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Research Article

The effect of ohmic, high-pressure carbon dioxide (HPCD), and maceration extraction methods on bioactive compounds of Iranian brown algae (*Nizimuddinia zanardini*)

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Abstract

Marine algae are a rich source of bioactive compounds with promising applications as nutrients, functional food ingredients, and therapeutic agents. This study aimed to investigate the effects of temperature and time variables in ohmic, HPCD, and maceration extraction methods on extraction yield, total phenolic content, total phlorotannin content, total flavonoid content, and antioxidant activity (DPPH radical scavenging) of the Iranian brown seaweed *Nizimuddinia zanardini*. The results showed that the highest total phenolic and phlorotannin contents were obtained using ohmic (45°C for 120 min: 960.7 mg GAE/g, 1016.17 mg PGE/g), while the lowest values were obtained with the maceration method (45°C for 24 h: 307.96 mg GAE/g, 281.83 mg PGE/g). Moreover, the maceration method at 45°C for 24 h yielded the highest total flavonoid content (242.67 mg QE/g). The HPCD method at 30°C for 60 min exhibited the highest antioxidant activity (DPPH scavenging: 83.08%). In terms of extraction yield, the ohmic method at 45°C for 120 min achieved the highest value (33.21%). The findings of this study indicate that among the three extraction methods, the ohmic method demonstrated the highest efficiency in extracting bioactive compounds from *Nizimuddinia zanardini*.

Keywords: Ohmic, HPCD, Algae, Antioxidant activity, Total phenolic content, *Nizimuddinia zanardini*

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1. Introduction

Seaweeds, due to their rich content of polysaccharides, vitamins, minerals, and polyunsaturated fatty acids (PUFAs), while being low in calories and fat, form a major part of the diet in Asia. In addition to their nutritional value as raw marine vegetables, seaweeds are also rich in bioactive compounds such as phlorotannins, phloroglucinols, carotenoids, fucoxanthin, and fucoidan, which encourage consumers to consciously choose healthy foods [1]. Potential applications of seaweed extract or whole seaweed include functional compounds to enhance the nutritional, textural, and sensory properties of various food products. Additionally, the addition of seaweed is also considered to improve properties related to the prevention of various diseases (such as obesity, high blood pressure, and diabetes) [2].

Seaweeds are classified into three main categories based on their chemical structure and pigment distribution. These categories are brown algae *(Phaeophyta*), red algae (*Rhodophyta*), and green algae (*Chlorophyta*), with brown seaweeds reported to have the highest levels of bioactive compounds [3].

Extraction is one of the fundamental stages in what is known as the "Global Five-Step Recovery Process." This process is a key protocol for isolating and recovering valuable compounds from natural sources. The main stages of this process include: (a) macroscopic pretreatment, (b) separation of large and small molecules, (c) extraction, (d) purification, and (e) formation of the final product [4]. The extraction of bioactive compounds can be performed using either traditional or modern methods. Traditional solvent extraction methods, such as maceration, boiling, thermal reflux, Soxhlet extraction, and others, have been widely used in industry in recent decades due to their operational simplicity and minimal equipment requirements. However, due to long processing times, solvent consumption, and high energy requirements, these methods are considered inefficient. Therefore, over time, the use of innovative technologies has been considered [5]. Novel methods, often classified as green methods, offer several advantages, including reduced solvent usage, shorter extraction times, and operation at lower temperatures compared to conventional methods [3]. The ohmic method (OH) and high-pressure carbon dioxide (HPCD) are examples of these methods.

Ohmic heating is based on passing an electric current through materials to heat them uniformly and rapidly. This technology allows for precise temperature control and can significantly impact enhance membrane permeability and promote electroporation in cellular tissues. This phenomenon plays a key role in facilitating molecular diffusion in plant tissues and is used in processes such as drying, pasteurization, and extraction of bioactive compounds [6, 7]. High-pressure CO_2 can be used for the extraction of bioactive compounds from algae. In the HPCD extraction method, a liquid or gas becomes supercritical when its temperature and pressure exceed the critical point, which for carbon dioxide is 31°C and 71 bar. CO_2 is non-toxic and safe, and its application under high pressure makes the cell membrane more permeable, reduces intracellular pH, and causes changes in the balance of intracellular electrolytes. Ultimately, this leads to the selective passage of certain substances through the cell membrane [8, 9].

Many researchers have focused on the extraction of bioactive compounds from seaweed and plant sources. Pereira et al. (2021) evaluated the potential of using ohmic heating for the extraction of bioactive compounds from *Gracilaria vermiculophylla* seaweed. Extraction using an electric field was conducted at 82°C and a frequency of 2-8 V/cm, and the results were compared with conventional extraction methods. The extraction yield using the ohmic method was higher after 1 hour compared to the conventional method, and the use of this method led to a 16%

increase in the extraction of phenolic compounds. These results indicate that ohmic heating offers the advantage of reducing extraction time [6]. Bhat et al. (2017) used ohmic and conventional heating methods, alternating with different temperature (60°C to 90°C) and time (1 to 5 min) combinations, to enzymatically treat bottle gourd (*Lagenaria siceraria*). They examined the effect of these methods on the total phenolic content (TPC). The highest increase in TPC was observed at 80°C for 4 minutes using ohmic heating and at 90°C for 5 min using conventional thermal treatment. The ohmic enzyme-treated samples showed the highest extraction of phenolic compounds and better color of the extract compared to the other samples [10]. Coelho et al. (2019) optimized the extraction of bioactive compounds from tomato by-products using the ohmic extraction method. The ohmic extraction experiments were conducted in medium electric fields with varying intensities to evaluate potential non-thermal effects on the extraction process and their impact on the properties of the bioactive compounds. The optimal conditions for polyphenol extraction using the ohmic method were found to be at 70°C for 15 min with 70% ethanol, resulting in a 58% increase compared to the control [11]. Gahruie et al. (2020) examined the extraction of bioactive compounds from saffron petals using maceration and three new extraction methods, including ohmic heating-assisted extraction, ultrasound-assisted extraction, and microwave-assisted extraction. Based on the overall data analysis, ohmic heating was recommended as an optimal method for extracting saffron petal extract with the highest amount of bioactive compounds [12]. In the study by Gavahian and Chu (2022), phenolic compounds in pineapple core were significantly higher in ohmic heating compared to the conventional treatment (using an extraction chamber similar to the ohmic system, along with a heating system based on hot oil bath at 100°C for 60 min). A similar trend was observed in antioxidant activity. Additionally, the yield related to the system was higher under optimal ohmic conditions compared to the conventional treatment [13]. In the study by Yin et al. (2015) on the extraction of phenolic compounds and flavonoids from *Sargassum horneri*, the results showed that, compared to conventional extraction, supercritical carbon dioxide extraction under optimal conditions had a better yield. The results also indicated that ethanol extraction provided a higher yield of phenolic and flavonoid compounds compared to conventional methods [14]. Several studies have compared the antioxidant activities of plant extracts obtained using supercritical fluid extraction and conventional methods. For example, Tsuda et al. (1995) studied the antioxidant activity of extracts from tamarind seed coats using supercritical carbon dioxide under various conditions. The results showed that with an increase in extraction pressure and temperature, the antioxidant activity of the extracts increased [15]. Palma and Taylor (1999) observed that the recovery of catechins and other phenolic compounds from grape seeds using supercritical carbon dioxide with methanol as a modifier was higher than with traditional solid-liquid extraction. Supercritical fluid extraction is faster and allows for selective separation of phenolic compounds by adjusting pressure and co-solvent concentration [16].

Examining process variables such as temperature and time is essential for enhancing the yield of bioactive compounds in food applications. Temperature increases the efficiency of extraction by enhancing solubility and diffusion rates; however, excessive heat can degrade sensitive compounds like antioxidants and vitamins. Along with temperature, extraction time affects performance and efficiency. Longer extraction times may improve yield but can reduce bioactive compounds and increase energy costs. Balancing time is crucial for both quality and overall cost-efficiency. The aim of this research is to investigate and compare three extraction methods—ohmic heating (OH), supercritical carbon dioxide (HPCD), and maceration—on the bioactive

compounds of the Iranian brown algae *Nizimuddinia zanardini*.

2. Materials and Methods

2.1. Preparation of Algae and Materials

The brown algae *Nizimuddinia zanardini* were collected in February 2022 from the Oman Sea, south of Iran (Chabahar Bay: 25°20'53" N and 60°28'1" E). The taxonomic group of the algae was determined by the Offshore Fisheries Research Center. After harvesting, the algae were washed twice with distilled water, sun-dried, ground, and then sieved using a 100-mesh sieve. For further extraction experiments, the samples were vacuum-sealed and stored in a cool, dry place. All chemicals, reagents, and solvents used were of analytical grade and were purchased from Merck KGaA (Germany) and Sigma-Aldrich (USA).

2.2. Extraction Methods

Based on preliminary experiments (extraction yield), a sample-to-solvent ratio of 1:10 was determined, and 50% ethanol was used for the extraction. The choice of 50% ethanol as the extraction solvent in this study was based on previous findings, where a 50% ethanol solution resulted in the highest total phenolic recovery from macroalgae compared to 30% and 70% ethanol solutions [25]. Prior to extraction, the mixtures were stirred for 30 min at room temperature and stirred at 200 rpm.

2.2.1. Maceration Extraction (ME)

In the maceration extraction method, the mixture was extracted at different temperatures (30°C, 45°C, and 60°C) in the dark for 12 and 24 h while stirring at 400 rpm. The resulting extract was then filtered using Whatman filter paper (No. 1). In the next step, to remove the solvent, the extracts were subjected to rotary evaporation (Rota Evaporator, IKA RV, 10) under vacuum distillation at 40°C to minimize the degradation of bioactive compounds. Finally, the extract was transferred to glass plates and dried in a freeze dryer at -70°C, turning it into a powder. The extract was then stored in dark glass containers at -18°C in the freezer until testing.

2.2.2. Ohmic Heating Extraction (OH)

This method was conducted using the ohmic extraction device at the Faculty of Agriculture, Shiraz University. The ohmic extraction unit consisted of a cylindrical chamber (with an internal diameter of 0.07 m and a length of 0.25 m) equipped with two titanium electrodes. The system was automated for voltage (0–300 V) and current (0–16 A), and temperature changes could be controlled, monitored, and recorded on a data sheet throughout the experiment. In this process, the samples were loaded into the chamber at a ratio of 1:10 (w/v) using 50% ethanol (Merck, Germany) as the solvent. Extraction was carried out by applying a constant voltage of 175 V between the two electrodes for 1 and 2 h at temperatures of 60°C and 45°C. Each extract was then filtered using Whatman filter paper (No. 1), and subsequently, to remove the solvent, the obtained extracts were subjected to vacuum distillation using a rotary evaporator (Rota Evaporator, IKA RV, 10). A temperature of 40°C was used at this stage to minimize degradation of bioactive compounds. Finally, the extracts were transferred to glass plates and freeze-dried at -70°C until converted into powder. The resulting powder was then stored in dark glass containers and kept in a freezer at -18°C until testing.

2.2.3. High-Pressure Carbon Dioxide Extraction (HPCD)

This method was carried out using the HPCD extraction device in the food science and technology laboratory pilot unit at Islamic Azad University, Shiraz Branch, Marvdasht. The device included a CO_2 cylinder containing carbon dioxide with 96% purity. It also featured an incubator chamber to control potential pressure increases and regulate temperature. The liquid carbon dioxide

was delivered by specialized CO_2 piston pumps (High pressure, Maximator Pump M111) made in Germany. During the experiment, the extraction chamber pressure was continuously monitored using a pressure gauge, and the temperature was controlled using a Memmert incubator. All fittings and the extraction chamber were made of stainless steel, and the high-pressure outlet valve was a needle-type valve manufactured by Butech. The supercritical fluid was pumped through a passage filled with the sample. Therefore, the extraction chamber was pressurized with the desired fluid using the pump. The fluid was then recovered, and the sample was removed from the cell for extract separation. The system was also equipped with a high-pressure passage for holding the sample and a container for collecting the extract and co-solvent, if necessary. In this method, extraction was carried out for 1 and 2 h at a pressure of 150 bar and temperatures of 30°C, 45°C, and 60°C. Each extract was then filtered using Whatman filter paper (No. 1). Subsequently, to remove the solvent, the obtained extracts were subjected to vacuum distillation using a rotary evaporator (Rota Evaporator, IKA RV, 10). A temperature of 40°C was used during this stage to minimize degradation of bioactive compounds. Finally, the extracts were transferred to glass plates and freeze-dried at -70°C until a dry powder was obtained. The resulting powder was stored in dark glass containers and kept in a freezer at -18°C until testing.

2.3. Determination of Extracted Bioactive Compounds and Extraction Yield

All phytochemical analyses of the extracts were performed in triplicate (n = 3). All freeze-dried extracts were dissolved in ethanol, prepared at a stock concentration of 1 mg/mL, and used for the analyses.

2.3.1. Total Phenolic Content (TPC) and Total Phlorotannin Content (TPhC) Analyses

TPC and TPhC were determined using the Folin–Ciocalteu reagent method as described by Garcia-Vaquero et al. (2021), with slight modifications [17]. A total of 100 µL of the sample was mixed with 2 mL of sodium carbonate solution (2% w/v Na_2CO_3). After 2 min, 100 µL of Folin–Ciocalteu reagent (1 M) was added to all mixtures. The reaction mixtures were then incubated for 30 min at room temperature in the dark. The absorbance of the reactions was measured at a wavelength of 765 nm using a spectrophotometer (Lambda 35 UV/VIS spectrometer, USA).

Distilled water was used as a blank, and gallic acid (purity >97.5%) at concentrations of 100–1200 mg/L and phloroglucinol (purity >99%) at concentrations of 100–1500 mg/L were used as standards for TPC and TPhC, respectively. The TPC results were expressed as mg of gallic acid equivalent per g of dry algae (mg GAE/g), and the TPhC results were expressed as mg of phloroglucinol equivalent per g of dry algae (mg PGE/g).

2.3.2. Total Flavonoid Content (TFC)

Flavonoid content was determined based on the protocol described by Čagalj et al. (2021), with slight modifications [18]. A volume of 1000 µL of the sample was mixed with 50 µL of 10% aluminum chloride (w/v), 50 µL of 1 M sodium acetate, and 1400 µL of distilled water. The mixture was left at room temperature for 30 min before measurement.

The absorbance was measured using a spectrophotometer at a wavelength of 415 nm. Distilled water was used as the blank. A standard curve (1–1100 mg/L) was plotted using quercetin and the results were expressed as mg of quercetin equivalent per g of dry algae (mg QE/g).

2.3.3.Antioxidant Activity Based on DPPH Radical Scavenging

The Diphenyl-2-Picryl-Hydrazil (DPPH) radical scavenging activity was measured based on the protocol described by Garcia-Vaquero et al. (2021) [17]. A total of 700 µL of a 100 µM methanolic

DPPH solution was added to 700 µL of the sample, and the mixtures were incubated at room temperature in the dark for 20 min. The absorbance of the reactions was measured against the blank (methanol without DPPH solution) at a wavelength of 517 nm using a spectrophotometer. The control sample contained 2 mL of methanol and 2 mL of DPPH solution. The percentage of DPPH radical inhibition was calculated using Equation 1.

Equation 1. $$DPPH\ \% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where:- A_{blank}(Absorbance of the blank sample) , - A_{sample} (Absorbance of the sample containing the ethanol extract)

2.3.4. Determination of Extraction Yield

The extraction yield was determined by calculating the amount of extracted solid material according to the method described by Sharifi and Khoshnoudi-Nia (2022) (Equation 2) [9].

Equation 2. $$Yield\ (\%) = [(mass\ of\ solid\ (g) / mass\ of\ raw\ material\ (g)] \times 100$$

2.3.5. Statistical Analysis

The effects of time and temperature on the extracted phytochemical compounds (TPC, TPhC, and TFC), antioxidant activity (DPPH), and extraction yield were analyzed using a multivariate general linear model in SPSS software version 25.0. Further differences were analyzed using Tukey HSD post-hoc tests. Differences were considered statistically significant at p-values < 0.05. The results were expressed as mean ± standard deviation. Graphs were plotted using Excel 2019.

3. Results and Discussion

3.1. Total Phenolic Content (TPC) of N. zanardini Extracts

Fig 1. shows the effect of different extraction conditions on the TPC of extracts from all three methods. In the maceration method, no specific increasing or decreasing trend was observed between different temperatures and times. The highest TPC was achieved at 30°C and 24 h (469.15 mg GAE/g). In contrast, the lowest TPC was observed at 45°C and 24 h, compared to the other methods and conditions, with a value of 307.96 mg GAE/g. In the High-Pressure Carbon Dioxide Extraction (HPCD) method, the highest TPC was extracted at 60°C and 60 min (817.48 mg GAE/g), while the lowest TPC was observed at 30°C and 120 min (371 mg GAE/g). The Ohmic Heating (OH) extraction method, one of the most recent extraction techniques, significantly enhances the extraction of bioactive compounds by increasing the permeability of the cell walls in algae. The results showed that this method, at 45°C and 120 min, achieved the highest TPC extraction compared to the other two methods and all conditions (960.7 mg GAE/g). These findings highlight the strong influence of the electric field in facilitating the extraction process. The electric field effectively disrupts the cell wall, increasing the permeability of solvents into the cells, thus leading to a higher extraction of bioactive compounds [19].

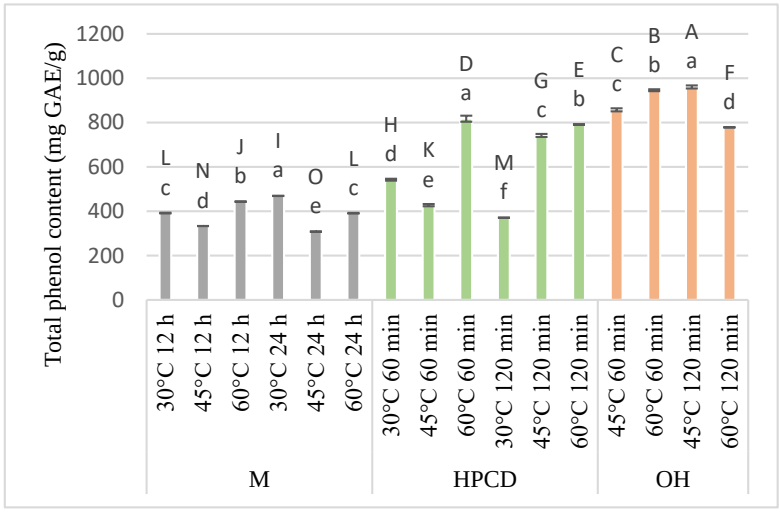

Fig 1. Effect of extraction methods on total phenolic content (TPC) of *N. zanardini* extracts. M: maceration extraction, HPCD: high pressure carbon dioxide extraction, OH: ohmic extraction. Lowercase letters indicate significant differences within each method and uppercase letters indicate significant differences among all methods.

Numerous studies have shown that time and temperature are key factors in the extraction of phenolic compounds from various natural sources, including algae, plants, and fruits. These factors, by influencing the physical and chemical properties of the samples, can affect the yield and quality of the extraction of bioactive compounds. Abdelhamid et al. (2018) reported that using a temperature of 50°C along with 50% ethanol for the extraction of phenolic compounds from three species of brown algae effectively contributed to an increase in phenolic content and antioxidant activity. Temperature and extraction time interact with each other, and higher temperatures typically increase the extraction rate; however, the use of excessively high temperatures may lead to the degradation of heat-sensitive compounds [20]. Gavahian and Chu (2022) stated that temperature has a significant effect on TPC. Phenolic compounds decreased when the extraction temperature exceeded 80°C and the processing time was more than 30 min. This reduction may be attributed to thermal degradation to these heat-sensitive compounds [13]. Studies have revealed that at temperatures above 60°C, the extraction yield of phenolic compounds decreases. This reduction may be due to the degradation of phenolic compounds or polymerization and oxidation reactions occurring at high temperatures. Ospina et al. (2017) reported that with an increase in temperature above 50°C, the amount of phenolic compounds extracted using the supercritical carbon dioxide method decreased. These changes are mostly attributed to undesirable chemical reactions caused by higher temperatures [21]. Pereira et al. (2016) also showed that increasing both time and temperature led to a higher yield of phenolic compound extraction from purple potatoes, and the interaction of these two factors had a significant impact on improving the extraction efficiency [22]. Additionally, other studies have indicated that temperature significantly affects extraction yield. For example, Goto et al. (2015) found that increasing the temperature from 40°C to 60°C led to an increase in the yield of fucoxanthin extraction from marine algae, while at temperatures above 60°C, the yield decreased [23]. An increase in temperature leads to a decrease in the density of supercritical carbon dioxide, resulting in reduced solvent power.

However, higher temperatures also increase the vapor pressure of solutes in the extracted materials and enhance the mass transfer of the solvent. Quitain et al. (2013) and Getachew et al. (2018) both reported higher extraction yields at a lower temperature of 40°C, which was explained by the increased density of supercritical carbon dioxide [24, 25]. However, Kanda et al. (2014) revealed that a higher temperature of over 60°C resulted in a greater extraction yield. They noted that elevated temperatures can aid in breaking down cell walls, thereby allowing easier access to bioactive compounds [26]. In a study by Sharifi et al. (2019), the amount of phenolic compounds extracted from barberry increased with longer extraction time and higher pressure. Additionally, the amount of phenolic compounds rose as the extraction time increased from 20 to 60 min. This increase in phenolic concentration at 60 min of extraction was attributed to the release of cell wall-bound phenolics, polymerization reactions, and the formation of new compounds [8].

3.2. The Total Phlorotannin Content (TPhC) of *N. zanardini* Extract

Phlorotannins belong to a group of polyphenols that are abundantly found in brown algae and play a significant role in a wide range of metabolic functions. Phlorotannins are synthesized through the acetate-malonate pathway [27].

Fig 2. shows the effect of different extraction conditions on the TPhC of the extracts from all three methods. In the maceration method, no specific increasing or decreasing trend was observed between different temperatures and times. The highest TPhC was observed at a temperature of 30°C and an extraction time of 24 h (463.17 mg PGE/g). In contrast, the lowest TPhC was observed at a temperature of 45°C and an extraction time of 24 h, compared to the other two methods and all conditions (281.83 mg PGE/g). In the supercritical carbon dioxide extraction method, the highest TPhC was observed at a temperature of 60°C and an extraction time of 60 min (855.04 mg PGE/g), while the lowest amount was observed at a temperature of 30°C and an extraction time of 120 min (352.75 mg PGE/g). The results of extraction using ohmic heating showed that this method at 45°C and 120 min provided the highest TPhC extraction compared to the other two methods and all conditions (1016.17 mg PGE/g). At 60°C and 60 min, the extraction amount reached 999.04 mg PGE/g, which was still higher than the other methods *($p < 0.05$)*. These results indicate that the electric field in this method helps to break down the cell walls of the algae and facilitates solvent penetration into the cells, leading to a significant increase in TPhC extraction [19].

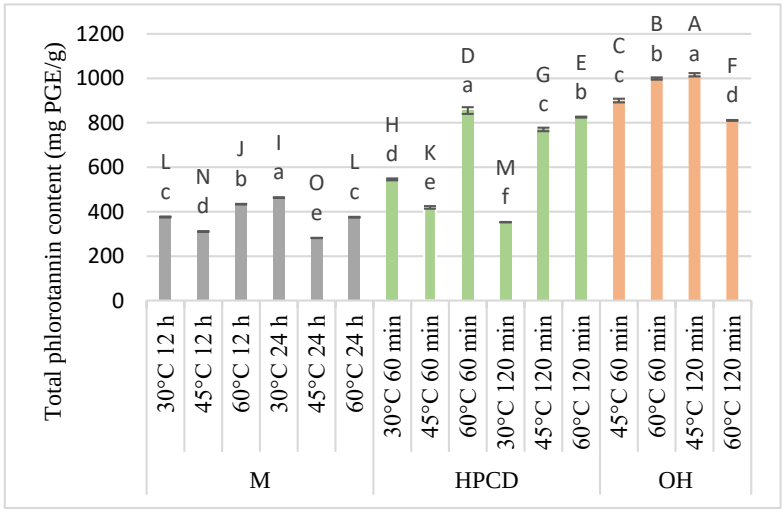

Fig 2. Effect of extraction methods on total phlorotannin (TPhC) content of *N. zanardini* extracts. M: maceration extraction, HPCD: high pressure carbon dioxide extraction, OH: ohmic extraction. Lowercase letters indicate significant differences within each method and uppercase letters indicate significant differences among all methods.

According to the existing literature, TPhC has not been extracted from algae using the ohmic heating and HPCD methods; however, the results can be compared with the results of phenolic compounds and measurements carried out by other methods. In line with the findings of the present study, different extraction methods have a significant impact on TPhC from algae. These results are consistent with previous research, particularly the study by Dang et al. (2018), which demonstrated that the use of different extraction methods affects the yield of bioactive compounds such as tannins and phlorotannins. In that study, investigations revealed that the concentration of polyphenols obtained through enzymatic extraction was significantly lower compared to that obtained using the maceration method. This difference is potentially due to the solvent's affinity for releasing tannins and phlorotannins [28]. In the present study, the maceration method showed that increasing temperature and time effectively influenced the TPhC content, which aligns with previous findings indicating that both time and temperature positively affect the extraction of bioactive compounds such as phlorotannins. A similar trend was observed in the study by Dang et al. (2018), which reported that prolonged maceration time led to increased concentrations of bioactive compounds [28].

3.3. Total Flavonoid Content (TFC) of *N. zanardini* Extract

Flavonoids have been reported as antioxidants and absorbers of a wide range of reactive oxygen species. It is well established that flavonoids represent a large group of phenolic compounds, including flavonols, flavones, catechins, proanthocyanidins, anthocyanidins, and isoflavonoids, all of which exhibit a wide range of biological activities [28].

Fig 3. illustrates the effect of different extraction conditions on the TFC of extracts obtained by the three methods. In the maceration method, no specific increasing or decreasing trend was observed regarding the extraction time. However, in terms of temperature, an initial increase followed by a decrease in TFC was noted ($p < 0.05$), indicating that raising the temperature to 60°C reduces the efficiency of TFC extraction [29, 30]. The highest TFC was observed at 45°C and 24 h (242.67 mg QE/g), which was the highest among all methods and conditions. In the HPCD extraction method, no clear increasing or

decreasing trend was observed across different temperatures and extraction times. The highest TFC (174.62 mg QE/g) was obtained at 60°C and 120 minutes ($p < 0.05$), while the lowest flavonoid content was observed at 60°C, 60 min and 45°C, 120 min compared to the other two methods and all other conditions. In the ohmic heating method, the highest TFC extraction was achieved at 60°C and 120 min reaching 239.19 mg QE/g ($p < 0.05$).

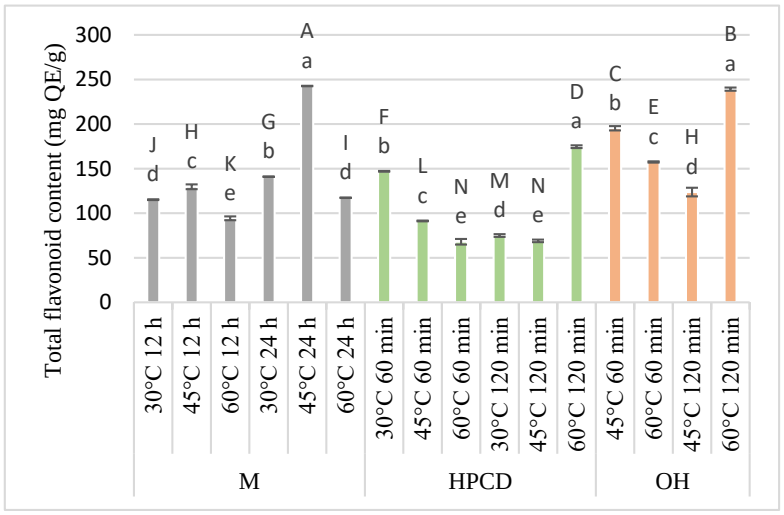

Fig 3. Effect of extraction methods on total flavonoid content (TFC) of *N. zanardini* extracts. M: maceration extraction, HPCD: high pressure carbon dioxide extraction, OH: ohmic extraction. Lowercase letters indicate significant differences within each method and uppercase letters indicate significant differences among all methods.

As Cox et al. (2010) showed, the extraction of flavonoids from brown algae, particularly species such as *Himanthalia elongata*, *Padina gymnospora*, and *Sargassum wightii*, resulted in higher total flavonoid content (TFC) compared to green and red algae. These findings were also clearly reflected in the present study, where significant amounts of TFC were extracted under optimal temperature and time conditions [31]. In a similar study, Pereira et al. (2016) reported that extraction time plays an important role in increasing TFC. Their results demonstrated that increasing both extraction time and voltage, the amount of extracted total flavonoids significantly increased [22].

3.4. Antioxidant Activity (DPPH) of *N. zanardini* Extract

The effect of different extraction conditions on the antioxidant activity of the extracts obtained through all three methods is shown in Fig 4. In the maceration method, the results indicated that there was no significant difference in antioxidant activity at 12 h across various temperatures ($p < 0.05$). The highest antioxidant activity in the maceration method was observed at 60°C and 24 h, reaching 46.45%. In the HPCD method, no clear increasing or decreasing trend was observed across different temperatures and durations. The highest antioxidant activity in this method was recorded at 30°C and 60 min, reaching 83.08%, which was the highest among all three methods and conditions. Conversely, the lowest antioxidant activity was observed at 60°C and 120 min, measuring 41.49%. The results of the ohmic heating extraction method showed that at 60°C and 60 min, the lowest antioxidant activity among all methods and conditions was recorded, with a value of 20.38%.

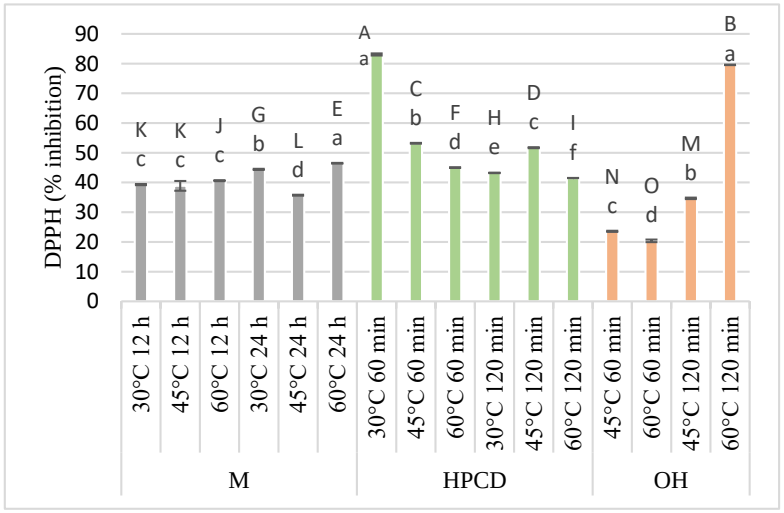

Fig 4. Effect of extraction methods on the antioxidant activity (DPPH) of *N. zanardini* extracts. M: maceration extraction, HPCD: high pressure carbon dioxide extraction, OH: ohmic extraction. Lowercase letters indicate significant differences in each method and uppercase letters indicate significant differences among all methods.

In the study by Gavahian and Chu (2022) on pineapple peel using the ohmic method, it showed that temperature, time, voltage, and frequency all influence antioxidant activity. According to the results, antioxidant activity decreased with increasing temperature, and this negative effect was particularly noticeable at temperatures above 90°C and with longer times. They also investigated that ohmic heating extraction can save 50% of the time and 80% of the energy compared to traditional methods. In this study as well, the use of the ohmic heating extraction (OH) method demonstrated similar advantages in reducing time and increasing extraction efficiency [13]. Coelho et al. (2019) also examined the effect of temperature and ethanol concentration on the antioxidant activity of tomato waste using the ohmic heating method and concluded that a temperature of 70°C is more suitable for extracting phenolic compounds with high antioxidant capacity. In this study, higher temperatures also showed similar results in increasing antioxidant activity. These results indicate the reverse effect of temperature at very high temperatures on phenolic compounds and antioxidant activity [11]. In the study by Guía-García et al. (2021) on the antioxidant evaluation of *Rhus microphylla* and *Myrtillocactus geometrizans* fruits, aqueous extracts using the ohmic method were significantly affected by time [32]. Syrpas et al. (2018) demonstrated that in supercritical carbon dioxide extraction from wild cyanobacteria, pressure had the greatest impact on yield, followed by time and temperature. The interaction effect of pressure and temperature had a synergistic impact. Extraction yield maximized at pressures between 30 and 50 MPa and temperatures above 50°C [33]. Supercritical fluid extraction is considered one of the most suitable methods for producing extracts with high antioxidant activity. Yepez et al. (2002) demonstrated the possibility of obtaining odorless and tasteless extracts from coriander with high antioxidant activity using supercritical carbon dioxide extraction. Extraction under moderate conditions (45°C and 177 bar), with a CO_2 density close to 0.74 g/mL, resulted in extracts with high antioxidant activity and yield [34]. Zancan et al. (2002) conducted a study to demonstrate the effect of temperature, pressure, and the addition of a co-solvent on the extraction kinetics and antioxidant activity of

ginger extract. The best results in terms of antioxidant activity were obtained when extraction was performed with a modifier at lower temperatures, lower pressures, and relatively long extraction times [35].

3.5. Extraction Yield of *N. zanardini* Extract

Extraction yield is related to the nature of the solvent (polarity and pH), extraction time, temperature, and the type of sample [36].

Fig 5. shows the effect of different extraction conditions on the extraction yield of the extracts from all three methods. In the maceration method, the highest extraction yield was observed at 60°C and 12 h (27.66%). The lowest extraction yield was observed at 45°C and 12 h (23.47%). In the supercritical carbon dioxide extraction method, the highest extraction yield was observed at 30°C and 120 min (26.76%), while the lowest extraction yield compared to the other two methods and all conditions was observed at 60°C and 120 min (14%). The ohmic heating extraction method, due to its ability to increase the permeability of algal cell walls, significantly increased the extraction yield. The results showed that this method at 45°C and 120 min had the highest extraction yield compared to the other two methods and all conditions (33.21%). These results demonstrate the strong influence of the electric field in facilitating the extraction process. The electric field can effectively cause the degradation of cell walls and increase the permeability of solvents into the cells, thereby resulting in higher extraction of bioactive compounds and improved extraction yield [19].

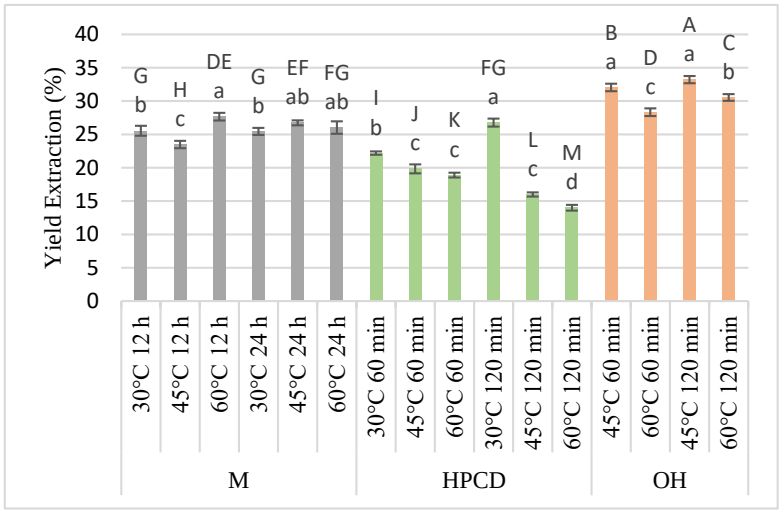

Fig. 5. Effect of extraction methods on extraction efficiency (EY) of *N. zanardini* extracts. M: maceration extraction, HPCD: high pressure carbon dioxide extraction, OH: ohmic extraction. Lowercase letters indicate significant differences within each method and uppercase letters indicate significant differences among all methods.

In the study by Silva et al. (2021) on nine brown macroalgae species, ethanol extracts exhibited the highest extraction yield percentages for all algal species, with values ranging from 14.6% to 38.8%. Among them, the extract obtained from *Undaria pinnatifida* showed the highest yields. This result was attributed to the high polarity of ethanol, which can facilitate the non-selective extraction of various algal components such as proteins and polysaccharides, thereby resulting in a higher recovery rate [36]. Shahidi et al. (2020), in their study on *Lepidium sativum* (garden cress) seeds using the maceration method at low temperatures and prolonged extraction times, achieved an extraction yield of approximately 7%. Several previous studies

have also highlighted the positive effect of extended extraction time on extraction yield [9, 30]. Olivares-Molina and Fernández (2016), in a study on brown algae, reported the highest extraction yield using enzymes (37.72%). In the present study, the ohmic heating method at 45°C and 120 min produced a similar yield (33.21%), showing good agreement with their findings [37]. Pereira et al. (2021) demonstrated that increasing extraction time enhances the yield of bioactive compounds [6]. Guía-García et al. (2021) also reported that the use of electric current in extraction increases the yield of bioactive compounds. Similarly, in this study, the ohmic method utilizing electric current showed the highest extraction yield [32].

4. Conclusions

The results of this study indicate that all three extraction methods—ohmic heating, high-pressure carbon dioxide, and maceration—were effective in extracting bioactive compounds from the brown alga *Nizimuddinia zanardini*. However, the specific characteristics of each method varied depending on the extraction conditions. The ohmic heating method, through the application of an electric field and increased cell permeability, yielded the highest levels of phenolic compounds, phlorotannins, and overall extraction efficiency, and was identified as the most effective method in this study. This method, due to its faster and more efficient extraction—especially at higher temperatures and shorter durations—is a suitable option for the industrial-scale extraction of bioactive compounds. In contrast, the maceration method is recommended for extracting compounds such as flavonoids because of its simplicity, low cost, and minimal equipment requirements. Additionally, the HPCD method is appropriate for extracting compounds with high antioxidant activity from this alga. Overall, the selection of an appropriate extraction method depends on the type of target bioactive compound, process conditions, and scale of operation, which can be considered for various applications in the food and pharmaceutical industries.

Conflict of Interest

No conflict of interest has been declared by the authors.

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مقاله پژوهشی

اثر روش‌های استخراج اهمیک، کربن دی اکسید با فشار بالا (HPCD) و ماسراسیون بر ترکیبات زیست‌فعال جلبک قهوه‌ای ایرانی (*Nizimuddinia zanardini*)

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چکیده

جلبک‌های دریایی منبعی سرشار از ترکیبات زیست‌فعال با کاربرد امیدوارکننده به عنوان مواد غذایی با خواص عملکردی و آثار درمانی هستند. هدف از این پژوهش بررسی اثرات متغیرهای دما و زمان در روش‌های استخراج اهمیک، کربن دی اکسید با فشار بالا (HPCD) و ماسراسیون بر بازده استخراج، محتوای فنول کل، فلوروتانین کل، فلاونوئید کل و فعالیت آنتی‌اکسیدانی (برحسب مهار رادیکال DPPH) عصاره جلبک قهوه‌ای ایرانی *Nizimuddinia zanardini* بود. نتایج نشان دادند که بیشترین و کمترین محتوای فنلی و فلوروتانین کل مربوط به روش‌های اهمیک (۴۵ °C در ۱۲۰ min: ۹۶۰/۷mg GAE/g، ۱۰۱۶/۱۷ mg PGE/g) و ماسراسیون (۴۵ °C در ۲۴ h: ۳۰۷/۹۶ mg GAE/g، ۲۸۱/۸۳ mg PGE/g) به ترتیب بودند. هم‌چنین روش ماسراسیون در دمای ۴۵ °C و زمان ۲۴ h بالاترین محتوای کل فلاونوئید را به‌دست آورد (۲۴۲/۶۷ mg QE/g). روش HPCD در دمای ۳۰ °C و زمان ۶۰ min از نظر فعالیت آنتی‌اکسیدانی (DPPH) با مقدار ۸۳/۰۸٪ بالاترین جایگاه را به‌دست آورد. از نظر بازده استخراج نیز روش اهمیک در دمای ۴۵ °C و زمان ۱۲۰ min مقدار بالاتری داشت (۳۳/۲۱٪). نتایج این تحقیق نشان داد در بین سه روش، روش اهمیک کارایی بیشتری در استخراج ترکیبات زیست‌فعال جلبک *Nizimuddinia zanardini* دارد.

واژه‌های کلیدی: اهمیک، کربن دی اکسید با فشار بالا، جلبک، فعالیت آنتی‌اکسیدانی، فنول کل، *Nizimuddinia zanardini*

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