

Extracting Polyphenols from *Saccharina angustata* by Two Methods

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The aim of the study was to evaluate the efficiency of two non-thermal extraction techniques, specifically ultrasound-assisted extraction (UAE) and dynamic maceration (DM), for recovering polyphenols from brown seaweed, *Saccharina (S.) angustata*. Given the thermolabile nature of many phenolic compounds, both methods were chosen for their ability to operate at low temperatures while preserving bioactivity. Moreover, two different solid-to-solvent ratios were tested (1 : 5 and 1 : 10, plant material to ethanol, w/v) to assess their influence on extraction efficiency. Total polyphenol content (TPC; Folin-Ciocalteu colorimetric method) and antioxidant activity (AA; DPPH assay) of the resulting extracts were analyzed to compare the effectiveness of these techniques. Importantly, the study is the first quantification of TPC and AA in *S. angustata*, highlighting a pioneering effort in the scientific investigation of this species. Our results revealed that the extract obtained using DM at a 1 : 10 solid-to-solvent ratio exhibited the highest values for extraction efficiency ($42.20 \pm 1.60\%$), TPC (2.61 ± 0.73 mg GAE.g⁻¹ DW), and AA ($11.69 \pm 2.48\%$). In contrast, the highest AA expressed as Trolox Equivalent Antioxidant Capacity (0.61 ± 0.01 mg TEAC.g⁻¹ DW) was observed in the extract obtained using DM at a 1 : 5 solid-to-solvent ratio. However, statistical analysis showed no significant differences ($P > 0.05$) in either TPC or AA between the extracts produced by UAE and DM, regardless of the solid-to-solvent ratio applied. Despite similar yields, UAE proved more time-efficient, requiring only 30 minutes compared to the 24-hour duration of DM. Nevertheless, DM remains a cost-effective and accessible option when ultrasonic equipment is unavailable.

Keywords: brown algae, *Saccharina angustata*, polyphenol content, antioxidant capacity, extraction approaches

1 Introduction

In recent years, marine macroalgae have garnered considerable scientific interest as rich reservoirs of bioactive compounds with diverse health-promoting properties. Among them, brown seaweeds (Phaeophyta) have emerged as particularly valuable due to their distinctive biochemical profile, which includes complex polysaccharides, phlorotannins, fucoxanthin, and iodine (Afonso et al., 2019). Compared to green and red algae, brown seaweed generally contains higher concentrations of potent antioxidants (Generalić Mekinić et al., 2019). Within this group, members of the order Laminariales, commonly referred to as kelp, play a critical role in marine ecosystems and represent an important resource in aquaculture and coastal economies. These kelps are cultivated in East Asia and harvested from

natural populations in Europe and North America to produce alginate, a polysaccharide widely used in pharmaceuticals, food products, and various industrial applications (Ye et al., 2015). Among these species, *Saccharina (S.) angustata* (previously known as *Laminaria angustata*) is harvested in Japan, where it accounts for approximately 15% of the country's total *Saccharina* production (Borlongan et al., 2019). It holds notable ecological and economic importance. Ecologically, it contributes to the maintenance of genetic diversity and the structural integrity of marine ecosystems by providing habitat and food for a wide range of marine organisms. Commercially, *S. angustata* is a valuable resource in kelp breeding programs and aquaculture, and it is increasingly utilized as a functional food ingredient due to its rich profile of bioactive compounds (Zhang et al., 2021).

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Its environmental adaptability and potential for high value bioproducts underscore its significance in both natural ecosystems and commercial applications. From a molecular standpoint, *Saccharina* species are particularly rich in phlorotannins, i.e., polyphenolic compounds unique to brown algae, composed of phloroglucinol (1,3,5-trihydroxybenzene) units. These include structural subclasses such as fucols, phlorethols, fucophlorethols, fuhalols, and eckols, all of which have been shown to exhibit strong antioxidant activity (AA) (Zhang et al., 2025). In addition to phlorotannins, *Saccharina* spp. have also been reported to contain flavonoids such as catechins, including epicatechin and epigallocatechin, which further contribute to their bioactive profile (Machu et al., 2015). Moreover, *Saccharina* species produce various carotenoid pigments, most notably fucoxanthin, a xanthophyll that has demonstrated promising anticancer activity (Wang et al., 2025). Although direct studies on *S. angustata* are limited, substantial evidence from closely related *Saccharina* species, such as *S. japonica* (formerly *Laminaria japonica*) strongly supports the commercial potential of this genus. Their high content of bioactive compounds, encompassing antioxidants, polysaccharides, and pigments, underpins their growing use in food, cosmetic, and pharmaceutical applications.

In effect, phenolic compounds, including phlorotannins and catechins, are among the most important natural antioxidants. Their structures, comprising one or more hydroxylated aromatic rings, allow them to effectively scavenge free radicals and inhibit oxidative damage (Machu et al., 2015). Due to the structural diversity and varying solubility of polyphenols, the efficiency and selectivity of their extraction from seaweed biomass are strongly influenced by both the choice of solvent and the extraction method employed (Duan et al., 2024). Among various solvents, ethanol is frequently used in this context due to its low toxicity, flammability, and environmental impact, as well as its recognition as a food-grade, green solvent (Frohlich et al., 2022). Importantly, its amphiphilic nature allows it to dissolve both polar and nonpolar constituents, making it suitable for extracting a wide range of phenolics (Otero-Guzman & Andrade-Pizarro, 2025). However, extraction efficiency is not solely determined by the choice of solvent. Variables such as ethanol concentration, extraction time and temperature, material-to-solvent ratio, and the employed technique (e.g., maceration, ultrasonic-assisted extraction, or reflux) also play critical roles (Nguyen-Kim et al., 2021). Previous studies have demonstrated that these parameters can affect not only the total yield of phenolic compounds but also the antioxidant capacity of the resulting extracts. For example, methods that enhance cell wall disruption, such as sonication or thermal treatment, may

significantly improve extraction efficiency, whereas milder techniques may better preserve thermolabile compounds (Liu et al., 2022). Therefore, optimization of extraction protocols is essential to fully harness the bioactive potential of seaweed-derived polyphenols.

The present study aims to evaluate and compare two ethanol-based extraction methods at two different sample-to-solvent ratios (1 : 5 and 1 : 10, w/v) applied to *Saccharina* (*S.*) *angustata*, with a focus on total polyphenol content and antioxidant activity. By identifying the most effective approach, this work seeks to contribute to the development of sustainable and efficient extraction strategies, ultimately supporting the use of *Saccharina* spp. as a source of functional ingredients in food, pharmaceutical, and cosmetic applications.

2 Material and Methods

2.1 Biological Material

In this study, we used commercially available brown seaweed *S. angustata*, sourced from Japan, as this species is neither naturally occurring nor cultivated in our local region. Given that Japan is one of the primary producers of *S. angustata* (Borlongan et al., 2019), the experimental material was obtained from a reputable aquaculture supplier, Mitoku Co., Ltd., to ensure consistency, quality, and traceability throughout the study. For reproducibility purposes, the material originated from batch number 31810154. The taxonomic identity of the biomass relied on the documentation and product labeling provided by the supplier. In fact, Mitoku Co., Ltd. is a long-established company specializing in seaweeds products, and the species identification is in accordance with Japanese food-grade standards. Hence, no additional morphological or molecular identification (e.g., DNA barcoding) was performed. The species *S. angustata* was chosen based on our previous research (Čmíková et al., 2024), as well as the notable lack of direct scientific studies specifically addressing it. Upon arrival, the samples were hermetically sealed and stored in a dark, dry environment at approximately 18 °C until further processing.

2.2 Chemicals

All chemicals were of analytical grade, and were purchased from Sigma Aldrich (Saint Louis, MO, USA) and Reachem (Bratislava, Slovakia).

2.3 Preparation of Algal Extracts

Dried seaweed biomass *S. angustata* was ground into a fine powder using a Philips ProBlend 4 blender (model

HR2100/40), operating at an approximate blade speed of 20,000rpm for 30 seconds. The resulting material was sieved through a 1 mm mesh, and only a fraction smaller than 1 mm was used for extraction. The powdered biomass was stored in airtight bags at 4 °C for subsequent analysis. Moisture content of the powder before extraction was measured using a moisture analyzer DBS 60 3 (Kern & Sohn GmbH, Altstadt, Germany) and determined to be $8.08 \pm 0.23\%$. Ethanolic extracts were prepared using solvent-to-biomass ratios of 1 : 5 and 1 : 10 (w/v), with 50% ethanol (v/v) as the extraction solvent. The powdered seaweed was initially mixed with the solvent and blended using the same blender for 1 minute to enhance contact between the solvent and solid phase.

2.4 Ultrasound-Assisted and Conventional Extraction Methods

Extracts of the macroalgae were prepared using two different methods: ultrasound-assisted extraction (UAE) and dynamic maceration (DM), following protocol described by Song et al. (2025), with slight modifications. For UAE, the first set of samples was processed using a Branson Digital Sonifier Model 450 ultrasonic probe system, equipped with a 102C converter. The probe had a tip diameter of 13 mm and operated at a frequency of 20 kHz. The amplitude was set to 30%, To minimize thermal degradation caused by ultrasonic heat, the extraction temperature was carefully maintained below 30 °C using a water bath, and sonication was applied in pulsed mode (20 s on/10 s off) over a total duration of 45 minutes. The effective sonication time was approximately 30 minutes, resulting in a total energy delivery of 23 kJ. The estimated acoustic power density was 9.45 W.cm^{-2} . For DM, the second set of samples was stirred continuously at 300 rpm on a magnetic stirrer (New Brunswick Scientific, USA) for 24 hours at a constant temperature of 25 °C. The extraction solvent (50% ethanol) was not replaced during the process and remained static throughout the 24-hour period. To minimize ethanol evaporation, preserve solvent concentration, and protect the extracts from light, dark glass bottles with screw caps were used as extraction vessels. After extraction, the mixtures were filtered through cheesecloth and centrifuged (Rotina 420, Hettich Zentrifugen, Tuttlingen, Germany) at $2,150 \times g$ for 20 minutes at 4 °C to remove solid residues. The resulting supernatants were collected for further analysis.

2.5 Solvent Removal and Lyophilization

Following extraction, ethanol was removed under reduced pressure using a rotary evaporator RVO 400. The resulting solutions were then lyophilized in Telastar

LyoQuest-55 ECO. The moisture of all powder samples was detected using the moisture analyzer DBS 60 3 (Kern & Sohn GmbH, Altstadt, Germany). The determined dry matter content was used in further calculations.

2.6 Extraction Efficiency

The extraction yields were calculated using the following formula:

$$\text{extraction yield (\%)} = \left(\frac{\text{weight of the dry extract}}{\text{weight of the dry sample}} \right) \times 100$$

2.7 Total Polyphenols Content and Antioxidant Activity

Extracts for analysis of TPC and AA were prepared as follows: approximately 20 mg of each sample (in duplicate) was mixed with 1 mL of extraction solvent (methanol:water:acetic acid, 80 : 18 : 2, v/v/v), homogenized, and sonicated for 5 minutes at 50 °C. The mixture was then centrifuged at $15,616 \times g$ for 10 minutes at 20 °C using a Rotina 420 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). The resulting supernatant was carefully transferred to clean microtubes. An additional 1 mL of the extraction solvent was added to the remaining pellet, and the entire extraction procedure was repeated.

The TPC of the extracts was determined using the Folin-Ciocalteu colorimetric method and expressed as gallic acid equivalents (GAE), following the procedure described by Valková et al. (2021a; 2021b; 2022), with minor modifications. For each assay, 25 µL of the sample was mixed with 50 µL of distilled water and 25 µL of Folin-Ciocalteu reagent. After allowing the reaction to proceed for 6 minutes, 100 µL of 7.5% sodium carbonate solution was added. The mixtures were then incubated in the dark at room temperature for 90 minutes. Absorbance was measured at 765 nm using a spectrophotometer Promega Glomax Multi Detection Plate Reader.

The free radical scavenging activity of the samples was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following previously described procedures (Valková et al., 2021a; 2021b; 2022). In this method, 20 mL of the sample was mixed with 180 mL of DPPH solution (prepared by dissolving 0.025 g of DPPH in 100 mL of ethanol). After 30 min. incubation in the dark, the decrease in the solution absorbance was measured at 515 nm using a spectrophotometer Promega Glomax Multi Detection Plate Reader. DPPH inhibitory activity was expressed as the percentage inhibition (I %) of DPPH in the above assay system, with Trolox used as a positive control. Additionally, the antioxidant activity of the extracts was recalculated and expressed as Trolox Equivalent Antioxidant Capacity (TEAC), with the results

reported in mg TEAC per gram of dry weight (mg TEAC.g⁻¹ DW), allowing for standardized comparison between samples.

2.8 Statistical Analysis

All measurements were performed in triplicate, and results expressed as mean \pm standard deviation. Normality of data distribution was assessed using the Shapiro-Wilk test, and homogeneity of variances was verified using the Levene's test. Due to insufficient normality and the small sample size ($n = 3$), non-parametric analysis was performed using the Kruskal-Wallis test, followed by Dunn's post hoc test for multiple comparisons. All statistical analyses were performed using Prism version 8.0.1 (GraphPad Software, San Diego, CA, USA). A p -value of less than 0.05 was considered statistically significant.

3 Results and Discussion

In this study, UAE and DM yielded comparable total extraction efficiencies from *S. angustata*, with no statistically significant differences ($P > 0.05$) observed between the two methods (Fig. 1). Importantly, the extraction yields obtained

(31.5–42.2%) are consistent with those previously reported for brown algae (Sunarwidhi et al., 2022), supporting the validity of our results and suggesting that the applied extraction conditions were suitable for *S. angustata*. On the other hand, an interesting observation was that UAE did not lead to a higher extraction yield compared to traditional DM, which stands in contrast to the prevailing trend reported in the literature. This variability in extraction efficiency may, in part, be attributed to species-specific factors. Indeed, the performance of UAE can vary significantly among brown algae species, as evidenced by the wide range of extraction yields and bioactivities reported across different studies (Ummat et al., 2020). Another potential explanation for this discrepancy lies in the ultrasound frequency applied in our study. While higher frequencies have been shown to enhance extraction yields in brown seaweeds (Dinç et al., 2024), our experiments were conducted at 20 kHz, as the default setting of the sonicator. Although this lower frequency may have limited extraction efficiency, it also helps mitigate excessive heating during UAE, which can be advantageous

for preserving thermolabile compounds. Therefore, the absence of a significant difference in extraction yield is likely due to a combination of the lower ultrasound frequency and the specific biochemical and structural characteristics of *S. angustata*.

Essentially, extraction methods significantly influence both TPC and AA of a sample, which measurements provide valuable insight into its antioxidant potential (Noreen et al., 2017). The efficiency of phenolic compound recovery, however, depends on several factors including solvent composition and polarity, sample-to-solvent ratio, as well as pH, temperature, extraction time, and the use of mechanical assistance, such as ultrasound (Gil-Martín et al., 2022). In the present study, DM at a 1 : 10 ratio yielded the highest TPC (2.61 ± 0.73 mg GAE.g⁻¹ DW; Fig. 2) and AA as measured by DPPH inhibition ($11.69 \pm 2.48\%$; Fig. 3). Conversely, the extract obtained using DM at a 1 : 5 ratio exhibited the highest antioxidant capacity when expressed as TEAC, reaching a value of 0.61 ± 0.01 mg TEAC.g⁻¹ DW (Fig. 4). The observed discrepancy between DPPH inhibition (%) and TEAC values, particularly between extracts obtained at 1 : 5 and 1 : 10 solid-to-solvent ratios, may be attributed to differences in the concentration, composition, and reactivity of the extracted antioxidant compounds. In this view, some antioxidants may react rapidly with DPPH radicals and exhibit high percentage inhibition at low concentrations; however, if they are present in low quantities or if their radical-scavenging activity plateaus quickly, their overall contribution to TEAC values (calculated based on a standard calibration curve with Trolox) may be limited (Castro et al., 2006). This highlights that while % DPPH provides a useful snapshot at a fixed concentration, TEAC offers

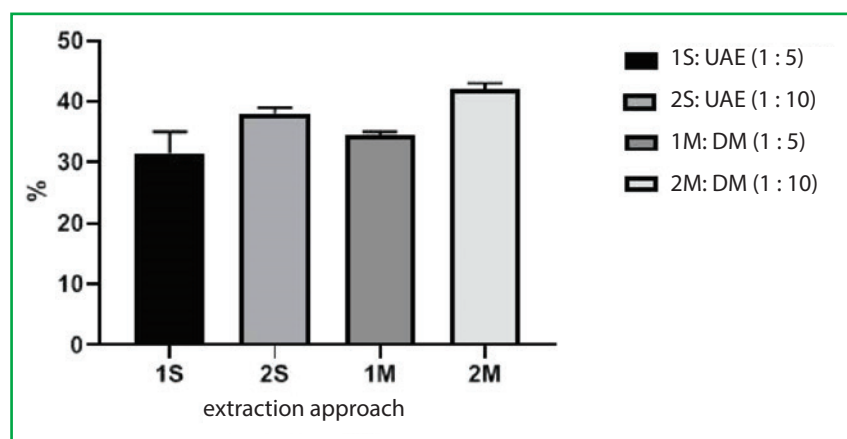


Figure 1 Comparison of extraction efficiency in *Saccharina angustata* extracts obtained using ultrasound-assisted extraction (UAE) and dynamic maceration (DM) at two sample-to-solvent ratios (1 : 5 and 1 : 10, w/v) no statistically significant differences were observed between the extraction methods or ratios ($p > 0.05$); data are presented as mean \pm standard deviation ($n = 3$)

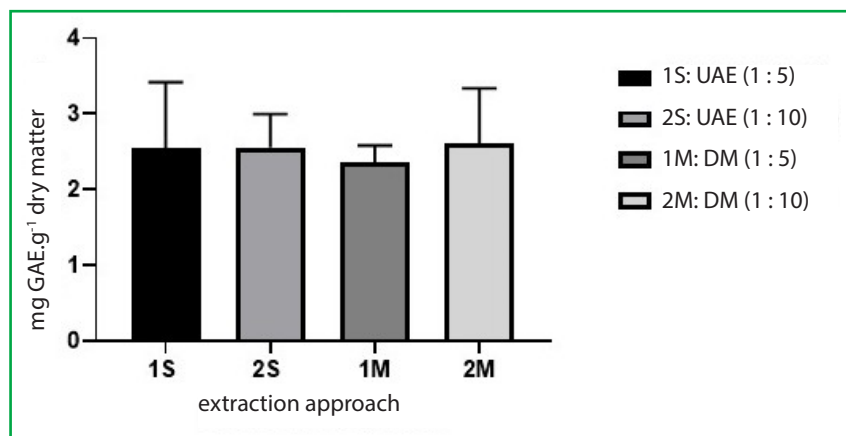


Figure 2 Comparison of total phenolic content (TPC) in *Saccharina angustata* extracts obtained using ultrasound-assisted extraction (UAE) and dynamic maceration (DM) at two sample-to-solvent ratios (1 : 5 and 1 : 10, w/v). TPC values are expressed as gallic acid equivalents (GAE). No statistically significant differences were observed between the extraction methods or ratios ($p > 0.05$). Data are presented as mean \pm standard deviation ($n = 3$)

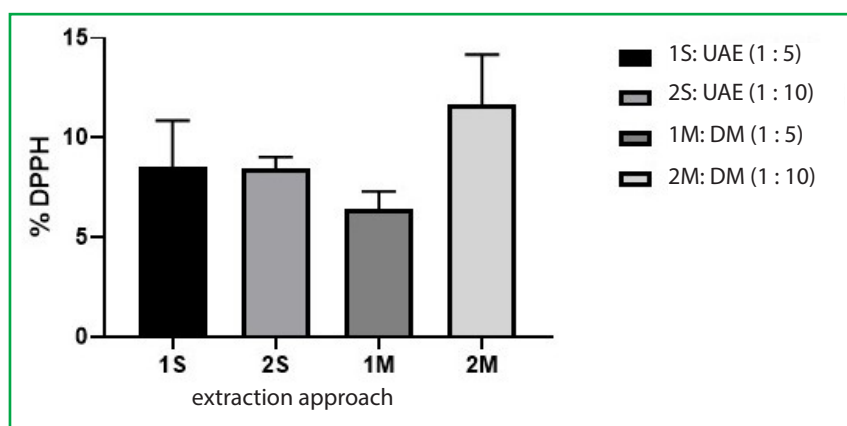


Figure 3 Comparison of antioxidant activity (AA) in *Saccharina angustata* extracts obtained using ultrasound-assisted extraction (UAE) and dynamic maceration (DM) at two sample-to-solvent ratios (1 : 5 and 1 : 10, w/v). Antioxidant activity is expressed as DPPH radical inhibition percentage (% inhibition). No statistically significant differences were observed between the extraction methods or ratios ($p > 0.05$); data are presented as mean \pm standard deviation ($n = 3$)

a more standardized and quantitative comparison of antioxidant capacity across samples. Most importantly, despite these observations, the differences among the various extraction techniques, irrespective of the sample-to-solvent ratios employed, were not statistically significant ($P > 0.05$). As a result, both approaches demonstrated comparable effectiveness in extracting TPC (Fig. 2) and AA (Fig. 3 and 4), suggesting that neither

method offered a distinct advantage under the tested experimental conditions. These findings are consistent with those of Kadam et al. (2015), who similarly reported no significant variation in TPC among species of brown seaweeds when different extraction techniques were applied. Although statistically significant differences in TPC and AA between the DM and UAE methods were not observed, UAE presents clear practical advantages.

Specifically, UAE achieves comparable results in just 30 minutes, compared to the 24 hours required for DM, markedly improving process efficiency. This substantial reduction in extraction time can lead to lower operational costs, including decreased energy consumption and labor demands, potentially offsetting the initial investment in ultrasonic equipment. Therefore, UAE represents a more time- and cost-efficient strategy for the extraction of bioactive compounds from seaweeds. Nonetheless, DM remains a viable and accessible alternative, particularly in settings where advanced extraction technologies are unavailable or where budgetary constraints preclude the use of ultrasonic systems. Importantly, this study represents the first documented assessment of TPC and AA in *S. angustata* under the specific experimental conditions applied. To the best of our knowledge, there are no prior studies in the literature that have investigated TPC and AA in *S. angustata*, highlighting the novelty of our research. In our experiment, the TPC was determined to be 2.4–2.6 mg GAE.g⁻¹ DW, which is consistent with earlier reports suggesting generally low TPC levels in *Saccharina* species. It is important to acknowledge that the Folin-Ciocalteu assay, employed in our study for TPC quantification, lacks complete specificity for phenolic compounds and can also react with a range of non-phenolic reducing agents. Consequently, the TPC values obtained should be regarded as indicative of the sample total reducing capacity rather than a precise determination of phenolic constituents. Nevertheless, this colorimetric method remains widely accepted and has been effectively utilized for TPC assessment in numerous natural matrices, as evidenced not only by our previous published peer-reviewed publications (Valková et al., 2021a;

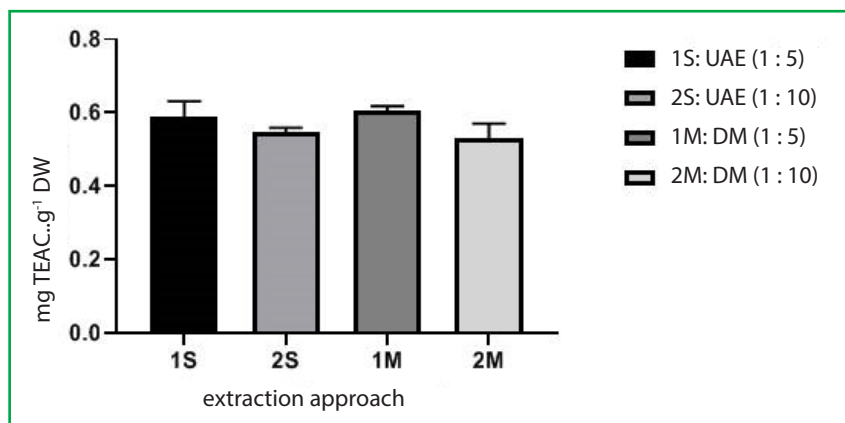


Figure 4 Comparison of antioxidant activity (AA) in *Saccharina angustata* extracts obtained using ultrasound-assisted extraction (UAE) and dynamic maceration (DM) at two sample-to-solvent ratios (1 : 5 and 1 : 10, w/v) antioxidant activity is expressed as Trolox Equivalent Antioxidant Capacity (TEAC); no statistically significant differences were observed between the extraction methods or ratios ($p > 0.05$); data are presented as mean \pm standard deviation ($n = 3$)

2021b; 2022), but also by other independent studies (Noreen et al., 2017; Lorenzo et al., 2020; Phuyal et al., 2020).

While specific data for *S. angustata* are lacking, similar trends have been observed in related species, as found in our study. For example, Heffernan et al. (2015) reported a TPC of 1.39 mg GAE.g⁻¹ DW in *L. digitata* using the DM method, while Kadam et al. (2015) recorded a TPC of 0.36 mg phloroglucinol equivalents (PGE).g⁻¹ in *L. hyperborea* via UAE. These findings, like ours, reflect the relatively low phenolic content typically found in brown algae. Consistent with the low TPC, our results also revealed low AA, as expected due to the well-established correlation between phenolic content and antioxidant capacity. This relationship is further supported by Heffernan et al. (2015), who similarly observed limited radical scavenging activity in *L. digitata* using the DPPH assay, in line with our observations. It is important to note that comparing polyphenol content across macroalgal studies is challenging due to variability in extraction techniques, quantification methods, and data reporting.

Furthermore, several biological and environmental factors including species, seasonal variation, algal age, geographic origin, and ecological conditions can significantly influence the phytochemical profile and phenolic composition of marine algae (Machu et al., 2015; Generalić Mekinić et al., 2019).

The optimization of extraction methods without the application of heat is particularly important when isolating polyphenols from brown algae, which contain thermolabile phlorotannins (Ummat et al., 2020). For this reason, the main objective of our study was the extraction of polyphenols without heat application. Purposely, we selected UAE and DM which allow for efficient extraction of polyphenols at low temperatures. In fact, UAE can significantly enhance the release of phenolic compounds by disrupting cell walls through acoustic cavitation, thereby increasing extraction yield (Ummat et al., 2020). On the other hand, DM is an extraction technique based on diffusion, where organic solvents are selected according to their polarity to extract target compounds (Sunarwidhi et al., 2022).

Furthermore, the 1 : 10 solid-to-solvent ratio demonstrated a slight advantage across both extraction methods employed in this study. Stirring at this ratio yielded the highest maximum recovery; however, it also exhibited greater variability. In contrast, sonication produced consistent and reliable results at both ratios, with particularly stable outcomes at 1 : 10. Given the minimal differences in average values, sonication appears to be the more suitable option when consistency and reproducibility are priorities. The solid-to-solvent ratio plays a critical role in the efficient extraction of bioactive compounds from algae, particularly phenolic compounds. Brown algal cell walls are rich in complex polysaccharides (e.g., alginates, fucoidans), which can impede the release of phenolics. An optimized solvent ratio enhances mass transfer, solvent penetration, and solubilization of target molecules, thereby improving extraction efficiency without oversaturating the system. An optimized solvent ratio can help improve solvent diffusion and extraction efficiency without oversaturating the system (Generalić Mekinić et al., 2019). Although it is commonly assumed that increasing the solid-to-solvent ratio enhances polyphenol extraction, our findings suggest otherwise. A higher ratio did not result in a significantly greater yield, aligning with literature indicating that extraction efficiency of phenolic compounds from brown algae is influenced by multiple factors (Santos et al., 2019) and it plateaus once equilibrium is reached. This behavior is particularly relevant when using ethanol, a polar solvent effective for extracting a broad spectrum of phenolic compounds. However, beyond a certain threshold, the mass transfer driving force diminishes, and further solvent addition yields no significant improvement (Taha et

al., 2024). Therefore, optimizing extraction parameters such as ethanol concentration, temperature, time, and solid-to-solvent ratio is essential for maximizing phenolic recovery from brown algae (Santos et al., 2019).

4 Conclusions

This study presents the first report on the effect of UAE and DM on polyphenols content in brown macroalga, *S. angustata*. Among the two extraction methods and two different solid-to-solvent ratios (1 : 5, 1 : 10, w/v) employed, none of these approaches yielded significantly ($P > 0.05$) higher extraction efficiency, TPC or greater AA in these extracts. Hence, it can be concluded that both methods are equally effective under the conditions applied in this study. This suggests that the selection between UAE and DM may be driven more by practical factors, such as equipment availability, cost-effectiveness, and scalability, rather than extraction efficiency alone. Moreover, the comparable performance of DM reinforces its potential as a simple and accessible method, particularly suited for the gentle extraction of thermolabile bioactive compounds in contexts where advanced technologies like UAE may not be feasible. The outcomes of this study are particularly relevant to the nutraceutical and functional food sectors, and similar investigations should be carried out using other commercially valuable edible or non-edible macroalgae.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Martina Lukáčová: conceptualization, data curation, investigation, methodology, software, resources, validation, visualization, writing – original draft, writing – review & editing; Mária Adamkovičová: data curation, formal analysis, investigation, methodology; Eva Kováčiková: data curation, investigation, methodology, software, validation; Hana Ďúranová: conceptualization, funding acquisition, investigation, project administration, software, resources, supervision, visualization, writing – original draft, writing – review & editing

AI and AI-Assisted Technologies Use Declaration

No generative AI tools/AI-assisted technologies were used during the preparation of the manuscript.

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