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Exploring the In Vitro Antioxidant Activity of Organic Extracts from the Red Alga Laurencia obtusa Found Along the Chabahar Coast

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A B S T R A C T

Objective: The discovery of antioxidant substances with great nutraceutical value and minor side effects for applications in preventive medicine, pharmaceuticals, and the food industry is a rapidly growing area of research. Consequently, algae, particularly marine macroalgae, are increasingly recognized as valuable sources of bioactive compounds with properties such as antioxidant, antitumor, and antihypertensive effects. This makes them ideal for use in the food and pharmaceutical industries, and they have attracted significant research interest. The algae along the Chabahar coast are rich in potential for future research. They are a potential resource for several studies. This study investigated the antioxidant characteristics of the red alga *Laurencia obtusa* collected from Chabahar Bay *in vitro*.

Methods: Algae were collected from the intertidal zone of Chabahar at a certain depth during the peak growth season. The process of using seawater and then fresh water for washing was followed by drying and grinding the algae. The extraction process was conducted using a maceration technique with an algae-to-solvent ratio of 1:10 over a 24-hour period. The solvents used were methanol, hexane, dichloromethane, and chloroform, each at concentrations of 0.01, 0.1, and 1 mg/mL. Antioxidant activity was assessed *in vitro* through DPPH free radical scavenging, ferrous ion chelating activity, and reducing power assays, with each assay performed in triplicates. In the DPPH and reducing power assays, ascorbic acid served as the positive control, while EDTA was used as the positive control in the chelating assay. Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Data processing was performed using GraphPad Prism 7 software, and the graphs and determination of IC_{50} were determined in Excel by applying linear regression of the different concentrations against the responses to obtain the linear formula.

Results: The assays indicated that the chloroform extract (1.38 mg/mL) exhibited the lowest IC_{50} value and the highest DPPH free radical scavenging activity for *L. obtusa*. The scavenging activity of the chloroform extract was significantly greater ($p < 0.0001$) at all tested concentrations than that of the hexane and dichloromethane extracts. Conversely, the methanol extract exhibited the lowest radical-scavenging activity ($p < 0.05$). In the chelating assay, the IC_{50} value for chloroform (1.95 mg/mL) was higher than that for hexane (2.61 mg/mL), dichloromethane (4.86 mg/mL), and methanol (5.44 mg/mL) ($p < 0.05$). The highest reducing power was observed in the hexane extract, followed in decreasing order by the chloroform, methanol, and dichloromethane extracts. It was concluded that the increase in concentration of the active substance increased the antioxidant activity of the extracts under study, with significant differences noted at 1 mg/mL compared to other concentrations in the different treatments ($p < 0.05$).

Conclusion: Low-polarity organic solvents are better solvents than high-polarity solvents for extracting antioxidants from L. obtusa, as they yield extracts with the highest in vitro activity. The extracts contain antioxidant compounds with a high electron-donating capacity and metal ion-chelating power. It is advisable to identify and purify the antioxidant compounds found in the chloroform and hexane extracts of *L. obtusa.*

Keywords: *Laurencia obtusa*, Marine algae, Antioxidant properties, Free radicals, Chelating, Reduction power

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Introduction

Algae encompass a broad array of diverse organisms belonging to various phylogenetic groups. Macroalgae are categorized into three primary divisions: Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyceae (brown algae) [1, 2]. In general, algae are photosynthetic organisms similar to plants and are typically aquatic. These organisms do not have genuine roots, stems, leaves, or vascular tissues and feature basic reproductive structures.[3]. Marine algae are categorized into three broad groups based on their color: brown (Phaeophyta), red (Rhodophyta), and green (Chlorophyta) algae. Nevertheless, this classification is determined not only by color but also by other fundamental distinctions, including ultrastructural and biochemical characteristics, such as photosynthetic pigments, storage compounds, and cell wall composition.[4].

Algae are exposed to a wide range of environmental stresses, including light, rapid temperature fluctuations, osmotic stress, and desiccation. Nevertheless, they are rarely affected by photodynamic damage, free radicals, or other potent oxidizing agents [5]. Most researchers consider marine algae to contain a broad spectrum of bioactive compounds that can act as potent antioxidants and exhibit antibacterial, antiviral, and antifungal activities [6, 7]. The antioxidant activity of algae is attributed to the presence of phenolic compounds, terpenes, acetogenins, indoles, and volatile halogenated hydrocarbons [8,9]. These natural antioxidant compounds have attracted considerable attention as alternatives to synthetic antioxidants for preventing oxidation in food systems [10,11].

Many research studies have highlighted the antioxidant capabilities of marine algae [12-16]. These studies indicate that each algal species possesses various antioxidant properties, ranging from weak to very strong, and each new species requires independent investigation to determine the antioxidant value of its extract.

A variety of algal species can be found along the shores of Chabahar Bay, many of which have yet to be explored for their antioxidant capabilities. Among these is *Laurencia obtusa*, a type of red seaweed (Rhodophyta) from the Rhodomelaceae family, which is commonly found in warm and temperate waters around the world [17, 18]. This species typically grows on rocky substrates, often at depths ranging from 0.5 to 20 m in areas characterized by strong water movement [17, 19]. As a primary producer, *L. obtusa* contributes significantly to coastal productivity and provides essential habitats and nursery grounds for various macroinvertebrates [20]. Furthermore, this species plays a crucial ecological role in chemical defense by producing secondary metabolites that deter herbivores, such as crabs, sea urchins, and fish [17, 21]. *Laurencia obtusa* is renowned for producing a remarkable diversity of halogenated secondary metabolites, particularly sesquiterpenes, diterpenes, C_{15}-acetogenins, and triterpenes [18, 22]. The halogenated nature of these compounds, especially brominated derivatives, is a distinctive feature of the genus *Laurencia*. These compounds have a wide range of potential pharmaceutical applications, including anticancer [23-25], antioxidant [26-28], antimicrobial [28, 29], gastroprotective, immunomodulatory [30,31], apoptotic [24,32], and anti-inflammatory activities [31]. The antioxidant properties and selective cytotoxicity of L. obtusa against cancer cells, coupled with its rich nutritional profile, render it a promising candidate for the development of bioactive compounds for nutraceutical and pharmaceutical applications.

The antioxidant potential of *Laurencia obtusa* varies considerably based on extraction methods and geographical origin, with studies reporting DPPH radical scavenging activity ranging from 8.2% to 60.8% and total phenolic content between 7.83 and 54.1 mg GAE/g dry weight [26, 27, 33]. Compared to other Laurencia species, *L. papillosa* demonstrates comparable or slightly higher activity, with generally higher phenolic content (45-60 mg GAE/g) and strong DPPH scavenging properties [32,34]. Similarly, *L. catarinensis* and *L. majuscula* show significant antioxidant capacity, although activity levels vary, with IC_{50} values of 5.59 µg/mL for *L. catarinensis* and 14.3 µg/mL for *L. majuscula* [29].

Among other red algal genera, Gracilaria species, including *G. caudata*, demonstrate DPPH scavenging activity of up to 85% with a phenolic content of 50-120 mg GAE/g, considerably higher than *that of L. obtusa* [35, 36]. *Hypnea musciformis* has significant

potential, exhibiting 65-70% DPPH scavenging activity at a concentration of 5 mg/mL, which is comparable to that of synthetic antioxidants [37]. In contrast, *Palmaria palmata* exhibits moderate activity similar to *L. obtusa* (40-50% at 2 mg/mL) [38], while Porphyra species show lower activity (20-35% at 2 mg/mL) with a phenolic content of 5-15 mg GAE/g [39]. *Jania rubens* demonstrated moderate potential comparable to *L. obtusa*, with 35-45% DPPH scavenging activity at 1 mg/mL [40].

The antioxidant activity of *L. obtusa* is significant and varies depending on the geographical region. Therefore, research on this seaweed species in the geographical area of the Oman Sea is essential. In Iran, its distribution occurs more or less in Hormozgan and Sistan and Baluchestan provinces during autumn, winter, and spring [41]. The lack of sufficient information regarding the antioxidant properties of algae in this region prompted this study on *Laurencia obtusa*. Consequently, this study examined the antioxidant properties of organic extracts from *Laurencia obtusa* under *in vitro* conditions.

Materials and Methods

Algal Collection and Extraction

Approximately 3 kg of fresh samples of the red algae *Laurencia obtusa* were gathered from the Chabahar coast, located at geographical coordinates 60°37'45" E longitude and 25°17'45" N latitude, with an elevation of 10 meters above sea level. Sampling was performed by hand separation at the lowest tide in autumn 2023. After removing the unusable components, the samples were repeatedly rinsed with saline seawater to eliminate potential impurities. Seaweeds were set on the polystriofoam container and transferred immediately to the laboratory of the university within 30 min. Subsequently, they were rinsed once with fresh water, freeze-dried using a freeze-dryer (Jalteb, Iran), and ground into a powder using an electric mill (Model: AR110O10). For extract preparation the next day, 5 g of dried algal powder was extracted at a 1:20 ratio (algae: organic solvent) in 100 mL of the following solvents selected based on their polarity and efficacy in recovering antioxidant compounds from *Laurencia obtusa* using the maceration method at room temperature: a) Methanol (polarity index: 5.1), the most polar solvent employed, was selected primarily for extracting hydrophilic antioxidant compounds such as polyphenols and flavonoids [27, 28]. b) Chloroform (polarity index: 4.1) has been extensively utilized for antioxidant extraction from *L. obtusa* because of its effectiveness in recovering compounds of moderate polarity, including flavonoid aglycones and terpenoids [27, 28]. C) Dichloromethane (polarity index: 3.1) is an intermediate-polarity solvent used to extract a broad range of secondary metabolites [19]. d) Hexane (polarity index: 0.1), the least polar solvent, was specifically chosen to extract lipophilic antioxidant compounds, including carotenoids and non-polar terpenes [42]. The submerged algae were sonicated for 3 min at 50% power and then shaken on a magnetic stirrer for 24 h to extract the bioactive compounds from the algae. The supernatant was filtered using 0.45 µm membrane filters. Subsequently, the solvents were evaporated under a chemical fume hood, and the collected samples were stored at -20°C until further analysis. The extract was prepared in triplicate.

Antioxidant Activity

DPPH Free Radical Scavenging Assay

To evaluate the antioxidant activity, stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals were used, following the procedure outlined by Brand-Williams et al. [43]. A total of 2 mL of the extract was combined with 2 mL of a 0.16 mM methanolic DPPH radical solution. This mixture was thoroughly agitated using a vortex mixer set at 2500 rpm for one minute and then incubated in the dark at room temperature for 30 min. The absorbance of the solution was recorded at 517 nm using a spectrophotometer. The ability of the extract to scavenge DPPH free radicals was determined using formula (1) and expressed as a percentage of RSA.

Formula (1): $RSA\% = [(A_{control} - A_{sample})/A_{control}] \times 100$

Asample represents the absorbance of the sample measured after a certain period, and Acontrol denotes the absorbance of the DPPH solution in the absence of

the sample. Ascorbic acid (2 mg/mL) was used as a positive control.

Iron-chelating activity

Chelating activity was assessed as described by Dinis et al. [44]. The extracts were prepared at various concentrations (0.01, 0.1, 0.5, and 1 mg/mL). A volume of 3.7 mL of the extract was combined with 0.1 mL of ferric chloride and allowed to stand for 3 min. Then, 0.2 mL of ferrozine was added, and the mixture was thoroughly shaken. After a 10-minute incubation at room temperature, the absorbance was measured at 562 nm. A negative control, excluding the samples, was prepared using the same procedure. EDTA (0.1 mg/mL) served as a positive control. Chelating activity was determined using Equation (2):

Formula (2): Inhibition percentage of ferrous ion

$$= [(A0 – A1)/A0] \times 100$$

The absorbance of the control is denoted by A0, and A1 indicates the absorbance of the extract or control. The control group consisted of $FeCl_2$, ferrozine, and distilled water.

Reducing Power Test

To assess the reducing power, a modified version of the method described by Oyaizu [45] was employed. Solutions of the extract at different concentrations (0.01, 0.1, 0.5, and 1 mg/mL) were prepared, and 1 mL of each solution was combined with 1 mL of 0.2 M phosphate buffer at pH 6.6. Subsequently, 1 mL of 1% potassium ferricyanide was added, and the mixture was incubated at 50°C for 20 min. After incubation, 1 mL of 10% trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 5000 rpm for 10 min. Two milliliters of The resulting supernatant was, mixed with 2 mL of distilled water, and 0.4 mL of 0.1% ferric chloride solution was added. The absorbance was measured at 700 nm after 10 min. An increase in absorbance indicated an increase in reducing power. Ascorbic acid (0.02 mg/mL) was used as a positive control.

Statistical analysis

Following the confirmation of variance homogeneity via Levene's test and normality through the Shapiro-Wilk test, a one-way analysis of variance was conducted to assess significant differences. Tukey's test was used to compare the means across different groups. The analyses were performed in triplicate, and the results are presented as mean ± standard deviation. Significance calculations and graph plotting were performed using GraphPad Prism (version 7) and Microsoft Excel. The IC50 values for DPPH and metal chelation were calculated using Excel 2018 by linear regression of four different concentration points against the antioxidative properties and obtaining the line equation.

Results

The results of the antioxidant capacity assay using the DPPH free radical scavenging test for *L. obtusa* (Figure 1) showed that the scavenging of DPPH free radicals increased as the concentration of the extract increased. At a concentration of 1 mg/mL, all extracts showed greater DPPH free radical scavenging activity than at other concentrations ($p<0.0001$). Additionally, when comparing solvents, the chloroform extract exhibited a significantly higher DPPH free radical scavenging capacity than the other extracts at all concentrations. Conversely, the methanol and dichloromethane extracts displayed the lowest DPPH free radical-scavenging capacity compared to the other extracts. Concentrations lower than 0.5 mg/mL of these two extracts did not show any significant difference ($p>0.05$).

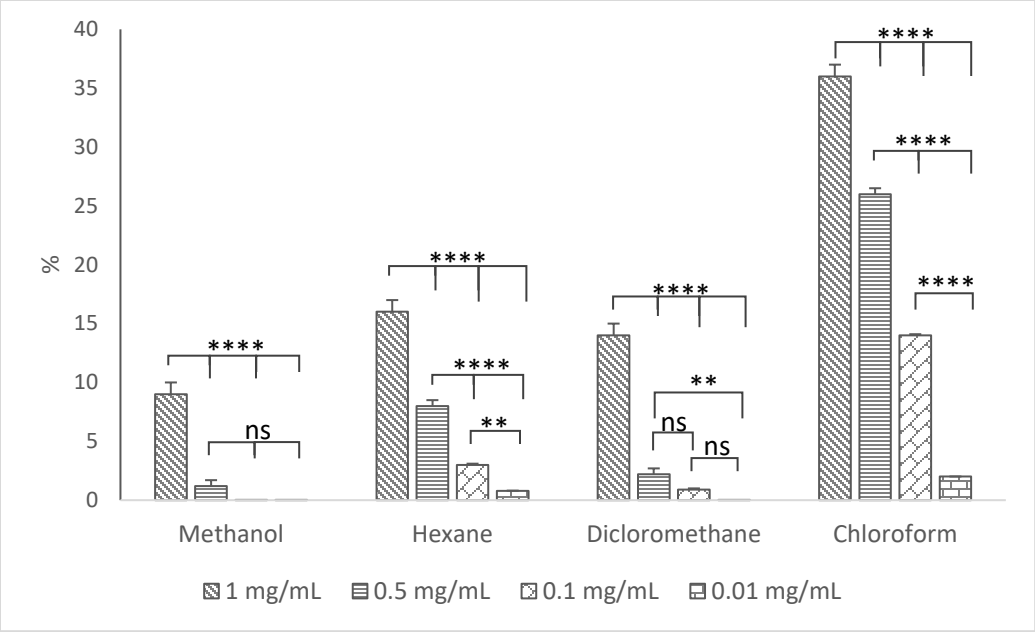
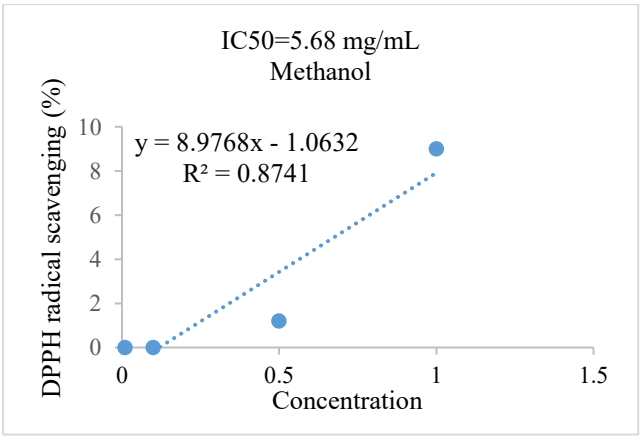
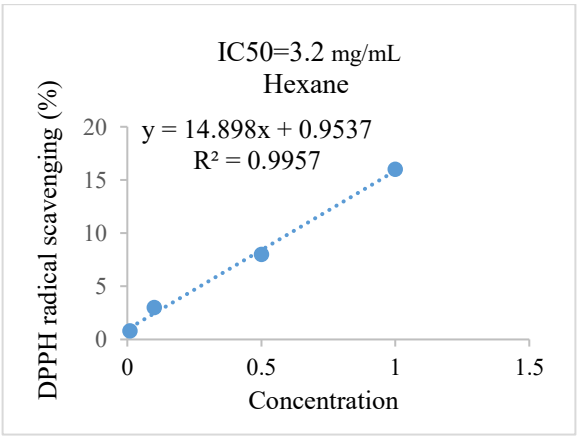


Figure 1: DPPH free radical scavenging activity (%) of organic extracts of the seaweed *Laurencia obtusa* at various concentrations. ** and **** showed significant difference at $p<0.01$ and $p<0.0001$, respectively. No significant difference was observed (“ns ”).

The IC50 value calculations indicated that chloroform, with an IC50 of 1.38 mg/mL, exhibited the greatest activity. Hexane (IC50 = 3.12 mg/mL) and dichloromethane (3.77 mg/mL) exhibited moderate activity. Methanol, with an IC50 of 5.65 mg/mL, demonstrated the weakest DPPH free radical scavenging effect (Figure 2).





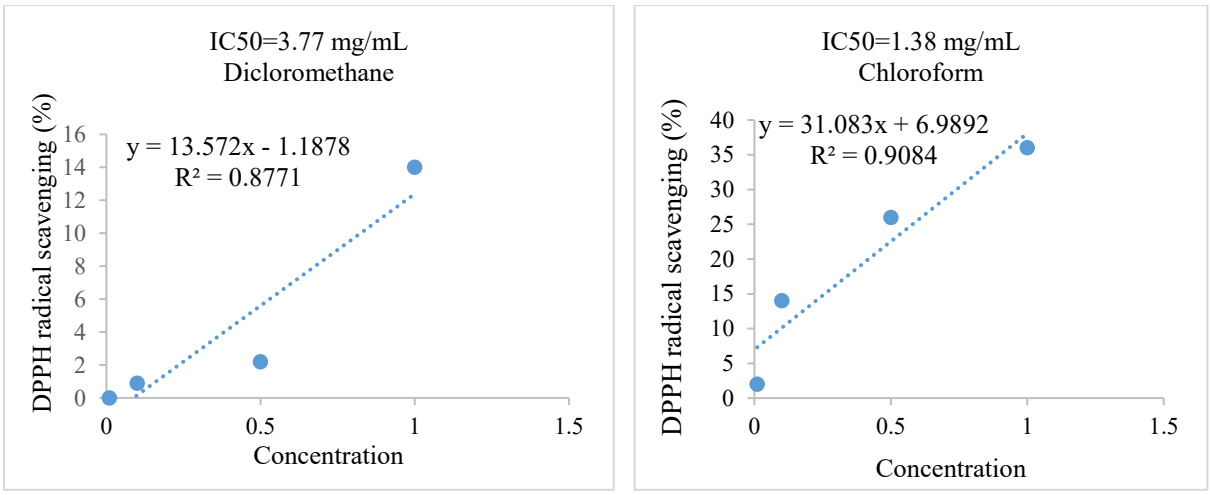


Figure 2: Linear regression graphs for estimating the IC_{50} of DPPH free radical scavenging by methanolic, hexane, dichloromethane, and chloroform extracts of *L. obtusa*.

The chelating properties of four different *L. obtusa* extracts (methanol, hexane, dichloromethane, and chloroform) were assessed over a concentration range of 0.01–1 mg/mL, and the findings were presented as a percentage of chelation (Figure 3). All extracts demonstrated a consistent relationship in which chelation increased with higher doses. Contrary to our initial expectations, the methanol extract demonstrated the weakest metal-chelating efficacy across most tested concentrations, exhibiting the lowest capacity to sequester metal ions, even at 1 mg/mL. In contrast, the chloroform extract displayed the most potent activity, achieving the maximum chelating percentage at the highest concentration tested. The hexane and dichloromethane extracts showed intermediate activities, although neither reached the efficacy of the chloroform extract. The chelating activity showed a statistically significant difference between the lowest and the most effective concentration of 1 mg/mL ($P < 0.0001$). This result highlights that the concentration of the extracting solvent plays a crucial role in determining the metal chelation effectiveness of the algal biomass.

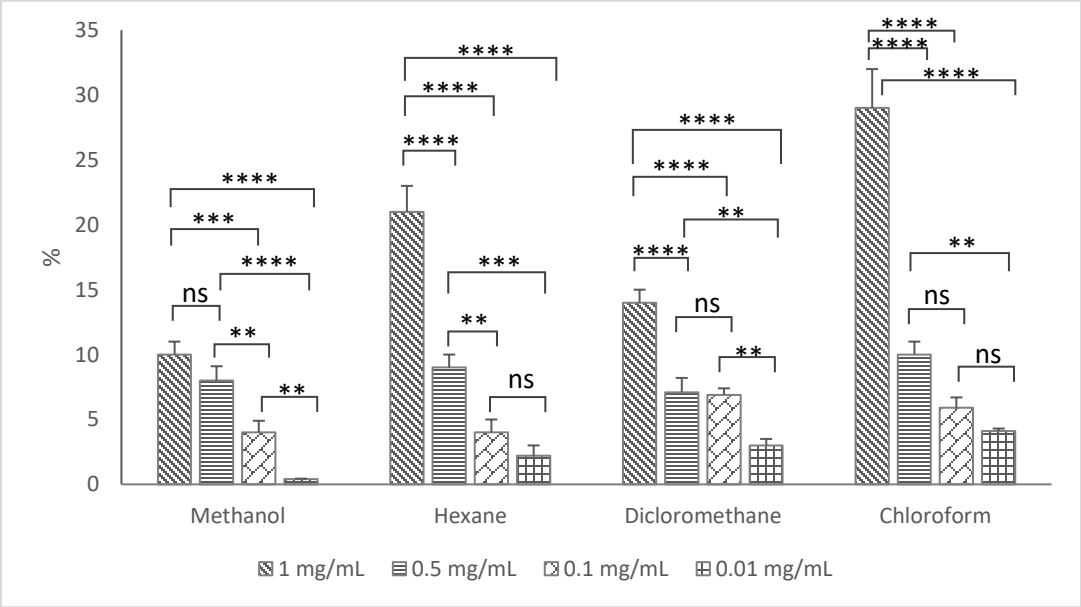


Figure 3: Chelating activity of organic extracts from *L. obtusa* at different concentrations. **, *** and **** showed significant difference at p<0.01, p<0.001 and p<0.0001, respectively. No significant difference was observed (“ns ”).

Furthermore, as the extract concentration increased, the chelating effect of the extracts increased with greater chelating capacity. Based on the IC50 values, the chelating effect was as follows: chloroform > hexane > dichloromethane > methanol. Therefore, the chelating capacity was the highest in chloroform and the lowest in methanol (Figure 4).

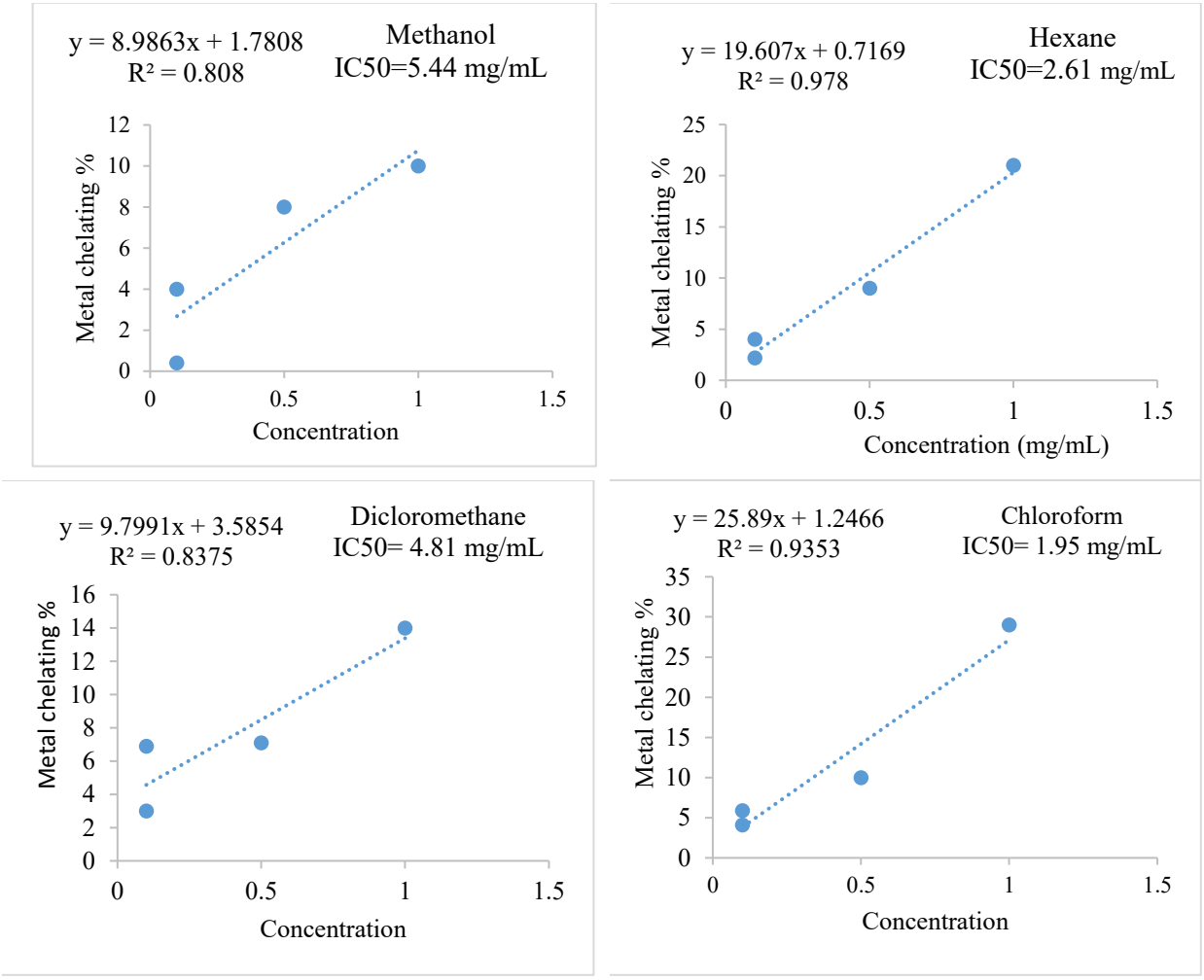


Figure 4: Linear regression graphs for estimating the IC_{50} of metal chelating activity by methanolic, hexane, dichloromethane, and chloroform extracts of *L. obtusa*.

The reducing power of the algal extracts was quantified by spectrophotometric absorbance, with higher absorbance values indicating greater reducing activity. This assessment was conducted at concentrations of 0.01, 0.1, 0.5, and 1 mg/mL (Figure 5). All extracts exhibited a concentration-dependent increase in their reducing power. Among the samples analyzed, the methanol extract consistently exhibited the lowest absorbance values across all concentrations, indicating the weakest reducing potential. Conversely,

the hexane extract displayed the highest reducing power, reaching a maximum absorbance of approximately 0.35 at 1 mg/mL, which markedly exceeded the values of the other extracts. The dichloromethane and chloroform extracts demonstrated moderate reducing activities, with their absorbance values remaining intermediate between those of the methanol and hexane extracts throughout the concentration range. The difference in reducing power between the highest extract concentration and the other concentrations was statistically significant ($P < 0.0001$).

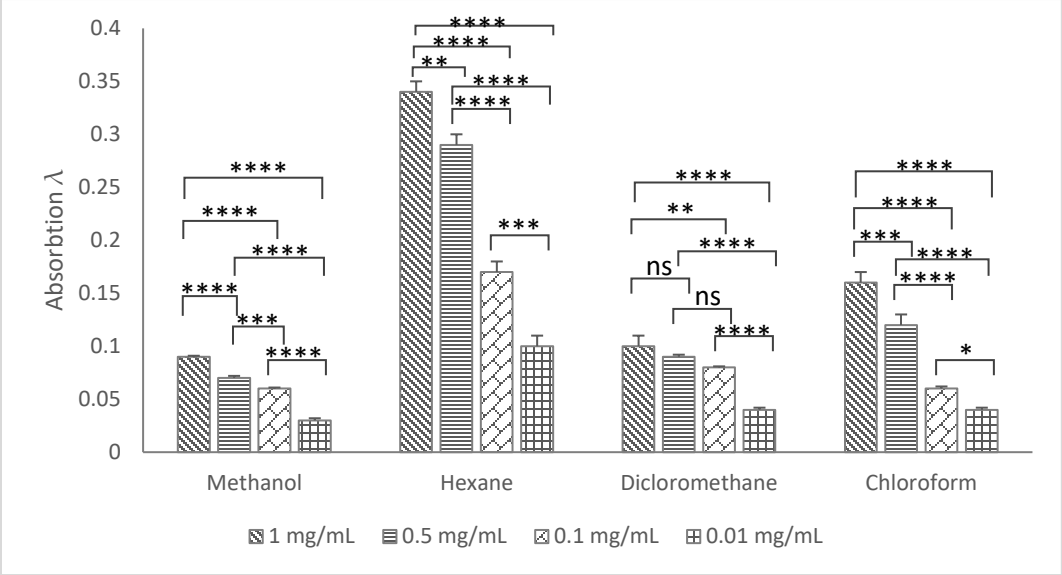


Figure 5: Reduction power of organic extracts from *L. obtusa* at different concentrations. *, **, *** and **** showed significant difference at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$, respectively. No significant difference was observed ("ns ").

Discussion

There are multiple methods for determining antioxidant activity. The chemical complexity of extracts often consists of various compounds with different functional groups, polarities, and chemical behaviors, which can lead to discrepancies in the results depending on the assay used. Consequently, applying multiple approaches to evaluate the antioxidant capacity of extracts is both useful and essential [46]. Based on the results obtained from the antioxidant capacity assays, *L. obtusa* possesses high potential for iron ion reduction and electron donation, iron chelation, and DPPH free radical-scavenging activities.

The DPPH assay provides fundamental information on the radical-scavenging activity of plant extracts. This technique is favored for assessing antioxidant activity because of its shorter duration compared to other assays. The IC50 value calculation results indicated that chloroform, with an IC50 value of 1.38 mg/mL, demonstrated the greatest activity, whereas methanol, with an IC50 of 5.65 mg/mL, exhibited the weakest DPPH free radical scavenging effect. Previous research has shown that IC50 values can differ based on the algal species and solvent used. The antioxidant activity of *L. obtusa* has been evaluated using various methods. El-Shenody et al. [27] reported a DPPH radical scavenging activity of 8.2% for *L. obtusa* and a total antioxidant capacity of 168.9 mg ascorbic acid

equivalent. In a separate investigation, Al Monla et al. [33] found that the protein extract from *L. obtusa* gathered along the Lebanese coast showed a DPPH radical scavenging activity of 60.8% at a concentration of 500 µg/mL, which was notably greater than that of the green alga *Ulva lactuca*. Demirel et al. [28] evaluated the antioxidant properties of different solvent extracts and essential oils derived from *L. obtusa* and *L. obtusa* var. pyramidata collected from the Turkish coast using DPPH, ABTS, and β-carotene bleaching assays. Their findings revealed that the chloroform extracts from both species demonstrated the most significant antioxidant activity. Some species predominantly contain non-polar and semi-polar antioxidant compounds; therefore, solvents with moderate polarity, such as chloroform and ethyl acetate, exhibit the highest antioxidant activity. The strong effectiveness of chloroform in scavenging DPPH radicals observed in this study can be attributed to the moderate polarity of the antioxidant compounds found in the algae tested. As chloroform is both a polar and non-polar solvent, it can be a more suitable solvent than solvents such as methanol for extracting existing compounds. Studies have demonstrated that the choice of solvent plays a decisive role in the efficiency of antioxidant compound extraction. Demirel et al. [28] conducted a systematic study to explore how three solvents with varying polarities, methanol (polarity index of 5.1), chloroform (polarity index of 4.1), and hexane (polarity index of 0.1), influence the extraction of antioxidant compounds from *L. obtusa*. Their findings revealed that among the solvents tested, the chloroform extract of *L. obtusa* demonstrated the greatest antioxidant activity [28]. This extract demonstrated ABTS radical scavenging activity between 42-44% and β-carotene bleaching inhibition between 64-66%, which were significantly higher than those of the methanolic and hexane extracts [28]. Chloroform, with a polarity index of 4.1, is considered the "optimal point" for extracting phenolic compounds of moderate polarity. These compounds, which are the most significant antioxidant agents, are extracted most efficiently within this polarity range [28]. Chloroform selectively extracts flavonoid aglycones (sugar-free form), which often exhibit stronger antioxidant activity than their polar glycosides [28]. Furthermore, chloroform effectively removes sugars, proteins, and other polar compounds that may interfere with antioxidant assays [26]. Although the extraction yield with chloroform is lower than that with methanol, the chloroform extract contains the highest concentration of effective antioxidant compounds, resulting in higher specific activity [28].

Compared to other Laurencia species, *L. papillosa* exhibits antioxidant activity comparable to or slightly higher than that of *L. obtusa*. Murad et al. [32] reported that sulfated polysaccharides extracted from *L. papillosa* possess strong antioxidant activity with significant DPPH radical scavenging ability. Al-Enazi et al. [29] conducted an evaluation of the antioxidant activity of *L. catarinensis* and *L. majuscula* utilizing the DPPH method. The results indicated that *L. majuscula* exhibited DPPH radical scavenging activity of 77.07%, with an IC_{50} value of 14.3 µg/mL. In comparison, *L. catarinensis* demonstrated a scavenging activity of 77.6% with an IC_{50} of 5.59 µg/mL [29]. Both species showed higher antioxidant activity than *L. obtusa*.

Compared to other red algae, species of the genus Gracilaria typically exhibit higher antioxidant activity than *L. obtusa*. Sousa et al. [35] found that sulfated polysaccharides derived from *Gracilaria caudata* exhibited an 85% DPPH radical scavenging activity at a concentration of 1 mg/mL, with an IC_{50} value close to 0.5 mg/mL. The total phenolic compound content in Gracilaria species usually ranges between 50-120 mg gallic acid equivalent per gram, which is significantly higher than that of *L. obtusa* [36]. *Palmaria palmata* exhibits moderate antioxidant activity, similar to that of *L. obtusa*. According to Yuan et al. [38], extracts from *P. palmata* demonstrated DPPH radical scavenging activity ranging from 40% to 50% at a concentration of 2 mg/mL, with the total phenolic content being approximately 15-25 mg of gallic acid equivalent per gram, which is comparable to *that of L. obtusa*. Species of Porphyra, including *P. yezoensis* and *P. umbilicalis*, generally show lower antioxidant activity compared to *L. obtusa*. Research shows that the DPPH radical scavenging activity of these species varies between 20% and 35% when the concentration is 2 mg/mL, and the total phenolic content is between 5 and 15 mg of gallic acid equivalent per gram. [39]. *Jania rubens* displayed moderate antioxidant activity comparable to that of *L. obtusa*. Karabay-Yavasoglu et al. [40] J. rubens extracts demonstrated a DPPH radical scavenging activity ranging from 35% to 45%

at a concentration of 1 mg/mL, with a phenolic compound content of about 30 mg of gallic acid equivalent per gram.

The bioactive compounds responsible for the antioxidant activity in *L. obtusa* have been investigated. Phenolic compounds and flavonoids are the most important antioxidant constituents of *L. obtusa*. Farghl et al. [26] confirmed the presence of significant amounts of these compounds. Flavonoids play a protective role by neutralizing free radicals and inhibiting lipid peroxidation [47]. Additionally, the β-carotene content of *L. obtusa* was determined to be 0.51 mg per 100 g dry weight [26]. Carotenoids protect against oxidation by quenching singlet oxygen and inhibiting peroxyl radical formation [27]. In contrast, Lajili et al. [30] demonstrated that a sulfated polysaccharide extracted from *L. obtusa* possesses significant antioxidant activity, increasing GSH levels and reducing TBARS in the gastric tissue of rats. This polysaccharide, with a high molecular weight (336,900 g/mol) and sulfate content of 28.2%, exhibited an antioxidant IC_{50} value equivalent to 24 µg/mL in the DPPH assay [30]. Furthermore, Monteiro et al. [23] identified numerous sesquiterpenes and diterpenes in L. obtusa, including laurene, cuparene, and various terpenoid derivatives, using mass spectrometry. Some of these compounds may also possess antioxidant activities.

In all algal species, the tested solvents showed a direct relationship between DPPH radical scavenging and extract concentration. Higher concentrations of the extracts led to increased activity of total antioxidant compounds [48]. The antioxidant efficacy of compounds, such as phenolic compounds, in plant extracts is contingent upon the type and concentration of these compounds, as well as the number and position of hydroxyl groups on their aromatic rings. An increase in the concentration of antioxidant compounds directly augments the capacity of various extracts to neutralize the free radicals. At elevated concentrations, the higher number of active groups, such as hydroxyl groups, in the reaction medium enhances the likelihood of hydrogen donation to free radicals, thereby improving the free radical scavenging efficacy of the extract [49].

In the chelating assay, similar to the free radical test, the chloroform solvent, with an IC50 of 1.96, exhibited the highest chelating power. The iron (Fe^{2+}) chelating ability of marine algae is probably related to the presence of active antioxidant compounds, such as phenolic compounds. These compounds inhibit Fe^{2+} by binding to it, reducing its sensitizing power, and consequently decreasing the redox potential [50]. Chew et al. [51] stated that red and brown algae have a high capacity to chelate Fe^{2+} due to the presence of phenolic compounds such as phlorotannins. The chelating ability is significantly influenced by the distinct structure of the antioxidant compounds and the quantity and arrangement of hydroxyl groups [52, 53]. However, it should be noted that antioxidant properties are not solely related to the presence of phenolic or polyphenolic compounds in the extracts.

Studies have demonstrated that metal-chelating activity is strongly influenced by the type of solvent used for extraction. Karimzadeh et al. [54] investigated the chelating activity of various extracts from the closely related species *Laurencia snyderiae*, indicating that the chloroform extract demonstrated the greatest activity, with an IC_{50} value of 0.027 mg/mL. This solvent can extract compounds of moderate polarity, such as flavonoid aglycones and specific phenolic compounds containing hydroxyl and carbonyl groups suitable for metal chelation [54]. Similarly, Demirel et al. [28] demonstrated that the chloroform extract of *L. obtusa* contains the highest concentration of effective phenolic compounds capable of chelating metals. Furthermore, species of the genus Laurencia are rich in halogenated compounds, some of which possess chelating properties [17]. In contrast, the methanolic extract, despite its higher extraction yield, exhibited lower chelating activity, likely due to the simultaneous extraction of inactive polar compounds and sugars that compete with the active constituents [54].

Additionally, compounds such as polysaccharides, proteins, and peptides present in the extracts possess metal ion-chelating ability [49]. In this study, the highest reducing power of the algae was obtained using a different solvent, which differed from the outcomes of the free radical scavenging and chelating techniques. Notably, hexane demonstrated the greatest reducing activity. The reducing power indicates the capacity of antioxidant compounds to donate electrons and reduce the oxidized substances. This characteristic

is usually assessed by the transformation of Fe^{3+} to Fe^{2+} in the Ferric Reducing Antioxidant Power (FRAP) assay, where an increase in absorption at 700 nm signifies improved reducing power [54]. In their comprehensive study on *L. obtusa* and its variety pyramidata, Demirel et al. [28] evaluated the reducing power of various extracts using the FRAP method and found that the hexane extract exhibited moderate activity (43.4% at 2 mg/mL).

Research on other Laurencia species has revealed the presence of additional antioxidant compounds. A study on *Laurencia undulata* by Li et al. [55] showed that 5-hydroxymethyl-2-furfural (5-HMF) possesses significant reducing power and can be developed as a novel marine antioxidant. Furthermore, floridoside and di-isofloridoside isolated from *L. undulata* demonstrated antioxidant properties, including reducing power [56]. Studies have shown that chelating activity and reducing power are influenced by specific structural factors. Compounds with multiple hydroxyl groups in adjacent positions (e.g., flavonoids with ortho-dihydroxyl groups) typically exhibit higher activity [56]. The presence of a carbonyl group adjacent to the hydroxyl group can also enhance the stability of metal complexes or improve their electron-donating ability [54]. Additionally, compounds of medium molecular weight, such as those extracted with chloroform, often show higher activity than compounds with either high or low molecular weights [28].

Compared to other red algal species, sulfated polysaccharides from *Gracilaria caudata* have been reported to exhibit a high reducing power, with an IC_{50} of approximately 0.5 mg/mL [35]. Souza et al. [37] demonstrated that sulfated galactans from *Hypnea musciformis* possess significant metal chelating activity and reducing power, although their activity was lower compared to *L. obtusa*. Finally, Yuan et al. [38] reported that *Palmaria palmata* extracts have moderate reducing power, which is comparable to the results obtained for *L. obtusa*.

The differences in the reducing power of the extracts can be explained by the diversity in the types of compounds extracted by each solvent. The antioxidant activities of various phenolic and polyphenolic compounds differ according to their chemical structure. Additionally, during the extraction process, other compounds that dissolve well in alcoholic solutions are extracted along with phenolic compounds. Some of these compounds act as electron donors, leading to a greater reduction in ferric ions through electron acceptance, which in turn enhances the absorption intensity of the solution [57]. Studies have indicated that algal extracts rich in phenolic and flavonoid compounds exhibit potent antioxidant activity. Consequently, the ability to donate electrons (for reducing power) is more closely linked to the activity of specific antioxidant groups within these bioactive compounds [58, 59].

Conclusion

The analysis of *Laurencia obtusa* confirmed that this red alga possesses significant antioxidant potential. Among all the solvents tested, the chloroform extracts consistently demonstrated the highest specific antioxidant activity owing to the optimal polarity of chloroform, which selectively extracts moderately polar phenolic compounds and flavonoid aglycones while excluding interfering polar compounds. The antioxidant properties of *L. obtusa* extracts position this alga as a promising natural resource for pharmaceutical applications, including as gastroprotective agents. In the food industry, these extracts have potential as natural food preservatives, functional food ingredients, nutraceutical products, and natural colorants. Future studies should focus on bioassay-guided fractionation and structural analysis of active compounds using advanced spectroscopic methods to identify the specific molecules responsible for the observed antioxidant effects. Comprehensive *in vivo* efficacy studies in animal models are needed to validate the antioxidant effects, establish dose-response relationships, and assess pharmacokinetic profiles and safety margins. Comprehensive mechanistic studies should assess the anti-inflammatory effects by targeting the inhibition of pro-inflammatory mediators and suppression of the NF-κB and MAPK signaling pathways. Oxidative stress protection should be assessed in disease models, including hepatotoxicity, neurotoxicity, and cardiovascular conditions. Protection against macromolecular damage, including DNA, protein, and lipid oxidation, requires systematic evaluation using validated biomarkers. The synergistic effects of purified compounds or extracts with existing

antioxidants should be explored for their potential enhanced protective activity. Standardized extraction protocols must be developed for industrial-scale-up, followed by stability studies and formulation development to ensure commercial viability. Ultimately, progression to clinical trials for specific applications, such as gastric protection and antioxidant supplementation, will determine the therapeutic potential of this valuable marine resource.

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Authors' contributions

Ali Taheri: Proposed idea, supervised the research, reviewed and edited the manuscript; Sedigh Mohammadi was responsible for gathering the data, conducting the experiment, and writing the initial draft of the manuscript.

Conflict of interests

The authors declare that they have no conflicts of interest.

Ethical approval

This study did not involve humans or animals for experimentation; therefore, ethical approval was not applicable.

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