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Determination of Total Phenolic Amounts of Chloroform, Acetone and Methanol Extracts of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.*

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ABSTRACT

The present study was aimed to determine the total phenolic amounts of the bioactive contents resulting from the extraction of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.* with different solvents such as chloroform, acetone and methanol. Total phenolic content was determined using the Folin-Ciocalteu method. Samples (12.5%) were extracted with chloroform, acetone, and methanol, then filtered and the solvents were evaporated. Absorbance was measured at 760 nm. Total phenolic content was expressed as gallic acid equivalents (GAE). The total phenolic contents obtained from chloroform, acetone and methanol extracts of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.* were determined as 136.40±1.93 mg GAE/g extract -3.61±0.18 mg GAE/g extract -7.83±0.03 mg GAE/g extract, 5.25±0.07 mg GAE/g extract -4.27±0.1 mg GAE/g extract -14.5±0.06 mg GAE/g extract and 15.7±0.27 mg GAE/g extract -2.6±0.04 mg GAE/g extract -14.84±0.11 mg GAE/g extract, respectively. Differences between total phenolic values were found to be statistically significant ($p<0.05$). The extraction yields obtained from chloroform, acetone and methanol extracts of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.* were determined as 2.2±0.06%-4.35±0.07%-6.68±0.14%, 2.96±0.02%-4.33±0.09%-9.74±0.16% and 14.96±0.24%-10.03±0.004%-19.03±0.3%, respectively ($p<0.05$). Biochemical parameters such as dry matter, protein, ash and lipid of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.* were found to be 94.05±0.01% -20.92±0.35% -26.55±1.77% -1.36±0.01%, 94.1±0.005%-17.04±0.04%-28.11±0.06% -2.66±0.05% and 95.1±0.05%- 23.01±0.07%- 9.59±0.787%-25.5±0.21%. The soluble protein concentrations of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.* in distilled water were determined as 66.45±0.5mg/g, 83.38±0.88 mg/g and 115.95±0.89 mg/g, respectively. In conclusion, chloroform and methanol extracts of *Schizochytrium sp.* had good extraction yield and phenolic content. On the other hand, the chloroform extract of *Sargassum sp.* had the highest phenolic content, while the extraction yield was at the lowest level. Acetone extracts of the 3 species tested exhibited low phenolic activity.

Keywords: *Sargassum sp.*, *Ulva sp.*, *Schizochytrium sp.*, Different solvents, Total phenolics.

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Introduction

Algae and their secondary metabolites, as primary producers in the aquatic ecosystem, play a crucial role in the environmental cycle and are widely utilized across various sectors. Macroalgae are known as three groups depending on pigmentation, namely brown (2000 species), red (7300 species) and green (1500 species) [1]. FAO stated that 28.6% (122.58 million tons) of world aquaculture production is macroalgae cultivation [2]. Cai et al. revealed that the tonnage and economic contributions of brown algae and other algae such as red and green to the world macroalgae production are 47.3%-52% and 52.7%-48%, respectively [3]. Additionally, the researcher has shown that green macroalgae contribute the lowest (less than 1%) to the world macroalgae production. Yu et al. stated that *Schizochytrium sp.* is an important source of antioxidant compounds such as phenolics and DHA product [4]. La et al. stated that phenolic compounds and n-3 PUFAs in *Schizochytrium sp.* can regulate the antioxidant function of animals [5].

Since macroalgae are exposed to various abiotic and biotic stresses during their life cycle in the ecosystem, they produce secondary metabolites to have strong defense systems. Phenolic compounds are indicators of stress algae metabolism [6]. Jégou et al. pointed out that macroalgae are rich in phytochemicals including antioxidants [7]. Researchers have reported that macroalgae contain carotenoids, lipids, dietary fiber, proteins, minerals, vitamins, and phenolic compounds, which are biologically active metabolites [8]. Wang et al. revealed that strong relations between phenolic compounds and antioxidant activities were found [9]. Phenolic compounds having antioxidant potential have been intensely investigated in recent years. Phenolic compounds are considered one of the most important classes of natural antioxidants [10]. Researchers have stated that phenolic compounds increase the antioxidant capacity of algae and that algae also contain phenolic compounds that are beneficial to human health [7]. Sadeghi et al. showed that applications of extracted phenolic compounds were pharmaceutical, biomedical, cosmetic industry, packaging industry, food industry and textile industry [11].

Algae attract attention due to their bioactive properties. The study aimed to reveal the total phenolic contents as well as their biochemical compositions, extraction yields and soluble protein concentrations of *Ulva sp.* (Chlorophyta), *Sargassum sp*. (Ochrophyta) and *Schizochytrium sp,*

Materials and Methods

The *Sargassum* sp., *Ulva* sp., and *Schizochytrium* sp. meals used in this study was sourced from commercial suppliers, namely Akuamaks (Turkey) and Fuzhou Wonderful Biological Technology Co. Ltd. (China). Solvents with varying polarities, including chloroform, acetone, and methanol, were employed for the extraction of *Sargassum sp*., *Ulva sp*., and *Schizochytrium sp*. meals.

Total Phenolic Content

The total phenolic content was determined using the Folin-Ciocalteu method as described by Singleton et al. [12]. Briefly, the samples were extracted with chloroform, acetone, and methanol at a concentration of 12.5 g/100 mL by stirring at room temperature for 24 hours. The resulting extracts were filtered through Whatman No. 1 filter paper, and the solvents were evaporated using a rotary evaporator. Each extract (1 mg/ml) was added to a universal bottle, followed by 1 mL of Folin-Ciocalteu reagent. The mixture was thoroughly mixed, and after 3 minutes, 3 ml of sodium carbonate solution was added. The mixture was then left to stand in the dark at room temperature (25°C) for 2 hours, as the reaction is sensitive to light. Absorbances were measured at 760 nm using a UV-1280 Shimadzu spectrophotometer. A gallic acid (GA)standard curve curve (0.0625,0.125,0.25,0.5 and 1 mg GA/ml) shown in Figure 1, was used in the calculations. Total phenolic content was expressed as gallic acid equivalents (mg GAE/g extract). Extraction yield (w/w) was used as an indicator of solvent efficiency. Total phenolic contents of *Sargassum sp*., *Ulva sp.* and *Schizochytrium sp.* were determined in triplicate.

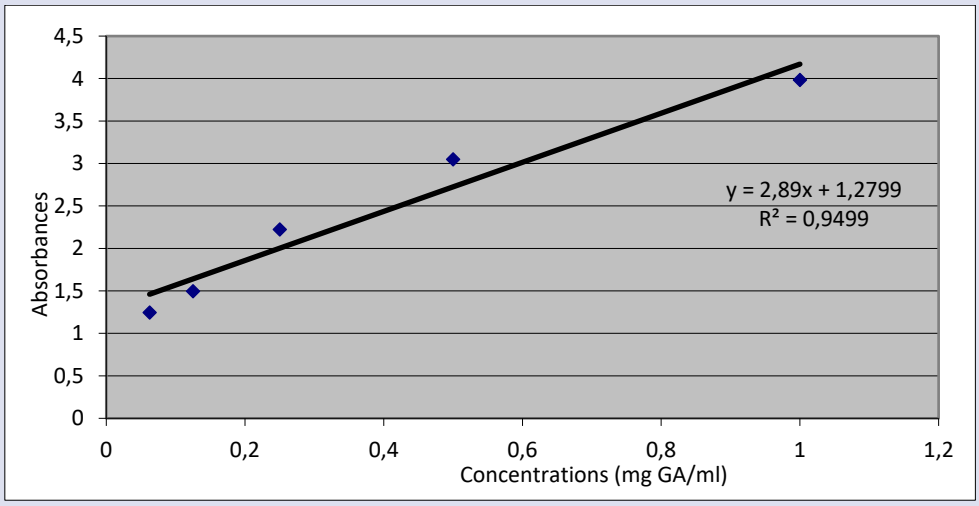

Figure 1. Standard curve obtained from different concentrations of GA.

Biochemical Compositions

Dry matter analysis was based on the principle of evaporating the moisture in the samples at 105 °C. For ash analysis, the samples were incinerated at 550 °C for 4 hours, cooled in a desiccator, and then weighed using a scale with a sensitivity of 0.0001 g. Lipid analysis was conducted using the chloroform–methanol extraction method described by Bligh and Dyer [13]. Protein content was determined following standard AOAC procedures [14], which involve three main steps: digestion, distillation, and titration. During digestion, the samples were incinerated at 420 °C. This was followed by the distillation step, and finally, titration was carried out using 0.1 N HCl. The volume of 0.1 N HCl consumed during titration was used in the calculations. Biochemical compositions of *Sargassum sp*., *Ulva sp.* and *Schizochytrium sp.* were determined in triplicate

Soluble Protein Contents

Soluble protein concentrations of *Sargassum sp*., *Ulva sp*. and *Schizochytrium sp.* were determined in triplicate using the dye-binding method developed by Bradford [15] (Bio-Rad Protein Assay, Cat. No: 5002). Following the procedure provided with the Bio-Rad kit, absorbance was measured at 595 nm using a Shimadzu UV–1280 spectrophotometer. The absorbance values were calculated based on a BSA (Bovine Serum Albumin) standard curve.

Statistical Analyses

Analyses were performed in triplicate. Statistical analyses were performed using SPSS 17.0 software. Duncan's multiple comparison test was applied at a 5% significance level. The results were expressed as mean ± standard error (SE).

Results

In current study, the total phenolic amounts of the bioactive extracts obtained with different solvents such as chloroform, acetone and methanol from *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp*. were determined and the

results are given in Table 1. Also, the biochemical compositions, extraction yields obtained from different solvents and the soluble protein concentrations of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.* were determined.

Table 1. Total phenolic contents of algae extracted in different solvents (mg GA/g extract)

	Solvents		
Species	**Chloroform**	**Acetone**	**Methanol**
Sargassum sp.	136.40±1.93^{c}	3.61±0.18^{a}	7.83±0.03^{b}
Ulva sp.	5.25±0.07^{b}	4.27±0.1^{a}	14.5±0.06^{c}
Schizochytrium sp.	15.7±0.27^{c}	2.6±0.04^{a}	14.84±0.11^{b}

a,b,c show statistical differences (p<0.05)

The total phenolic contents obtained from chloroform, acetone and methanol extracts of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.* were determined as 136.40±1.93 mg GAE/g extract-3.61±0.18 mg GAE/g extract-7.83±0.03 mg GAE/g extract, 5.25±0.07 mg GAE/g extract-4.27±0.1 mg GAE/g extract-14.5±0.06 mg GAE/g extract and 15.7±0.27 mg GAE/g extract-2.6±0.04 mg GAE/g extract-14.84±0.11 mg GAE/g extract, respectively. Differences between total phenolic values were found to be statistically significant (p<0.05). The highest total phenolic content was observed in the chloroform extract of *Sargassum sp.* while the lowest total phenolic contents were in the acetone extracts of all tested species. The lowest total phenolic content was found in acetone group of *Schizochytrium sp.* The extraction yields of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.* extracted with different solvents are given in Table 2. The extraction yields obtained from chloroform, acetone and methanol extracts of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.* were determined as 2.2±0.06%-4.35±0.07%-6.68±0.14%, 2.96±0.02%-4.33±0.09%-9.74±0.16% and 14.96±0.24%-10.03±0.004%-19.03±0.3%, respectively (p<0.05). The highest extraction yield was determined in the methanol extract of *Schizochytrium sp.* The all extracts of *Schizochytrium sp.* have higher extraction yields according to the extracts of *Sargassum sp.* and *Ulva sp.*

In study, the biochemical compositions of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.* were also determined (Table 3). Biochemical parameters such as dry matter, protein, ash and lipid of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.* were found to be 94.05±0.01%-20.92±0.35%- 26.55±1.77%- 1.36±0.01%, 94.1±0.005%-17.04±0.04%- 28.11±0.06%- 2.66 ± 0.05% and 95.1±0.05%- 23.01±0.07%- 9.59±0.787%- 25.5±0.21% (p<0.05). The highest dry matter, protein, ash and lipid amounts were observed in *Schizochytrium sp.* (95.1±0.05%), *Schizochytrium sp.* (23.01±0.07%), *Ulva sp.* (28.11±0.06%) and *Schizochytrium sp.* (25.5±0.21%).

Table 2. The extracted yields of algae extracted in different solvents (%)

	Solvents		
Species	**Chloroform**	**Acetone**	**Methanol**
Sargassum sp.	2.2±0.06^{a}	4.35±0.07^{b}	6.68±0.14^{c}
Ulva sp.	2.96±0.02^{a}	4.33±0.09^{b}	9.74±0.16^{c}
Schizochytrium sp.	14.96±0.24^{b}	10.03±0.004^{a}	19.03±0.3^{c}

a,b,c show statistical differences (p<0.05)

Table 3. Biochemical compositions of algae (% dry basis)

Species	**Biochemical Compositions**			
	Dry Matter	**Protein**	**Ash**	**Lipid**
Sargassum sp.	94.05±0.01^{a}	20.92±0.35^{b}	26.55±1.77^{b}	1.36±0.01^{a}
Ulva sp.	94.1±0,005^{a}	17.04±0.04^{a}	28.11±0.06^{c}	2.66±0.05^{b}
Schizochytrium sp.	95.1±0.05^{b}	23.01±0.07^{c}	9.59±0.787^{a}	25.5±0.21^{c}

a,b,c show statistical differences (p<0.05)

The soluble protein concentrations of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.* in distilled water were determined as 66.45±0.5mg/g, 83.38±0.88 mg/g and 115.95±0.89 mg/g, respectively p<0.05). The lowest and highest amounts of the soluble protein concentrations were *Sargassum sp.* (66.45±0.5mg/g) and *Schizochytrium sp.* (115.95±0.89 mg/g), respectively.

Discussions

In the present study, the total phenolic contents of bioactive extracts obtained from *Sargassum sp.*, *Ulva* sp., and *Schizochytrium* sp. using different solvents such as chloroform, acetone, and methanol were determined. In addition, the biochemical compositions, extraction yields from different solvents, and soluble protein concentrations of *Sargassum sp.*, *Ulva sp.*, and *Schizochytrium sp*. were investigated.

El-Baky et al. [16] found that total phenolic content of *Ulva lactuca* was 4.6±0.58 mg GAE/g extract. Kumar et al. [17] determined phenolic contents in three green algae were 32.57-61.69 mg/g dry weight. Gaffney et al. [18] found that the highest total phenol content of *Schizochytrium sp.* was 3.1 ± 0.1 mg GAE/g. Çelenk [19] revealed that total phenolic contents of Chlorophyta and Ochrophyta species were 126.3-6.3 mg GAE/g and 477.2-3.3 mg GAE/g, respectively. Puspita et al. [20] found that soluble total phenolic content of viscozyme extract of the brown alga *Sargassum muticum* was 6.4% of dry weight. Güner [21] determined that total phenolic contents of brown and green macroalgae were 33.20±1.41 mg GAE/g methanol extract and 2.34±0.1 mg GAE/g (chloroform extract)-25.58±1 mg GAE/g (methanol extract), respectively. Yılmaz et al. determined that the highest and lowest phenolic contents of *Gongolaria barbata* (Ochrophyta) were 2.29±0.01 mg GAE/g extract and 0.41±0.01 mg GAE/g extract, respectively [8]. The total phenolic contents of Sargassum vulgare and Ulva intestinalis extracted using acetone, ethanol, chloroform, and methanol were determined to be 0.004 mg GAE/100 g-0.003 mg GAE/100 g-0.003 mg GAE/100 g-0.003 mg GAE/100 g and 0.003 mg GAE/100 g-0.002 mg GAE/100 g-

0.003 mg GAE/100 g-0.004 mg GAE/100 g, respectively [22]. Gür and Polat [23] found that phenolics ranged between 34.6-106.05 mg GAE/g dry weight. Santos et al. [24] determined that the lowest and highest total phenolic contents of *Sargassum muticum* were 1752 ± 46.3 mg GAE/L and 440.8 ± 20.4 mg GAE/L, respectively. Researchers have indicated that the observed differences in total phenolic content can be attributed to multiple factors, including environmental conditions, algal species, geographical origin, physiological variations, the choice of solvents, extraction conditions, nutrient availability, and the growing season [22].

Elnabris et al. [25] found that extraction yields of *Ulva lactuca, Enteromorpha compressa Padina pavonica* were 17%, 7.3% and 5.2%, respectively. Güner [21] showed that extraction yields of brown and green macroalgae were 228 mg (%0,21-chloroform)-3902 mg (%3,6-methanol) and 195 mg (%0,22-chloroform)- 927 mg (%1,1-methanol), respectively. Puspita et al. [20] revealed that extraction yields of aqueous extract and enzyme extract were 26.5 ± 4.7% of dry algal material and 32.6 ± 4.9% of dry algal material, respectively. Park et al. [26] showed that the extraction yields of brown macroalgae varied from 68.40% to 81.88%. Hashem et al. [27] showed that the extraction yield depends on the solvent polarity.

Researcher showed that biochemical compositions such as ash, lipid and protein of *Ulva sp.* and *Sargassum sp.* were 28.77-29.6% ; 0.38-3.4% ; 9.24-33.6% and 26.95-27.94% ; 0.91-1.37% ; 20.6-20.69%, respectively [28,29]. Øverland et al. [30] showed that ash, lipid and protein changes of green, brown and red macroalgae were 11-55% ; 0.3-2.8% ; 3.2-35.2%, 15-45% ; 0.3- 9.6% ; 2.4-16.8% and 12-42.2% ; 0.2-12.9% ; 6.4-37.6%, respectively. Naz et al. [31] showed that ash, lipid and protein amounts of green and brown macroalgae were 12.19-17.68% ; 1.74-4.84% ; 5.56-6.70% and 13.19 - 21.38% ; 4.31-5.83% ; 9.75-11.45%, respectively. Gür and Polat [23] determined ash, lipid and protein values of macroalgae were 3.12-77%, 0.25 - 6.35% and 2.94 - 6.15%. Allen et al. [32] revealed that *Schizochytrium* sp. is rich source of lipid and docosahexaenoic acid (DHA). Researcher showed that lipid, PUFA, EPA and DHA amounts of *Schizochytrium sp.* were 46-78%, 46.96%, 0.72% and 37.63%, respectively [33]. De Lima Valença et al. [34] revealed that protein, lipid, DHA and EPA amounts of the genus *Schizochytrium sp.* were 17% , 53% , 27.20% DHA and 0.28% EPA, respectively. Park et al. [26] showed that ash contents of macroalgae ranged from 16.79% to 26.02%.

Literatures showed that ash contents of macroalgae depend on the species, geographical, environmental factors, the presence of various mineral components and the amount of mineral absorbed [35]. Chakraborty and Bhattacharya [36] pointed out that the lipid contents of macroalgae can change according to the amounts of the elements in their environment. Ahmad et al. [37] revealed that brown macroalgae have high lipid contents than those of red and green macroalgae species. The differences in the protein amounts obtained from macroalgae could be due to factors such as geographic area, species, maturity and seasons [38]. Macroalgae contain between 8% and 47% protein by dry weight. Øverland et al. [30] showed that protein amounts of green and red macroalgae were higher than that of brown macroalgae. The researcher has demonstrated that biochemical differences can exist even within the same species. Variations in the ash, lipid, and protein contents of macroalgae may be attributed to factors such as species type, geographical location, season, sampling site, water quality, light intensity, salinity, temperature, and species-specific characteristics. [29]. Bernaerts et al. [39] stated that the observed differences may be due to the difference in the analysis methods used in the studies.

The soluble protein concentrations of *Sargassum sp.*, *Ulva sp.* and *Schizochytrium sp.* in distilled water were determined as 66.45±0.5mg/g, 83.38±0.88 mg/g and 115.95±0.89 mg/g, respectively ($p<0.05$). The lowest and highest amounts of the soluble protein concentrations were *Sargassum sp.* (66.45±0.5mg/g) and *Schizochytrium sp.* (115.95±0.89 mg/g), respectively. El-Sayed et al. [40] reported that soluble protein concentrations can vary depending on factors such as harvest time and cell degradation. Soluble proteins are associated with an increase in nitrogenous compounds, including nitrates, free amino acids, ammonia, nitrites, and short-chain peptides.

In conclusion, the chloroform and methanol extracts of *Schizochytrium sp.* demonstrated good extraction yields and notable phenolic content. Conversely, the chloroform extract of *Sargassum sp.* exhibited the highest phenolic content, despite having the lowest extraction yield. The acetone extracts of all three tested species showed low phenolic activity. Algae are globally recognized as sustainable resources with high bioactive potential. In this context, further optimization studies are needed on culturable algae, taking into account the factors that influence phenolic compound levels, extraction efficiency, soluble protein content, and overall biochemical composition. The data generated from such studies will enhance industrial interest in algae and broaden their range of applications.

Conflicts of interest

There are no conflicts of interest in this work.

Ethical Approval Statement

Since no human or animal subjects were used in this study, ethical approval was not required.

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