

Phytochemical profile and antioxidant potential of sea buckthorn leaves: A comprehensive analysis of nutrients and bioactive compounds

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Abstract

Background: Sea buckthorn (*Hippophae rhamnoides* L.) is a valuable plant with diverse health benefits attributed to its rich phytochemical composition and medicinal properties. In this study, the chemical composition and antioxidant potential of sea buckthorn leaves were investigated using a hydroethanolic extract. Proximate analysis, mineral content determination, antioxidant assays (DPPH and ABTS), and antihemolytic activity were employed to assess the nutritional and functional properties of the extract.

Results: Proximate analysis revealed significant amounts of carbohydrates (61.51 g/100 g), proteins (12.49 g/100 g), and fats (13.4 g/100 g). The mineral composition showed high calcium, sodium, and magnesium levels, with potassium being the lowest among the major minerals. The total phenolic content (TPC) was 131.34 mg GAE/g extract DW, and the total flavonoid content (TFC) was 43.00 mg CE/g extract DW. Antioxidant activities linked to the phenolic profile were notable, with DPPH and ABTS assays showing IC₅₀ values of 2.10 mg/g DW and 0.46 mg/g DW, respectively. The phenolic profile, dominated by quinic and rutin, contributed to the observed strong antioxidant activity. Furthermore, the extract exhibited potent reducing power and anti-hemolytic activity.

Conclusion: The findings of this study highlight the potential of sea buckthorn leaves as a valuable source of nutrients and bioactive compounds with potent antioxidant properties. Further research is warranted to explore the specific mechanisms of action and potential applications of these compounds in various health-promoting products.

KEYWORDS

antioxidant properties, LC-ESI-MS, minerals, phenolic profiling, sea buckthorn leaves

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides* L. or *Elaeagnus rhamnoides* L.) belongs to the *Elaeagnaceae* family and counts eight subspecies.¹

Native to Europe and Asia, it is now widely spread around Russia, China, Canada, and America.² Several studies reported a long history of sea buckthorn used as a traditional, functional, and medicinal plant.^{3,4} Furthermore, several articles on sea buckthorn reported

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numerous nutritional and bioactive compounds, including sugars, vitamins (B1, B2, B6, B11, C, E, and K), lipids, fatty acids, free amino acids, proteins, minerals (K, Ca, P, Fe, Zn, Mn, and Cu), and phenolic compounds. More than 200 natural bioactive compounds have predominantly been identified, including acids, flavonoids, polysaccharides, sugar alcohols, organic acids, phytosterols, and antioxidant enzymes.⁵ These, known also as secondary metabolites, are associated with several biological properties comprising cytoprotective, chemotherapeutic, chemoprotective, antibacterial, anticancer, cardiovascular protectant, antihyperglycemic, antipsoriatic, anti-inflammatory, anti-angiogenic, antiproliferative, antiobesity, and antioxidant activities.^{6–8} Antioxidant activity has been well-studied from a different angle, including the inactivation of reactive oxygen species (ROS), free radicals scavenging, chelation of pro-oxidative transition metals, and reducing power.⁹ Thus, these bioactive compounds share various therapeutic effects against several pathological diseases such as diabetes, cardiovascular diseases, psoriasis disease, and different types of cancer.^{8–11} The different plant parts and products of sea buckthorn, including fruits, seeds, leaves, pulp, peel, and twigs, consumed in various forms such as pulp and seed oil, fruit puree, leaf tea, and dietary supplements have been the object of many research studies over the past years due to its healthy properties. For example, the seeds of sea buckthorn have been reported to be used as a natural dietary supplement for eggs, meat, and other foods.¹² Furthermore, sea buckthorn leaf extracts, especially their flavonoid derivatives, have been reported to exert beneficial effects on diet-induced obese mice through the management of metabolic disorders such as inflammation, hepatic steatosis, and insulin resistance.¹³ Sea buckthorn products and by-products have been increasingly valued worldwide due to their natural bioactive compounds and health-promoting effects. Much attention has been devoted to the chemical and biological properties of sea buckthorn from different varieties in different areas of the world.¹⁴ Although the well-being and health benefits of sea buckthorn have been reported, especially on fruits, very little has been done on the chemical profile and bioactivity of sea buckthorn leaves. Similarly, in Algeria, this wild species distributed in the Mitidja plain, situated in the north of the country, was mainly used as a hedge plant. However, there is a lack of reports on the Algerian sea buckthorn in terms of bioactive compounds and nutritional and functional properties. Therefore, this study aimed to determine for the first time the chemical composition, antioxidant, and antihemolytic properties of sea buckthorn leaf extracts collected from the north-center of Algeria, to provide better insight into the nutritional and health benefits of consuming and valorizing sea buckthorn leaves, thus positioning them as potential and affordable healthy food options for the local community.

MATERIALS AND METHODS

Analytical grade standards, solvents, and reagents were used in the present study. DPPH, Folin–Ciocalteu reagent, gallic acid, and various standards were obtained from Sigma-Aldrich and Biochimica.

Sea buckthorn leaves were collected from the Mitidja region in Algeria during autumn. After drying, the leaves were macerated with 70% ethanol. The combined extracts were concentrated using a rotary evaporator at 40°C and then freeze-dried.

The proximate composition of sea buckthorn samples was determined according to the methods described by the Association of Official Analytical Chemists¹⁵ and following Biel and Jaroszewska method.¹⁶ Moisture content was determined by oven-drying at 105°C, while ash content was determined by incineration at 550°C. Crude fat was determined by Soxhlet extraction with hexane. Crude fiber was determined using a fiber analyzer. Determination was performed in triplicate. Carbohydrates were estimated according to the following formula:

$$\text{Carbohydrates \%} = 100 - \text{Crude protein} + \text{Crude fat} + \text{Ash} + \text{Crude fiber}$$

The mineral composition of the sea buckthorn leaves was determined following the method previously reported by Biel and Jaroszewska¹⁷ with some modifications. 500 mg of air-dried and ground sea buckthorn leaves were calcinated at 550°C for 8 h, dissolved in 0.6 M HCl, then heated at 60°C for 15 min. Then, the obtained solution was filtered and diluted to a final volume of 50 mL. Sodium and potassium were analyzed by flame photometry, while calcium and magnesium were determined using titrimetric method. Copper, iron, and nickel were measured using atomic absorption spectroscopy.¹⁵

Total phenolic content (TPC)

The total phenols of sea buckthorn leaves were quantified by spectrophotometric method at 765 nm according to Morgenstern et al.¹⁸ with some modifications using the Folin–Ciocalteu assay. Extraction of phenolics was done using 70 % ethanol at room temperature as previously described. TPC was calculated from the calibration curve of gallic acid.

Total flavonoids content (TFC)

The total content of flavonoids expressed as mg of catechin equivalents (CE) per g of dried matter of sea buckthorn leaves was determined by using aluminum chloride and sodium nitrite.¹⁹ Briefly, 500 μ L of the extract was mixed with 150 μ L of sodium nitrite and 2 mL of distilled water. After 6 min, 150 μ L of 10% aluminum chloride and 2 mL of 1 M sodium hydroxide were added. The absorbance was measured at 510 nm. The calibration curve was prepared using catechin.

Phenolic compounds were performed using LC-ESI-MS following Mighri et al.²⁰ Mass spectrometric analysis was performed using a quadrupole mass spectrometer in negative ion mode. For analysis, a C18 column was used, with a mobile phase consisting of 0.02% acetic acid in a mixture of H₂O/ACN (1:1) with linear isocratic elution

at a flow rate of 0.4 mL/min, and a temperature of 40°C, with an injection volume of 20 µL. The phenolic compounds were identified by comparing their mass spectra and retention times to 20 standards of 98% purity.

The antioxidant potential of the sea buckthorn extracts was assessed using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according to the method of Sánchez-Moreno et al.²¹ Briefly, the DPPH solution was prepared by dissolving 4 mg of DPPH powder in 100 mL methanol. Then, 1950 µL of DPPH was added to 50 µL of various concentrations of the extracts. The absorbance of samples was measured at 515 nm. Ascorbic and gallic acids were used as positive controls.

The antioxidant potential of the sea buckthorn extracts was also assessed by using the ABTS radical scavenging assay following the previous method of Dudonné et al.²² with some modifications. Ascorbic and gallic acids were used as the positive controls. The ABTS stock solution was prepared from 7 mM ABTS and 2.45 mM ammonium persulfate. After 16 h in the dark at room temperature, the stock solution was diluted with ethanol to an absorbance of 0.7 (±0.02). Then, 50 µL of the extracted sample was mixed with 1.5 mL ABTS⁺ solution for 3 min. The absorbance was determined at 734 nm.

The reducing power of sea buckthorn leaf extracts was measured according to the method described by Karagözler et al.²³ with some modifications. 1 mL of the extract at different concentrations was mixed with 2.5 mL of phosphate buffer solution 0.2 M (pH 6.6) and 2.5 mL of potassium ferrocyanide (1%). The mixture was incubated at 50°C for 20 min. After cooling down to room temperature, 2.5 mL of TCA (trichloroacetic acid) (10%) was added to stop the reaction. After centrifugation for 10 min at 3000 rpm, 2.5 mL of the supernatant was added to 2.5 mL of distilled water and 0.5 mL of ferric chloride. The absorbance was measured at 700 nm using water as a blank.

The anti-hemolytic activity of the leaf extract was assessed using the standard method described by Karim et al.²⁴ Briefly, 10 mL of blood from a voluntary person was collected and centrifuged for 10 min at 3000 rpm. The supernatant was removed, and the pellet was re-suspended in saline solution (0.9% NaCl) after being washed twice with PBS (0.2 M, pH 7.4). The same volume of the mixture extracted in PBS and erythrocyte suspension and incubated at room temperature for 5 min. After, 0.2 mL of H₂O₂ solution and 0.82 M PBS were added to the reaction mixture. The samples were again incubated at 37°C for 3 h. Then, the samples were centrifuged at 3000 rpm for 10 min and the absorbance of the supernatant was determined at 540 nm. Ascorbic acid was used as positive control.

RESULTS AND DISCUSSION

The bioactivity of plants has been widely studied because of their traditional, nutritional, and beneficial potential properties which are attributed mostly to their major nutrients including carbohydrates, proteins, lipids, vitamins, minerals, and phytochemical constituents. These phytochemicals, or natural bioactive compounds, include polyphenols, flavonoids, and phytosterols.⁵ At present, among the thousands of research articles on sea buckthorn plants, there have been

very few nutritional and bioactivity investigations done, especially on leaves.^{8,25,26}

This research study used hydroethanolic extract of sea buckthorn to study the chemical composition and the *in vitro* antioxidant effect. The composition of the extracting solution (70% ethanol) was selected based on the methods previously reported applied to sea buckthorn.²⁷ The extraction yield was 181.1 mg/g. Proximate analysis of sea buckthorn leaves was investigated to identify their major nutrients and energy content. In 100 g of dried leaves, the proximate analysis composition of the samples revealed 3.00 ± 0.29 g moisture and 61.51 g carbohydrates, of which 6.00 ± 0.02 were total fiber, followed by 13.4 ± 1.98 g of fat and 12.49 ± 0.13 g of total protein. These nutrients accounted for 404.6 kcal/100 g DW. Also, 6.60 ± 0.13 g ash/100 g dry matter was recorded in the sea buckthorn leaves. The protein content was consistent with the average values of proteins (12.03 g/100 g) reported by Kashif and Ullah²⁸ but it was lower than some other studies.^{16,29} The relevant protein content, along with its carbohydrate content and low moisture content, suggests the value of this product as a feed. Ash, protein, fat, and fiber contents were in concordance with those reported by Jaroszewska et al.³⁰ and showed higher concentrations of macroelements in sea buckthorn leaves than in the other parts of this plant. In the present study, proximate contents of the dried leaves showed low values of moisture (3%) and consequently a higher proportion of dry matter as compared to most fresh vegetables. The high values of dry matter are a consequence of the drying methods used. This may lead to preserving the quality and nutrient content in the leaves and extending shelf life.³¹ Fresh leaves may contain up to 71% moisture that can be reduced largely upon drying, reaching values of 4.7%^{28,32} for air-dried leaves, well in agreement with the values obtained in the present study.

Seven minerals were quantified in this study, including four major elements (calcium, potassium, sodium, and magnesium) and some relevant minor elements (copper and iron). Nickel was also investigated but was below the detection limit.

Among the major elements, calcium, sodium, and magnesium were the major mineral nutrients in sea buckthorn leaves, ranging from 600 to 905 mg/100 g DW (Table 1). Potassium was the least

TABLE 1 Mineral composition of sea buckthorn leaves extract (mg/100 g DW).

	Macroelements
Calcium (Ca)	904.70 ± 3.66
Sodium (Na)	842.60 ± 10.58
Magnesium (Mg)	841.26 ± 1.13
Potassium (K)	598.20 ± 4.42
	Microelements
Iron (Fe)	15.22 ± 0.07
Copper (Cu)	0.38 ± 0.03
Nickel (Ni)	ND

Note: Values are expressed as mean ± SD, n = 3. DW dry weight, SD standard deviations, ND not detected, ≤ 2 mg/100 g DW.

macroelement, being 1.5 times lower than the other major elements. Mineral content evaluation in sea buckthorn leaves revealed a high concentration of calcium, sodium, and magnesium followed by potassium. Calcium was the dominant element, with a concentration of 904.71 mg/100 g DW. These findings are in agreement with those reported by Tkacz et al.,³³ in which calcium was the dominant mineral, and the lowest percentage of macroelements was for potassium (964.97 mg Ca/100 g vs. 706.16 mg K/100 g). Sabir et al.³⁴ reported a different ranking on the major minerals with the highest concentration of potassium followed by magnesium and calcium. A similar ranking on the mineral content was also reported by Biel et al.,³⁵ with values of potassium three times higher than those found in the present study, with an average level of 1830 mg/100 g DW. The Ca:Mg ratio in the leaves of sea buckthorn was below 3, which was considered as the limit value of magnesium deficiency and may increase the risk of several diseases such as cancer, cardiovascular disease, and diabetes.³⁶ However, the values for both calcium and magnesium in the current study were three times lower than those reported in other studies, with an average level of 320 mg/100 g DW and 280 mg/100 g DW, respectively. Variable factors might affect mineral component contents including genetic, climatic, and soil characteristics.^{16,35} Regarding micronutrients, iron content was the highest (15.22 mg/100 g), followed by copper (0.38 mg/100 g). The copper level was consistent with the results of Jaroszewska et al.³⁷ who reported that the leaves of the sea buckthorn contained 0.39 mg Cu/100 g DW but much lower than that reported by Kashif and Ullah,²⁸ who found 3.93 mg/ 100 g DW, which is 10 times higher compared to the results mentioned above. A large variability has been also reported for iron content with levels as high as 22.4 mg/ 100 g DW²⁸ or as low as 4.60 mg/ 100 g DW.³⁷ As previously described for macrominerals, the content of these minor elements is strongly affected by soil characteristics.

The TPC of extracts, as determined by the Folin-Ciocalteu method, was 131.34 ± 0.07 mg GAE/ g extract DW, corresponding to 23.79 mg GAE/ g leaf DW, while the TFC was 43.00 ± 0.08 mg CE /g extract DW, corresponding to 7.79 mg CE/ g leaf DW. The TPC values reported in the literature vary widely. A broad similar observation to the values reported in the present study was reported by Bitová et al.,³⁸ who found a total phenolic content ranging from 71.6 to 103 mg GAE/g DW for samples from the Czech Republic, and by Cho et al.³⁹ who reported values ranging from 86.2 to 116.7 mg GAE/g DW on samples from South Korea at different harvesting times. Another Korean study⁴⁰ reported a variation in total polyphenol yield according to the ethanol ratio with the used solvent, in which 80% EtOH gave a TPC content of 285.6 mg GAE/g DW. Šně et al.⁴¹ also found that the TPC extracted with ethanol was higher than the current study's values (123.78 and 165.76 mg GAE/g DW). However, some other studies reported much lower total phenolic contents. For example, the ethanolic extract samples studied by Michel et al.⁴² contained half (65 mg GAE/g DW) of the value of the phenolics reported in the present study.

Similarly, the TPC recorded on leaves of four Romanian varieties ranged from 41.60 to 48.12 mg GAE/g DW. In addition, Korekar

et al.⁴³ reported that the TPC average value in the leaves was 63.30 mg GAE/g DW. Noteworthy, this TPC content was three-fold and two-fold higher than that Upadhyay et al.⁷ found in the aqueous and hydroalcoholic leaf extract of sea buckthorn, respectively. In addition, and surprisingly, Jaroszewska and Biel³⁷ reported average values extremely lower than that found in the present study, ranging from 7.06 to 10.69 mg/g DW on sea buckthorn leaves from Poland. Significant differences between the content of the phenolic compounds of sea buckthorn, method of extraction, varieties, and parts were reported elsewhere.^{44,45} Based on the TPC values of leaves measured in the current study and the cited literature, the TPC may vary according to the species, cultivars, genetic diversity, the method of extraction and quantification used, period, the part of the plant and the extracting solvent,^{18,38,43,46} type of locality and the year of harvest.³²

The TFC found in the studied samples was 43.00 ± 0.08 mg CAE/g DW. In another research study, reported by Criste et al.,¹⁴ the values ranged from 31.53 to 36.58 mg quercetin (QE)/g of fresh weight for four sea buckthorn varieties. The TPC of hydroethanolic extracts of sea buckthorn leaves from Poland was much lower (2.54 mg quercetin/g DW) than other values reported in the literature. On the contrary, TFC content in two distinct leaves extract of sea buckthorn collected from North-West India⁷ was 2- to 3-fold higher than that found in the present study.

LC-ESI-MS analysis tentatively identified 15 phenolic compounds in the sea buckthorn leaves hydroethanolic extract. These compounds can be grouped into six phenolic acids including quinic, gallic, o-coumaric, protocatechuic, rosmarinic acids and chlorogenic, and nine flavonoids including epicatechin, rutin, quercetin-3-o-galactoside, naringin, quercitrin, apigenin-7-o-glucoside, quercetin, naringenin and luteolin (Table 2). The higher phenolic compounds in the LC-ESI-MS analysis of sea buckthorn leaf extracts were quinic acid

TABLE 2 Amounts of phenolic compounds identified in Algerian sea buckthorn leaves.

Compounds	mg/100 g DW
Quinic acid	2637.84
Rutin	933.45
Naringin	399.11
Epicatechin	320.75
Chlorogenic acid	225.63
Hyperoside (quercetin-3-o-galactoside)	135.14
Quercitrin (quercetin-3-o-rhamnoside)	123.54
Apigenin-7-o-glucoside	59.07
Luteolin	49.15
Salviolinic acid	39.31
Naringenin	16.64
Protocatechuic acid	12.77
Rosmarinic acid	12.61
o-coumaric acid	11.99
Quercetin	5.36

and rutin, while the content of quercetin was very low. Furthermore, results showed that quinic acid was the dominant phenolic acid while rutin was the dominant flavonoid. The results showed a three-fold increase in the content of quinic acid compared with that of rutin in the leaf ethanolic extracts. In addition, results showed that quinic acid represents more than 50 % of the phenolic acids and flavonoids identified in the extract. In this regard, it has been stated that sea buckthorn is well known as a valuable source of polyphenols, including flavonoids, hydrolysable tannins, and phenolic acids,^{47,48} which have been also reported to have relevant biological properties such as antioxidant, antibacterial, and anti-inflammatory activities. Most of the results reported large variations of the identified phenolic compounds in the various plant parts of sea buckthorn. The most abundant flavonoid in sea buckthorn leaves was quercetin.⁴ Studies by Bittová et al.³⁸ reported the presence of catechin, gallic acid, p-coumaric acid, caffeic acid, ferulic acid, rutin (quercetin 3-rutinoside), and quercitrin (quercetin 3-rhamnoside) in sea buckthorn leaves. In another study, authors identified six phenolic compounds including rutin, quercetin-3-galactoside, quercetin-3-glucoside, myricetin, quercetin, and kaempferol.⁴⁹ Moreover, results of another preparation made from leaves reported the following compounds: catechin, epicatechin, ellagic acid, apigenin, and six flavonols among its quercetin and rutin detected. From all the leaf tea samples compared, ellagic acid was reported to be the major compound.⁵⁰ Such great variation of phenolic compounds as reported also by several studies^{14,51,52} was in concordance with the results obtained in the present study. For instance, Criste et al.¹⁴ reported that quercetin was predominant at levels ranging from 152 to 733 mg/100 g in leaf extracts. The later result was in accordance with the content of quercitrin present at 682 mg/100 g. In addition to that, quercitrin content was reported to increase over time. Besides quercitrin, quercetin-3-galactoside but not gallic acid was identified in the present study. Such variation in sea buckthorn composition may be due to many factors including extraction methodology, climatic conditions, harvesting time, cultivars, and subspecies.

Antioxidant activity, as assessed by DPPH and ABTS, is presented in Table 3 by the inhibitory concentration 50 (IC₅₀) in comparison with ascorbic acid and gallic acid as positive controls. The extract prepared using 70% EtOH exhibited a dose-dependent DPPH scavenging activity, with an IC₅₀ of 2.10 mg/g DW. Similarly, the percentage of inhibition of the sea buckthorn leaves extract (70% EtOH) by ABTS assay increased in a dose-dependent manner. The hydroethanolic extract exhibited 50% inhibition by ABTS at a value of 0.46 mg/g DW. The hydroethanolic extract showed strong ABTS free radical scavenging

TABLE 3 ABTS, DPPH radical scavenging activities in the sea buckthorn leaves as determined by IC₅₀.

Elements	ABTS (mg/g DW)	DPPH (mg/g DW)
Extract	0.46 ± 0.02	2.10 ± 0.01
Ascorbic acid	0.10 ± 0.01	2.34 ± 0.01
Gallic acid	0.02 ± 0.004	0.98 ± 0.02

Note: Values are expressed as mean ± SD, n = 3.

activity compared with the IC₅₀ values obtained for ascorbic acid (0.10 mg/g DW) and gallic acid (0.02 mg/g DW).

The DPPH-free radical scavenging capacity of hydroethanolic extract was comparable to the capacity of ascorbic acid with IC₅₀ values of 2.34 mg/g DW. However, gallic acid showed a much more powerful radical scavenging activity when evaluated through DPPH assay, with IC₅₀ of 0.98 mg/g DW. The tested hydroethanolic extract exhibited the highest antioxidant activity at 0.2 mg/mL (80%). However, the IC₅₀ of the extract as measured by ABTS assay was 10 times higher than gallic acid and 40 times higher than ascorbic acid values. These results showed that sea buckthorn leaves extract exhibited a good capacity to neutralize the DPPH radical as compared to ascorbic acid and a low capacity to decrease ABTS radicals as compared to ascorbic and gallic acids. It has been described that both tests tend to have a good correlation,⁵¹ although this was not the case in the present study. DPPH is more soluble in an alcoholic medium than ABTS, and the antioxidant compounds already extracted through a hydroalcoholic solvent may react more easily with DPPH radical, offering a better radical scavenging capacity. The high antioxidant activity shown in the present study could be related to the high contents of phytochemicals in the leaf extracts such as phenolic acids and flavonoids, and most of the time specific phenolic compounds. Previous works indicate that polyphenols and flavonoids may have a protective effect against free radicals and their presence has been reported by many studies in leaves, berries, and seeds of sea buckthorn⁵² reporting a high free radical scavenging activity (IC₅₀ = 1.86 µg/mL). These results supported the hypothesis that phenolic compounds function in antioxidant pathways as reducing agents, hydrogen donors, and singlet-oxygen quenchers. In addition, the new research conducted by Shan et al.⁵³ on crude and purified sea buckthorn leaves revealed stronger antioxidant activity than the present sea buckthorn leaves extract with IC₅₀ values of 0.016 and 0.002 mg/mL, respectively.

The extract from sea buckthorn leaves had a higher reducing power than ascorbic acid at the same concentration as seen in Figure 1. Similar results were obtained in some previous works⁴⁹ and

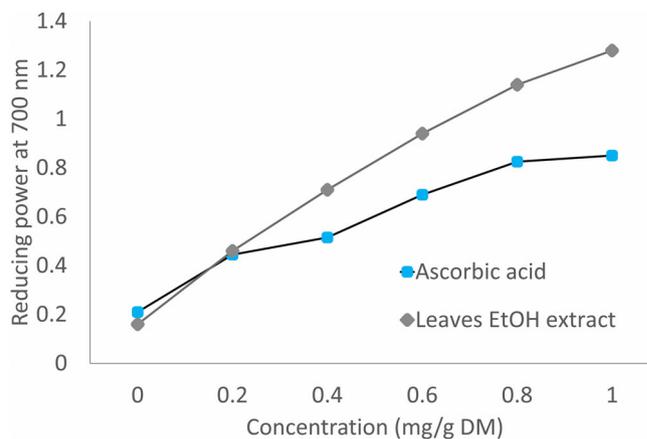


FIGURE 1 Dose-dependent reducing power activity of sea buckthorn ethanolic extracts. Data present means ± SD (n = 3).

can be attributed to the abundance of other natural bioactive compounds in the extract, mainly polyphenols. These natural bioactive compounds present in the sea buckthorn leaves may act as reducing agents and could donate electrons.

The FRAP assay was done to determine the ability to reduce ferricyanide Fe (III) to ferrous form Fe(II). A dose-dependent increase in the absorbance of the reaction for all the tested extracts and standards was observed (Figure 1). The results showed that increasing the sample concentration resulted in an increase in ferric-reducing activity. The hydroethanolic extract exhibited the highest reducing power at the maximum tested concentration of 1 mg/g DW.

To determine the protective effects of sea buckthorn leaf extracts, hemolysis was induced in human erythrocytes using hydrogen peroxide. Inhibition of hemolysis was evaluated to determine whether bioactive compounds, such as polyphenols, present in the hydroethanolic extract, were able to counterbalance oxidative damage on human erythrocytes. Increasing concentrations of the hydroethanolic extract exhibited a dose-dependent antihemolysis effect. IC₅₀ value for the leaf extract was 0.76 mg/mL compared to 0.51 mg/mL for ascorbic acid. Thus, sea buckthorn leaf extract exhibited significant antihemolytic activity, closer to the ascorbic acid. Similarly, previous research has reported a strong inhibitory effect of sea buckthorn extracts on hydrogen peroxide hemolytic effect.⁵⁴ This activity is likely attributed to the presence of polyphenols, such as quercetin, which have been reported to protect against oxidative hemolysis. Thus, such observations were explained by the preservation of the intact state membrane, interaction with membrane proteins and lipids,⁵⁵ inhibition or reduction of free radical levels, and increasing resistance of erythrocytes to oxidative damage. The lack of toxic effect of sea buckthorn extracts on human erythrocytes reinforces the antihemolytic activity of the extract against H₂O₂-induced hemolysis.

CONCLUSION

This study highlights the significant nutritional and antioxidant properties of Algerian sea buckthorn leaves for the first time. The mineral analysis showed substantial levels of calcium, sodium, and magnesium. The phenolic profile was particularly noteworthy, with a total phenolic content (TPC) of 131.34 mg GAE/g extract DW and a total flavonoid content (TFC) of 43.00 mg CE/g extract DW. The LC-ESI-MS analysis identified 15 phenolic compounds, dominated by quinic acid and rutin. These compounds contribute to the strong antioxidant activity, as confirmed by the DPPH and ABTS assays. The study also demonstrated that the extract exhibited potent reducing power and antihemolytic activity. These findings suggest that sea buckthorn leaves could be a valuable source of bioactive compounds in health-promoting applications, particularly in the food and pharmaceutical industries.

Further research is recommended to explore their mechanisms of action, which could pave the way for new applications and products derived from sea buckthorn leaves. This study sets a foundation for

future investigations into the health benefits and potential uses of this remarkable plant.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data sets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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