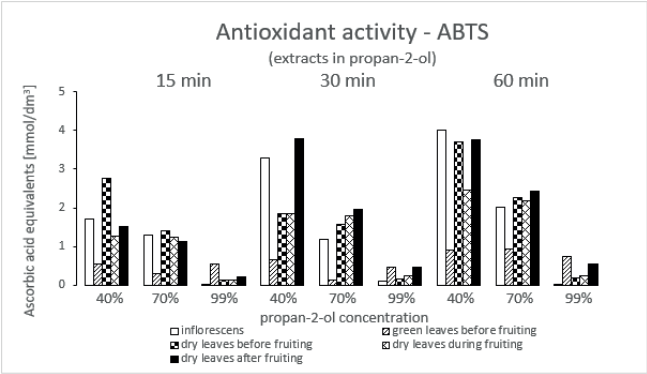

FIGURE 8. Antioxidant activity evaluated using the ABTS method and expressed as ascorbic acid equivalents (AAE; mmol/dm³) of propan-2-ol extracts of all evaluated parts of *Coryllus avellana* – the extracts were prepared in 40%, 70%, and 99% propan-2-ol by ultrasound-assisted extraction (15, 30, or 60 min)

The presented results show that the highest antioxidant potential determined by the DPPH method was found for extracts prepared from leaves dried after fruiting, extracted for 60 min in 99% methanol (AAE 0.763 mmol/dm³), leaves dried before fruiting, extracted for 60 min in 99% methanol (AAE 0.758 mmol/dm³), leaves dried after fruiting, extracted for 60 min in 70% methanol (AAE 0.756 mmol/dm³), leaves dried after fruiting, extracted for 60 min in 70% propan-2-ol (AAE 0.754 mmol/dm³), leaves dried after fruiting, extracted for 30 min in 70% propan-2-ol (AAE 0.751 mmol/dm³), leaves dried after fruiting, extracted for 30 min in 70% ethanol (AAE 0.743 mmol/dm³), leaves dried before fruiting, extracted for 60 min in 70% ethanol (AAE 0.744 mmol/dm³), leaves dried before fruiting, extracted for 15 min in 70% propan-2-ol (AAE 0.740 mmol/dm³), leaves dried before fruiting, extracted for 60 min in 70% methanol (AAE 0.741 mmol/dm³) and for extracts obtained from leaves dried after fruiting, extracted for 60 min in 70% ethanol (AAE 0.740 mmol/dm³).

After the application of the ABTS method, the highest antioxidant potential was found for extracts prepared from leaves dried before fruiting, extracted 60 min in 40% propan-1-ol (AAE 6.217 mmol/dm³), leaves dried after fruiting, extracted for 60 min in 40% propan-1-ol (AAE 5.253 mmol/dm³), inflorescences extracted for 60 min in 40% propan-1-ol (AAE 4.030 mmol/dm³), inflorescences extracted for 60 min in 40% propan-2-ol (AAE 4.009 mmol/dm³), inflorescences extracted for 60 min in 40% ethanol (AAE 4.005 mmol/dm³), leaves dried after fruiting, extracted for 30 min in 40% propan-2-ol (AAE 3.789 mmol/dm³), leaves dried after fruiting, extracted for 60 min in 40% propan-2-ol (AAE 3.757 mmol/dm³), inflorescences extracted for 60 min in 70% ethanol (AAE 3.641 mmol/dm³), leaves dried before fruiting, extracted for 60 min in 40% propan-2-ol (AAE 3.711 mmol/dm³), and for extracts obtained from leaves dried after fruiting, extracted for 30 min in 40% propan-1-ol (AAE 3.630 mmol/dm³).

DISCUSSION

With the growing human awareness of the causes of premature aging, the cosmetic market attempts to meet the consumers' demands by offering effective anti-aging products for the skin. The main active ingredients in these products are antioxidants, a large group of compounds including, among others: flavonoids, phenolic acids, and vitamins, which have the ability to neutralize free radicals [22, 23]. They are added to various types of creams, as well as in preparations used in beauty salons. The growing interest in natural cosmetics has popularized the addition of natural antioxidants to cosmetics during production. Many of the most applied antioxidants are those isolated from plants.

The reason why it was decided to study the leaves and inflorescences of *C. avellana* was the high values of antioxidant activity of hazelnuts presented in literature. It was decided to evaluate the antioxidant activity of the extracts of other morphological parts of the plant depending on the growing season and solvents applied for the extraction of *C. avellana* L. For this purpose, male inflorescences and leaves harvested before, during, and after fruiting were used. To determine their antioxidant potential, 2 methods based on spectrophotometric measurements, i.e. DPPH and ABTS, were applied.

In this study, the highest antioxidant potential determined by the DPPH method was found for extracts prepared from leaves dried after fruiting, extracted for 60 min in 70%, 99% methanol or in 70% propan-2-ol or ethanol of the same concentration, as well as extracted for 30 min either in 70% ethanol or propan-2-ol. Moreover, high antioxidant activity was also found for extracts obtained from leaves dried before fruiting, extracted for 60 min in 70% or 99% methanol and in 70% ethanol, as well as those extracted for 15 min in 70% propan-2-ol.

Analysis of the antioxidant potential determined using the ABTS method showed that the highest antioxidant potential in extracts prepared from leaves dried before fruiting, extracted for 60 min in 40% propan-1-ol or in 40% propan-2-ol, for extracts from leaves dried after fruiting, extracted either for 30 min or 60 min in 40% propan-1-ol as well as in 40% propan-2-ol. High activity was also observed for extracts from inflorescences in 40% propan-1-ol or propan-2-ol as well as in 40% or 70% ethanol, all extracted for 60 min.

Delgado et al. analyzed the total phenolic content and antioxidant potential of the hazelnut (*C. avellana* L.) against other

types of nuts [24]. Hazelnut raw materials were harvested by hand from the ground in early September 2008 in Bragança, northern Portugal, dried for 5 days, and then the fruits were frozen to –20°C. After thawing, the woody pericarp was removed and the seeds were crushed in a mill. The rest of the nuts, i.e. almonds, walnuts, peanuts, and pine nuts, were purchased from the local market and also crushed. Hazelnuts were divided into 5 samples, which were extracted in different solvents. The highest antioxidant value was shown by extracts extracted for 24 h in 80% (v/v) acetone, so these conditions were also applied to other nuts.

To test the ability to neutralize free radicals, the DPPH method was applied. The highest values of gallic acid (GA) equivalent and thus the highest concentration of phenols were achieved using the aqueous extracts, while the lowest using the methanolic extracts. The highest free radical scavenging capacity was observed for extracts prepared in acetone. No enhancement of antioxidant potential with increasing extraction time was observed. Of all the raw materials tested, hazelnuts had the highest antioxidant content, while walnut had the highest phenolic content. The lowest antioxidant properties were recorded for almonds, which, according to the authors, could be due to the fact that these nuts were tested without the peel [24]. Based on the obtained results, undiluted propan-1-ol was the least effective solvent for the above-mentioned extracts. It is also worth noting that the vegetative stage of the plant has a significant impact on the antioxidant potential of the extracts in short-chain alcohols.

Ghirardello et al. compared the phenolic content and antioxidant activity of hazelnut extracts prepared in different solvents [25]. Ethanol, methanol, and acetone were applied as extractants. Antioxidant potential was evaluated using the DPPH and ABTS methods. Similar to the results obtained by Delgado et al., the best extraction was achieved by using the application of 80% acetone at 50°C, while ethanol at the same concentration at 80°C proved to be the least effective solvent [24].

Pelvan et al. analyzed the phenolic content and antioxidant properties of hazelnut extracts [26]. The raw material consisted of crushed: natural hazelnut, roasted hazelnut without a peel, and roasted hazelnut peel. Samples were extracted with acetone, water, and acetic acid for 30 min. Total phenolic content was presented in GA equivalents (mg GA/g sample) and the free radical scavenging capacity was determined using the DPPH and ABTS methods (mmol trolox/g sample). In the study, 22 compounds were detected in the raw materials, i.e. flavonoids, phenolic acids and other derivatives in various concentrations in almost all samples. Whole hazelnuts and roasted peel contained the most GA and vanillic acid, while roasted seeds contained a lot of syringic acid and vanillic acid. The raw materials were ranked in descending order of phenolic acid content in the following order: roasted nut peel, whole hazelnut, and roasted hazelnut without peel (lowest content). The results suggest, similar to the findings of Delgado et al. [24], that the removal of the peel during the roasting process can have a very strong effect on the low phenolic content. Analysis of antioxidant properties of the extracts evaluated using the DPPH method showed that the roasted hazelnut peel had the highest antioxidant properties, while the roasted nut had the lowest. These results were confirmed by the ABTS method [26].

Taş and Gökmen compared the antioxidant capacity evaluated using the DPPH method of various types of roasted and unprocessed nuts with or without peel [27]. The extract of unprocessed hazelnut with peel reached even 84.9–93.6% of antioxidant activity. Authors, similar to Pelvan et al. [26] confirmed the fact that nut skins, usually removed by roasting, are the most valuable source of antioxidants [27].

Schmitzer et al. analyzed the phenolic content and antioxidant potential of fresh and roasted fruits of 6 European types of hazel *C. avellana* L. ("Pauetet", "Tonda Gentile Romana", "Negret", "Toda Gentile delle Langhe", "Barcelona", and the local variety "Istrska dolgoplodna leska") [28]. All raw materials were collected in 2008 in the garden of the Biotech Faculty in Maribor. The extracted materials were divided into 3 groups: nuts peeled by hand and soaked in warm water, whole fruits with skin, and seeds roasted in an oven for 15 min at 140°C. Methanol was used for extraction. Total phenol content was presented as GA equivalent in mg/kg. To determine the free radical scavenging capacity, the DPPH method was applied. The highest phenolic content was shown in extracts from whole nuts of the "Barcelona" variety, and the lowest – in roasted "Toda Gentile delle Langhe" hazelnuts. The extracts of the peeled nuts of the latter variety were also characterized by the lowest antioxidant activity. The highest antioxidant value was determined in "Tonda Gentile Romana" whole nut extracts. In all the tested varietes, there was a high correlation between the high content of phenolic compounds and antioxidant potential. Similar to studies by Pelvan et al. [26] and Taş and Gökmen [27], a decrease in antioxidant activity was observed with the removal of the skin from the seeds. Contrary to Pelvan et al. [26], Schmitzer et al. reported no significant difference in antioxidant properties between roasted and unroasted hazelnuts [28].

Whole hazelnut and green leave extracts were studied by Alasalvar et al. [29]. The extracts were prepared in 80% (v/v) ethanol and 80% (v/v) acetone and antioxidant activity by the DPPH method as well as phenolic, tannin, and phenolic acid content were evaluated. The leaves extracted in acetone showed the highest content of phenols, tannins, and antioxidant activity. In contrast, the ethanolic extract of the nut had the lowest free radical scavenging potential. Five phenolic acids were detected in the other extracts, with the highest amount similar to the results reported by Pelvan et al. [26]. The authors suggest that leaves of *C. avellana* may be a valuable source of antioxidants [29].

A similar analysis of antioxidant properties using the DPPH method and evaluation of the content of phenols and phenolic acids was performed by Shahidi et al. [30]. The tested raw material consisted of walnut, hazel leaf, seed skin, hard shell, and leafy hazelnut cover. They observed that the hazelnut itself showed the lowest antioxidant activity. The shell, seed skin, leafy cover, and hazel leaves were identified as the more valuable sources of antioxidants. In all extracts, the presence of

five phenolic acids was demonstrated, similar to Alasalvar et al. [29]. Just like in the study by Schmitzer et al. [28], a strong correlation between the presence of these acids in the raw material and antioxidant properties was found. The results suggest that hazelnut by-products seem to be a rich source of antioxidants [30].

Demchik et al. studied the antioxidant activity and phenolic content of other morphological parts of *C. avellana* tree and the nuts. Its leaves, nuts, shell, and green nut cover were analyzed. The raw materials were frozen to –80°C, then ground and extracted for 30 min in 80% ethanol [31]. It was confirmed – identical to the study of Shahidi et al. – that the phenolic content in hazelnut was lower than in shell, green cover, and leaves [30]. The highest antioxidant potential was found for leaves and green covers. The lowest free radical scavenging capacity among the raw materials tested were observed for hazelnuts. Similarly to the results of other studies, Demchik et al. stated that the presence of phenolic compounds significantly affects the antioxidant potential [31].

In summary, *C. avellana* seems to be rather underestimated by the cosmetic industry. It is a plant rich in various active ingredients with high antioxidant potential. Based on the obtained results it can be assumed that extracts from *C. avellana* L. could enrich the cosmetics market by producing anti-aging cosmetics containing natural antioxidants.

CONCLUSIONS

1. *Corylus avellana* L. is a valuable source of antioxidants.
2. The growing season has a great effect on its antioxidant activity. Among the examined parts of *C. avellana*, the highest activity was found in extracts of dried leaves harvested before and after fruiting.
3. The obtained antioxidant potential of the extracts was highly affected by the extractant used and by the extraction time.
4. Ultrasound-assisted extraction belonging to the so-called green techniques is a valuable method that can be used to obtain extracts with a high antioxidant potential.
5. The antioxidant properties of *C. avellana* make it a potentially effective ingredient in anti-aging preparations.

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