Comparative Analysis of Biological Activities and Phenolic Content between Fresh and Steamed Sargassum fusiforme in Different Extraction Solvents

Hyun-Hwa Lee 1, Jin-Sol Kim 1, Jun-Han Jeong 1, Sook-Young Lee 2,* and Chun-Sung Kim 3, *

1 Department of Biology, Chosun University, Gwangju 61452, Republic of Korea
2 Marine Bio Research Center, Chosun University, Wando 59146, Republic of Korea
3 Department of Oral Biochemistry, Chosun University, Gwangju 61452, Republic of Korea
* Correspondence: seedbank@chosun.ac.kr (S.-Y.L.); cskim2@chosun.ac.kr (C.-S.K.);
Tel.: +82-10-8610-6739 (S.-Y.L.); +82-62-230-7088 (C.-S.K.); Fax: +82-61-555-1260 (S.-Y.L.);
+82-62-608-5300 (C.-S.K.)

Abstract: Sargassum fusiforme (SF), a perennial brown, is often steamed for consumption. SF contains many bioactive compounds; however, the effect of steaming these compounds is unknown. This study investigates phenolic component contents and antioxidant and antiaging activities of fresh (SF) and steamed (SSF) extracts in different solvents (95% ethanol, 95% methanol, hot water). Total polyphenol and flavonoid content was highest in SSF methanol (9.76 mg TAN/g) and SF methanol extracts (13.42 mg QUE/g). The DPPH radical scavenging activity was highest in SF methanol extracts (IC50 = 5.83 µg/µL), whereas the FRAP activity was higher in SSF extracts. Methanol SSF extract exhibited higher SOD, CAT, and APX activities. Collagenase inhibition activity was higher in all SSF extracts, whilst tyrosinase inhibition activity was highest in SSF methanol extract (30.5%). Analysis of phenolic compound contents revealed that 4-hydroxybenzoic acid content was highest in SF methanol extract, whilst steaming increased naringenin and naringin contents in ethanol extracts. Finally, antioxidant activity of both extracts showed a positive correlation with polyphenol content. Overall, these results suggested that SSF ethanol and methanol extract had higher phenolic component content and antioxidant and antiaging activities. Thus, SSF can be potentially used in health functional foods and cosmetic products.

Keywords: Steamed sargassum fusiforme; antioxidant; antiaging; phenolic content

1. Introduction

Sargassum fusiforme (SF) is a perennial brown algae belonging to the Sargassaceae family. It is commonly known as Tot in South Korea, Hijiki in Japan, and Yang qi cai in China [1]. The cultivation of SF is widely distributed along the coasts of Asian countries, including South Korea, Japan, and China; in South Korea, the highest distribution is found along the southern and western coasts and on Jeju Island [2,3]. In South Korea, it is collected for consumption in either fresh, pretreated, or dried forms, with the latter two attenuating its astringent taste or increasing its storability [4,5]. The mean total production of SF in South Korea over the past five years (2016–2020) was 38,069 tons, and based on the outstanding quality, approximately 1800 tons were exported to Japan and China, making South Korea one of the major exporters of SF [6,7]. In Asian countries, including Japan and China, SF is mostly used as food, whereas its consumption is low in western countries such as Australia and the UK [8], thus implying the need to investigate the broad utility and applicability of SF.

SF contains an abundance of amino acids, glutamic acid, and aspartic acid; neutral polysaccharides, laminaran, and fucoidan; as well as dietary fibers and minerals. It also contains bioactive compounds such as phlorotannin, sterol, and various pigments, making
it an outstanding medicinal material as well as a source of dietary fibers [9,10]. Notably, SF has been reported to inhibit breast cancer cell proliferation, promote osteoblast activity, suppress adipocyte differentiation, and reduce total cholesterol and blood pressure. It has also been reported to have protective effects on the skin mediated by fucoidan, which inhibits photoaging, as well as by other polysaccharides, which inhibit melanin biosynthesis and suppress UV-induced oxidative stress [11–17].

Although marine algae are consumed in either fresh form or after subjecting them to cooking modes such as soaking, boiling, blanching, and steaming [18,19], SF has long been consumed after steaming or boiling rather than in the fresh form in Asia [5,18]. Steaming, the process of using steam to cook an ingredient, has been frequently used for cooking SF in South Korea. Compared to other cooking methods that involve heating (boiling and blanching), steaming prevents the destruction of useful components in the ingredient [20]. Notably, this method is also advantageous as it increases the elution of such useful components, including minerals, trace elements, and polysaccharides, by inducing increases or decreases in the components [21]. Nevertheless, previous studies have mostly focused on the bioactivity of fresh SF, and only a few studies have investigated its polyphenol content, antioxidant effects, and antiaging activities after subject to the different cooking modes.

Moreover, the useful components in marine algae, such as polysaccharides and phenolic compounds, are affected by not only the cooking process but also the extraction time and temperature, sample-to-solvent ratio, and extraction method (proportion and type of the extraction solvent) [22,23]. Notably, depending on the properties of the compound, certain solvents could be more effective than others; for example, semipolar solvents, such as ethanol and methanol, are more effective in extracting pigments and polyphenols, whereas polar solvents, such as water, are more effective in extracting polysaccharides and proteins [24,25]. Thus, the type and yield of the extracted compounds from marine algae may vary according to the extraction solvent type and method used; the various pretreatments necessary for marine algae to be fit for consumption may alter its components and their bioactivities [26,27]. Therefore, this study investigated the changes in bioactivity of SF due to steaming by comparatively analyzing the polyphenol, flavonoid, and phenolic contents as well as the antioxidant and antiaging activities between fresh and steamed SF in different extraction solvents. The results are anticipated to contribute towards the exploration of novel applications for steamed SF and in developing steamed SF-based materials for health functional foods and cosmetic products.

2. Materials and Methods

2.1. Sample Preparation

_Sargassum fusiforme_ (SF) was obtained from Wando-gun, Jeollanam-do, South Korea. The material was washed with distilled water three times to remove salt and dried at 65 °C for two days. To prepare steamed SF (SSF), fresh SF was steamed for 10 min using ≥95 °C water at the onset, followed by three washes with distilled water to remove salt, and then dried for 48 h at 65 °C. The dried SF and SSF samples were ground using a plant grinder (USC, Seoul, Republic of Korea) and stored at −20 °C for subsequent use in the extraction process.

2.2. Reagents

Both the 95% methanol and ethanol used for extraction were of the guaranteed reagent grade and purchased from Duksan General Science (Seoul, Republic of Korea). The Folin–Denis′ reagent used for measuring the total polyphenol and flavonoid contents was purchased from Fluka (Buchs, Switzerland). Sodium carbonate, tannic acid, quercetin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), BHT, 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), ascorbic acid, sodium acetate, and FeCl₃ were purchased from Sigma (St. Louis, MO, USA). All reagents used for measuring the antioxidant and antiaging activities were purchased from Sigma, except for the BSA protein assay kit (Thermo Fisher Scientific,
Waltham, MA, USA). The three reference materials used in the LC-MS/MS analysis—4-hydroxy benzoic acid, naringenin, and naringin—were purchased from Sigma, while the solvents, including acetonitrile and methanol, used in HPLC were purchased from Sigma.

2.3. Extract Preparation

Distilled water (400 mL) was added to the 8 g of dry SF and SSF samples and the mixtures were allowed to stand for 1 h at 24–26 °C. Then, hot water extraction at 80 °C was performed for 3 h. To prepare ethanol and methanol extracts, 80 mL 95% ethanol and methanol were added to the 8 g of SF and SSF powders, and the extraction was performed by shaking at 25 °C and 120 rpm for 24 h. The hot water extract was filtered using Whatman No. 2 filter paper and enriched at 70 °C using a rotary evaporator (V/V2000; Heidolph, Schwabach, Germany). The ethanol and methanol extracts were centrifuged at 4 °C, 3000 rpm for 20 min and enriched at 38 °C. The extracts were stored at −20 °C for subsequent use. The extraction yields of SF and SSF were calculated as follows:

\[
\text{Yield} (\%) = \left[\frac{A}{B}\right] \times 100, \tag{1}
\]

A: The weight of the dried extracts
B: The weight of the dried samples

2.4. Measurement of Total Polyphenol Content

The total polyphenol content in SF and SSF extracts was measured using a modified Folin–Denis’ method [28]. To 80 µL of 10 mg/mL SF and SSF extracts, 80 µL of Folin–Denis’ reagent was added, and the mixture was allowed to react at 24–26 °C for 3 min. Then, 80 µL of 10% sodium carbonate was added to the mixture and allowed to react for 1 h. Thereafter, the absorbance was measured at 760 nm using a Multiskan Go (1510, Thermo scientific). The total polyphenol content in the SF and SSF extracts was estimated based on a calibration curve drawn using tannic acid (TAN).

2.5. Measurement of Total Flavonoid Content

The total flavonoid content in SF and SSF extracts was measured using the method described by Nieva Moreno et al. [29] with a few modifications. To 5 µL of 10 mg/mL SF and SSF extracts, 5 µL of 10% aluminum nitrate, 5 µL of 1 M potassium acetate, and 235 µL of 95% methanol were added, and the mixture was allowed to react at room temperature 24–26 °C for 40 min. Thereafter, the absorbance was measured at 415 nm using a Multiskan Go. The total flavonoid content in the SF and SSF extracts was estimated based on a calibration curve drawn using quercetin (QUE).

2.6. Measurement of DPPH Radical Scavenging Activity

To measure the DPPH radical scavenging activity of the SF and SSF extracts, the reductive activity of the sample based on the electron donor effect on DPPH was measured. To 0.98 mL of 150 µM DPPH solution, 0.02 mL of 20 mg/mL SF and SSF extracts were added, and the mixture was left in the dark for 30 min. Thereafter, the absorbance was measured at 517 nm [30]. A synthetic antioxidant, BHT, was used as the positive control. The difference in absorbance between the sample and the negative control was estimated as percentage (%), and the DPPH radical scavenging activity was calculated as follows:

\[
\text{DPPH radical scavenging activity} (\%) = \left[1 - \frac{A}{B}\right] \times 100 \tag{2}
\]

A: Absorbance of the sample group
B: Absorbance of the negative control

2.7. Measurement of ABTS Radical Scavenging Activity

To measure the ABTS radical scavenging activity of the SF and SSF extracts in different solvents, 250 µL of 7 mM ABTS solution containing 140 mM potassium persulfate and
22 mL ethanol were mixed and allowed to react in the dark for 16 h. Then, 10 µL of 10 µg/µL SF and SSF extracts and 200 µL of ABTS solution were mixed and incubated at 24–26 °C for 3 min. Finally, the absorbance was measured at 734 nm [31]. Ascorbic acid was used as the positive control, and the ABTS radical scavenging activity was calculated as follows:

\[
\text{ABTS radical scavenging activity (\%) = \left(1 - \frac{A}{B}\right) \times 100}
\]

A: Absorbance of the sample group  
B: Absorbance of the negative control

2.8. Measurement of Ferric Reducing Antioxidant Power (FRAP) Activity

To measure the FRAP activity of the SF and SSF extracts in different solvents, the FRAP solution was prepared by mixing 300 mM sodium acetate (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl₃ in a 10:1:1 ratio. To 150 µL of FRAP solution, 10 µL of 10 µg/µL SF and SSF extracts were added and allowed to react at 37 °C for 30 min. Then, the absorbance was measured at 593 nm [32]. Ascorbic acid was used as the positive control, and the FRAP activity was calculated as follows:

\[
\text{FRAP activity (\%) = \left(1 - \frac{B}{A}\right) \times 100}
\]

A: Absorbance of the sample group  
B: Absorbance of the negative control

2.9. Measurement of the Antioxidant Enzyme Activity

2.9.1. Superoxide Dismutase (SOD) Activity

To measure SOD activity, the method described by Beauchamp and Fridovich [33] was used with a few modifications. Briefly, the reduction rate of nitro blue tetrazolium (NBT) was measured using the superoxide anion produced by the reaction between xanthine and xanthine oxidase. To 0.2 mL of the solution containing 50 mM potassium phosphate pH 7.0, 3 mM EDTA, 3 mM xanthine, and 0.75 mM NBT, 20 µL of SF and SSF extracts were added, and the mixture was allowed to react at 25 °C for 10 min. Then, 0.3 unit/mL xanthine oxidase was added, and the absorbance was measured at 560 nm. The SOD activity was calculated as follows:

\[
\text{SOD units/mg = \left[\frac{(A - B) \times 50}{B}\right]/\text{Sample volume} \times \text{Total volume}/\text{Pr (mg/mL)}}
\]

A: Absorbance of the sample group  
B: Absorbance of the negative control  
Pr: Concentration of the sample protein

2.9.2. Catalase (CAT) Activity

To measure catalase activity, the method described by Aebi [34] was used, wherein the amount of reduced hydrogen peroxide (H₂O₂) substrate is measured. To 0.2 mL of the solution containing 50 mM potassium phosphate pH 7.0 and 10 mM H₂O₂, 20 µL of the SF and SSF extracts were added, and the absorbance was measured at 240 nm every 30 s for 2 min. The catalase activity was calculated using the following formula, where 43.6 is the H₂O₂ extinction coefficient at 240 nm:
Catalase activity (Unit/mg) = \[
\frac{(A - B)}{43.6} \times \frac{\text{Total volume}}{\text{Sample volume}} / \text{Pr (mg/mL)}
\] (6)

A: Absorbance of the sample group
B: Absorbance of the negative control
Pr: Concentration of the sample protein

2.9.3. Ascorbate Peroxidase (APX) Activity

APX is a key enzyme involved in H$_2$O$_2$ removal alongside catalase. APX activity was measured using the method described by Nacano and Asada [35] with a few modifications, wherein the ascorbate is oxidized to measure the level of H$_2$O$_2$ reduction. To 0.2 mL of the solution containing 50 mM potassium phosphate pH 7.0, 0.5 mM ascorbate, 0.1 mM EDTA, and 0.1 mM H$_2$O$_2$, 20 µL of SF and SSF extracts were added, and the mixture was allowed to react at 37 °C for 5 min. Then, the absorbance was measured at 290 nm. The APX activity was calculated using the following formula, where 2.8 is the ascorbate extinction coefficient at 290 nm.

\[
\text{APX activity (Unit/mg)} = \frac{(A - B)}{2.8} \times \frac{\text{Total volume}}{\text{Sample volume}} / \text{Pr (mg/mL)}
\] (7)

A: Absorbance of the sample group
B: Absorbance of the negative control
Pr: Concentration of the sample protein

2.10. Measurement of Antiaging Activity

2.10.1. Collagenase Inhibitory Activity

To 50 µL of 1 mg/mL SF and SSF extracts, 12.5 µL of the solution containing 0.1 M Tris-HCl pH 7.5 and 4 mM CaCl$_2$ and 75 µL of 0.5 mg/mL collagenase were added, and the mixture was allowed to react at 37 °C for 20 min. The reaction was terminated by the addition of 0.25 mL of 6% citric acid. Finally, 750 µL of ethyl acetate was added, and the upper aqueous phase was obtained from the mixture. To a 96 well plate, 0.2 mL aliquot of the supernatant was added, and the absorbance was measured at 320 nm. The collagenase inhibitory activity was measured as follows [36,37]:

\[
\text{Collagenase inhibitory activity (%) = \frac{1 - (A - AB)}{(C - CB)} \times 100}
\] (8)

A: Absorbance of the sample group
AB: Absorbance of the sample group without the enzyme
C: Absorbance of the control group
CB: Absorbance of the control group without the enzyme

2.10.2. Elastase Inhibitory Activity

The elastase inhibitory activity was measured using the method described by Cannell et al. [38]. To 50 µL of 1 mg/mL SF and SSF extracts, 50 µL of a solution containing 0.5 mg/mL N-succinyl-(Ala)$_3$-p-nitroanilide dissolved in 50 mM Tris-HCl, pH 8.6 was added, and the mixture was allowed to react at 37 °C for 5 min. The absorbance was measured at 410 nm. Next, 50 µL of the solution containing 0.2 unit/mL porcine pancreas elastase prepared using 50 mM Tris-HCl, pH 8.6 was added and the mixture was allowed to react at 37 °C for 5 min. The absorbance at 410 nm was measured again, and the elastase inhibitory activity was calculated as follows [37]:

\[
\text{Elastase inhibitory activity (%) = \frac{A - (B - C)}{A} \times 100}
\] (9)

A: Absorbance of the control group with the enzyme
B: Absorbance of the sample group with the enzyme
2.11. Measurement of Tyrosinase Inhibitory Activity

The tyrosinase inhibitory activity was measured using the method described by Yagi et al. [39] with a few modifications. A mixture of 70 \(\mu\)L of 1.5 mM 3,4-dihydroxy-L-phenyl-alanine (L-DOPA) dissolved in 0.1 M potassium phosphate buffer (pH 6.8), 60 \(\mu\)L of 0.1 M potassium phosphate buffer (pH 6.8), and 20 \(\mu\)L of 1 mg/mL SF and SSF extracts was prepared and allowed to react for 5 min at 37 \(^\circ\)C. Then, 50 \(\mu\)L of 500 U/mL mushroom tyrosinase was added, followed by incubation for another 5 min at 37 \(^\circ\)C. Finally, the absorbance was measured at 490 nm, and the tyrosinase inhibitory activity was calculated as follows [37]:

\[
\text{Tyrosinase inhibitory activity (\%) = \frac{A - (B - C)}{A} \times 100}
\]  

A: Absorbance of the control group with the enzyme  
B: Absorbance of the sample group with the enzyme  
C: Absorbance of the sample group without the enzyme

2.12. Analysis of Phenolic Contents

The phenolic compounds in the SF and SSF extracts in different solvents were quantified using LC-MS/MS (4000 Q-Trap, AB Sciex, MA, USA; LC 20A System, Shimadzu, Kyoto, Japan). Three phenolic compounds, 4-hydroxy benzoic acid, naringenin, and naringin, were used in the quantification process, with acetonitrile and methanol as solvents for the analysis. In 1 mL of each extraction solvent, 20 ng of SF and SSF extracts were dissolved, the mixture was centrifuged at 4 \(^\circ\)C, and 3000 rpm for 10 min, and the supernatant separated and diluted 40-fold with distilled water. After analyzing 10 \(\mu\)L of this solution in a Gemini C18 column (3 \(\mu\)m, 110 Å, 50 mm \(\times\) 2.0 mm), the conversion was performed. Each sample was analyzed at least three times.

2.13. Statistical Analysis

Each experiment was repeated at least three times to obtain the mean and standard error of the mean values. Data were analyzed using the IBM SPSS Statistics (version 25; SPSS Inc., Chicago, IL, USA). To test the significance of each experimental result, ANOVA was performed with Duncan’s multiple test; statistical significance was set at \(p < 0.05\). In addition, the results of total polyphenol and flavonoid contents, antioxidant activities (DPPH and ABTS radical scavenging activities; FRAP activity; and SOD, CAT, and APX activities) of SF and SSF extracts were subjected to the following analyses using MetaboAnalyst 5.0 (http://www.metaboanalyst.ca 16 October 2022): principal component analysis (PCA) with biplot, variable importance in projection (VIP) score analysis, and correlation analysis. The variables that showed a correlation with the antioxidant activity of SF and SSF extracts in different solvents were investigated.

3. Results

3.1. Extraction Yield of SF and SSF Extracts in Different Solvents

In this study, 95% ethanol, 95% methanol, and hot water extracts of SF and SSF were prepared, and the extraction yield of SF and SSF in the different solvents ranged between 8–25% and 2–19%, respectively (Table 1). The highest yield was obtained for water extracts for both SF and SSF at 25.0% and 19.0%, respectively. Compared to methanol extract, the yield of water extract was 3.9-fold higher for SF and 6.6-fold higher for SSF.
Table 1. The extraction yields of fresh and steamed *Sargassum fusiforme* extracts.

| Solvent   | SF       | SSF      |
|-----------|----------|----------|
| Ethanol   | 8.5 ± 0.3 b | 7.8 ± 0.2 b |
| Methanol  | 6.4 ± 0.5 b | 2.9 ± 0.1 c  |
| Water     | 25.0 ± 2.2 a | 19.0 ± 0.9 a |

SF: *S. fusiforme* extract, SSF: steamed *S. fusiforme* extract. The different lowercase letters represent significant differences in the same column (*p* < 0.05).

Meinita et al. [10] reported that the content of carbohydrates in SF was higher than the other components (40–62%, dry weight). Dobrinčić et al. [25] found that monosaccharides, proteins, and minerals in brown algae are highly polar and thus easily extracted in water. In addition, Farvin et al. [40] examined 26 marine algae species, including *Sargassum asperifolium*, *Sargassum angustifolium*, and *Sargassum boveanum*, in different extraction solvents (50% ethanol, absolute ethanol, and water) and demonstrated a higher yield for water extracts, wherein proteins and the soluble and polar components were eluted in abundance. Thus, the higher yield of SF and SSF water extracts compared to the other two solvent extracts may be attributed to the high dissolution level of large amounts of carbohydrates in SF. The findings suggest that hot water extraction is more suitable to ensure an efficient yield for SF and SSF.

3.2. Total Polyphenol and Flavonoid Contents in SF and SSF Extracts in Different Solvents

Polyphenols include phenolic acid, flavonoid, tannin, and lignin, and marine algae contain an abundance of polyphenols and secondary metabolites, including flavonoids [41]. The polyphenols and flavonoids in marine algae are essential for survival as they help clear UV-induced reactive oxygen species and support the structural integrity in the sea. Moreover, these components are also beneficial for human health owing to their high nutritional level and have a role in disease prevention [42,43].

Analysis of the total polyphenol contents in the SF and SSF ethanol, methanol, and water extracts showed that the polyphenol content range across extraction solvents was 4–8 mg TAN/g for SF and 4–10 mg TAN/g for SSF (Figure 1a). The highest total polyphenol content was detected in methanol extracts for both SF and SSF (9.8 mg TAN/g), compared to that of ethanol and water extracts. Interestingly, the highest total polyphenol content in SSF methanol extract was 1.3-fold higher than that of SF methanol extract. The total polyphenol content in SF and SSF ethanol extracts was 4.5 mg TAN/g and 4.8 mg TAN/g, respectively, without a significant difference between SF and SSF content. The lowest total polyphenol content at 4.3 mg TAN/g was detected in SSF water extract. The highest total polyphenol content observed in SF and SSF methanol extracts observed in the present study is in line with the findings of Lee et al. [44], who reported that the SF methanol extract showed higher total polyphenol content than 70% ethanol and water extracts. In addition, phenolic compounds combined with proteins or cellulose have been found, and heat treatment of food was reported to increase the phenolic content through the release of these combined phenolic compounds [45]. This could explain the higher total polyphenol content in methanol and ethanol extracts of SSF than for SF in the present study.

The total flavonoid content range across extraction solvents was 0–14 mg QUE/g for SF and 0–13 mg QUE/g for SSF (Figure 1b). The SF and SSF water extracts had the lowest total flavonoid content (1.0 mg QUE/g and 0.4 mg QUE/g, respectively), whereas the SF methanol (13.4 mg QUE/g) and SSF ethanol (12.8 mg QUE/g) extracts showed the highest total flavonoid content. The total flavonoid content in the SSF ethanol extract was 1.4-fold higher than that of the SF ethanol extract. The total flavonoid content in water extract was 1.0 mg QUE/g for SF and 0.4 mg QUE/g for SSF, at markedly lower levels compared to other extracts. Sujatha et al. [46] measured the total flavonoid content in different extraction solvents (aqueous, ethanol, methanol, and acetone) for *Sargassum swartzii* and found the highest content in the methanol extract (26.9 mg QE/g), followed by acetone, ethanol, and...
aqueous extracts, which is in agreement with the higher total flavonoid content detected in methanol and ethanol extracts in the present study.

![Figure 1](image_url)

Figure 1. Total polyphenol (a) and flavonoid (b) contents in ethanol, methanol, and water extracts of fresh and steamed Sargassum fusiforme. SF: S. fusiforme extract, SSF: Steamed S. fusiforme extract, TAN: Tannic acid, QUE: Quercetin. The different lowercase letters represent significant differences between solvents within the same sample ($p < 0.05$).

Total flavonoid contents varies depending on the determination method used (aluminum nitrate or aluminum chloride assay), the standard compounds used for calculation (quercetin, rutin, morin, etc.), and the difference in absorbance among individual flavonoids in each extract [47,48]. In this study, an aluminum nitrate colorimetric assay was used for flavonoid content determination. This assay was reported to be specific for measuring flavonols and flavones [49]. Consequently, the assay indicated that the flavonoids in the SF and SSF extracts were mainly flavonols and flavones. Due to its cost-effectiveness, rapidity, and easy repeatability [48], this assay is widely used for measuring total flavonoid content; however, it faces the difficulty of not being able to determine all flavonoid types [50–52].

The total polyphenol and flavonoid contents in methanol and ethanol extracts were higher for SSF compared with SF. This may be attributed to the changes in polyphenol and flavonoid contents of SF extracted in different solvents due to heat treatment. Moreover, the highest polyphenol and flavonoid contents were observed in the methanol SF and SSF extracts, indicating that they could potentially contribute to antioxidant activity.

3.3. DPPH Radical Scavenging Activity in SF and SSF Extracts in Different Solvents

The IC$_{50}$ of DPPH radical scavenging activity ranged 5–69 µg/mL for SF and 8–26 µg/mL for SSF extracted in different solvents. The SF and SSF methanol extracts showed the highest activity, followed by ethanol and water extracts. The highest IC$_{50}$ value of activity for SF and SSF methanol extracts were 5.8 µg/µL and 8.4 µg/µL, respectively (Table 2). The IC$_{50}$ values of activity for SSF ethanol extract was 19.8 µg/µL, which was 1.76-fold higher than that for SF ethanol extract. In addition, the lowest activity was shown by water extracts, with SSF (IC$_{50}$ = 25.4 µg/µL) showing 2.7-fold higher activity than SF (IC$_{50}$ = 68.5 µg/µL).

In agreement with the findings of the present study, Sobuj et al. [53] reported that the DPPH radical scavenging activity for solvent-extracted Sargassum corifolium and Hypnea pannosa was in the order of methanol > ethanol > water. In another study by Sasadara et al. [54], the DPPH radical scavenging activity of the red algae Gracilaria sp. was high in 100% ethanol and methanol extracts at IC$_{50}$ = 13.60 µg/mL and IC$_{50}$ = 38.03 µg/mL, respectively, whereas the lowest activity was detected in water extract (IC$_{50}$ = 622.30 µg/mL). Cox et al. [55] found that the DPPH radical scavenging activity of the H. elongate methanol extract at 100 µg/mL according to the pretreatment method was 75% for the fresh sample and 52.5% for the sample steamed for 45 min, indicating the possibility that heat treatments, such as steaming, boiling, and microwaving, destroy the thermally unstable antioxidants. In contrast to these reports, the DPPH radical scavenging activity was higher in steamed
SF than that of fresh SF in the present study, implying that conditions such as the steaming duration and extraction solvent influence the DPPH radical scavenging activity in SF and SSF. In this study, the DPPH radical scavenging activity was determined as IC$_{50}$ using a general method based on expressing the discoloration percentage of the solution after the reaction. It was reported that the initial absorbance of the colored radical is reduced when a reaction with a potential antioxidant occurs and a change of absorbance depends on the reaction time and conditions, reactivity, solvent used, and concentration of the tested phenol [56,57]. Since a single point was measured here, it is necessary to analyze the DPPH radical scavenging activity of SF and SSF extracts by monitoring the entire reaction time.

**Table 2.** The DPPH, ABTS, and FRAP IC$_{50}$ values of fresh and steamed *Sargassum fusiforme* extracts.

| Solvent     | DPPH (IC$_{50}$ = µg/µL) SF | ABTS (IC$_{50}$ = µg/µL) SF | ABTS (IC$_{50}$ = µg/µL) SSF | FRAP (IC$_{50}$ = µg/µL) SF | FRAP (IC$_{50}$ = µg/µL) SSF |
|-------------|-------------------------------|-----------------------------|-------------------------------|-----------------------------|-----------------------------|
| Ethanol     | 34.8 ± 0.3$^b$                | 19.8 ± 1.2$^b$              | 13.9 ± 1.3$^a$                | 11.3 ± 0.3$^b$              | 17.4 ± 0.2$^c$              |
| Methanol    | 5.8 ± 0.1$^a$                 | 8.4 ± 0.1$^a$               | 14.1 ± 0.9$^a$                | 6.7 ± 0.1$^a$               | 6.1 ± 0.3$^a$               |
| Water       | 68.5 ± 3.0$^c$                | 25.4 ± 1.1$^c$              | 18.5 ± 0.5$^b$                | 56.5 ± 0.6$^c$              | 11.5 ± 0.2$^b$              |

Ascorbic acid: 0.5 ± 0.1 0.2 ± 0.1 0.2 ± 0.1

SF: *S. fusiforme* extract, SSF: Steamed *S. fusiforme* extract. The different lowercase letters represent significant differences in the same column ($p < 0.05$).

### 3.4. ABTS Radical Scavenging Activity in SF and SSF Extracts in Different Solvents

The IC$_{50}$ of ABTS radical scavenging activity ranged 13–19 µg/µL for SF and 6–57 µg/µL for SSF in ethanol, methanol, and water extracts (Table 2). Although the activity did not significantly vary between SF ethanol and methanol extracts (IC$_{50}$ = 13.9 µg/µL and 14.1 µg/µL, respectively), the highest activity was detected in ethanol extract. For SSF, the highest activity was detected in methanol extract (IC$_{50}$ = 6.7 µg/µL) and the lowest in water extract (IC$_{50}$ = 56.5 µg/µL), with a similar trend as that for DPPH radical scavenging activity. The ethanol extracts of SF and SSF showed similar levels of ABTS radical scavenging activity, with IC$_{50}$ values of 13.9 µg/µL and 11.3 µg/µL, respectively. However, the SSF methanol extract showed 2.1-fold higher activity than that of SF methanol extract, whereas SSF water extract showed the lowest activity that was 3-fold lower than that of SF water extract.

Lee et al. [44] measured the ABTS radical scavenging activity in different SF extracts and found that the activity was lower in SF water extract, obtained by high-pressure extraction, (IC$_{50}$ = 1.64 mg/mL) than that of SF ethanol and methanol extracts (IC$_{50}$ = 0.90 mg/mL and 0.96 mg/mL, respectively); thus, they reported that the radical scavenging activity of SF was dependent on the extraction solvent. Sobuj et al. [58] extracted *Padina tetrastromatica* and *Gracilaria tenuistipitata* using different extraction solvents (methanol, ethanol, and water) and showed that the ABTS radical scavenging activity was the highest in methanol extracts, followed by ethanol and water extracts; moreover, they observed a positive correlation between the ABTS radical scavenging activity and the total phenolic content, suggesting that the total phenolic content is a critical determinant of the ABTS radical scavenging activity. Similar to these reports, SF and SSF water extracts showed lower DPPH and ABTS radical scavenging activities than those in ethanol and methanol extracts in the present study. The increase in total polyphenol content in SSF ethanol and methanol extracts due to steaming may have had an effect on the ABTS radical scavenging activity, resulting in the higher activity in SSF compared to SF extracts.

Juliao et al. [59] measured the ABTS radical scavenging activity of seaweeds (*Treptacanthha abies-marina*, *Cystoseira humilis*, *Asparagopsis armata*, and *Asparagopsis taxiformis*) under different drying conditions; shade-dried extracts demonstrated 20–50% lower activity compared to sun-dried extracts. As the ABTS radical scavenging activity may vary according to the drying method, it is necessary that future studies compare the activity under drying conditions and methods other than those used in our study (65° C for 48 h).
3.5. FRAP Activity in SF and SSF Extracts in Different Solvents

The IC$_{50}$ of FRAP activity ranged 6–18 $\mu$g/µL for SF and 3–10 $\mu$g/µL for SSF across the three extraction solvents (Table 2). For SF, the activity was the highest in methanol extract (IC$_{50}$ = 6.1 $\mu$g/µL), followed by water and ethanol extracts. For SSF, similar levels of activity were observed in ethanol and methanol extracts at IC$_{50}$ values of 3.5 $\mu$g/µL and IC$_{50}$ = 4.2 $\mu$g/µL, respectively. In all three extraction solvents, the FRAP activity was higher for SSF than that for SF, indicating that steaming increased the FRAP activity. Notably, the SSF ethanol and methanol extracts showed 4.9-fold and 1.4-fold higher levels of FRAP activity than that of respective SF extracts, whereas their water extracts had a similar activity level (IC$_{50}$ = 10.0 $\mu$g/µL and 11.5 $\mu$g/µL, respectively).

In agreement with the findings of the present study, Fonseca et al. [60] showed that the FRAP activity in ethanol extract of C. abies-marina was increased by 2-fold but decreased by 1.7-fold in its water extract after steaming. In addition, the FRAP activity in aqueous and ethanol extracts of C. abies-marina exhibited a similar trend as that of the total phenolic content. Similar to these findings, the SSF methanol extract with a high phenolic content showed a high level of FRAP activity in the present study. Rajauria et al. [61] measured the FRAP activity for heat-treated (85, 95, 100, 110, and 121 $^\circ$C for 15 min) Laminaria saccharina, Laminaria digitata, and Himanthalia longate and found that all three marine algae species showed an increase in FRAP activity up to 110 $^\circ$C compared to that of the respective fresh samples, followed by a decrease at the higher temperature. The DPPH radical scavenging activity was also shown to increase according to the heat treatment, suggesting that heat treatment increases the antioxidant contents and activities in marine algae. In contrast, Amorim-Carrilho et al. [62] evaluated the antioxidant activity in brown algae Himanthalia longate according to the cooking process and found no significant difference between the dried sample and the 40 min steamed sample, and the cooking (steaming) time was hypothesized to have a negative effect on the antioxidant contents. These results suggest that the FRAP activity could vary according to the sample type, cooking time and temperature, and extraction solvent. In this study, the FRAP activities of SF and SSF extracts were investigated after steaming at 95 $^\circ$C for 10–15 min; thus, it is considered necessary to observe changes in FRAP activity according to steaming time and temperature.

3.6. Antioxidant Enzyme Activity in SF and SSF Extracts in Different Solvents

All biological organisms, from plants to marine algae, produce reactive oxygen species that induce oxidative stress during the process of electron transfer, and thus they possess a variety of defense components to remove such species and for self-protection. These include antioxidants such as glutathione and ascorbic acid as well as antioxidant enzymes such as SOD, CAT, and APX [63,64].

The SOD, CAT, and APX activities were measured for the SF and SSF extracts. The SOD activity of SF and SSF methanol extracts was 3.12 unit/mg and 9.95 unit/mg, respectively, which was higher than that of the other extracts (Figure 2a). In all extraction solvents, the SOD activity was higher for SSF than that for SF, with 3.6-fold, 3.2-fold, and 1.4-fold higher activity in SSF ethanol, methanol, and water extracts compared to that of respective SF extracts. The CAT activity showed a similar trend as the SOD activity (Figure 2b). The CAT activity was higher in methanol extracts of both SF and SSF compared to that of their ethanol and water extracts. The CAT activities in SSF methanol (1.84 unit/mg) and ethanol extracts were 2.9-fold and 1.7-fold higher, respectively, than that of corresponding SF extracts. On the contrary, the CAT activities in SF and SSF water extracts were 0.05 unit/mg and 0.02 unit/mg, respectively, indicating a markedly lower level than that of ethanol and methanol extracts. The APX activity, similar to SOD and CAT activities, was the highest in methanol extracts. The APX activity levels in SF extracts decreased according to the extraction solvent in the order methanol, ethanol, and water (2.38, 1.56, 1.54 unit/mg, respectively), whereas that for SSF extracts decreased in the solvent order methanol, water, and ethanol (5.01, 2.05, 1.63 unit/mg, respectively). Notably, the highest APX activity (5.01 unit/mg) was shown by the SSF methanol extract at a 2.1-fold higher level than the
SF methanol extract (Figure 2c). The APX activity in SSF ethanol extract was 1.3-fold higher than that of SF ethanol extract, whereas the activity did not significantly vary between their water extracts.

Seaweed is considered a rich source of several types of protein and protein derivatives, such as enzymes, peptides, glycoproteins, lectins, and mycosporine-like amino acids [65]. The chemical composition of seaweed depends on the seaweed species and can be altered according to the extraction method, such as heat treatment, and extraction time [66]. Temperature is a major factor that has to be taken into consideration during the extraction of seaweed protein as it affects enzyme activity, protein integrity, and solubility of cellular components, including cell wall polysaccharides [67]. High temperatures can also lead to the degradation or formation of compounds, protein denaturation, and catalysis of reactions, including the hydrolysis of polysaccharides and proteins into smaller molecules [68].

In this study, although protein denaturation in SF and SSF water extracts was a possibility, the results of quantifying SOD, CAT, and APX activities suggested that numerous antioxidants in both extracts contributed to the removal of active oxygen. Lee et al. [69] examined SOD-like activity in ethanol extracts of Codonopsis lanceolata subjected to different numbers of steaming processes and found the highest activity at 72.2% in the sample after seven processes compared to that of the control; based on these findings, the elution level of bioactive substances in Codonopsis lanceolata was considered to be influenced by steaming. In addition, Kang et al. [70] measured the SOD-like activity in the water extracts of Polygonatum sibirium subjected to different numbers of steaming process and reported that the activity varied according to the origin of the plant and the number of steaming processes; thus, that the change in the SOD-like activity was attributed to the change in the phenolic content in the plant based on the extraction method, number of steaming processes involved, and the variation in the habitat geography. In the present study, the methanol extract, with high total polyphenol content and high phenolic content after steaming of SF, also exhibited high levels of SOD, CAT, and APX activities, implying that a combination of steaming and methanol extraction effectively extracts the SF compounds with an outstanding level of antioxidant activity.

Choi et al. [71] extracted Acer mono bark at different extraction temperatures, durations, and pressures. They reported that the SOD-like activity was higher at 80 °C compared to that at 40 °C (at a fixed extraction duration and pressure), indicating that extraction temperature has a bigger influence on SOD-like activity than that of the extraction duration and pressure. However, as the activities of SOD, CAT, and APX in the SF and SSF extracts were measured at only two extraction temperatures in this study, more temperature points should be used in future studies.

Figure 2. Antioxidant enzyme activities of fresh and steamed Sargassum fusiforme extracts. (a) SOD, (b) CAT, and (c) APX activity. SF: S. fusiforme extract, SSF: Steamed S. fusiforme extract. The different lowercase letters represent significant differences between solvents within the same sample (p < 0.05).
3.7. Collagenase and Elastase Inhibitory Activities in SF and SSF Extracts in Different Solvents

Skin aging is a natural phenomenon that accompanies physiological aging, but it may be accelerated by environmental factors, such as UV radiation, which turns the skin dry and thin with reduced elasticity, resulting in the formation of wrinkles [72]. Collagen is one of the most abundant proteins in the human body that determines the elasticity of the dermis along with elastin. The degradation of collagen and elastin by collagenase and elastase, respectively, is known to induce wrinkle formation [73].

To determine the antiaging activity of SF and SSF in different extraction solvents, the collagenase and elastase inhibitory activities were measured. The collagenase inhibitory activity in SF was higher in ethanol (28.7%) than that of methanol (25.6%) and water (23.1%) extracts, whereas for SSF, highest activity was observed in methanol extract (56.9%). Notably, the collagenase inhibitory activity in SSF methanol extract was the highest and 2.2-fold higher than that of SF methanol extract (Table 3). Similarly, the collagenase inhibitory activities in ethanol and water extracts were 1.1-fold and 1.3-fold higher for SSF than SF, indicating that the activity increased in all extraction solvents after steaming.

Susano et al. [74] evaluated the antiaging activity of five different fractions obtained from Carpomitra costata and found a high collagenase inhibitory activity at IC\textsubscript{50} = 7.2 µg/mL for the ethyl acetate fraction, which was reportedly correlated with high levels of phenolic content and DPPH radical scavenging activity. Similarly, the SF and SSF methanol and ethanol extracts in the present study showed a high collagenase inhibitory activity compared to their water extracts, which may be attributed to the high levels of total phenolic content and DPPH radical scavenging activity in the methanol and ethanol extracts that affect the collagenase inhibitory activity.

Table 3. Collagenase, elastase, and tyrosinase inhibitory activities of fresh and steamed Sargassum fusiforme extracts.

| Solvent     | Collagenase Inhibitory Activity (%) | Elastase Inhibitory Activity (%) | Tyrosinase Inhibition Activity (%) |
|-------------|-------------------------------------|----------------------------------|-----------------------------------|
|             | SF        | SSF        | SF        | SSF          | SF        | SSF          |
| Ethanol     | 28.7 ± 0.9 \textsuperscript{a} | 32.0 ± 0.3 \textsuperscript{b} | 68.6 ± 0.1 \textsuperscript{a} | 44.7 ± 0.1 \textsuperscript{a} | 14.9 ± 2.3 \textsuperscript{c} | 25.1 ± 1.1 \textsuperscript{b} |
| Methanol    | 25.6 ± 3.4 \textsuperscript{ab} | 56.9 ± 4.9 \textsuperscript{a} | 39.8 ± 0.2 \textsuperscript{b} | 16.0 ± 0.7 \textsuperscript{b} | 23.3 ± 0.8 \textsuperscript{b} | 30.5 ± 0.1 \textsuperscript{a} |
| Water       | 23.1 ± 0.1 \textsuperscript{b} | 28.9 ± 0.5 \textsuperscript{b} | 14.0 ± 0.1 \textsuperscript{c} | 12.4 ± 0.3 \textsuperscript{c} | 28.2 ± 0.2 \textsuperscript{a} | 22.9 ± 1.7 \textsuperscript{b} |

SF: S. fusiforme extract, SSF: Steamed S. fusiforme extract. The different lowercase letters represent significant differences in the same column (p < 0.05).

The elastase inhibitory activity in SF and SSF ranged 14–69% and 12–45%, respectively, across the different extraction solvents. For both SF and SSF, the elastase inhibitory activity was higher in ethanol extract than that of methanol or water extracts (Table 3). The highest elastase inhibitory activity was observed in the SF ethanol extract (68.6%), which was 1.5-fold higher than that of the SSF ethanol extract (44.7%). The SF methanol and water extracts also showed a higher elastase inhibitory activity (39.8% and 14.0%, respectively) than corresponding SSF extracts (16.0% and 12.4%, respectively). Arguelles et al. [75] examined the elastase inhibitory activity in the acidified methanol extract of Sargassum aquifolium and found that the activity increased with an increase in the extract concentration, due to the increase in phenolic content (100–300 µg gallic acid/mL). Similarly, the SF and SSF extracts in the present study showed a high elastase inhibitory activity in ethanol and methanol extracts, which were shown to have high total polyphenol and flavonoid contents, whereas the lowest elastase inhibitory activity was found in water extracts. These results suggest that extraction using methanol or ethanol could be most suitable for extracting the compounds with antiwrinkle effects from SF and SSF.

3.8. Tyrosinase Inhibitory Activity in SF and SSF Extracts in Different Solvents

UV exposure elicits melanin production by melanocytes found in the basal layer of the skin to prevent UV-induced skin damage, and tyrosinase is known to facilitate the production of melanin. An increase in melanin production could cause pigmentation, such
as freckles, sun spots, and age spots. Hence, inhibition of tyrosinase activity is crucial in skin whitening [76].

SF water extract showed the highest tyrosinase inhibitory activity at 28.2%, followed by methanol and ethanol extracts at 23.3% and 14.9%, respectively. For SSF, the methanol extract showed the highest activity at 30.5%, followed by ethanol and water extracts at 25.1% and 22.9%, respectively. SSF methanol and ethanol extracts showed 1.3-fold and 1.7-fold higher tyrosinase inhibitory activity, respectively, than corresponding SF extracts (30.5% vs. 23.3% and 25.1% vs. 14.9%). On the contrary, the tyrosinase inhibitory activity in SF water extract (28.2%) was 1.2-fold higher than that of SSF water extract (22.9%).

In a study examining the tyrosinase inhibitory activity in 11 species of marine algae of the Sargassum family as with SF [77], the activity was the highest in 70% ethanol extracts at 50.07 mg kojic acid (KAE)/g and 52.65 mg KAE/g for S. fillicinum and S. yendoi, respectively; however, the total phenolic and flavonoid contents in the two marine algae species were not high, and the study reported that the tyrosinase inhibitory activity was greatly influenced by the phenolic composition rather than the content. Similarly, the total polyphenol content in the present study was lower in the SF water extract than that of the SF methanol extract, whereas the tyrosinase inhibitory activity was the highest in the SF water extract, indicating that the tyrosinase inhibitory activity could be influenced by non-phenolic compounds. The evaluation of tyrosinase inhibitory activity according to the extraction solvent showed that the activity was the highest in the SF water extract (28.2%) and SSF methanol extract (30.5%), with SF mostly exhibiting a higher activity than SSF, suggesting an influence of steaming on the antioxidant activity as well as the whitening effect based on the changes in the bioactive substances in SF.

3.9. Analysis of Phenolic Compounds in SF and SSF Extracts in Different Solvents

Considering its antimicrobial activity as well as high stability and solubility in water, 4-hydroxybenzoic acid is mainly used in drugs, such as antithyroid agents, [78] and cosmetic products [79]. Naringin and naringenin, the aglycon form of naringin, are abundantly found flavones in citrus fruits [80] and reportedly have antioxidative, anti-inflammation, and antiobesity effects in mouse models [81]. Naringenin is converted into various flavonoids, such as catechins, flavonols, and anthocyanidin, through enzymatic mechanisms [82]. The measurement of 4-hydroxybenzoic acid, naringenin, and naringin in SF and SSF was deemed valuable in this study because the identification of these functional components enhances the applicability of SF in cosmetic and pharmaceutical industries.

To determine the phenolic content changes in SF and SSF extracts in different solvents, 4-hydroxybenzoic acid, naringenin, and naringin were measured, and the results are presented in Table 4. The 4-hydroxybenzoic acid content in SF was the highest in its methanol extract (10.46 µg/g), which was approximately 10-fold higher than that of the water extract (1.05 µg/g). The 4-hydroxybenzoic acid content in the SSF ethanol extract was negligible, whereas that of the SSF methanol extract was 298.08 µg/g, which was approximately 6.5-fold lower than that of the SF methanol extract.

| Solvent  | 4-hydroxy Benzoic Acid (µg/g) | Naringenin (µg/g) | Naringin (µg/g) |
|----------|-------------------------------|------------------|-----------------|
|          | SF               | SSF               | SF              | SSF           | SF              | SSF           |
| Ethanol  | 4.60 ± 0.08 b      | ND                | 0.21 ± 0.03     | 0.41 ± 0.07    | 298.08 ± 10.54 | 517.98 ± 9.64 |
| Methanol | 10.46 ± 0.11 a     | 1.62 ± 0.03       | ND              | ND            | ND              | ND            |
| Water    | 1.05 ± 0.04 c      | 1.72 ± 0.08       | ND              | ND            | ND              | ND            |

SF: S. fusiforme extract, SSF: Steamed S. fusiforme extract, ND: Not detected. The different lowercase letters represent significant differences in the same column ($p < 0.05$).

In a study examining the 4-hydroxybenzoic acid content in hot water and ethanol extracts of Codonopsis lanceolata, the content decreased by 2.3-fold and 1.7-fold, respectively after steaming, which was reportedly due to the loss of stability during the heat treat-
ment [83]. Similarly, in the present study, the lower 4-hydroxybenzoic acid content in the SSF methanol extract compared to that of the SF methanol extract is presumed to be due to the steaming treatment. In addition, the total polyphenol content in the SSF methanol extract was approximately 1.3-fold higher than that of the SF methanol extract; based on this, the lower 4-hydroxybenzoic acid content in the SSF methanol extract was attributed to antioxidants other than those extracted from SSF.

Next, naringenin was detected only in the ethanol extracts for both SF and SSF, with an approximately 2-fold higher level in the SSF ethanol extract (0.41 µg/g) than the SF ethanol extract (0.21 µg/g). Tanna et al. [41] found that the naringenin content in the methanol extract of the brown algae *Stoechospermum polyodioides* was 3 µg/g, which was considerably higher than the content in the SSF ethanol extract in the present study. However, naringenin was not detected in the methanol extracts of the brown algae *Sargassum linearifolium*, *Iyengaria stellata*, and *Spatoglossum asperum*, indicating that the naringenin content may vary across different species of marine algae.

Lastly, naringin, as in the case of naringenin, was detected only in the ethanol extracts, with 298.08 µg/g in SF and 517.98 µg/g in SSF extract. According to Ko et al. [84], naringenin and naringin are nonpolar polyphenol compounds that are mainly extracted using organic solvents such as methanol and ethanol. Consistently, naringenin and naringin were not detected in the SF and SSF water extracts in the present study, and ethanol was deemed more suitable for the extraction of naringenin and naringin from SF. In addition, the total flavonoid content was approximately 1.4-fold higher in the SSF ethanol extract than that of the SF ethanol extract, presumably due to the higher contents of naringenin and naringin in the SSF ethanol extract. As such, changes of phenolic compounds in SF were observed according to the method of extraction and the cooking (steaming) process, and steaming along with ethanol or methanol extraction seems more suitable for the extraction of phenolic compounds from SF.

Zhao et al. [85] evaluated the phenolic compound in *Sargassum fusiforme* ethanol extract under different drying conditions. They reported a higher retention of 4-hydroxybenzoic acid content in freeze-dried samples. They also reported that the content of 4-hydroxybenzoic acid was affected by the drying temperature. Thus, it is required to examine the phenolic compound content of SF and SSF extracts under various drying conditions.

### 3.10. PCA of the Total Polyphenol and Flavonoid Contents and the Antioxidant Activities in SF and SSF Extracts

Principal component analysis (PCA) was used to analyze the total polyphenol and flavonoid contents as well as the antioxidant activities (DPPH and ABTS radical scavenging activities, FRAP activity, and SOD, CAT, and APX activities) and compare the patterns across SF and SSF extracts in different solvents. The PCA results showed a variation of 76.7% and 16.5% for PC1 and PC2, respectively, which accounted for approximately 93.2% of the variation in bioactivity between SF and SSF (Figure 3a). The PC1 appears to be mainly correlated with the antioxidant activity and thus determines the distribution of SF and SSF extracts along the x-axis. The PC2 is correlated with the total polyphenol and flavonoid contents as well as the antioxidant enzyme (SOD, CAT, and APX) activities, and differentiate between the SSF2, SF3, and SSF3 groups and the SF1, SF2, and SSF1 groups. The SF and SSF water extracts were found within the same ellipse, and based on the antagonistic direction of arrows for the total polyphenol and flavonoid contents and the antioxidant activities seen in Figure 3b, the antioxidant activities in the SF and SSF water extracts were determined to be low, whereas the total polyphenol content, DPPH, and ABTS radical scavenging activities, and antioxidant enzyme activities were high in the SSF methanol extract.
In the multivariate analysis, a VIP score greater than 1 indicates a factor with an influence on the group differentiation [86]. The VIP scores of SF and SSF exceeded 1 for the DPPH radical scavenging activity (2.14) and SOD activity (1.48), indicating that these factors are useful for differentiating SF and SSF in different extraction solvents (Figure 4a).

A positive correlation \( r = 0.586–0.898 \) was found between the total polyphenol content and the DPPH and ABTS radical scavenging activities and antioxidant enzyme (SOD, CAT, and APX) activities for SF and SSF, with a significantly high positive correlation \( r = 0.898, p = 0.015 \) between the total polyphenol content and the APX activity (Figure 4b). You et al. [87] reported a high positive correlation between the phenolic content and the DPPH and ABTS radical scavenging activities based on the increase in these activities with an increase in the phenolic compound contents in the extracts of 19 Sargassum species. Similarly, the SF and SSF extracts in the present study showed a positive correlation between

![Figure 3](image-url)  
**Figure 3.** Score plot (a) and biplot (b) for PCA analysis of the different solvent extracts of *Sargassum fusiforme* for antioxidant determinants. SF1: *S. fusiforme* ethanol extract, SF2: *S. fusiforme* methanol extract, SF3: *S. fusiforme* water extract, SSF1: Steamed *S. fusiforme* ethanol extract, SSF2: Steamed *S. fusiforme* methanol extract, SSF3: Steamed *S. fusiforme* water extract, TP: Total polyphenol content, TF: Total flavonoid content.

![Figure 4](image-url)  
**Figure 4.** VIP scores of PLS-DA analysis (a) and correlation matrix (b) between total polyphenol, flavonoid, content, and antioxidant activities according to the different solvent extracts of *Sargassum fusiforme*. The intensity of red and blue colors represents positive and negative correlations. TP: Total polyphenol content, TF: Total flavonoid content.
the total polyphenol content and the ABTS radical scavenging activity and antioxidant enzyme activities, suggesting a close association between the total polyphenol content and the antioxidant activity in SF and SSF.

4. Conclusions

Macroalgae contain various bioactive compounds that are affected by many factors, including the cooking method, extraction conditions, season, abiotic stress, and cultivation time [22,23,88]. Thus, it is paramount to investigate seaweed-derived and seaweed extracts under various conditions of the cooking process (time and temperature) and using different extraction solvents. Here, the total polyphenol and flavonoid contents, the antioxidant and antiaging activities, and the phenolic compounds in SF and SSF extracts were comparatively analyzed using different solvents. Total polyphenol and flavonoid contents were found to be highest in SF and SSF methanol extracts. Moreover, in most cases, SSF ethanol and methanol extracts exhibited higher levels of antioxidant and antiaging activities and contained more phenolic compounds compared to SF extracts. The results of this study collectively suggest that the phenolic contents in SF extracts are modified according to the extraction solvent and the steaming process, which ultimately affects the antioxidant activity in the SSF extract. This indicates that the phenolic contents and antioxidant and antiaging activities can be improved through the steaming process. Thus, the SSF extract might be useful as a key ingredient in healthy and functional foods or cosmetic products. In conclusion, these results indicate the importance of further exploration of the application and functionality of SF.

Author Contributions: S.-Y.L. and C.-S.K. Conceived the study; H.-H.L., J.-S.K. and J.-H.J. Performed the experiments and analyzed the data; S.-Y.L. and H.-H.L. Writing—original draft; H.-H.L., S.-Y.L. and C.-S.K. Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Korea Institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries, grant number 20210656.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data reported are available in the Figure 4.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Liu, J.; Luthuli, S.; Yang, Y.; Cheng, Y.; Zhang, Y.; Wu, M.; Choi, J.I.; Tong, H. Therapeutic and nutraceutical potentials of a brown seaweed Sargassum fusiforme. Food Sci. Nutr. 2020, 8, 5195–5205. [CrossRef]
2. Wang, W.; Lu, J.B.; Wang, C.; Wang, C.S.; Zhang, H.H.; Li, C.Y.; Qian, G.Y. Effects of Sargassum fusiforme polysaccharides on antioxidant activities and intestinal functions in mice. Int. J. Biol. Macromol. 2013, 58, 127–132. [CrossRef]
3. Lee, K.J.; Kang, E.H.; Yoon, M.; Jo, M.R.; Yu, H.; Son, K.T.; Jeong, S.H.; Kim, J.H. Comparison of Heavy Metals and Arsenic Species in Seaweeds Collected from Different Regions in Korea. Appl. Sci. 2022, 12, 7000. [CrossRef]
4. Park, K.E.; Jang, M.S.; Lim, C.W.; Kim, Y.K.; Seo, Y.W.; Park, H.Y. Antioxidant activity on ethanol extract from boiled-water of Hizikia fusiformis. Appl. Biol. Chem. 2005, 48, 435–439.
5. Yun, S.J. History and Recipe of Use of Seaweed in Korea. In Proceedings of the the EASDL Conference, Yongin, Republic of Korea, 1–13 April 2007.
6. Statistics Korea. 2020 Fishery Production Trend Survey Results. Available online: http://kosis.kr (accessed on 27 July 2021).
7. Tanaka, K.; Ohno, M.; Largo, D.B. An update on the seaweed resources of Japan. Bot. Mar. 2020, 63, 105–117. [CrossRef]
8. Yokoi, K.; Konomi, A. Toxicity of so-called edible hijiki seaweed (Sargassum fusiforme) containing inorganic arsenic. Regul. Toxicol. Pharmacol. 2012, 63, 291–297. [CrossRef]
9. Kwon, Y.R.; Kwang, Y.S. Quality characteristics of Hijikia fusiformis extracts with different extraction method. Korean J. Food Preserv. 2015, 22, 70–77. [CrossRef]
10. Meinita, M.D.N.; Harwanto, D.; Sohn, J.H.; Kim, J.S.; Choi, J.S. Hizikia fusiformis: Pharmacological and Nutritional Properties. Foods 2021, 10, 1660. [CrossRef]
66. Yangthong, M.; Ruensirikul, J.; Kaneko, G. The Hot-Water Extract of Sargassum sp. as a Feed Ingredient for Spotted Scat (Scatophagus argus Linnaeus, 1766) Reared in Songkhla Lake: Effects on Growth, Feed Efficiency, Hematological Data and Body Composition. *Fishes* 2022, 7, 170. [CrossRef]

67. O’Connor, J.; Meaney, S.; Williams, G.A.; Hayes, M. Extraction of protein from four different seaweeds using three different physical pretreatment strategies. *Molecules* 2020, 25, 2005. [CrossRef]

68. Plaza, M.; Turner, C. Pressurized hot water extraction of bioactives. *TrAC-Trends Anal. Chem.* 2015, 71, 39–54. [CrossRef]

69. Lee, H.K.; Choi, O.Y.; Choi, D.B.; Choi, H.S. Antioxidative and antimicrobial effects of ethanol extract of *Codonopsis lanceolata* by steaming times. *Korean J. Food Nutr.* 2021, 34, 107–113.

70. Kang, M.W.; Chang, J.P.; Doh, E.S.; Kil, K.J.; Yoo, J.H. Antioxidant activities of water extracts from steamed *Polygonati rhizoma*. *Korean J. Herb.* 2017, 32, 33–40. [CrossRef]

71. Choi, W.Y.; Jeong, M.H.; Lee, H.Y. Optimization of extraction process for enhancement of antioxidant activity of *Acer mono* bark. *J. Appl. Bot.* *Food Qual.* 2014, 87, 46–55.

72. Alves, A.; Sousa, E.; Kijjoa, A.; Pinto, M. Marine-derived compounds with potential use as cosmeceuticals and nutricosmetics. *Molecules* 2020, 25, 2536. [CrossRef] [PubMed]

73. Lee, S.H.; Kim, Y.C. Antioxidant Capacity and Anti-Wrinkle Efficacy of Rhamnus davurica Methanol Extract in a Cell-free System. *J. Investig. Cosmetol.* 2021, 17, 1–7.

74. Susano, P.; Silva, J.; Alves, C.; Martins, A.; Gaspar, H.; Pinteus, S.; Mouga, T.; Goettert, M.I.; Petrovski, Ž.; Branco, L.B. Unravelling the dermatological potential of the brown seaweed *Carpomitra costata*. *Mar. Drugs* 2021, 19, 135. [CrossRef] [PubMed]

75. Arguelles, E.; Sapin, A.B. Chemical composition and bioactive properties of *Sargassum aquifolium* (Turner) C. Agardh and its potential for pharmaceutical application. *Philipp. J. Sci.* 2021, 151, 9–24.

76. Oliva, C.; Solano, F. New insights into the active site structure and catalytic mechanism of tyrosinase and its related proteins. *Pigment. Cell Melanoma Res.* 2009, 22, 750–760. [CrossRef]

77. Baek, S.H.; Cao, L.; Jeong, S.J.; Kim, H.R.; Nam, T.J.; Lee, S.G. The comparison of total phenolics, total antioxidant, and anti-tyrosinase activities of Korean *Sargassum* species. *J. Food Qual.* 2021, 2021, 6640789. [CrossRef]

78. Rousset, B. Antithyroid effect of a food or drug preservative: 4-hydroxybenzoic acid methyl ester. *Experientia* 1981, 37, 177–178. [CrossRef]

79. Del Olmo, A.; Calzada, J.; Nuñez, M. Benzoic acid and its derivatives as naturally occurring compounds in foods and as additives: Uses, exposure, and controversy. *Crit. Rev. Food Sci. Nutr.* 2017, 57, 3084–3103. [CrossRef]

80. Tripoli, E.; La Guardia, M.; Giammanco, S.; Di Majo, D.; Giammanco, M. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. *Food Chem.* 2007, 104, 466–479. [CrossRef]

81. Alam, M.A.; Subhan, N.; Rahman, M.M.; Uddin, S.J.; Reza, H.M.; Sarker, S.D. Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. *Adv. Nutr.* 2014, 5, 404–417. [CrossRef] [PubMed]

82. Cotas, J.; Leandro, A.; Monteiro, P.; Pacheco, D.; Figueirinha, A.; Gonçalves, A.M.; da Silva, G.J.; Pereira, L. Seaweed phenolics: From extraction to applications. *Mar. Drugs* 2020, 18, 384. [CrossRef] [PubMed]

83. Coelho, C.H.; Seo, Y.C.; Choi, W.Y.; Lee, C.G.; Kim, D.U.; Chung, J.Y.; Chung, H.C.; Park, D.S.; Ma, C.J.; Lee, H.Y. Enhancement of antioxidative activity of *Codonopsis lanceolata* by stepwise steaming process. *Korean J. Med. Crop Sci.* 2012, 20, 238–244. [CrossRef]

84. Ko, M.J.; Kwon, H.L.; Chung, M.S. Optimum conditions for extracting flavanones from grapefruit peels and encapsulation of extracts. *Korean J. Food Sci. Technol.* 2014, 46, 465–469. [CrossRef]

85. Zhao, T.; Dong, Q.; Zhou, H.; Yang, H. Drying kinetics, physicochemical properties, antioxidant activity and antidiabetic potential of *Sargassum fusiforme* processed under four drying techniques. *LWT* 2022, 163, 113578. [CrossRef]

86. Zengin, G.; Sinan, K.I.; Mahoomodally, M.F.; Angeloni, S.; Mustafa, A.M.; Vittori, S.; Maggi, F.; Caprioli, G. Chemical composition, antioxidant and enzyme inhibitory properties of different extracts obtained from spent coffee ground and coffee silverskin. *Foods* 2020, 9, 713. [CrossRef]

87. You, S.; Jang, M.; Kim, G.H. Inhibition of nitric oxide and lipid accumulation by *Sargassum* sp. seaweeds and their antioxidant properties. *Korean J. Food Process.* 2021, 28, 288–296. [CrossRef]

88. Generalić Mekinić, I.; Šimat, V.; Botić, V.; Crnjac, A.; Smoljo, M.; Soldo, B.; Soldo, B.; Ljubenkov, I.; Čagalj, M.; Skroza, D. Bioactive phenolic metabolites from Adriatic brown algae *Dictyota dichotoma* and *Padina pavnica* (*Dictyotaceae*). *Foods* 2021, 10, 1187. [CrossRef]