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Effect of extraction methods on the antioxidant properties of water hyacinth, *Eichhornia crassipes*

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ABSTRACT

Water hyacinth, *Eichhornia crassipes*, is a pharmaceutical aquatic plant that has created many environmental problems due to its being non-native to Anzali Wetland in Iran. This study aimed to compare the antioxidant activities of the leaf, stem, and root extracts of water hyacinth prepared by solvent extraction (SE), and ultrasound-assisted extraction (UAE) methods. The extraction process in the UAE method was performed using a water bath ultrasound device at a frequency of 42 kHz and a power of 70 watts in 60 min. The total phenol content, DPPH radical scavenging activity, total antioxidant capacity, and ferric-reducing antioxidant power in different extracts were evaluated. The highest amounts of total phenol contents in both SE and UAE methods were related to the water and water/ethanol extracts of the leaf (27.32 ± 0.24 and 25.29 ± 1.49 µg GAE/g DW, respectively) by the UAE method which did not exhibit significant differences with the water extract of the leaf by SEM ($p > 0.05$). The water/ethanol, and ethanol leaf extracts by the UAE method (0.84 ± 0.02, and 0.77 ± 0.03%), and the water leaf extract by SE method (0.76 ± 0.05) respectively displayed the highest free radical scavenging activity which did not show significant differences with the water leaf extract by UAE method, the water/ethanol, ethanol leaf extracts by SE method, and the water stem extract by UAE method ($p > 0.05$). In addition, in the total antioxidant capacity assay, the water/ethanol extracts of the leaf (27.75 ± 1.74 and 26.88 ± 3.73 30 mg AAE/g DW) respectively by UAE and SE methods showed the highest amounts of total antioxidant capacity exhibiting significant differences from other treatments ($p < 0.05$). On the other hand, the highest amounts of the ferric reducing antioxidant power were related to the water leaf extract by SE method (73.06 ± 13.30 mg AAE/g DW) and the water/ethanol, ethanol leaf extracts by UAE method (70.95 ± 2.47 and 69.42 ± 8.40) respectively displayed significant differences from other treatments ($p < 0.05$). In general, the UAE method was more efficient than the SE method. In addition, water/ethanol solvent with a ratio of 50:50 was the best solvent for extracting in the UAE method. According to the results, the invasive water hyacinth plant was found to be a suitable option for the extraction of natural antioxidant compounds. Moreover, the extract of this plant can be used as a natural preservative to increase the shelf life of seafood products.

Keywords: *Eichhornia crassipes*, Antioxidant properties, Water hyacinth, Extraction methods, Bioactive compounds.
Article type: Research Article.

INTRODUCTION

Nowadays, humans are involved with a high variety of different diseases and the high costs of chemical drugs for treatment (Rabiepour *et al.* 2024). So that, oxidative stress increases cells' production of free radicals due to an imbalance between reactive oxygen species (ROS) and antioxidant defense (Sindhi *et al.* 2013). Therefore, considering these problems and the harmful effects of synthetic drugs on human health, turning to natural treatment is an effective approach in this field (Haque *et al.* 2023). Hence, to increase the health and longevity of humans, researchers are seeking to discover and extract new natural compounds to produce products with added value (Rabiepour & Babakhani 2023). Therefore, the consumption of a food diet rich in antioxidants may help

boost the body's antioxidant defenses to fight reactive oxygen species, oxidative stress, and reduce the risk of related diseases (Giampieri *et al.* 2014; Zhang & Tsao 2016; Shahzamani *et al.* 2023; Houldsworth 2024). Antioxidants are the body's main active defense mechanism as free radical scavengers (Yehye *et al.* 2015). Synthetic antioxidants are used to delay lipids oxidation and increase the food products shelf life. Recently, the use of some of these antioxidants as food additives has been severely restricted due to the risks to consumer health (Girgih *et al.* 2013; Kim 2013). Therefore, using natural antioxidants with safe and dietary sources as an alternative is essential (Udenigwe & Aluko 2012). Accordingly, many researchers have moved towards active and raw materials extracted from plants (Afsharnezhad *et al.* 2017). Natural plant antioxidants are mainly polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and acetylbones), carotenoids (xanthophylls and carotenes), and vitamins (vitamins E and C; Manach *et al.* 2004; Baiano & Del Nobile 2016). Plants are considered one of the sources of biologically active compounds, and plant extracts have many benefits for human health. For instance, they show a wide range of biological effects such as antioxidant, anti-inflammatory, anti-bacterial, anti-viral, anti-aging, anti-cancer, etc. (Manach *et al.* 2004; Mendiola *et al.* 2008; Peng *et al.* 2014; Zheng *et al.* 2016; Naghdi *et al.* 2021; Bouzroud *et al.* 2023; Changaee *et al.* 2023). Hence, these effects and properties cause their use in food and pharmaceutical industries (Ebrahimi *et al.* 2024). Aquatic plants have particular importance in ecosystem sequencing (Milberg 1982), and Anzali Wetland is a unique and valuable aquatic ecosystem that contains a wide variety of aquatic plants and animals (Hassanzadeh *et al.* 2021). In recent years, this wetland has been under serious pressure from environmental and human problems (Vesali Naseh *et al*. 2012). The most important factors causing environmental crises in Iranian wetlands, especially Anzali Wetland, are population growth and urbanization, as well as agricultural, industrial, and tourism activities (Hargalani *et al.* 2014; Ziarati *et al.* 2015; Esmaeilzadeh *et al.* 2016), drainage and drying, discharge of various industrial, agricultural, household, and urban pollutants and nutrients to the wetland, sedimentation of incoming water sediments, occupation of wetland lands and turning them into arable lands, uncontrolled hunting, non-native animal and plant species (Tavakoli & Sabetraftar 2003; Ottinger *et al.* 2017; Sarkheil *et al.* 2021). Invasive species threaten local natural resources, biodiversity, ecological and agricultural environment, forests, pastures, and fisheries processing and provide lasting damage. In today's world, bio-invasions have created serious environmental and economic damage on a local and global scale (Achaval *et al.* 1979; Andersen 2005). One of the most invasive plant species is water hyacinth (Abba & Sankarannair 2024). Water hyacinth, *Eichhornia crassipes* is one of the perennial and free-floating aquatic plants. It belongs to the Commelinales order and the Pontederiaceae family, which is widespread in tropical and subtropical countries (Parsons *et al.* 2001; Ajithram *et al.* 2021). *E. crassipes* is one of the plants with high absorption power of heavy elements and metals in aquaponic systems and aquatic ecosystems (such as wetlands; Dixit *et al*. 2007; Aqdas & Hashmi 2023). Due to its compatibility with a wide range of environmental factors, including acidity, electrical conductivity, and temperature, this plant can be effective in wastewater treatment systems to improve water quality by reducing organic and inorganic elements (Delgado *et al.* 1995; Singh *et al.* 2023). However, the proliferation of water hyacinths in nutrient-rich waters has become a global concern (Hashem *et al.* 2020). The first report on the presence of water hyacinth in Iran referred to October 2012 during a study on the flora composition of paddy fields and aquatic ecosystems in Guilan Province, Iran (Mozaffarian & Yaghoubi 2015). This plant has an efficient asexual reproductive system (Center & wright 1991), covering lakes and rivers and blocking waterways, disrupting water transportation, agricultural products, tourism activities, and irrigation of farms. It can also reduce dissolved oxygen levels in water bodies, leading to reduced biological quality and water quality and reduced aquatic production (Gao & Bo 2004; Gunnarsson & Petersen 2007; Laranjeira & Nadais 2008; Shanab & Shalaby 2012). On the other hand, *E. crassipes* has many advantages and uses. This plant can be used in animal feed and alternative fuel source (Patel 2012), fertilizer production (Khaket *et al.* 2012; Dushimeyesu *et al.* 2023; Karouach *et al.* 2024), alternative feed and improvement of growth in fishes (Andriani *et al.* 2023; Ariyanto *et al.* 2023; Islama *et al.* 2023), production and development of natural fiber-based biocomposites (Mahardika *et al.* 2023), and development of nanotechnology (Fitria *et al.* 2023; Kalaivani & Ravi 2023). In addition, it can be considered a good source for producing natural bioactive compounds and new drugs to treat various diseases (Tulika *et al.* 2017; Anusiya *et al.* 2020). The leaves and stems of *E. crassipes* are a significant source of stigmasterol and antioxidant compounds that may pave the way to evaluate the potential use of this fast-growing species (Silva *et al.* 2015). In various research, the bioactive properties of this plant, including anti-cancer and antioxidant (Noufal *et al.* 2023), antibacterial (Kavinkumar *et al.* 2023), anti-inflammatory (Raju *et al.* 2023), antifungal (Ratnani *et al.* 2024), antiparasitic (Elagib 2020),

hepatoprotective (Prasanth *et al.* 2021), and diabetic wound healing (Firdaus *et al.* 2021) properties have been investigated. Extraction represents the first step in the research of medicinal plants and the preparation of extracts from plants is the starting point for the separation and purification of chemical components present in plants (Mandal *et al.* 2007; Jha & Sit 2022). Nowadays, for the extraction of bioactive compounds from plants, green and environmentally friendly extraction methods have very favorable characteristics compared to traditional and usual extraction methods in producing a pure product (Eskilsson & Björklund 2000; He *et al.* 2009; Arshad *et al.* 2024). Meanwhile, the extraction method and the quality of the obtained extract play a very important role in the subsequent measurements and evaluations of the plant. In the extraction method, attention should be paid to several factors, including the plant matrix, the cost-effectiveness of the method, achieving a favorable yield from the final product, the shortness of the extraction process, environmental friendliness, and many other factors (Bitwell *et al.* 2023). Many solvents have been used to extract antioxidants from foods and herbs. The choice of solvents should be based on the chemical nature and polarity of the antioxidant compounds of extracts. More phenols, flavonoids, and anthocyanins are water-soluble antioxidants. Polar and intermediate solvents, such as water, ethanol, methanol, propanol, acetone, and their aqueous mixtures, are extensively used for the extraction of these groups of bioactive (De Camargo *et al.* 2016; Van Tang *et al.* 2016). Currently, various methods such as solvent extraction (SE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), and other techniques are used for the extraction of bioactive compounds (Gill & Qiu 2020; Sanjeewa *et al.* 2023). In this research, we used solvent extraction (SE) and ultrasound-assisted extraction (UAE) methods. The extraction of bioactive compounds by the UAE method as an environmentally friendly method has attracted much attention (Thompson & Doraiswamy 1999). In this method, sound waves (20 kHz to 10 MHz) destroy the cellular structure of the plant, which enables the release of compounds. In addition, the main factor in the UAE method is the cavitation phenomenon (Picó 2013; Tiwari 2015). The advantages of the UAE method could be mentioned as increasing extraction efficiency and speed as well as elevating mass transfer (Singla & Sit 2021), maintaining the quality of the extract (Dzah *et al.* 2020), less need for solvent, time and energy (Herrera & Luque de Castro 2004; Zhang *et al.* 2009; Buvaneshwaran *et al.* 2023), upraising contact between plant sample matrix and solvent molecules (Altemimi *et al.* 2016; Quintero Quiroz *et al.* 2019). The present study aimed to evaluate the effect of different extraction methods on the antioxidant activity of various water hyacinth extracts.

MATERIALS AND METHODS

Chemical materials used include distilled water, ethanol (Lian Fidar Kia manufacturing company, Iran), methanol (Merck, Germany), sulfuric acid (Merck, Germany), sodium phosphate (Merck, Germany), ammonium molybdate (Merck, Germany), sodium carbonate (Merck, Germany), Folin Ciocalteu′s (Sigma-Aldrich, America), 2,2-Diphenyl-1-picrylhydrazyl (Sigma-Aldrich, USA), potassium phosphate buffer (Merck, Germany), potassium free cyanide (Merck, Germany), trichloroacetic acid (Merck, Germany), iron chloride (Merck, Germany), gallic acid (Merck, Germany).

Collection and preparation of plant samples

For this study, fresh samples of water hyacinth were identified and collected from the protected area in Anzali Wetland (southwest of the Caspian Sea, Guilan Province, Iran). Then, to remove epiphytes, sands, and sediments, the samples were washed with the wetland's water and then with fresh drinking water to remove impurities. Afterward, they were transferred to the Fisheries Products Processing Laboratory at the Faculty of Natural Resources, University of Guilan and placed in an oven (Behdad Medical Equipment Company, BM55E model, Iran) for drying at 40 °C to dry completely. The dried samples were pulverized using an electric mill (Hardstone model GCS2700W, England). Then the powdered samples were placed in a zippered plastic bag and were stored in the refrigerator at 4 °C until further testing.

Extraction methods

Two methods were used to extract water hyacinth: (i) Solvent extraction (SE) and (ii) Ultrasound-assisted extraction (UAE) methods (Wang *et al.* 2009; Liu *et al.* 2010; He *et al.* 2013).

Extraction of different parts of water hyacinth using solvent extraction (SE) method

In this method, 2 g of dried and groundwater hyacinth powder was placed in 50 mL of extraction solvent containing water/ethanol in ratios (water: ethanol) of 0:100, 50:50, and 100:0. Then, they were stirred by a shaker

(Perzan Pajooh, model 3008, Iran) for 6 h at 150 rpm and room temperature. The samples were passed through Whatman No. 40 filter paper and were centrifuged for 20 min at 1000 rpm using a centrifuge (Sanat Pardaz Dena Company, model FD-50005-BT, Iran). Finally, the obtained extracts were stored in the refrigerator in dark bottles until further evaluation. Each extraction was performed in three replications (Wang *et al.* 2012).

Extraction of different parts of water hyacinth using ultrasound-assisted extraction (UAE) method

The extraction process was performed using a water bath ultrasound device at a frequency of 42 kHz and a power of 70 watts for 60 min. Briefly, 2 g of water hyacinth powder was added to 50 mL solvent, including water/ethanol in ratios (water: ethanol) of 0:100, 50:50, and 100:0. Then mixed by a shaker for 20 min at 150 rpm and room temperature. The extracts obtained were passed through filter paper and centrifuged. Finally, the extracts were stored in the refrigerator and dark bottles until further evaluation. Each extraction was performed in three replications (Kadam *et al.* 2015).

Evaluation of antioxidant properties of extracts

Evaluation of the antioxidant properties of the extracts included total phenol content (TPC), free radical scavenging activity (DPPH), total antioxidant capacity (TAC), and ferric-reducing antioxidant power (FRAP).

Determination of total phenol content (TPC)

The amount of total phenol content (TPC) of water hyacinth extracts was measured according to the method described by Taga *et al.* (1984). In this method, 100 µL of the extract was mixed with 2 mL of 2% Na_2Co_3 and was placed at room temperature for 2 min. Then, 100 µL of 50% Folin Ciocalteu′s reagent was added, mixed, and placed in the dark for 30 min at room temperature. Samples absorption was measured at 720 nm using an ELISA Reader (Winooski Company, model VT 05404-0998, America) device. The results were expressed as µg gallic acid equivalent per gram dry weight (µg GAE/g DW).

Determination of free radical scavenging activity (DPPH)

Evaluation of antioxidant activity using stable radicals 2, 2 diphenyl-1-pipicryl-hydrazyl (DPPH) was measured according to the method described by Brand Williams *et al.* (1995). At first, 1 mL of the extract was added to 1 mL of 0.16 mM free radical methanol solution of DPPH, then mixed well and kept at room temperature and in the dark for 30 min. Afterward, the absorbance of the samples was read in the ELISA Reader (Winooski Company, model VT 05404-0998, America) device at 517 nm. The extract's free radical scavenging activity (DPPH) was calculated using the following formula. The results were expressed as a percentage (%) of radical scavenging activity (RSA).

$$\text{Radical scavenging activity (\%)} = [1-(A_{\text{sample}} - A_{\text{sample blank}})/A_{\text{control}})] \times 100$$

Where A_{sample} = Absorbance of the DPPH solution plus test sample, A_{control} = Absorbance of the DPPH solution without sample, and $A_{\text{sample blank}}$ = Absorbance of the sample without DPPH solution

Determination of total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) of water hyacinth extracts was measured according to the method described by Prieto *et al.* (1999). For this test, 2 mL of the extract was mixed with 2 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and placed in glass tubes with lids. Then, the solution was placed in a water bath (95 °C) for 90 min. After cooling the samples at room temperature, the absorption of the samples was read in the ELISA Reader (Winooski Company, model VT 05404-0998, America) device at 695 nm. The results were expressed as mg ascorbic acid equivalent per gram dry weight (mg AAE /g DW).

Determination of ferric-reducing antioxidant power (FRAP)

The ferric-reducing antioxidant power **(**FRAP) of water hyacinth extracts was determined according to the method described by Chew *et al.* (2008). Briefly, 0.1 M potassium phosphate buffer with pH = 6.6 (2.5 mL) and 1% potassium ferric cyanide (2.5 mL) were mixed with 1 mL of the extract. This solution was stored at 50 °C for 20

min. Then, 2.5 mL trichloroacetic acid was added to this solution. Afterward, 2.5 mL water and 0.5 mL iron chloride ($FeCl_3$; 0.1%) were added to 2.5 mL of the mixture. The solution was kept at a constant temperature for 30 min to form a dye. The absorbance of the samples was read in the ELISA Reader (Winooski Company, model VT 05404-0998, America) device at a wavelength of 700 nm, and FRAP was expressed in mg of ascorbic acid equivalent per gram dry weight (mg AAE /g DW).

Statistical analysis

We used SPSS version 18 to perform statistical analysis, and Excel software version 2013 to draw graphs. In this way, to check the significance of the tests, the obtained data were analyzed using the one-way analysis of variance (ANOVA) method to check the responses in each method separately. Then, the average comparison was done using Duncan's test at the level of 95% probability ($p < 0.05$).

RESULTS

The highest amount of total phenol content (TPC) in the SE method was obtained in the water extract of leaf (24.35 ± 2.52 µg GAE/g DW), exhibiting a significant difference from other treatments ($p < 0.05$). Also, the lowest amounts of total phenol content were respectively related to the ethanol extract of the root (4.23 ± 0.77), the ethanol extract of the stem (5.80 ± 0.32), and the water/ethanol extract of root (7.26 ± 0.34) indicating significant differences from other treatments ($p < 0.05$). The highest amount of total phenol content in the UAE method was obtained in the water extract of the leaf (27.32 ± 0.24 µg GAE/g DW), displaying a significant difference from other treatments ($p < 0.05$). However, the lowest amounts of total phenol content in the UAE method were related to the ethanol extract of the root (4.34 ± 1.39) and the ethanol extract of the stem (6.05 ± 0.69) respectively, revealing significant differences from other treatments ($p < 0.05$). The highest amounts of total phenol contents in both methods were related to the water and water/ethanol extracts of the leaf (27.32 ± 0.24 and 25.29 ± 1.49 µg GAE/g DW, respectively) by UAE method which did not exhibit significant difference with the water extract of the leaf by SE method ($p > 0.05$). However, the lowest amounts of total phenol contents in both SE and UAE methods were related to the ethanol extract of the root (4.23 ± 0.77 and 4.34 ± 1.39 µg GAE/g DW) by SE and UAE methods respectively; the ethanol extract of the stem (5.80 ± 0.32) by SE method; the ethanol extract of stem (6.05 ± 0.69) by UAE method; and the water/ethanol extract of the root (7.26 ± 0.34) by SE method exhibiting significant differences from other treatments ($p < 0.05$). The total phenol test results are shown in Table 1.

Table 1. Comparison of the effect of SE and UAE methods on the amount of the total phenol content (TPC) among the water, water/ethanol, and ethanol extracts from different parts (leaf, stem, and root) of water hyacinth, *E. crassipes*. The results were expressed as µg gallic acid equivalent per gram dry weight (µg GAE/g DW).

Treatments **Extraction methods**	**Leaf-Water**	**Leaf-Water/ethanol**	**Leaf-Ethanol**	**Stem-Water**	**Stem - Water/ethanol**	**Stem - Ethanol**	**Root-Water**	**Root - Water/ethanol**	**Root - Ethanol**
SE method	24.35 ± 2.52^{Ba}	20.60 ± 3.36^{Cb}	15.09 ± 1.70^{Dc}	19.12 ± 3.87^{Cb}	13.46 ± 1.58^{Dc}	5.80 ± 0.32^{FGd}	13.09 ± 1.54^{Dc}	7.26 ± 0.34^{EFGd}	4.23 ± 0.77^{Gd}
UAE method	27.32 ± 0.24^{Aa}	25.29 ± 1.49^{ABb}	18.95 ± 1.42^{Cc}	19.72 ± 0.81^{Cc}	13.51 ± 0.65^{Dd}	6.05 ± 0.69^{FGf}	10.20 ± 1.41^{Ee}	8.31 ± 1.07^{EFe}	4.34 ± 1.39^{Gf}

Values were expressed as the mean ± standard deviation. Large non-eponymous letters indicate significant differences between all treatments at the 95% confidence level ($p < 0.05$), and small non-eponymous letters in each row indicate significant differences in each extraction method at the 95% confidence level ($p < 0.05$).

The results of the free radical scavenging activity are shown in Table 2. The water extract of the leaf (0.76 ± 0.05 %), the water/ethanol extract of the leaf (0.72 ± 0.05), and the ethanol extract of the leaf (0.71 ± 0.003) respectively exhibited the highest amounts of free radical scavenging activity in SE method. However, no significant difference was found with the water extract of the stem ($p > 0.05$). In addition, the ethanol extract of the root (0.22 ± 0.01%) and the water/ethanol extract of the root (0.29 ± 0.05) respectively revealed the lowest amounts of free radical scavenging activity exhibiting significant differences from other treatments ($p < 0.05$). The water/ethanol extract

of the leaf (0.84 ± 0.02%) and the ethanol extract of the leaf (0.77 ± 0.03) displayed the highest free radical scavenging activity in UAE method. However, no significant difference was found with the water extract of the leaf and the water extract of the stem ($p > 0.05$). Moreover, the ethanol extract of the root (0.37 ± 0.06), the ethanol extract of the stem (0.46 ± 0.04), and the water/ethanol extract of the root (0.47± 0.03 %) had the lowest amounts of free radical scavenging activity in UAE method with significant differences from other treatments ($p < 0.05$). In both SE and UAE methods, the water/ethanol, ethanol leaf extracts by UAE method (0.84 ± 0.02, 0.77 ± 0.03 %) and the water leaf extract by SE method (0.76 ± 0.05) respectively exhibited the highest free radical scavenging activity, while did not display significant differences with the water leaf extract by UAE method, the water/ethanol, ethanol leaf extracts by SE method and the water stem extract by UAE method ($p > 0.05$). However, the ethanol and water/ethanol extracts of the root by SE method (0.22 ± 0.01 and 0.29 ± 0.05 %) respectively led to the lowest free radical scavenging activity, with significant differences from other treatments ($p < 0.05$).

Table 2. Comparison of the effect of SE and UAE methods on the amount of free radical scavenging activity (DPPH) between the water, water/ethanol, and ethanol extracts from different parts (leaf, stem, and root) of water hyacinth, *E. crassipes*. The results were expressed as a percentage (%) of radical scavenging activity (RSA).

Treatments / **Extraction methods**	Leaf-Water	Leaf-Water/ethanol	Leaf- Ethanol	Stem-Water	Stem - Water/ethanol	Stem - Ethanol	Root-Water	Root - Water/ethanol	Root - Ethanol
SE method	0.76 ± 0.05^{ABa}	0.72 ± 0.05^{BCab}	0.71 ± 0.003^{BCab}	0.65 ± 0.07^{Cb}	0.49 ± 0.02^{Dc}	0.38 ± 0.05^{Ed}	0.47 ± 0.02^{Dc}	0.29 ± 0.05^{Fe}	0.22 ± 0.01^{Fe}
UAE method	0.72 ± 0.03^{BCbc}	0.84 ± 0.02^{Aa}	0.77 ± 0.03^{ABab}	0.70 ± 0.01^{BCbc}	0.65 ± 0.03^{Cc}	0.46 ± 0.04^{DEde}	0.55 ± 0.12^{Dd}	0.47 ± 0.03^{DEde}	0.37 ± 0.06^{Ee}

Values were expressed as the mean± standard deviation. Large non-eponymous letters indicate significant differences between all treatments at the 95% confidence level ($p < 0.05$). and small non-eponymous letters in each row indicate significant differences in each extraction method at the 95% confidence level ($p < 0.05$).

In the case of total antioxidant capacity, in the SE method, the highest amount was related to the water/ethanol extract of the leaf (26.88 ± 3.73 mg AAE /g DW), with a significant difference from other treatments ($p < 0.05$). In addition, the lowest amounts of total antioxidant capacity were obtained in the ethanol extract of the stem (1.36 ± 0.87), the water extract of the root (3.96 ± 0.67), and the water extract of the stem (4.16 ± 0.62) with significant differences from other treatments ($p < 0.05$). In the case of the UAE method, the highest amount of total antioxidant capacity was related to the water/ethanol extract of the leaf (27.75 ± 1.74 mg AAE /g DW), with a significant difference from other treatments ($p < 0.05$). However, the lowest amounts of total antioxidant capacity were related to the ethanolic extract of the stem and the water extract of the root (2.27 ± 0.33 and 2.27 ± 1.13), the water extract of the stem (2.50 ± 0.48) and the water/ethanol extract of the stem (3.38 ± 0.80) respectively, with significant differences from other treatments ($p < 0.05$). In the case of both SE and UAE methods, the water/ethanol extracts of the leaf (27.75 ±1.74 and 26.88 ± 3.73 mg AAE /g DW) respectively by UAE and SE methods exhibited the highest total antioxidant capacity with significant differences from other treatments ($p < 0.05$). However, the ethanol extract of the stem by SE method (1.36 ± 0.87), the ethanol extract of the stem (2.27 ± 0.33) by UAE method, the water extract of the root (2.27 ±1.13) by UAE method, the water extract of the stem (2.50 ± 0.48) by UAE method and the water/ethanol extract of the stem (of 3.38 ± 0.80 mg AAE /g dry weight) by UAE method respectively displayed the lowest amounts of total antioxidant capacity with significant differences from other treatments ($p < 0.05$). The results of the total antioxidant capacity test are shown in Table 3. According to Table 4, the water leaf extract by SE method (73.06 ± 13.30 mg AAE /g DW) exhibited the highest amount of ferric-reducing antioxidant power with a significant difference from other treatments ($p < 0.05$). In addition, the ethanol extract of the root (2.22 ± 0.34), the water stem extract (3.41 ± 0.74), the water root extract (4.54 ± 0.97), the water/ethanol root extract (4.59 ± 1.29) and the water/ethanol stem extract (7.74 ± 1.82) respectively displayed the lowest amounts of ferric reducing antioxidant power with significant differences from other treatments ($p < 0.05$). The water/ethanol and ethanol leaf extracts (70.95 ± 2.47 and 69.42 ± 8.40 mg AAE /g DW respectively) led to the highest amounts of ferric-reducing antioxidant power by UAE method with significant differences from other treatments ($p < 0.05$). However, the ethanol extract of the root (2.16 ± 0.19),

the water extract of stem (2.50 ± 0.69), the water/ethanol extract of the root (4.54 ± 1.03), and the water extract of the root (5.50±0.89) respectively exhibited the lowest amounts of ferric reducing antioxidant power with a significant difference from other treatments ($p < 0.05$). The highest amounts of ferric reducing antioxidant power in both SE and UAE methods were related to the water leaf extract by SE method (73.06 ± 13.30 mg AAE /g DW) and the water/ethanol, ethanol leaf extracts by UAE method (70.95 ± 2.47 and 69.42 ± 8.40 respectively) with significant differences from other treatments ($p < 0.05$). However, the lowest amounts of ferric-reducing antioxidant power in both SE and UAE methods were obtained in the ethanol extract of the root by UAE method (2.16 ± 0.19) and the ethanolic extract of the root by SE method (2.22 ± 0.34), the water extract of the stem (2.50 ± 0.69) by UAE method, the water stem extract (3.41 ± 0.74 mg AAE /g DW) by SE method, the water root extract (4.54 ± 0.97) by SE method, the water/ethanol extract of the root (4.54 ± 1.03) by UAE method, the water/ethanol root extract (4.59 ± 1.29) by SE method, the water root extract (5.50 ± 0.89) by UAE method, and the water/ethanol stem extract (7.74 ± 1.82) by SE method respectively with significant differences from other treatments ($p < 0.05$).

Table 3. Comparison of the effect of SE and UAE methods on the amount of total antioxidant capacity (TAC) between the water, water/ethanol, and ethanol extracts from different parts (leaf, stem, and root) of water hyacinth, *E. crassipes*. The results were expressed as mg ascorbic acid equivalent per gram dry weight (mg AAE /g DW).

Treatments / **Extraction methods**	Leaf-Water	Leaf-Water/ethanol	Leaf- Ethanol	Stem-Water	Stem - Water/ethanol	Stem - Ethanol	Root-Water	Root - Water/ethanol	Root - Ethanol
SE method	7.67 ± 0.94^{Ec}	26.88 ± 3.73^{Aa}	12.13 ± 1.53^{CDb}	4.16 ± 0.62^{FGde}	5.80 ± 0.91^{EFcd}	1.36 ± 0.87^{He}	3.96 ± 0.67^{FGde}	5.82 ± 0.75^{EFcd}	12.61 ± 0.72^{CDb}
UAE method	21.81 ± 0.29^{Bb}	27.75 ± 1.74^{Aa}	12.16 ± 1.54^{CDcd}	2.50 ± 0.48^{GHe}	3.38 ± 0.80^{GHe}	2.27 ± 0.33^{GHe}	2.27 ± 1.13^{GHe}	13.77 ± 1.79^{CDc}	10.72 ± 0.99^{CDd}

Values were expressed as the mean± standard deviation. Large non-eponymous letters indicate significant differences between all treatments at the 95% confidence level ($p < 0.05$). and small non-eponymous letters in each row indicate significant differences in each extraction method at the 95% confidence level ($p < 0.05$).

DISCUSSION

Antioxidants have attracted the most attention among all the compounds extracted from plants. Antioxidants from natural sources are valuable bioactive compounds with well-demonstrated potential for use in the food industry (Lourenço *et al*. 2019). These compounds reduce oxidation by their ability to suppress free radicals and thus provide more significant improvement and stability of food systems (Luo *et al.* 2010; Aware *et al.* 2019). Synthetic antioxidants have been limited due to their toxicity and risks to human health (Weisburger 1999). Thus, efforts to identify antioxidants of plant origin have increased. Due to its low toxicity and relatively low cost, ethanol is used as a solvent to extract phenolic compounds from plants (Zhou *et al.* 2017; Yang *et al.* 2019). Therefore, in this study, ethanol and water were used. Moreover, research results have shown that the mixture of solvents could help extracting antioxidant compounds. For instance, using a binary solvent, such as ethanol-water, could be more effective than a mono-solvent system (water or pure ethanol; Wong *et al.* 2014). Assay for the Folin Ciocalteu′s reagent is an extensive method for quantifying phenolic compounds. The mechanism of this method is electron transfer (ET; Abramovi˘c *et al.* 2018). Measuring total phenol by this reagent is simple and convenient and has become a common method for studying phenol antioxidants from fruits, vegetables, and herbs (Singleton *et al.* 1999; Magalhaes *et al.* 2007; Yoo *et al.* 2012). Bodo *et al.* (2004) studied the antioxidant properties of water hyacinth. The ethanolic extract of the leaf contained more polyphenol compounds than that of the stem, which was similar to the results of Liu *et al.* (2016) and the present study. The research results of Borzouei & Tabarsa (2017) indicated that leaf metabolites of *E. crassipes* had the highest amount of total phenol compared to roots, similar to the present study results. In a study, Soto-Maldonado *et al.* (2022) examined the antioxidant and antimicrobial capacity of *Maytenus boaria* leaves by SE method. The best values of total phenolic content were observed in ethanolic extract, in disagreement with the present study. The water extract of the leaf in the present study showed the highest amount of total phenol content in the SE method.

Table 4. Comparison of the effect of SE and UAE methods on the amount of ferric reducing antioxidant power (FRAP) among the water, water/ethanol, and ethanol extracts from different parts (leaf, stem, and root) of water hyacinth (*E. crassipes*). The results were expressed as mg ascorbic acid equivalent per gram dry weight (mg AAE /g DW).

Treatments **Extraction methods**	**Leaf-Water**	**Leaf-Water/ethanol**	**Leaf-Ethanol**	**Stem-Water**	**Stem - Water/ethanol**	**Stem - Ethanol**	**Root-Water**	**Root - Water/ethanol**	**Root - Ethanol**
SE method	73.06 ± 13.30^{Aa}	50.79 ± 3.51^{Cc}	60.36 ± 7.97^{Bb}	3.41 ± 0.74^{Gde}	7.74 ± 1.82^{EFGde}	12.92 ± 0.94^{EFd}	4.54 ± 0.97^{FGde}	4.59 ± 1.29^{FGde}	2.22 ± 0.34^{Ge}
UAE method	29.58 ± 9.82^{Db}	70.95 ± 2.47^{Aa}	69.42 ± 8.40^{Aa}	2.50 ± 0.69^{Gd}	23.23 ± 1.90^{Db}	14.81 ± 3.16^{Ec}	5.50 ± 0.89^{FGd}	4.54 ± 1.03^{FGd}	2.16 ± 0.19^{Gd}

Values were expressed as the mean± standard deviation. Large non-eponymous letters indicate significant differences between all treatments at the 95% confidence level ($p < 0.05$). and small non-eponymous letters in each row indicate significant differences in each extraction method at the 95% confidence level ($p < 0.05$).

Fitriansyah *et al*. (2018) studied the extraction of various parts of *Phyllanthus emblica* prepared by maceration extraction method using solvents with different polarities such as n-hexane, ethyl acetate, and ethanol. Phenol compound in the stem bark extract of *P. emblica* exhibited antioxidant activity higher than the leaf and fruit extracts, while in our study, the leaf extract displayed a higher total phenol content than the stem and root. In the study conducted by Powthong & Suntornthiticharoen (2023), the phenolic content in ethanol extract of water hyacinth was higher than in aqueous extract. In the present study, the amounts of phenol in the aqueous extracts of different parts of the plant were higher in both extraction methods. Jang *et al*. (2019) compared the antioxidant activity of different parts of the lotus, *Nelumbo nucifera*, such as flowers, seeds, and roots. The total phenolic content was the highest in flowers, followed by leaves, seeds, and roots, which correlated with their antioxidant activities. However, in the present study, the leaf extract showed the highest total phenol content among the leaf, stem, and root extracts. In addition, the results of the research conducted by Tyagi & Agarwal (2017), showed that the leaves of water hyacinth contain the highest amount of total phenol, in agreement with the results of current study. Poorhashemi *et al.* (2020) worked on the parameters of extraction and optimization of antioxidant and phenolic compounds by UE method and ethanol solvent from *Myristica fragrans* seeds. Their results showed that UAE method is appropriate for extracting antioxidant and phenolic compounds from the plant. In the present study, UAE method led to better results than SE method. Li *et al.* (2007) compared the antioxidant activity of *Agrimonia pilosa* using the ultrasound and the soxhlet methods. Their results showed that both methods are effective in extracting plant antioxidant compounds. Chemat *et al.* (2004) investigated the antioxidant properties of cumin seed extract using both traditional and ultrasound extraction methods. Their results showed that the efficiency and quality of plant extracts by ultrasonic extraction were better and faster than the traditional extraction method, similar to the present study. In another study, Khalili *et al.* (2016) studied the effect of different extraction methods on the antioxidant activity of the shoots of *Crocus Caspius* reporting that the ultrasound extract led to a higher phenolic content than the soaking extract. In the present study, the highest amount of total phenol was attributed to UAE method. In a research, Dias *et al.* (2017) compared two methods, i.e., soxhlet and ultrasound-assisted with four types of solvents to extract the pepper Dedo de moça, reporting the high effectiveness of phenolic and antioxidant compounds obtained by ultrasound extraction. Ma *et al.* (2009) examined the extraction process of phenolic compounds from a type of citrus concluding that ultrasound significantly increased the extraction of phenolic compounds compared to the flood extraction method. Albu *et al.* (2004) reported that using UAE method elevated the carnosic acid extracted from rosemary. Martin *et al.* (2014) stated that the main reason for the higher amount of compounds extracted in UAE method was the sound cavitation created in the solvent, which was similar to the results of Zhang *et al.* (2009), Lianfu *et al.* (2008) and Rira *et al.* (2006). This indicates that the solvent's polarity increases the yield, and more polar compounds were found in these samples (Bodo *et al.* 2004). Naghdi & Babakhani (2018) investigated the extraction of antioxidant compounds from the algae, *Sargassum angustifolium*, *Astraulis Padina*, *Cystoseria merica*, and *Colopomenia sinuosa* with solvents of water, water /ethanol (50:50) and ethanol by UAE method, concluding that the aqueous algae extract contains highest amount of total phenol. In the present study, the water and water /ethanol extracts of the leaf contained higher phenolic compounds. One reason for the high TPC may be due to the color reaction of proteins in aqueous extracts with the Folin-Ciocalteu reagent, leading to an overestimation of total phenolic compounds (Surendraraj *et al.* 2013). The spectrophotometric assay measures the antioxidants' ability to reduce 2, 2-diphenyl-1-picrylhydrazyl (DPPH), commonly another radical not found in biological systems (Sharma & Bhat 2009). Changes in light absorption will be examined to evaluate the antioxidant potential through free radical scavenging of DPPH with experimental samples (Ebrahimzadeh *et al.* 2008). DPPH is a stable radical with a dark purple color that reacts with other radicals or decreasing agents to reduce the adsorption at 515 nm (Ricci *et al.* 2019; Martinez-Morales *et al*. 2020). This method is the simplest, so it is common in various laboratories and widely used (Foti *et al.* 2003). In a study by Tulika *et al.* (2017), qualitative phytochemical analysis and antioxidant activity of methanolic extracts of *E. crassipes* were investigated. Methanolic extracts of different parts of *E. crassipes* using the DPPH free radical scavenging method showed that the stem had the highest antioxidant activity, the leaves the least, and the root less activity than the stem. Moreover, in the research done by Malviya *et al*. (2010) *in vitro*, the antioxidant potential of aqueous extract of *Trapa natans* L. fruits rind was investigated, reporting that it exhibits significant antioxidant activity against free radicals. However, in the present study, the water/ethanol, ethanol leaf extracts by UAE method and the water leaf extract by SE method respectively showed the highest percentage of DPPH neutralization, while the ethanol and water/ethanol root extracts by SE method displayed the lowest free radical

scavenging power respectively. Enien *et al.* (2011) investigated the antioxidant activity of water hyacinth by inhibition method of DPPH, reporting significant antioxidant activity, attributed to the presence of hydroxyl groups and unsaturated bonds in its composition, with a high ability in inhibition of free radicals. Adam *et al.* (2009) examined the extraction of antioxidants from plants using the ultrasound method (ultrasonic bath and ultrasonic probe) and their antioxidant capacity by free radical scavenging. In two plants, *Ruta graveolens* L. and *Mentha longifolia* L., the ultrasound bath method exhibited higher efficiency than the ultrasound probe system. However, in *Coriandrum sativum* L., *Plantago lanceolata* L., *Achillea millefolium* L., and *Mentha spicata* L., the ultrasound probe system was more efficient than the ultrasound bath. Generally, in the present study, the UAE method was more efficient than the SE method. Abideen *et al.* (2015) investigated antioxidant activity in different parts of the *Phragmites karka* plant. Its leaves exhibited the highest free radical-scavenging ability, followed by stem and root, similar to the present results. Moreover, in a study by Eden *et al.* (2023), the leaf extract of *E. crassipes* had more DPPH scavenging activity than those of stem and root, consistent with our results. Total antioxidant capacity is one of the methods based on electron atom transfer used to study the antioxidant properties. However, nowadays, it has attracted less attention. Ganorkar *et al.* (2022) investigated the shikimic acid extraction from different morphological parts (stems, leaves, and roots) of water hyacinth (*E. crassipes*) using the UAE method. The stems showed the highest antioxidant activity, while in the present study, the leaves displayed the highest. The results of research by Hodhodi *et al.* (2021) showed that the highest total antioxidant activity of brown algae, *Sargassum angustifolium* extract was in association with the water/ethanol extract (30:70). However, in the present study, the water/ethanol leaf extract (50:50) revealed the highest total antioxidant activity. Baradaran *et al.* (2014) examined the antioxidant activity of different extracts of leaves and flowers of *Artemisia Annua*. Their results showed that the methanolic extract of the leaf had the highest total antioxidant activity, while the water extract of the flower was the lowest. However, in the present study, the highest activity was related to the water/ethanol leaf extract. Safari *et al.* (2013) investigated the antioxidant activity of green algae, *Chaetomorpha sp.* by immersion extraction method. Their results showed that the 70% acetone extract had the highest total antioxidant activity, while in the present study, the water/ethanol (50:50) extract of the leaf exhibited the highest. The FRAP is a simple method that produces fast and reproducible results (Benzie & Strain 1996; Niemeyer & Metzler 2003). This method can be readily used for aqueous and alcoholic extracts of various plants. Nowadays, it will successfully be used to study the antioxidant activity of pure chemical compounds and plant extracts. This method measures the ability of antioxidants to reduce ferric iron (Antovich *et al.* 2002). The evaluation of regenerative power shows the electron-giving ability of a compound (Yen & Chen 1995). In the results obtained by Awote *et al.* (2021), water hyacinth root extract showed a high ferric reducing antioxidant power compared to other parts. On the contrary, in our study, the root extract displayed the lowest ferric-reducing antioxidant power. Alam *et al.* (2020) evaluated the antioxidant activity of methanolic extract of *Nymphaea capensis* leaf by ferric-reducing antioxidant power. Their results showed that the elevated concentration of methanolic extract increased the reducing antioxidant power of iron. The soaking extract was more robust in the ferric-reducing antioxidant power. In the present study, the highest ferric-reducing antioxidant power was related to the water leaf extract by SE method. In another study, Surendraraj *et al.* (2013) investigated the antioxidant properties of parts of leaves, stems, and flowers of water hyacinth, reporting that the ethanolic extracts of flowers had the highest ferric-reducing antioxidant power, not consistent with our study. Furthermore, Kumar *et al.* (2008) reported that the methanolic extract of *Kappaphycus alvarezii* showed a high inhibitory power of the metal. Jayanthi & Lalitha (2011) investigated the ferric-reducing antioxidant power of *E. crassipes, in vitro*, so that, the aqueous extract showed a higher ferric-reducing antioxidant power, in agreement with the present work. Shukla *et al.* (2023) reported that the ethyl acetate extract of water hyacinth leaves had the highest ferric-reducing antioxidant power. However, in our study, the water extract of the leaf exhibited the highest amount of ferric-reducing antioxidant power. Also, Chanda & Kaneria (2012) studied extracting antioxidants from *Syzygium cumini* L. leaves by three different methods including sequential cold percolation extraction, decoction extraction, and maceration extraction methods, reporting that acetone extract by sequential cold percolation displayed maximum absorbance and also maximum ferric reducing antioxidant power. In the present study, the water extract of the leaf by SE method, as well as the water/ethanol and ethanol extracts of the leaf by UAE method exhibited the highest ferric-reducing antioxidant power among leaf, stem and root extracts respectively. Emsen & Doganit (2018) examined the ferric-reducing antioxidant power of the methanol and water extracts obtained from *Ceratophyllum demersum*, revealing that the water extract had more ferric-reducing antioxidant power than the methanol one. Thitilertdecha

et al. (2008) worked on the antioxidant activities of various extracts of the seeds and peel of *Nephelium lappaceum*. A higher amount of ferric-reducing antioxidant power was demonstrated in the methanol extract of peels and potential antioxidant activities than in the seed extracts which was not similar to the present study.

CONCLUSION

Anzali Wetland is one of the most beautiful and important aquatic ecosystems in Iran and the world. In recent years, this wetland has been affected by the uncontrolled growth of invasive and non-native aquatic plants, including water hyacinth, *E. crassipes*, which has caused many environmental and economic problems for this wetland and people. Water hyacinth is a medicinal plant due to the presence of bioactive compounds in it. Medicinal plants contain effective compounds, which have very useful properties in various fields. One of these effective and bioactive compounds is antioxidant, which is known as a functional element in increasing human health and reducing disease risks. Hence, to discover and use bioactive compounds such as antioxidants, it is necessary to prepare and carry out an extraction step. In today's world, green and environmentally friendly extraction techniques have attracted the attention of many researchers and industries to obtain the plants bioactive compounds. One of these new and environmentally friendly techniques is the ultrasound-assisted extraction (UAE) method. This method increases the efficiency of the extraction process and on the other hand, it is associated with less consumption of solvent, energy, and time. For this reason, it was chosen as a suitable method for extraction in this study. This study showed that the highest amounts of total phenol content in both SE method and UAE method were related to the water and water/ethanol extracts of the leaf (27.32 ± 0.24 and 25.29 ± 1.49 µg GAE/g DW, respectively) by the UAE method which did not exhibit significant differences with the water extract of the leaf by SE method ($p > 0.05$). Also, the water/ethanol, and ethanol leaf extracts by UAE method (0.84 ± 0.02, 0.77 ± 0.03 %) respectively, and the water leaf extract by SE method (0.76 ± 0.05) had the highest free radical scavenging activity. However, these extracts did not show significant differences with the water leaf extract by UAEM, the water/ethanol, and ethanol leaf extracts by SE method, and the water stem extract by UAE method ($p > 0.05$). However, in part of total antioxidant activity, the water/ethanol extracts of the leaf (27.75 ± 1.74 and 26.88 ± 3.73 mg AAE /g DW) respectively by UAE and SE methods showed the highest amounts of total antioxidant activity with significant differences from other treatments ($p < 0.05$). Moreover, the highest ferric-reducing power was related to the water leaf extract by SE method (73.06 ± 13.30) and the water/ethanol, ethanol leaf extracts by UAE method (70.95 ± 2.47 and 69.42 ± 8.40 mg AAE /g DW) respectively with significant differences from other treatments ($p < 0.05$). The UAE method was more efficient than the SE method. Also, water/ethanol solvent with a ratio of 50:50 was the best solvent for extracting by the UAE method. According to the results, the invasive water hyacinth plant was an appropriate option to extract natural antioxidant compounds for use in various industries. Furthermore, the extract of this plant can be used as a natural preservative to increase the shelf life of value-added seafood products. In fact, by using new technologies and processing methods, this plant can be used in sections including food, pharmaceutical, nutraceutical, medical, agriculture, and other industries, since different parts of this plant have multiple bioactive properties with various functions. Also, we suggest using water hyacinth extract in combination with nanotechnology as an effective and efficient preservation method in the food packaging industry.

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