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RESEARCH ARTICLE

The Effectiveness of Ultrasound-Assisted Extraction on Antioxidative Properties of Bract Leaves of Globe Artichoke

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ABSTRACT

Objective: The antioxidant-rich artichoke bracts leaves are a particularly waste of the food industry. Thus, it would be possible to utilize a cheap and natural material, which is industrial waste, instead of synthetic antioxidants. The present study aimed to extract from the bract leaves of globe artichoke by ultrasound-assisted extraction and to evaluate their antioxidant activities.

Materials and Methods: In this study, the effect of ultrasound-assisted extraction (UAE) on antioxidative properties was studied. The extracts were obtained from the leaves of the head part of the artichoke by using UAE and evaluated for their antioxidative properties. For this purpose, antioxidant activity methods were investigated for different extraction times. The results obtained were compared with standard antioxidants.

Results: The results obtained from this study showed that the shorter extraction time resulted in higher antioxidative properties. Accordingly, in plant extracts prepared by UAE-1, the highest total phenolic content value (193.80 µg pyrocathecol equivalent/mg extract), the highest total flavonoid content value (254.13 µg catechin equivalent/mg extract), the highest total chlorophyll content value (10.68 µg/mL) and carotenoid (0.57 µg/mL) were found. Similarly, UAE-1 extracts showed the best results in terms of free radical scavenging activity. Also, the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of UAE-1 (89.09%) was determined to be higher than the standard antioxidant α-Tocopherol (85.68%) and very close to another standard antioxidant butylated hydroxyanisole (91.98%).

Conclusion: UAE could be preferred instead of the traditional method (Soxhlet) as a shorter, eco-friendly, and low energy cost.

Keywords: Antioxidative properties; Ultrasound-assisted extraction; Plant extract; *Cynara scolymus* L.; Radical scavenging

INTRODUCTION

The increasing interest in the replacement of synthetic antioxidants with natural ones has opened the door to much research, particularly to the study plant sources and new antioxidants contained in these sources.[1,2]

Plant extracts are a generous source of bioactive compounds with medical features.[3] Reactive oxygen species are produced continuously during special metabolic events in the organism, especially various sources such as lipid peroxidation and mitochondrial cytochrome oxidase, or the result of exogenous sources including ultraviolet light, environmental toxins, and anticancer drugs.[4]

It is known that antioxidants have the feature of delaying or preventing bitter and other taste deterioration caused by oxidation when used in foods other than protecting the cell with its defense mechanism.[5] Flavonoids, polyphenolics, tocopherols, and ascorbic acid are the most important natural antioxidant groups. It is known that phenolic compounds have high antioxidant activity and their most important sources are found in plants.

In particular, the extraction of phenolic compounds from agricultural and industrial organic wastes has been one of the most important issues that many researchers are interested in. This is because extraction is the main step in isolating and using biocomponents.

The artichoke (*Cynara scolymus* L.), which grows in Southern Europe and the Mediterranean region and has wild forms in the countries in this basin, is a 50-150 cm tall herbaceous plant that blooms blue-purple flowers of the daisy-family. Artichoke contains some phenolic compounds.[6] The fact that artichoke is nutritious and beneficial to health is due to certain chemi-

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cal compositions, including these high levels of polyphenolic compounds and inulin. Previous studies exhibited that the extracts from artichoke have antioxidant[7], antiseptic[8], antibiotic,[9] and anticarcinogenic[10] effects. The antioxidant-rich artichoke bracts leaves are a particularly waste of the food industry. Thus, it would be possible to utilize a cheap and natural material, which is industrial waste, instead of synthetic antioxidants.

Soxhlet extraction, known as the traditional method, has been frequently used to obtain plant extracts containing bioactive compounds. However, situations such as taking a long time and requiring large amounts of toxic and expensive solvents limit the usability of this method. Environmentally friendly and effective extraction techniques are required in order to use bioactive plant extracts in food technology, pharmaceutical, and cosmetic formulations. In recent years, sustainable new extraction techniques have reduced extraction time, reduced solvent consumption, and improved the quality of extracts obtained. However, it has been observed in the literature that studies for extraction of phenolic compounds from various parts of the artichoke are carried out by traditional methods (Soxhlet or maceration).[6] Ultrasound-assisted extraction (UAE) is an important technique used in the pharmaceutical and food industry.[11] Ultrasonic energy produces many tiny bubbles in the liquid medium and causes the particles to break off by causing the solids to mechanically. The sound waves usually provide effective contact between the solid and solvent resulting in good recovery of the analyte. Ultrasonic application mechanically breaks down cell walls. With the mechanical destruction of the cell wall, intracellular components easily exit the cell and pass into the solvent.[12] Long sonication time may cause degradation of the compounds for isolation. Therefore, the processing time of UAE necessarily requires optimization.

The present study aimed to extract from the bract leaves of globe artichoke by UAE during different extraction times and to evaluate their antioxidant activities. For these purposes, different antioxidative properties were studied by optimizing the extraction time.

MATERIALS AND METHODS

Materials

The bract leaves of the artichoke (*Cynara scolymus* L.) plant used as research material in this study were supplied from the Istanbul local market in April 2018 and were thoroughly cleaned with distilled water and dried at room temperature for approximately 7 days in the dark. The leaves were cut into small pieces before extraction. All material was kept in the refrigerator at +4 °C until used.

Reagents and Solvents

All chemicals used in this study are of high-performance liquid chromatography purity and have been obtained from Merck, Sigma Aldrich, Fluka, and Riedel-de Haen companies.

Extraction Procedures: Ultrasound-Assisted Extraction

For UAE, 2.5 g of plant samples were taken into the tared glass beakers and completed with 25 mL of solvent (96% ethanol). Extraction was performed in the ultrasonic bath (Bandelin Electronic 320 w 35 kHz) for different extraction times such as 1, 5, 15, or 30 min, respectively. After this process, the extracts were centrifuged (10,000 rpm for 15 min) (Sigma 3K30). After extraction, the solvent was removed using a fume hood and extracts were obtained. The extracts obtained were kept at +4 °C in the refrigerator.

Determination of Antioxidative Properties

Total phenolic compound content was determined using the method of Slinkard-Singleton.[13] Total flavonoid content was determined by using a colorimetric method according to Zhishen et al.[14] Proline analysis was performed according to the simple modification of the method developed by Bates.[15] Anthocyanin content in dried leaves, has been determined by the modification of the method developed by Padmavati et al.[16] For determination of total chlorophyll and total carotenoid content, used method of Arnon.[17] β-Carotene bleaching method analysis was carried out according to the method developed by Bruni et al.[18] Ferric reducing test was performed according to the method of Oyaizu.[19] Metal chelating activity was determined according to the method of Decker and Welch.[20] 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability of the extracts was determined using the method of Brand-Williams et al.[21] 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radical scavenging ability of the extracts was determined using the method of the Arnao et al.[22] N, N-dimethyl-p-phenylenediamine (DMPD) cation radical scavenging ability was determined according to the method of Fogliano et al.[23] The method of Osawa and Namiki was studied for the measurement of total antioxidant capacity.[24]

RESULTS

Amount of Total Phenolic Compounds

The total phenolic content of the extracts prepared by subjecting the bract leaves of artichokes to different periods in an ultrasonic bath was determined. The results are shown in Table 1. Results are given as µg pyrocatechol equivalent of the phenolic compounds for each mg extract. The highest total phenolic con-

tent value was found in UAE-1 extract (193.80 µg pyrocatechol equivalent/mg extract).

Total Flavonoid Content

The total flavonoid content of the extracts prepared by subjecting the bract leaves of artichokes to different periods in an ultrasonic bath and using ethanol was given in Table 1. The highest flavonoid amount value was found in UAE-1 extract (254.13 µg catechin equivalent/mg extract). Total phenolic compound and flavonoid content results also decreased as the extraction time increased. As a result of the study, the highest antioxidant activity is observed in the extract which was applied for 1 min UAE. Therefore, as a result of the study, it can be concluded that antioxidant activity decreases as long-term ultrasound-assisted extraction can lead to the loss of these phytocomponents which have an antioxidant effect.

Proline Content

The proline content of plants is an indicator of stimulation of the pentose phosphate pathway. The pentose phosphate pathway is controlled by the synthesis of cytosolic proline. High proline content in plants is responsible for high phenolic compounds. For these reasons, proline analysis for edible plants is regarded as an indicator of their antioxidative properties. The total proline contents of the samples obtained from the artichoke leaves are given in Table 1. Among the proline contents of different extracts, the highest amount was found in UAE-30 extract (0.83 µg proline/mg extract). Research on the proline content of plants or food is a newly arising area therefore there is not much data available to compare the results of this study.

Anthocyanin Content

Anthocyanins are dark-coloured pigments extracted from plants. The results obtained from our study, the anthocyanin value of the leaves was 0.065 µmol/g.

Total Chlorophyll and Carotenoid Content

The total chlorophyll and carotenoid contents of the extracts obtained from the artichoke leaves are given in Table 2. The chlorophyll and carotene contents of artichoke leaf extract decreased when the extraction time increased. This situation could be an indication that long-term ultrasound exposure is damaging to these compounds. It was observed that the UAE-1 extract of the plant had the highest total carotenoid content (0.567 µg/mL), and the highest total chlorophyll content (10.68µg/mL).

β-Carotene Bleaching Test Results

The results obtained in the β-carotene bleaching method are given in Table 3. According to the results obtained in this method; two ethanol extracts UAE-15 (1.03), UAE-30 (1.04) of the artichoke leaves were higher than the positive control butylated hydroxyanisole (BHA). It was determined that UAE-1 (0.92) and UAE-5 (0.99) extracts also showed an effect close to BHA.

Ferric Reducing Test Results

In this section, in order to investigate the reduction capacity of the extracts of the bract leaves of artichoke in different concentrations prepared with ethanol to reduce Fe^{3+} to Fe^{2+} added to the tubes, the reducing power was tested by comparing it with standard antioxidants (BHA and α-Tocopherol). Results are shown in Figure 1. The increase in absorbance values is directly proportional to the amount of Fe^{2+} in the reaction medium. In the reducing power assay, the highest reducing power was exhibited by UAE-15 Ethanol extract (0.692 at 700 nm).

Chelating Activity on Ferrous Ion

Metal chelating activities based on inhibition of Ferrozin-Fe^{2+} complex formation in samples and standards are shown in Figure 2. UAE-1 (46.48%) showed the highest metal chelating activity at high concentrations (200 µg/mL). The metal chelating power increased as the extraction time decreased in the extracts created by ultrasound-assisted extraction. On the other hand, increasing the concentration increased the metal chelating power. When comparing extracts with ethylenediaminetetraacetic acid (EDTA), which is known to be a strong chelating agent, it has been observed that EDTA gives higher results compared to all extracts. In addition, according to results the amount of flavonoids and metal chelating activity decreases when the time of ultrasound-assisted extraction increases. Flavonoids, which are from the phenolic family, show strong metal-chelating properties compared to other phenolic compounds. Therefore, the decreasing total flavonoid content also support the results of the metal chelating activity.

DPPH Scavenging Ability

For the determination of DPPH radical scavenging activity in *Cynara scolymus* L. extracts, the extracts were prepared using UAE. The results obtained from samples and standards are shown in Figure 3. UAE-1 extract prepared by the one-min ultrasound-assisted method showed the highest results. According to the results, UAE-1 extract is very close to the standard antioxidant BHA (91.98%) with high antioxidant activity,

Table 1. Total flavonoids, phenolics and, proline amount of artichoke bracts for different extraction times.

Extract	Total Phenolic Content (µg pyrocatechol equivalent/mg extract)	Total Flavonoid Content (µg catechin equivalent/mg extract)	Proline Content (µg proline/mg extract)
UAE-1	193.80	254.13	0.43
UAE-5	188.95	223.30	0.60
UAE-15	182.38	214.97	0.82
UAE-30	182.14	211.08	0.83

UAE-1:Ultrasound-assisted extraction-1 min; UAE-5:Ultrasound-assisted extraction-5 min; UAE-15:Ultrasound-assisted extraction-15 min; UAE-30:Ultrasound-assisted extraction-30 min.

Table 2. Total chlorophyll and carotenoid amounts of bract leaves of artichokes (*Cynara scolymus* L.) for different extraction times.

Extract	Chlorophyll a (µg/mL)	Chlorophyll b (µg/mL)	Total Chlorophyll (µg/mL)	Total Carotenoid (µg/mL)
UAE-1	4.63	6.08	10.68	0.57
UAE-5	3.48	5.88	9.36	0.45
UAE-15	2.19	4.35	5.62	0.38
UAE-30	2.13	4.18	5.55	0.32

UAE-1:Ultrasound-assisted extraction-1 min; UAE-5:Ultrasound-assisted extraction-5 min; UAE-15:Ultrasound-assisted extraction-15 min; UAE-30:Ultrasound-assisted extraction-30 min.

Table 3. β–Carotene bleaching effects of bract leaves of artichoke (*Cynara scolymus* L.) for different extraction times.

Extract	RAA (60 min.)*	RAA (120 min.)*
UAE-1	0.92	0.84
UAE-5	0.99	0.89
UAE-15	1.03	0.96
UAE-30	1.04	0.97
BHA (Positive Control)	1	1
Negative Control (Linoleic Acid Emulsion)	0.14	0.13

UAE-1:Ultrasound-assisted extraction-1 min; UAE-5:Ultrasound-assisted extraction-5 min; UAE-15:Ultrasound-assisted extraction-15 min; UAE-30:Ultrasound-assisted extraction-30 min; RAA*: Relative Antioxidant Activity

while another high antioxidant activity standard, α-Tocopherol (85.68%), has an equally strong antioxidant activity.

ABTS Radical Scavenging Activity

The results of ABTS radical scavenging activities are shown in Figure 4. The ethanol extract UAE-5 (75.04%), which was extracted for 5 min in the studied extracts at a concentration of 200 µg/mL, exhibited the highest ABTS radical scavenging activity. Inhibition values increased in all extracts depending on the concentration. Trolox, a standard with a known antioxidant effect, showed an activity of 99.5% at all concentrations studied.

$DMPD^{+}$ Scavenging Ability

DMPD radical scavenging activities of all samples and standards are shown in Figure 5. At high concentrations (200 µg/mL), in the extracts using UAE 1 minute (UAE-1) has the highest inhibition values (72.02%). DMPD radical scavenging activity increased as the extraction time was shortened. When standard antioxidants were examined, the DMPD radical scavenging activity of ascorbic acid was found to be 98% (200 µg/mL). The synthetic standard antioxidant BHA showed an activity of 65.13% (200 µg/mL). Accordingly, when compared with standard antioxidants, UAE-1 (72.02%) showed higher activity than BHA.

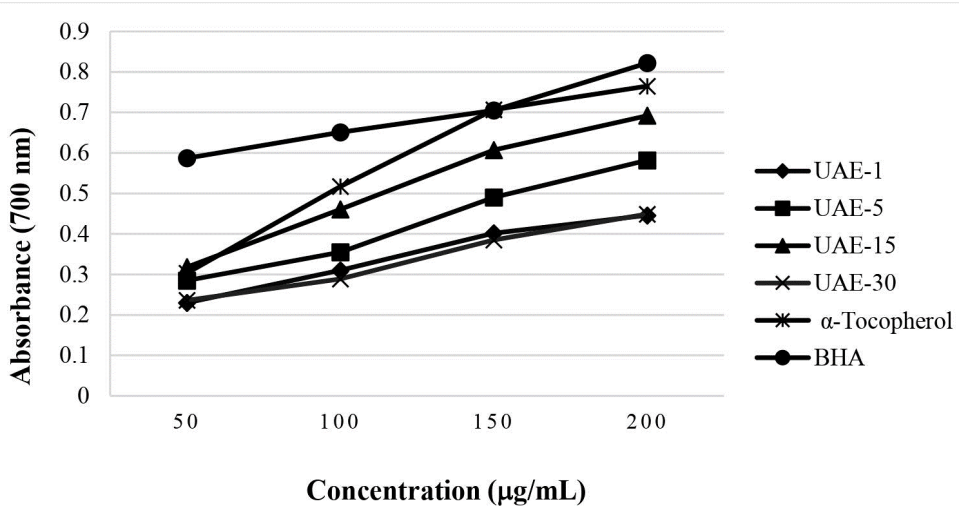

Figure 1. Reducing power of ethanol extracts obtained by UAE methods (1, 5, 15, and 30 min). UAE-1:Ultrasound assisted extraction-1 min; UAE-5:Ultrasound assisted extraction-5 min; UAE-15:Ultrasound assisted extraction-15 min; UAE-30:Ultrasound assisted extraction-30 min; BHA: Butylated hydroxyanisol.

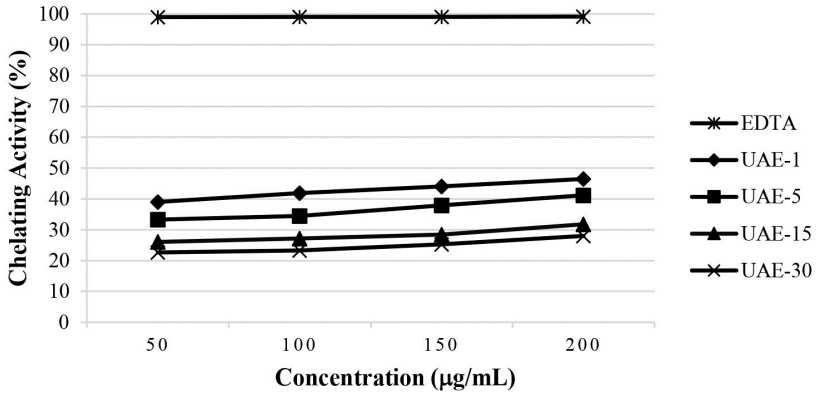

Figure 2. Chelating activities of ethanol extracts obtained by UAE methods (1, 5, 15, and 30 min). UAE-1:Ultrasound assisted extraction-1 min; UAE-5:Ultrasound assisted extraction-5 min; UAE-15:Ultrasound assisted extraction-15 min; UAE-30:Ultrasound assisted extraction-30 min; EDTA: Ethylenediaminetetraacetic acid

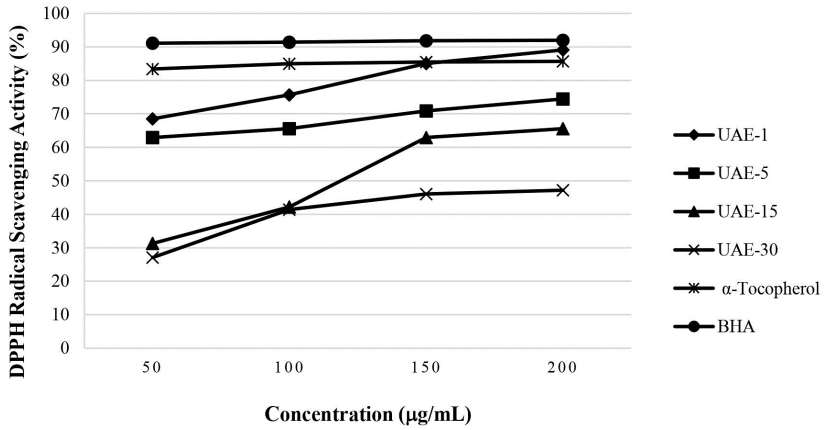

Figure 3. DPPH radical scavenging activities of ethanol extracts obtained by UAE methods (1, 5, 15, and 30 min). UAE-1:Ultrasound assisted extraction-1 min; UAE-5:Ultrasound assisted extraction-5 min; UAE-15:Ultrasound assisted extraction-15 min; UAE-30:Ultrasound assisted extraction-30 min; BHA: Butylated hydroxyanisol.

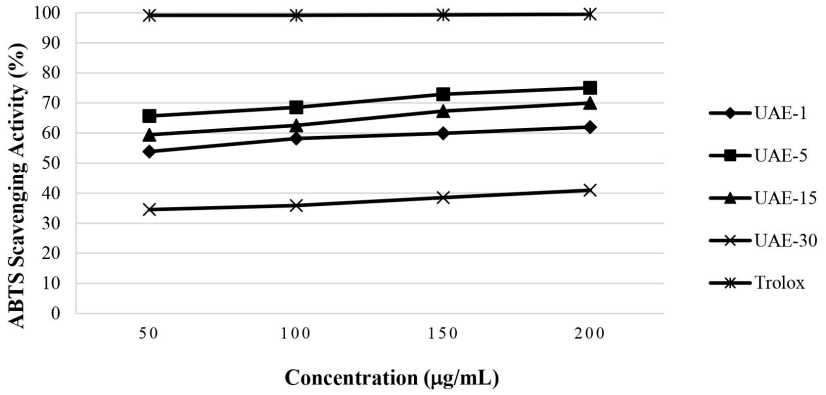

Figure 4. ABTS radical scavenging activities of ethanol extracts obtained by UAE methods (1, 5, 15, and 30 min). UAE-1:Ultrasound assisted extraction-1 min; UAE-5:Ultrasound assisted extraction-5 min; UAE-15:Ultrasound assisted extraction-15 min; UAE-30:Ultrasound assisted extraction-30 min.

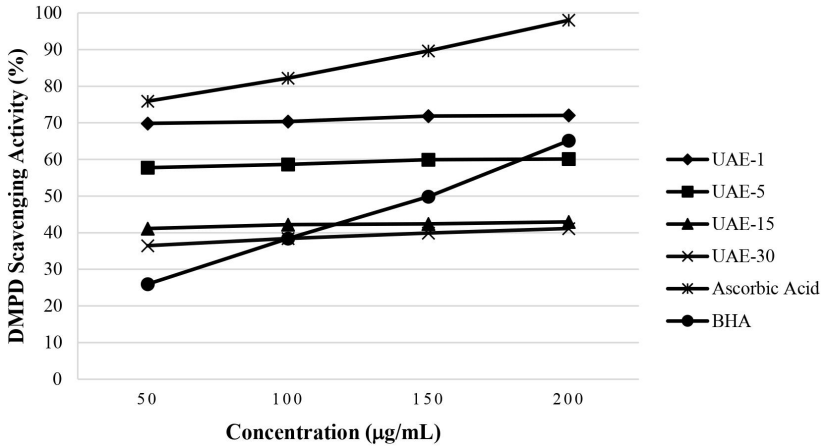

Figure 5. DMPD radical scavenging activities of ethanol extracts obtained by UAE methods (1, 5, 15, and 30 min). UAE-1:Ultrasound assisted extraction-1 min; UAE-5:Ultrasound assisted extraction-5 min; UAE-15:Ultrasound assisted extraction-15 min; UAE-30:Ultrasound assisted extraction-30 min; BHA: Butylated hydroxyanisol.

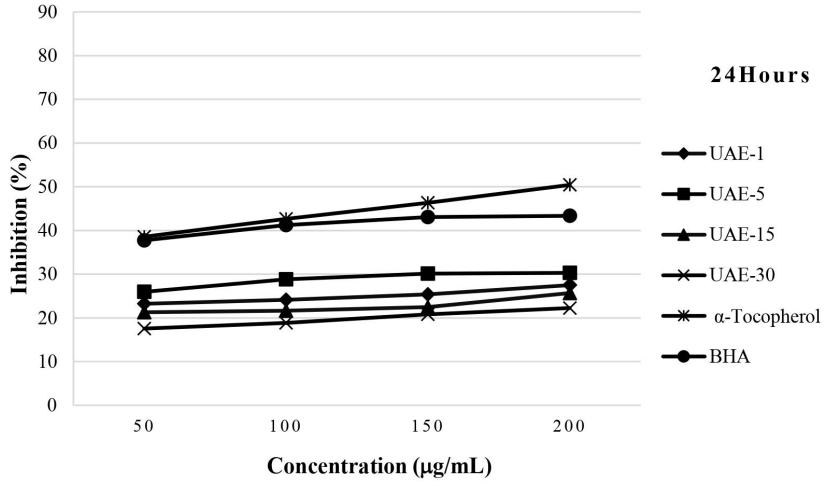

Figure 6. Antioxidative effect of ethanolic samples obtained by UAE (1, 5, 15, and 30 min) at the end of the first 24 h. UAE-1:Ultrasound assisted extraction-1 min; UAE-5:Ultrasound assisted extraction-5 min; UAE-15:Ultrasound assisted extraction-15 min; UAE-30:Ultrasound assisted extraction-30 min; BHA: Butylated hydroxyanisol.

Total Antioxidant Capacity

The total antioxidant activity was determined according to the thiocyanate method with slight modifications. Total antioxidant activity was determined by taking measurements at 24-hour intervals for 3 days. The effects of extracts and standards on linoleic acid peroxidation are shown in Figure 6. It was observed that UAE-5 (30.3%) extract had the highest activity at the end of the first 24 h. The antioxidant activity of this extract is close to the activity of standard antioxidants BHA (43.35%) and α-Tocopherol (50.42%). At the end of 48 h, the total antioxidant activity of the extracts started to decrease due to deterioration in the structure of the extracts over time. After 72 h, ultrasound-assisted extracts and standard antioxidants were no longer active.

DISCUSSION

The antioxidative properties of bract leaves of the artichoke plant (*Cynara scolymus* L.) were investigated using UAE in different antioxidant parameters. According to our results obtained from the study, extracts obtained from the leaves of the artichoke (*Cynara scolymus* L.) bract showed a high antioxidative effect. From these effects, the ethanol extract exhibited high reducing power, noteworthy phenolic content, and DPPH scavenging capability at the same conditions.

The total amount of phenolic compounds was found to be 193.80 µg pyrocatechol equivalent per mg of ethanol extract after one minute of extraction time. There are no studies conducted with bract leaves of artichoke. However, there are studies on the leaves of the artichoke plant. For example, Ben Salem et al.[25] extracted the leaves by maceration method using different increasing solvent polarities (such as hexane, butanol, ethyl acetate, 75% EtOH/H_2O, and aqueous. They found the maximum content of the total phenolic compound by using ethanol extracts. Their result corresponded to 54.54 mg gallic acid equivalent per g dry weight of extract. Our results are higher than the work of Ben Salem et al.[25] Kollia et al.[26] found the phenolic content of artichoke leaves as 1.45 mg GAE/g DW by UAE. All these results comply with our studies in terms of proving the presence of phenolic content in bract leaves of artichoke.

Stumpf et al.[27] prepared extracts from artichoke leaves by using three different extraction methods. The extracts were compared with their total phenolic compounds and total antioxidant capacities. Extraction according to the European Pharmacopoeia or UAE gave similar results when compared. However, the results obtained with hot water extraction are quite inadequate. This study shows us the importance of the UAE-method. Stumpf et al.[27] prepared artichoke leaf extract with 80% methanol by using UAE. All extracts were directly used for the determination of total phenolic concentration and antioxidant capacity. This study was showed that total phenolic content and total antioxidant capacity are closely related to the extraction method.

Reche et al.[28] performed mathematical modeling of UAE and they studied the kinetics of bioactive compounds obtained from artichoke by-products. In this study, an evaluation of an artichoke by-product rich in bioactive compounds by UAE and ethanol solvent was proposed. The effective diffusion coefficient exhibited temperature dependence, whereas the external mass transfer coefficient and the equilibrium extraction yield depended on both temperature and ultrasound power density. This study also supports our study.

Lavecchia et al.[29] extracted the artichoke residues they prepared using ethanol as a solvent in a thermostated bath at 60°C and determined the phenolic compounds in the stems and bracts by obtaining 51.10±0.74 and 24.58±0.57 mg gallic acid equivalent per g extracts, respectively. These values are very low when compared to our study. Ultrasound-assisted technology is known to act on plant tissues by the cavitational phenomenon induced at the solid/liquid interface. This effect facilitates the release of extractable compounds and enhances mass transport by disrupting the plant cell walls. Pasqualone et al.[30] analyzed the extracts obtained from three artichoke varieties (Opal, Capriccio, and Catanese) in terms of antioxidant parameters; in the UAE of samples, the extract showed a total phenolic content higher than non-ultrasound extraction method. The results were expressed as mg gallic acid equivalent per kg. Zuorro et al.[31] reported total polyphenol content in bracts (24.14 mg GAE/g) using a 50:50 ethanol-water mixture as an efficient extraction method, and solvent-extraction procedure. In our study, UAE-1 extract also exhibited the highest flavonoid amount value with 254.13 µg catechin equivalent per mg of extract. This result was very high when compared the other similar studies. Ben Salem et al.[25] found that ethanol extract had the highest value in terms of total flavonoid content (12±0.83 CE/g DW) by using the maceration method. These studies also determined the flavonoid content of the artichoke leaves, like our study. Petropoulos et al.[3] found very low flavonoid content in the edible head of artichoke. They studied eight different genotypes of artichoke heads and determined the highest value as 7.2 mg/g extract. The chlorophyll and carotenoid contents of our studied samples were also high. In order to make a comparison of chlorophyll and carotenoid contents, no studies were found in the literature with artichoke bracte leaves. Ben Salem et al.[25] measured that the β-carotene bleaching effect of ethanol extract (70.74%) is the highest inhibition rate and higher than butylated hydroxytoluene (BHT) (47.94%).

Flavonoids are a main class of polyphenols in plants. They are known as antioxidants and free radical scavengers. The antioxidant activity of plants has been correlated to the total flavonoid content. For this reason, in our study, especially free or cation radical scavenging capacity was found to be very high. DPPH radical scavenging activity of UAE-1 extract showed similar

activities to the standard antioxidants such as BHA and α-tocopherol, at all concentrations tested. DMPD cation radical scavenging ability of the extract was determined higher than BHA in all the concentrations studied. These results are very pleasing. In addition, in extraction time optimization studies, it is seen that an effective extraction takes place in a very short time such as one minute. Ergezer et al.[32] prepared artichoke bracte leaf extract with 80% ethanol by using the maceration method. They obtained the DPPH radical scavenging activity as 79.91 % after the 7th day of storage. The result we found in our study is that the DPPH radical scavenging activity is 89.09 % for the extract concentration of 200 µg/mL. This result is higher than α-tocopherol (85.68%). Mena García et al.[33] reported lower results of DPPH (26.59 ±0.62 mg TE/g) using a mixture of ethanol/water (50:50 v/v) and a microwave-assisted extraction method from the artichoke. Shallan et al.[34] showed that the antioxidant activity (DPPH) of Globe artichoke bracts extract in ethanolic solution was 6.42 mg/L. Quispe et al.,[35] determined the total phenolic compounds of artichoke bracts between 10.86 mg and 24.82 mg GAE/g in artichoke extracts prepared using ultrasound-assisted extraction and ethanol as solvent. The radical scavenging activity of DPPH was found between 15.49 mM and 38.65 mM trolox, and the trolox equivalent antioxidant capacity from 12.56 to 32.52 mM trolox, respectively.

To investigate the reducing capacity of Fe^{3+} to Fe^{2+}, the reducing power was tested by comparing the extracts with the standards. The increase in absorbance values is directly proportional to the amount of Fe^{2+} in the environment. The best activity among the prepared extracts was UAE-15 (0.692 at 700 nm). The good results obtained from ethanol extracts may suggest that the preparation of artichoke extracts in a moderately apolar solvent such as ethanol will have a more active effect in terms of reducing power than solvents with high polarity or high apolarity. In terms of ultrasound-assisted extraction, it can be argued that the 1-minute and 5-minute extraction times are not fully sufficient to reduce Fe^{3+}, while the 15-minute extraction period provides the necessary optimization for this reduction event. It can be said that in longer extractions such as 30 min, the extract may be damaged by sound waves and cause it to lose its reducing power feature. Ben Salem et al.[25] examined the reducing power parameter in artichoke leaf extracts in their study. For the reducing power test of *Cynara scolymus* L., they measured the absorbance at 515 nm in a UV-VIS spectrophotometer of the ferric reducing antioxidant power mixture prepared with extracts. expressed the antioxidant capacity of artichokes as trolox Equivalent. Among all extracts of artichoke in their work, the ethanol extract demonstrated a favorable iron-reducing capacity (527.79 µmol Fe^{2+}/mg dry extract). Thang et al.[36] studied and compared different extraction techniques to extract cynarine and chlorogenic acid (classical extraction, ultrasound-assisted extraction, enzyme-assisted extraction) from leaves of *Cynara scolymus* L. In addition, the extracts were also studied for antioxidant activities. The antioxidant activity of the artichoke extract was tested by the ferric-reducing antioxidant power method. They found the highest reducing power of ferric iron in pectinase enzyme extracts from artichoke leaves. The measurement of reducing power of ascorbic acid and artichoke extract from dried artichokes treated with pectinase enzymes was exhibited as 48.0 and 77.8 mg/L, respectively. Artichoke extract hydrolyzed by pectinase enzymes also had a higher radical scavenging capacity (IC_{50}=30 mg/L) compared to ascorbic acid (IC_{50}=11 mg/L). Wioletta Biel et al.[37] reported that the antioxidant capacity of artichoke extract was measured at 1060.8 µmol trolox/1 g dry matter.

UAE-1 (46.48%) showed the highest metal chelating activity at high concentrations (200 µg/mL). It was observed that the percentages of metal chelating activity increased as the extraction time was shortened in the extracts applied ultrasound-assisted extraction. Flavonoids from the phenolic family show strong metal chelating properties compared to other phenolic compounds. Therefore, the decrease in the total flavonoid content due to the increase in the extraction time also supports the results of the metal chelating activity. Among the analyzed extracts, the ethanol extract UAE-5 (75.04%), which was extracted for 5 min at a concentration of 200 µg/mL, exhibited the highest ABTS radical scavenging activity. It can be said that the ultrasonic extraction time, which gives the optimum value and can be considered ideal is 5 min in terms of the high value of ABTS radical scavenging activity of artichoke extracts prepared with ethanol. Kollia et al.[26] found ABTS radical scavenging activity in artichoke leaves as 1.25 mg TE/g DW by UAE in their study with artichoke leaves. Ben Salem et al.[25] also analyzed the scavenging activity of the artichoke extract and found that ethanol extract had a high activity. Sihem et al.[1] also demonstrated that the extracts of the head leaves obtained by using the maceration method had the highest activity. It was observed that UAE-5 (30.3%) extract had the highest total antioxidant activity at the end of the first 24 h. The antioxidant activity of this extract is close to the activity of standard antioxidants BHA (43.35%) and α-tocopherol (50.42%).

It is a very important detail that a waste material can be used instead of synthetic antioxidants in the food, pharmaceutical, or cosmetic industry. It is known that the bract leaves of the artichoke are a waste in local markets and the food industry. The high flavonoid content and high antioxidant activity of these leaves can be evaluated. Thus, the natural antioxidant needs of the food, pharmaceutical, or cosmetic industry can be met. For example, it may be possible for an inexpensive material to replace antioxidants with artificial and toxic properties in the industry by using it for purposes such as preserving properties, extending shelf life, adding nutritional value, flavoring, and coloring in foods. It was determined that extracts (UAE-1) prepared in 1 min in the ultrasound-assisted extraction method had the highest antioxidative properties in most of the parameters studied. Extracts prepared in 30 min (UAE-30) showed

the lowest inhibition values in most of the parameters studied. Therefore, it can be argued that the increase in the extraction time may cause the extract to lose its properties and lead to a decrease in its antioxidative properties.

CONCLUSION

UAE could be preferred instead of the traditional method as a shorter, eco-friendly, and low energy cost. For further studies, it may be recommended to isolate specific compounds from the extracts and optimize extraction methods, especially ultrasound-assisted extraction, through experimental design. Optimization can be achieved by examining the antioxidant activity in conditions where solvent, pH and temperature parameters change.

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