

Antioxidant potential of extracts from different parts of *Cichorium intybus* L.*

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ABSTRACT

Introduction: Free radicals are formed mainly as a result of environmental pollution, ultraviolet (UV) radiation, and stress. These factors contribute to the formation of oxidative stress, which is involved in the aging process of the skin, among other things. Antioxidants are compounds found naturally in plant products that have beneficial effects on human health. Natural antioxidants found in fruits, vegetables, and other plants have a positive anti-aging effect. Therefore, in recent years there has been an increased interest in plant raw materials to obtain antioxidants useful not only in cosmetology. One of these plants that is commonly grown in our country is chicory (*Cichorium intybus* L.). The aim of the study was to evaluate the antioxidant properties of alcoholic extracts obtained from 3 parts of *C. intybus* L. The influence of extraction time and solvent selection on the antioxidant activity of various parts of chicory was also investigated.

Materials and methods: The research material consisted of dried herb, stem, and root of *Cichorium intybus* L. Low molecular weight

alcohols, i.e. methanol, ethanol, isopropanol, and n-propanol at concentrations of 40%(v/v), 70%(v/v) and undiluted were used to obtain extracts using ultrasonic-assisted extraction. The antioxidant properties of the extracts were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis[3-ethyl-2,3-dihydrobenzothiazole-6-sulfonate] diammonium salt (ABTS) methods.

Results: Extracts from all parts of chicory showed antioxidant potential, but it varied depending on the part of chicory to be used. The highest activity was observed for herb extracts and the lowest for root extracts. Methanol seemed to be the best solvent for extraction to obtain the extracts characterized by high antioxidant capacity.

Conclusions: Based on the obtained results, it can be concluded that due to their antioxidant activity, *C. intybus* L. extracts, especially those obtained from the herb, can be suggested as components of natural cosmetics.

Keywords: antioxidant activity; alcoholic extracts; DPPH; ABTS; chicory (*Cichorium intybus* L.).

INTRODUCTION

Aging of the body is a natural and inevitable process. All tissues, cells, and organs degenerate over time. Many of them are constantly being repaired, replaced by new structures, or reconstructed. A significant portion of the metabolism is focused on this continuous repair process. However, as the body's ability to regenerate diminishes, irreversible changes occur that could be considered the aging process of tissues or the body [1, 2].

Both the onset and progression of aging are genetically determined or stimulated by environmental conditions. Intrinsic factors may include age, hormonal changes, and metabolic disorders. Extrinsic factors include ultraviolet (UV) radiation, environmental pollution, chemical and physical damage, and the effects of poor nutrition [3, 4].

The skin aging process is inextricably linked to numerous physiological changes in different skin layers as well as changes in physical and morphological properties. The skin becomes thin, gray, and dull. These lesions are most commonly found on the face, neck, and dorsal surface of the forearms and hands. A special feature of skin aging is the appearance of wrinkles.

They are formed due to a decrease in skin hydration and elasticity [5, 6].

Free radicals are produced in the human body as a result of natural cellular respiration, enzymatic reactions, viral or bacterial infections, and under the influence of exogenous factors such as UV radiation or environmental pollution [7, 8]. When produced in excess, they have a destructive effect on cells and cause many diseases. They also have a negative effect on the skin itself. They affect it by destroying intercellular cement, and collagen and elastin fibers. The skin becomes dry, less elastic, prone to irritation and wrinkles appear. Free radicals are dangerous not only for the human body, but also for the ingredients contained in cosmetics, as they can reduce the value of the preparation [7, 9].

In addition to its beneficial properties, oxygen can also be harmful. As a result of metabolic processes, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced which, when produced in appropriate amounts, regulate cellular processes. However, an excess of ROS and RNS can lead to oxidative stress in cells. This occurs due to an imbalance between the ROS and RNS produced and the body's ability to perform detoxification processes [8].

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Antioxidants are compounds found naturally in plant products that have beneficial effects on human health [10]. They prevent the harmful effects of free radicals, whose excessive concentrations can pose a serious threat to cellular structures. Antioxidants not only prevent the formation of free radicals, but also participate in the neutralization and removal of oxidative damage [11].

Antioxidants are stable compounds, so they do not become radicals after donating an electron. Their main function is to protect cells and tissues from any damage. They can also control the processes of free radical formation and prevent the action of toxic products of their metabolism [11, 12]. One of the main sources of antioxidants is various parts of plants. Therefore, in recent years there has been an increased interest in plant raw materials for their use in obtaining antioxidants for application, among others, in cosmetology. One of such plants, which is widely grown in our country, is chicory [13].

Cichorium intybus L., also known as chicory, is a herbaceous plant that grows in various parts of Europe, Africa, and Asia. It is a glandular, biennial plant with a tuberous taproot and a rosette of about 30–70 green leaves and usually reaches 20–150 cm in height. *Cichorium intybus* L. grows from many tall and hollow stems. Short and thick hairs can be found at the bottom of the stem. The buds are branched, rigid, and do not contain sap. The leaves are green with gray tints. They are elongated and lanceolate [13, 14]. Chicory flowers are light blue and open only on sunny days. In temperate climates, *C. intybus* L. not only grows wild [15], but is also cultivated in Europe. It can be found in meadows, fallows, wastelands, and along roadsides. It occurs in lowland areas, but also in lower mountain areas [16, 17].

The root, herb, flowers, and leaves of *C. intybus* L. contain many chemical compounds. Among others, chicory roots contain sap and sesquiterpene lactones or phenolic acids. The leaves contain vitamin A, the elements: Ca, K, Mg, Na, Fe, Cu, Mn, and Zn, as well as inulin and phenolic compounds. The flowers contain various sugars, coumarin derivatives, flavonoids, silicic acid, anthocyanins, and essential oils [18]. The plant also contains other compounds such as: choline, tannins, phytosterols, copper, lipids, proteins, amino acids, succinic acid, saponins, terpenoids, and flavonoids [19, 20].

Fresh and dried material is most commonly used for medicinal applications. The plant has a wide range of pharmacological and biological properties. In South Africa and India, chicory root is used as a coffee substitute or additive. Chicory coffee is also consumed in Poland. The seeds and leaves of the plant are considered hepatoprotective and blood-purifying in Iranian folk medicine. Chicory extract has been found to have immunotoxic, antibacterial, antidiabetic, antioxidant, detoxifying, and refreshing properties. Its phytochemical activity is due to the presence of such compounds as fructooligosaccharides, inulin and polyphenols, including caffeic acid derivatives or chlorogenic acid [13, 14, 15].

The aim of the study was to determine the antioxidant properties of extracts from dried stems, roots, and herbs of *C. intybus* L. using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis[3-ethyl-2,3-dihydrobenzothiazole-6-sulfonate] diammonium salt (ABTS) methods, to evaluate the effect of the applied solvent on the antioxidant activity of the obtained extracts, and

to evaluate the effect of ultrasonic-assisted extraction conditions on the antioxidant properties of *C. intybus* L. extracts.

MATERIALS AND METHODS

Reagents were purchased from the following companies: ABTS, DPPH, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from Sigma-Aldrich (USA), methanol, iso-propanol and n-propanol, sodium persulfate from Chempur, Piekary Śląskie (Poland), and ethanol from Linegal Chemicals, Warsaw (Poland).

The *C. intybus* L. used as research material was collected in August from meadows near Szczecin in the West Pomeranian Voivodeship. The herb with flowers, stem, and root of chicory was dried at room temperature in a well-ventilated room for about 4 weeks. The harvesting sites were characterized by rather dry soil, strong sunlight, and a long distance from road traffic.

The dry raw material was used to prepare extracts. Four short chain aliphatic alcohols including methanol, ethanol, iso-propanol, and n-propanol were used in 3 concentrations: 40%, 70%, and undiluted, were used as extractants. The ultrasound-assisted extraction procedure used has been described previously [21, 22]. This method is classified as a green extraction technique. Briefly, 10 cm³ of alcohol was added to 0.5 g of dry raw material placed in a glass test tube, the tubes were closed with plastic stoppers and placed in an ultrasonic bath (Sonic 0.5, Polsonic, Warsaw) for 15, 30 or 60 min. After ultrasonic extraction, the extracts were separated from the remaining plant material by filtration. A total of 36 extracts were prepared from each part of the chicory. After filtration, the clear extracts were transferred to stoppered plastic tubes and stored in the dark at room temperature until analysis.

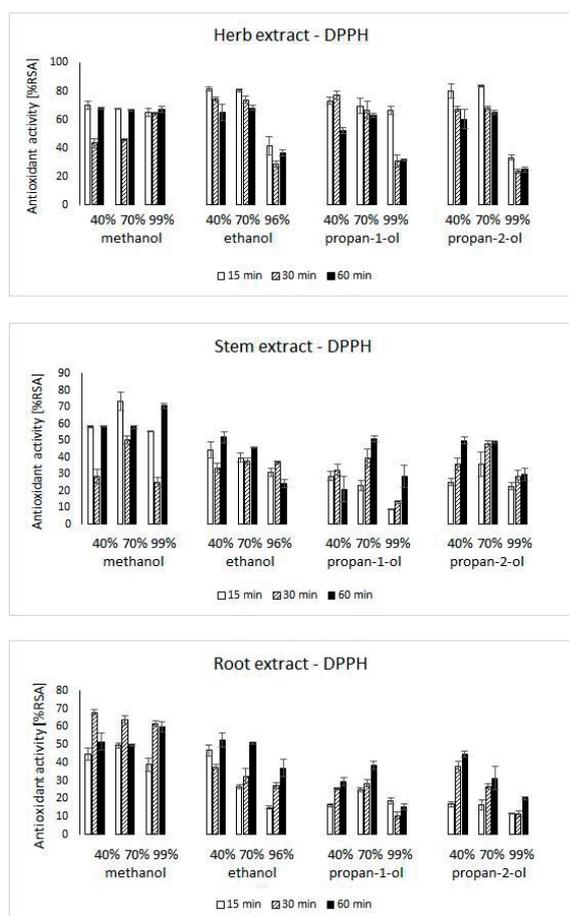
To evaluate the antioxidant activity of the prepared extracts, the DPPH and ABTS methods were applied as described previously [22, 23]. Both methods are based on spectrophotometric measurements. Trolox was used as a standard for both methods. Three samples were prepared from each extract. The antioxidant potential of the samples was expressed as the percentage of radical scavenging activity (%RSA), calculated according to the formulas presented in [23, 24]. Calibration curves for determination of antioxidant activity vs. Trolox concentration with DPPH method were $y = 176.3x + 6.75$, $R = 0.991$, and for ABTS method $y = 41.1x - 0.70$, $R = 0.998$.

Statistical analysis was performed using the Excel program of Microsoft Office 2019. The results are presented as arithmetic mean \pm standard deviation (SD). Linear regressions and correlation coefficients were calculated to establish the calibration curve of antioxidant activity vs. Trolox concentration and to evaluate the correlation between the results obtained with both methods.

RESULTS

The mean (\pm SD) antioxidant activities (%RSA) of *C. intybus* extracts in 4 short-chain alcohols of different concentrations, evaluated by the DPPH method, are presented in Figure 1. The

highest activity of herb extracts was found for extracts prepared in 40% and 70% ethanol and 70% isopropanol after 15 min of ultrasonic-assisted extraction: 81.4 ± 1.5 , 80.6 ± 1.0 , and 83.3 ± 0.6 %RSA, respectively. The highest antioxidant potential of stem extracts, 73.2 ± 5.5 and 70.7 ± 1.4 , was observed for extracts prepared in 70% methanol after 15 min extraction and in concentrated methanol after 60 min extraction, respectively. The highest activity of root extracts was found for extracts in 40%, 70%, and undiluted methanol after 30 min ultrasound assisted extraction: 67.9 ± 1.4 , 63.7 ± 2.6 , and 61.9 ± 1.6 %RSA, respectively.

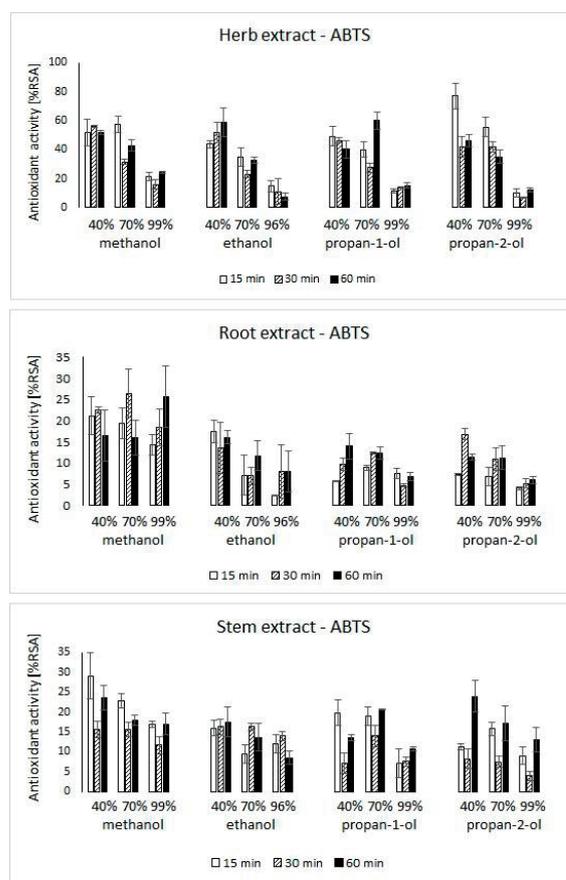


DPPH – 2,2-diphenyl-1-picrylhydrazyl; %RSA – radical scavenging activity

FIGURE 1. Antioxidant activities of *Cichorium intybus* herb, root, and stem extracts in 4 short-chain alcohols of different concentrations evaluated by DPPH method. The vertical lines represent the standard deviation

Figure 2 shows the mean (\pm SD) antioxidant potential expressed as %RSA of herb, root, and stem extracts of cichory in the same solvents evaluated by ABTS method. The highest activity of herbal extracts was found for extract in 40% isopropanol after 15 min extraction and in 70% n-propanol after 60 min extraction: 77.2 ± 9.0 and 60.2 ± 6.0 %RSA, respectively. The highest antioxidant potential of root extracts was observed for extracts in 40%, 70%, and undiluted methanol after 30, 30 and 60 min ultrasound-assisted extraction: 22.5 ± 0.7 , 26.5 ± 5.8 , and 25.7 ± 7.3 , respectively. For stem extracts, the highest activity was found for 15 min extraction in 40% methanol and

for extracts in 40% isopropanol and n-propanol after 60 min extraction: 29.1 ± 5.8 , 24.0 ± 3.9 and 23.7 ± 3.0 %RSA, respectively.



ABTS – 2,2'-azinobis[3-ethyl-2,3-dihydrobenzothiazole-6-sulfonate] diammonium salt; %RSA – radical scavenging activity

FIGURE 2. Antioxidant activities of *Cichorium intybus* herb, root, and stem extracts in 4 short-chain alcohols of different concentrations evaluated by ABTS method. The vertical lines represent the standard deviation

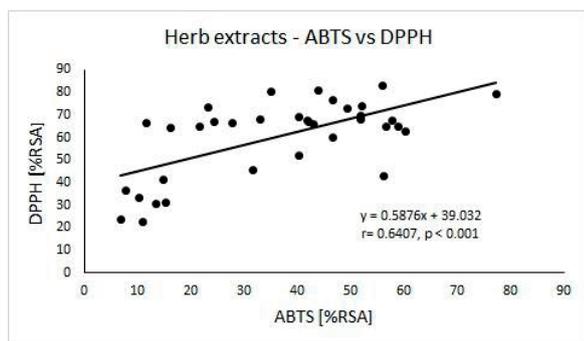
Correlation between antioxidant activities determined by DPPH and ABTS methods in herb, root and stem extracts are shown in Figures 3, 4, 5. A high, statistically significant correlation between the results obtained with these methods for extracts of all studied parts of *C. intybus* was found.

DISCUSSION

Cosmetology has always used the benefits of plants for face and body care. Plant active ingredients are widely used in cosmetics. They have moisturizing, drying, firming, astringent, toning, cleansing, regenerating, peeling, or nourishing effects on the skin. The various properties of herbs have been known for centuries. All the collected information was passed on to the following generations. It should be added that plant extracts were the source of many elixirs [16, 17].

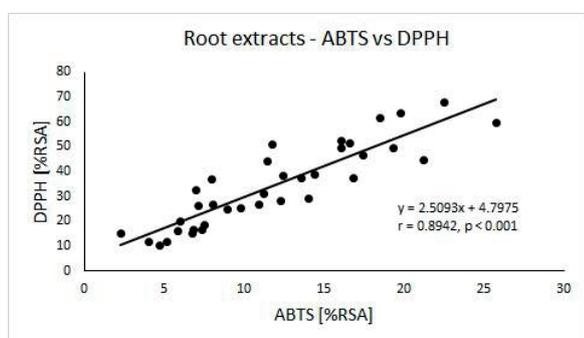
Nowadays, there are many new ways to produce cosmetic products. We can get practically everything in synthetic form. Nevertheless, people want to be closer to nature. We are increasingly

inclined towards natural plant ingredients. The most sought-after products are cosmetics that delay the aging process [16, 18].



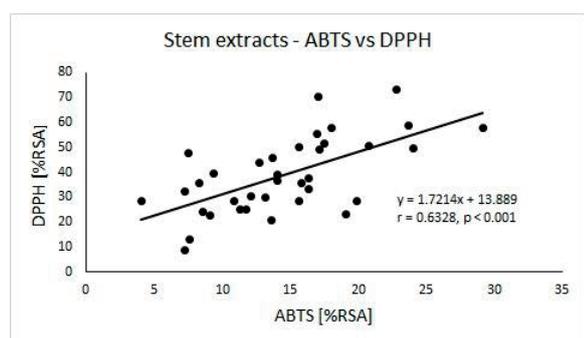
DPPH – 2,2-diphenyl-1-picrylhydrazyl; ABTS – 2,2'-azinobis[3-ethyl-2,3-dihydro-benzothiazole-6-sulfonate] diammonium salt; %RSA – radical scavenging activity

FIGURE 3. Relationship between the antioxidant activity of *Cichorium intybus* herb extracts determined by DPPH and ABTS methods



DPPH – 2,2-diphenyl-1-picrylhydrazyl; ABTS – 2,2'-azinobis[3-ethyl-2,3-dihydro-benzothiazole-6-sulfonate] diammonium salt; %RSA – radical scavenging activity

FIGURE 4. Relationship between the antioxidant activity of *Cichorium intybus* root extracts determined by DPPH and ABTS methods



DPPH – 2,2-diphenyl-1-picrylhydrazyl; ABTS – 2,2'-azinobis[3-ethyl-2,3-dihydro-benzothiazole-6-sulfonate] diammonium salt; %RSA – radical scavenging activity

FIGURE 5. Relationship between the antioxidant activity of *Cichorium intybus* stem extracts determined by DPPH and ABTS methods

Skin aging is a multifactorial process that is not yet fully understood. One of the most important external factors influencing skin aging is free radicals. They have a negative effect on the skin structure, sometimes leading to the induction of lesions [19, 20]. Antioxidants are compounds that neutralize

free radicals. Plants are a natural source of antioxidants, often with strong antioxidant activity [25]. In addition, products made from natural compounds frequently have greater activity than those made from synthetic substances and are safer [26, 27, 28].

An example of a plant rich in substances with antioxidant properties is chicory, also known as *C. intybus* L. Chicory has been used in folk medicine since ancient times. It is cultivated or grows wild throughout the world and is used as a diuretic, anti-inflammatory, and digestive aid. It contains many compounds with therapeutic properties. The most important of these are polyphenolic compounds such as flavonoids, very active in neutralizing free radicals [21, 28, 29].

In the present study, extracts were prepared in 4 short-chain alcohols of different concentrations using ultrasonic-assisted extraction for 15, 30 or 60 min. To evaluate the antioxidant activity of extracts from different parts of *C. intybus* L., 2 methods, i.e. DPPH and ABTS, were used. In the DPPH test, the highest antioxidant potential was observed mainly for methanol extracts (Fig. 1). The highest values were recorded for extracts from dried herbs, regardless of the extraction time or type of solvent used. The extract from the dried stem in 99% (v/v) isopropanol at an extraction time of 15 min had the lowest ability to neutralize free radicals. Among the extracts from various parts of *C. intybus* L. tested using the ABTS method, the highest antioxidant activity was observed for the extract from dried herb prepared in 40% (v/v) isopropanol at 15 min extraction. A significant correlation was found between the antioxidant activity of each chicory part tested using the DPPH and ABTS methods (Fig. 3, 4, 5), but the most consistent results were recorded for the root of *C. intybus* L. (Fig. 4).

Street et al. presented the results of the studies to evaluate the antioxidant potential of *Cichorium intybus* L. Two chicory varieties were tested: *C. intybus* var. *Silvestre* and *C. intybus* var. *Foliosum*. The antioxidant activity was expressed as protective activity against lipid peroxidation. Calculations were made to check the percentage reduction of hydroperoxide degradation products. The results presented suggest that chicory juices have a strong antiradical effect [30]. The antiradical potential of red chicory was also investigated using the DPPH radical and 3 reactions catalyzed by specific enzymatic sources of ROS. On a molar basis, the phenols of *C. intybus* var. *Silvestre* were as active as the reference compound Trolox in scavenging the synthetic DPPH radical. In another study, *C. intybus* was also found to exhibit hydrogen peroxide inhibition and ferrous ion chelation in addition to DPPH %RSA [30].

Jancic et al. determined biologically active compounds in *C. intybus* L. leaf samples from different locations in Montenegro, 7 from the natural state while 2 were grown in a greenhouse. The antioxidant activity was determined using 3 methods: DPPH, FRAP, and ABTS. Fresh raw materials were used for the study. The total antioxidant activity of the analyzed samples was $8.28 \pm 0.17 \mu\text{M}$ Trolox/g fresh sample for wild plants in the DPPH method and $18.38 \pm 0.12 \mu\text{M}$ Trolox/g in the ABTS method. In contrast, the activity of cultivated plants was almost twice as low. The total polyphenol content was also more than twice lower in the cultivated samples than in the wild chicory samples [31].

Janda et al. investigated *C. intybus* L. for its health-promoting properties. It was shown that all morphological parts of the plant

contain a large number of chemical compounds. The roots contain mainly sap, sesquiterpene lactones and guaianolides. They also contain phenolic compounds, vitamins, and sugars. The aerial parts are a rich source of essential oils containing valuable compounds such as: carvacrol, thymol, cinnamaldehyde, and camphor. *Cichorium intybus* L. has been shown to have antiviral, antibacterial and antifungal, antiprotozoal, hepatoprotective, antidiabetic, analgesic, anticancer, and anti-inflammatory properties. Most studies indicated that the beneficial effects of the extracts were related to their high antioxidant activity [13].

Li et al. investigated the effect of drying methods on the phenolic profile and antioxidant activity of *C. intybus* L. leaves. They found that different drying methods had significant effects on the total phenolic content of the leaves. The best results were obtained from leaves dried in hot air and the worst results were obtained from leaves dried in the shade. It can be concluded that if the herb, root, and stem were dried with warm air, their antioxidant activity would increase significantly [32].

In conclusion, the study conducted and a review of the literature may indicate that *C. intybus*, due to the antioxidant properties of extracts from different parts of the plant, can be an ingredient in cosmetic preparations with anti-aging effects. It would be advisable to consider extending the research with new extraction solvents and other process parameters in order to find even more active extracts.

CONCLUSIONS

1. Alcoholic extracts of selected parts of *C. intybus* L. show antioxidant properties as determined by DPPH and ABTS methods.
2. The antioxidant activity of the obtained extracts depends on the part of the plant used. The highest activity was found for extracts from the dried herb.
3. Extraction conditions, i.e. duration, choice of solvent, affect the antioxidant potential of the obtained extracts.

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