Effect of Different Solvents on the Extraction of Compounds from Different Parts of *Undaria pinnatifida* (Harvey) Suringar

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Abstract: Asian kelp (*Undaria pinnatifida*) is a brown alga mainly grown and consumed in East Asian countries such as China, Japan, and Korea. To date, studies on the various biological activities of Asian kelp have been conducted; however, studies focusing on the different parts of Asian kelp and their use for other than food are limited. Therefore, in this study, different parts of Asian kelp, namely, whole *U. pinnatifida* (UP), *U. pinnatifida* blade (UPB), *U. pinnatifida* stipe (UPS), and *U. pinnatifida* sporophylls (UPSP), were extracted by using different solvents including ethanol, methanol, and hot water. The antioxidant activity, antiaging effect, and phenolic content of the extracts were investigated. The results revealed that the UPB methanolic extract had significantly higher polyphenol and flavonoid contents, with the respective values of 5.61 mg TAN/g and 7.80 mg QUE/g, than the other extracts. The DPPH and ABTS radical scavenging activities and FRAP values were the highest in the 95% ethanolic (IC₅₀ 12.73 mg/mL), methanolic (63.83%), and ethanolic (75.75%) extracts of UPB, respectively. Superoxide dismutase, catalase, and ascorbate peroxidase enzymatic activities were also measured, and the results showed that the activities of these enzymes were higher in the UPB methanolic extract than in the other extracts. Similarly, the highest elastase and tyrosinase inhibitory activity was observed in the UPB methanolic extract. The highest phenolic content was found in the hot water extract of UP (4-hydroxybenzoic acid) and the ethanolic extracts of all parts (naringin and naringenin). The phenolic content of the Asian kelp differed depending on the parts and extraction solvents used. Finally, the antioxidant activity of the UPB extract showed a close correlation with the polyphenol and flavonoid contents. The methanol extract of UPB exhibited excellent antioxidant and antiaging activities, indicating its potential use in the development of functional materials using Asian kelp and in other new applications.

Keywords: *Undaria pinnatifida*; Asian kelp; phenolic content; antioxidant effect; antiaging activity

1. Introduction

Asian kelp (*Undaria pinnatifida* (Harvey) Suringar) is a brown seaweed belonging to the Alariaceae family. It mainly grows on rocky temperate coasts and is widely consumed in China, Japan, and Korea [1]. In Korea and Japan, it has been consumed for a long time under the name of ‘miyok’ and ‘wakame’, respectively [2]. In particular, Korean people drink seaweed soup after childbirth because it is helpful for purifying the blood [3]. Asian kelp can be divided into different parts, namely, the blade (lamina), midrib, stipe, sporophyll, and holdfast, which acts as a root [4]. In Korea and Japan, the blade and sporophyll of Asian kelp are highly consumed [5], whereas in China, the sporophyll and midrib are also consumed as food and traditional medicine for dropsy, stomach ailments,
and fistulas [6]. Currently, the blade (lamina) and midrib of Asian kelp are commonly used, whereas the rest are discarded; therefore, studies are needed for the efficient utilization of Asian kelp [7].

Asian kelp is rich in physiologically active substances, such as phenolic compounds (phlorotannin), polysaccharides (fucoidan and alginic acid), vitamins, and minerals [8]. It is also rich in viscous polysaccharides and soluble fiber, which helps in dieting owing to its low calories, and is effective in preventing diabetes [9]. Phlorotannin extracted from Asian kelp sporophyll has anti-inflammatory and antioxidant effects [10]. Fucoidan extracted from Asian kelp sporophyll has antioxidant [11], anticancer [12], and antibacterial [1] effects. Moreover, Asian kelp sporophyll polysaccharides have been reported to exert physiological activities such as a hyperglycemia-alleviating effect [13] and an inhibitory effect on fat accumulation in high-fat-induced mice [14]. In addition, previous studies have focused on the antioxidant activity of Asian kelp; however, there are only a few studies on the antioxidant effect, antiaging activity, and phenolic content of the different parts of Asian kelp.

Several secondary metabolites were identified from seaweeds and it has been proved that these metabolites have a skin protection ability, for example, via anti-inflammatory, antioxidant, moisturizing, photo-protective, and regenerative properties [15,16]. In addition, during aging, the extracellular matrix proteins are sensitive to the intense activity of proteolytic enzymes such as elastases and collagenase, which causes observable changes in the skin, such as a loss of skin elasticity and wrinkling of skin [15]. To prevent skin aging, the inhibition of the collagenase and elastase activities by natural compounds is one of the promising approaches. It has been proved that the plant extracts have been extensively used to possess anti-elastase and anti-collagenase activities [15,17]; however, to date, there was little knowledge of the inhibitory enzymatic activities of seaweed extracts. Due to the wide range of these bioactive compounds present in macroalgae, the examination of marine algae-derived and new seaweeds extracts is very important [15,18,19].

It is known that compared with land plants, seaweeds are more difficult to collect, to remove salt from, and to store. The ingredients and physiological effects of seaweed vary greatly depending on the extraction conditions, extraction solvent, and seaweed type [20]. Moreover, the nutrient content of seaweed is affected by abiotic (habitat, season, water temperature, harvest time, and processing method) and biotic (life history, size, reproductive stage, and seaweed species) factors [21]. The morphological characteristics of Asian kelp, such as the midrib length, stipe length, blade division degree, and sporophyll location, are affected by various environmental factors [22], and it has been reported that Asian kelp differs in its lipid composition depending on the region where it grows [23].

There are several solvents used for the extraction of natural products, such as hot water and organic solvents (methanol and ethanol); however, the selection of the solvent is based on the polarity of the compounds [24]. The content and function of the dissolved bioactive substances vary depending on the extraction solvent used; therefore, in this study, we analyzed the antioxidant effect, antiaging activity, and phenolic content of the different parts (blade, stipe, and sporophyll) of Asian kelp extracted with hot water, methanol, and ethanol. The findings of this study will provide basic data for the development of functional materials using Asian kelp, and to promote the efficient use and exploration of new applications of Asian kelp.

2. Results and Discussion

2.1. Extraction of Different Parts of Asian Kelp with Different Solvents

Different parts of Asian kelp, namely, the UP, UPB, UPS, and UPSP, were extracted with different solvents including 95% ethanol, 95% methanol, and hot water. For all the Asian kelp parts, the highest extraction yield was achieved using hot water (4.5–35.6%), compared with that obtained with ethanol (0.7–2.3%) and methanol (1.5–9.2%) (Table 1). The UP hot water extract showed the highest yield (35.6%), which was 7.91, 1.21, and 17 times higher than the yields of the UPB, UPS, and UPSP extracts, respectively. It has been
found that the fucoidan (polysaccharide) from *Sargassum hystrix* by ethanolic extraction showed a lower yield when compared to other methods such as with HCl, CaCl$_2$, and EDTA. It has been reported that the reason for a lower yield in the ethanolic extraction might be because the ethanol has two kinds of polarities such as polar (OH) and nonpolar (alkyl) groups, and that the fucoidan might dissolve in the polar group, thus leading to a low yield in the ethanolic extract [25]. Previous studies reported that Asian kelp is rich in water-soluble compounds, such as soluble polysaccharides, carbohydrates, peptides, and proteins [26–28], which are less susceptible to ethanol and methanol. Another study, in red algae (*Porphyra tenera*), found that among the different extraction methods (100 °C water, 37 °C water, and 70% ethanol) the result showed that the 100 °C hot water method indicated the highest yield in differently processed laver [29]. This might be the reason for the high extraction yield that occurred in the hot water extract. These results suggest that hot water extraction is the most suitable method for the extraction of Asian kelp with a high yield.

**Table 1.** Extraction yield of different parts of *Undaria pinnatifida*.

<table>
<thead>
<tr>
<th>Seaweed Part</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95% Ethanol</td>
</tr>
<tr>
<td>UP</td>
<td>2.1 ± 0.3 $^a$</td>
</tr>
<tr>
<td>UPB</td>
<td>2.2 ± 0.3 $^a$</td>
</tr>
<tr>
<td>UPS</td>
<td>2.3 ± 0.3 $^a$</td>
</tr>
<tr>
<td>UPSP</td>
<td>0.7 ± 0.2 $^b$</td>
</tr>
</tbody>
</table>

UP: whole *U. pinnatifida*, UPB: *U. pinnatifida* blade, UPS: *U. pinnatifida* stipe, UPSP: *U. pinnatifida* sporophylls. The different letters (a–d) indicate a significant difference ($p < 0.05$).

### 2.2. Analysis of Total Polyphenol and Flavonoid Content in Different Solvent Extracts of Different Parts of Asian Kelp

The total polyphenol and flavonoid content in different solvent extracts of different parts of Asian kelp was analyzed. The results showed that the content of polyphenols and the flavonoid content varied depending on the seaweed habitat, harvest time, and extraction solvent polarity [30,31]. Among the different solvent extracts of the different parts of Asian kelp, the methanolic extracts exhibited the highest total polyphenol content, followed by the ethanol and hot water extracts (Figure 1A). Among the methanolic extracts, the UPB extract had the highest total polyphenol level, which was 1.20, 1.89, and 7.58 times higher than that of the UPS, UP, and UPSP extracts, respectively. Among the ethanolic extracts, the UPSP extract showed the highest total polyphenol content (2.53 ± 0.02 mg TAN/g), followed by the UPS (2.32 ± 0.05 mg TAN/g), UP (1.69 ± 0.01 mg TAN/g), and UPB (1.54 ± 0.05 mg TAN/g) extracts. Among the different solvent extracts, the hot water extracts showed the lowest total polyphenol content, ranging between 0.59 and 0.95 mg TAN/g. Koivikko et al. [32] analyzed eight different extraction solvents consisting of different polarities to extract the polyphenols from *Fucus vesiculosus*. The results showed that using 70% acetone was a more suitable method for the polyphenol compound extraction when compared to the water extract for most of the seaweed species. In addition, they reported that the phenolic compounds had a greater solubility in the polar organic solvent than in water. Moreover, in another study, they found that for polyphenol extraction, the most effective solvents were aqueous mixtures of methanol, ethanol, and acetone [29]. A similar result was obtained in this study in that the highest total polyphenol content was achieved in methanol and ethanol. From these results, it is shown that for the highest polyphenolic yield the methanolic and ethanolic extraction methods are the most suitable.
Additionally, they reported that the difference might be due to the difference in the geo-
egengineering of the methanolic extracts.

Among the ethanolic extracts, the UP extract exhibited the highest flavonoid concentration, which was 1.08, 2.54, and 4.53 times higher than that of the UPB, UPS, and UPSP extracts, respectively. Among the three extraction methods, the hot water extraction yielded the lowest flavonoid content, ranging between 0.008–0.59 mg QUE/g. This result supports the total polyphenol content. Similar results were obtained in red algae and several seaweeds, in that the methanolic extract showed the highest total flavonoid content when compared to other solvents [33–37].

Based on the total polyphenol and flavonoid content results, methanolic extraction is the most suitable method for the extraction of Asian kelp, with the highest yield of secondary metabolites. The phlorotannins and total flavonoid content were analyzed from the methanolic extract of brown algae *Fucus vesiculosus* collected from various regions and the results showed that the total flavonoid content varied from 15.6 to 26.4 mg QUE/g DW. Additionally, they reported that the difference might be due to the difference in the geographical regions and reproductive phase [37]; however, in this study, the total flavonoid content of the *U. pinnatifida* methanolic extract ranged from 0.008 to 7.8 mg QUE/g DW. From these results, it is shown that the flavonoid content in the brown algae depends not
only on the extraction solvent, but also might vary due to the collection site, breeding season, and salinity. In addition, it is shown that the presence of a significant amount of flavonoids in brown algae suggests that it has antioxidant activities, namely, the scavenging of reactive oxygen species, and an inhibition of lipid peroxidation.

2.3. DPPH Radical Scavenging Activity of Different Parts of Asian Kelp Extracted with Different Solvents

Among the different solvent extracts, the methanolic and ethanolic extracts showed the highest DPPH radical scavenging activities. The results showed that the different solvent extracts exhibited different DPPH scavenging activities (Table 2). The DPPH scavenging activity of the methanolic extracts of the different parts of Asian kelp ranged from IC\(_{50}\) 12.61–27.49 mg/mL. Among the ethanolic extracts, the UPB (IC\(_{50}\) 12.73 ± 1.10 mg/mL) and UP (IC\(_{50}\) 17.42 ± 1.29 mg/mL) extracts showed the highest DPPH scavenging activity, followed by the UPSP (IC\(_{50}\) 29.03 ± 1.45 mg/mL) and UPS (IC\(_{50}\) 29.41 ± 1.08 mg/mL) extracts. The DPPH scavenging activity of the methanolic extract was slightly higher when compared to the other two solvents. A previous study has reported that phenolic compounds are the primary contributors to the antioxidant activity of various seaweeds [38]. In this study, the highest percentage of inhibition occurred in the methanolic extract which was slightly higher when compared to the other two solvents. In addition, compared to the IC\(_{50}\) value of the ascorbic acid (positive control), the values of all the Asian kelp extracts showed lower DPPH scavenging activity (Table 2).

Table 2. DPPH half-maximal inhibitory concentration (IC\(_{50}\)) of different solvent extracts of different parts of Undaria pinnatifida.

<table>
<thead>
<tr>
<th>Seaweed Part</th>
<th>DPPH Radical Scavenging Activity (IC(_{50}) = mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95% Ethanol</td>
</tr>
<tr>
<td>UP</td>
<td>17.42 ± 1.29 (b)</td>
</tr>
<tr>
<td>UPB</td>
<td>12.73 ± 1.10 (a)</td>
</tr>
<tr>
<td>UPS</td>
<td>29.41 ± 1.08 (c)</td>
</tr>
<tr>
<td>UPSP</td>
<td>29.03 ± 1.45 (c)</td>
</tr>
</tbody>
</table>

Ascorbic acid 1.10 ± 0.01

UP: whole U. pinnatifida, UPB: U. pinnatifida blade, UPS: U. pinnatifida stipe, UPSP: U. pinnatifida sporophylls. The different letters (a–d) indicate a significant difference (\(p<0.05\)).

2.4. ABTS Radical Scavenging Activity of Different Solvent Extracts of Different Parts of Asian Kelp

ABTS radical scavenging activity showed different trends from those of the DPPH radical scavenging activity. The results revealed that the extracts prepared using different solvents had different ABTS scavenging activities, whereas ascorbic acid at different concentrations (0.1, 0.25, and 0.5 \(\mu g/\mu L\)) had an ABTS scavenging activity ranging from 11.59–19.81% (Table 3). Among the different solvent extracts, the methanolic extracts showed the highest ABTS scavenging activity, especially the UPB (63.83 ± 0.34%) and UP
(41.72 ± 0.59%) methanolic extracts. In contrast, the ethanolic extracts of all parts of the Asian kelp did not show a high ABTS scavenging activity. Among the methanolic extracts, the UPB extract (63.83 ± 0.34%) had the highest ABTS scavenging activity, followed by the UP (41.72 ± 0.59%), UPS (38.06 ± 0.17%), and UPSP (29.44 ± 0.45%) extracts; the UPB extract showed the highest ABTS scavenging activity, which was 1.53, 1.68, and 2.17 times higher than that of the UP, UPS, and UPSP extracts, respectively. The hot water extracts showed a different trend from that of the methanolic extracts. Although the ABTS scavenging activity was high in the UPB methanolic extract, the hot water extracts of the UPS (56.10 ± 0.67%) and UPSP (41.57 ± 1.17%) showed significantly higher ABTS scavenging activities. The ABTS scavenging activity of the hot water extracts of the different parts of Asian kelp ranged from 25.06–56.10%. This corroborates the results of the DPPH scavenging activity assay, in which different solvent extracts showed different DPPH scavenging activities. Cho [39] measured the ABTS scavenging activity of the ethanolic extracts of four types of seaweeds, and the results showed that at 1 mg/mL, *Porphyra tenera*, *Sargassum fusiforme*, and *Enteromorpha linza* extracts showed ABTS scavenging activity values of 16.96 ± 1.40%, 18.65 ± 2.20%, 34.83 ± 1.65%, and 13.65 ± 1.10%, respectively, which were lower than those of the ethanolic extracts of the different parts of Asian kelp in the present study (10.59–32.61%). These results suggest that both methanolic and hot water extracts of Asian kelp have the highest ABTS scavenging activities.

Table 3. ABTS radical scavenging activities (%) and FRAP values of different solvent extracts of different parts of *Undaria pinnatifida*.

<table>
<thead>
<tr>
<th>Seaweed Part (10 µg/µL)</th>
<th>ABTS Radical Scavenging Activity (%)</th>
<th>FRAP Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95% Ethanol</td>
<td>Methanol</td>
</tr>
<tr>
<td>UP</td>
<td>32.61 ± 2.11 c</td>
<td>41.72 ± 0.59 b</td>
</tr>
<tr>
<td>UPB</td>
<td>41.65 ± 0.44 b</td>
<td>63.83 ± 0.34 a</td>
</tr>
<tr>
<td>UPS</td>
<td>50.35 ± 1.11 a</td>
<td>38.06 ± 0.17 c</td>
</tr>
<tr>
<td>UPSP</td>
<td>10.59 ± 0.92 d</td>
<td>29.44 ± 0.45 d</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ascorbic acid (µg/µL)</th>
<th>ABTS radical scavenging activity (%)</th>
<th>FRAP value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>16.65 ± 0.18</td>
<td>19.81 ± 0.18</td>
</tr>
<tr>
<td>0.25</td>
<td>11.59 ± 0.18</td>
<td>16.65 ± 0.18</td>
</tr>
<tr>
<td>0.5</td>
<td>11.59 ± 0.18</td>
<td>16.65 ± 0.18</td>
</tr>
<tr>
<td>0.1</td>
<td>16.65 ± 0.18</td>
<td>19.81 ± 0.18</td>
</tr>
<tr>
<td>0.25</td>
<td>11.59 ± 0.18</td>
<td>16.65 ± 0.18</td>
</tr>
<tr>
<td>0.5</td>
<td>11.59 ± 0.18</td>
<td>16.65 ± 0.18</td>
</tr>
</tbody>
</table>

UP: whole *U. pinnatifida*, UPB: *U. pinnatifida* blade, UPS: *U. pinnatifida* stipe, UPSP: *U. pinnatifida* sporophylls. The different letters (a–d) indicate a significant difference (*p* < 0.05).

2.5. FRAP Activity of Different Solvent Extracts of Different Parts of Asian Kelp

The different solvent extracts of the different parts of Asian kelp showed different FRAP values. Among the ethanolic and methanolic extracts, the UPB and UPS extracts exhibited the highest FRAP values, whereas ascorbic acid at different concentrations (0.1, 0.25, and 0.5 µg/µL) had FRAP values of 47.63–81.04% (Table 3). Among the ethanolic extracts, the highest FRAP value was achieved in the UPB extract (75.75 ± 0.15%), followed by the UPS (60.33 ± 0.47%), UP (46.92 ± 0.09%), and UPSP (36.21 ± 0.06%) extracts. Following the ethanolic extracts, the second highest FRAP value was found in the methanolic extracts. The FRAP value of the methanolic extracts of the different parts of Asian kelp ranged from 50.11 ± 0.34 to 69.54 ± 0.20%. Among the methanolic extracts, the UPB extract showed the highest FRAP value, which was 1.32, 1.36, and 1.39 times higher than that of the UPS, UP, and UPSP extracts, respectively. The lowest FRAP value was observed in the hot water extracts. For all parts of the Asian kelp, the FRAP value was lower in the hot water extract than in the ethanolic and methanolic extracts. This supports the results of the DPPH scavenging activity assay. These results indicate that the ethanolic and methanolic extracts of the different parts of Asian kelp have the highest ABTS scavenging activities.
2.6. Analysis of the Antioxidant Enzymatic Activity of Different Solvent Extracts of Different Parts of Asian Kelp

Plants have antioxidant enzymes, such as SOD, CAT, and APX, which can eliminate free radicals [40]. SOD converts reactive oxygen species, such as superoxide (O$_2^-$), into H$_2$O$_2$, and CAT and APX convert H$_2$O$_2$ into water and oxygen. The SOD, CAT, and APX activities of different solvent extracts of the different parts of Asian kelp were measured. The results showed that the methanolic extracts of all parts of the Asian kelp showed higher antioxidant enzymatic activities than the ethanolic and hot water extracts. Among the methanolic extracts, the UPB, UPS, and UP extracts showed significantly higher SOD activity (Unit/mg) than the UPSP extract (Figure 2A); the UPB extract exhibited the highest SOD activity, which was 1.34, 2.24, and 3.15 times higher than that of the UPS, UP, and UPSP extracts, respectively. Among the ethanolic extracts, the UPSP extract (4.34 ± 0.45 Unit/mg) had the highest SOD activity, followed by the UP (3.33 ± 0.64 Unit/mg), UPB (2.51 ± 0.50 Unit/mg), and UPS (2.44 ± 0.45 Unit/mg) extracts. Among the hot water extracts, the UPSP extract showed the highest SOD activity (0.62 ± 0.27), but there was no significant difference in SOD activity between the extracts of the different parts.

**Figure 2.** SOD, CAT, and APX activities of different parts of Undaria pinnatifida. (A) SOD, (B) CAT, and (C) APX activities. UP: whole U. pinnatifida, UPB: U. pinnatifida blade, UPS: U. pinnatifida stipe, UPSP: U. pinnatifida sporophylls. The different letters (a–d) indicate a significant difference (p < 0.05).
The CAT activity (unit/mg) showed a similar trend to that of the SOD activity. For all parts of the Asian kelp, the methanolic extracts showed higher CAT activity than the ethanolic and hot water extracts (Figure 2B). Among the methanolic extracts, the UPB (2.18 ± 0.17 Unit/mg), UPS (1.59 ± 0.35 Unit/mg), and UP (1.02 ± 0.09 Unit/mg) extracts showed a higher CAT activity (Unit/mg) than the UPSP extract (0.27 ± 0.14). This finding supports the results of the SOD activity assay; however, among the ethanolic extracts, the UP (0.53 ± 0.03 Unit/mg) and UPSP (0.53 ± 0.06 Unit/mg) extracts exhibited the highest CAT activity, whereas the UPS extract showed the lowest (0.32 ± 0.03 Unit/mg). In contrast, the hot water extracts of all parts of the Asian kelp did not show high CAT activity.

The APX activity (Unit/mg) showed a similar trend to that of the SOD and CAT activities. Among the different solvent extracts, the methanolic extracts of all parts of the Asian kelp showed significantly higher APX activity (Figure 2C) than the ethanolic and hot water extracts, which showed a more or less similar APX activity. Among the methanolic extracts, the UP, UPB, UPS, and UPSP extracts had APX activity values of 2.00 ± 0.31, 5.05 ± 0.57, 4.89 ± 0.62, and 2.70 ± 0.34 Unit/mg, respectively. The UPB extract had the highest APX activity, which was 1.03, 1.87, and 2.53 times higher than that of the UPS, UPSP, and UP extracts, respectively. In contrast to the results of the SOD and CAT activity assays, the second highest APX activity was shown in the hot water extracts. Among the hot water extracts, there were no significant differences between the different parts of Asian kelp. The ethanolic extracts also showed a similar trend to that of the hot water extracts, but exhibited much lower APX activity than the methanolic extracts. Taken together, these results suggest that the methanolic extracts of all parts of Asian kelp have high SOD, CAT, and APX activities.

2.7. Collagenase and Elastase Inhibitory Activities of Different Solvent Extracts of Different Parts of Asian Kelp

Ultraviolet light causes oxidative stress on the skin and causes the rapid signs of aging, such as wrinkles, elasticity loss, and freckles [41]. The collagen and elastin present in the dermal layer of the skin maintain skin firmness and elasticity by crosslinking, but it has been reported that they are degraded by collagenase and elastase [42]. Analysis of the collagenase, elastase, and tyrosinase inhibitory activities of the different solvent extracts of the different parts of Asian kelp revealed varying degrees of activities according to the solvents and seaweed parts used. Among these enzyme inhibitory activities, the collagenase inhibitory activity was the highest. The highest collagenase inhibitory activity was found in the UP (65.8 ± 2.5%) and UPSP (43.9 ± 1.8%) ethanolic extracts as well as the UPB (68.9 ± 1.9%) and UPSP (43.2 ± 0.8%) methanolic extracts. Interestingly, the collagenase inhibitory activity was observed in the ethanolic extracts of all parts of the Asian kelp, as shown in Table 4. The lowest collagenase inhibitory activity was found in the hot water extracts of all parts of the Asian kelp. In the UPB methanolic extract and the UPB hot water extract, the collagenase inhibitory activity was not detected. Freitas et al. [43] analyzed the collagenase inhibitory activity of ethanolic and water extracts of \textit{Fucus spiralis}, and their results showed that the ethanolic extract (IC\textsubscript{50} = 89.9 µg/mL) had a higher collagenase inhibitory activity than the water extract (IC\textsubscript{50} = 4.4 µg/mL). A similar result was obtained in this study, in which the ethanolic extract showed higher collagenase inhibitory activity (13.7–65.8%) than the hot water extract (6.6–31.6%). Fitton et al. [44] analyzed the collagenase inhibitory activity of Asian kelp; they found that the extract contained 89% fucoidan and that a 0.1 mg/mL Asian Kelp extract showed 99% inhibitory activity. They also found that the antiaging activity was affected by components such as proteins and amino acids, which varied according to the habitat, extraction conditions, and analysis method [45]. These results indicate that ethanol is the most suitable extraction solvent for obtaining an Asian kelp extract with a high collagenase inhibitory activity; however, the percent of collagenase inhibitory activity might vary according to the kelp’s habitat, extraction conditions, and analysis method.
Table 4. Collagenase, elastase, and tyrosinase inhibitory activities of different parts of Undaria pinnatifida.

<table>
<thead>
<tr>
<th>Seaweed Part</th>
<th>Collagenase Inhibitory Activity (%)</th>
<th>Elastase Inhibitory Activity (%)</th>
<th>Tyrosinase Inhibitory Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95% Ethanol</td>
<td>Methanol</td>
<td>Water</td>
</tr>
<tr>
<td>UP</td>
<td>65.8 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.1 ± 0.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31.6 ± 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>UPB</td>
<td>21.4 ± 1.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>68.9 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>UPS</td>
<td>13.7 ± 1.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>14.2 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>UPSP</td>
<td>43.9 ± 1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.2 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.6 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

UP: whole U. pinnatifida, UPB: U. pinnatifida blade, UPS: U. pinnatifida stipe, UPSP: U. pinnatifida sporophylls. ND: not detected. The different letters (a–d) indicate a significant difference (p < 0.05).

In contrast to the collagenase inhibitory activity, elastase inhibitory activity was observed in all solvent extracts of all parts of the Asian kelp; however, unlike the collagenase inhibitory activity, the elastase inhibitory activity was significantly lower in the UP (22.3 ± 0.4%) and UPSP (26.3 ± 0.1%) ethanolic extracts, and the UPB and UPSP methanolic extracts showed a higher collagenase inhibitory activity than elastase inhibitory activity (Table 4). Interestingly, the UPS methanolic extract did not exert collagenase inhibitory activity, but showed an elastase inhibitory activity of 23.6 ± 0.3%. Among the hot water extracts (excluding the UPSP extract), the UP (31.6 ± 0.2%) and UPS (14.2 ± 0.3%) extracts showed higher collagenase inhibitory activity than elastase inhibitory activity, whereas the UPB did not exert collagenase inhibitory activity. The elastase inhibitory activity of the UPB methanolic extract was 9.60 ± 0.2%. Castejón et al. [15] measured the polyphenol content of Alaria esculenta, Palmaria palmata, and Ulva lactuca hot-water extracts. Compared to the other species, A. esculenta showed the highest polyphenol content, which was responsible for its elastase inhibitory activity. In this previous study, the A. esculenta hot-water extract showed the highest polyphenol content, whereas in the present study, the UPB methanolic extract had the highest polyphenol content. These results suggest that the suitable extraction solvent for the analysis of elastase inhibitory activity may vary based on the species. Moreover, in the evaluation of the antiaging activity of the different solvent extracts of the different parts of Asian kelp, the UPB methanolic extract showed excellent collagenase and elastase inhibitory activities, indicating that methanolic extraction is the most suitable method for extracting functional compounds that can prevent the signs of skin aging by inhibiting wrinkle formation.

2.8. Tyrosinase Inhibitory Activity of Different Solvent Extracts of Different Parts of Asian Kelp

Tyrosinase is an enzyme that promotes the production of melanin, which is known as a black and brown pigment, in the basal layer of the skin [46]. The inhibition of tyrosinase activity is involved in skin whitening by controlling the melanin pigmentation, as excessive melanin synthesis can cause skin pigmentation (such as spots, freckles, age spots), aging, and cancer [47]. In the ethanolic extracts of the Asian kelp, the tyrosinase inhibitory activity was significantly lower than the collagenase and elastase inhibitory activities. In addition, tyrosinase inhibitory activity was not detected in the UPB and UPSP ethanolic extracts (Table 4). In contrast, in the UP and UPS methanolic extracts, the tyrosinase inhibitory activity was significantly higher than the collagenase and elastase inhibitory activities. In the hot water extracts of all parts of the Asian kelp, the tyrosinase inhibitory activity was lower than the collagenase and elastase inhibitory activities. Hassan et al. [48] analyzed the tyrosinase inhibitory activity of various solvents (n-hexane, ethyl acetate, aqueous fraction, and ethanolic) extracts of Padina australis. The results showed that the ethanolic and aqueous fractions showed the lowest tyrosinase inhibitory activities, with respective values of 0.09 ± 0.01 and 0.20 ± 0.03 mg ascorbic acid equivalents (AAE)/g. A similar result was obtained in the current study, in which the ethanolic extracts of all parts of the Asian kelp exhibited the lowest tyrosinase inhibitory activity. Moreover, the UP (36.0 ± 1.8%) and UPB (34.9 ± 0.7%) methanolic extracts showed the highest tyrosinase inhibitory activities. These results imply that methanolic extraction is a suitable method for extracting compounds with a potential use as skin whitening materials.
2.9. Analysis of Phenolic Compound Content of Different Solvent Extracts of Different Parts of Asian Kelp

4-Hydroxybenzoic acid has various medicinal properties, such as having antibacterial [49], anti-inflammatory [50], and hypoglycemic [51] effects. Simple phenolic compounds also serve as precursors in polyphenol biosynthesis [52]. Parabens, known as the esters of 4-hydroxybenzoic acid, are synthetic preservatives mainly used in cosmetics and pharmaceuticals [53]. Naringin is a glycoside containing naringenin as an aglycon flavonoid, and is mainly found in citrus fruits. Naringenin is known as an early precursor in all flavonoids biosynthesis [54,55], and it has anticancer, anti-gastric ulcer [56], lipid metabolism-promoting, and antioxidant [57] effects. It exerts an anti-lipolytic effect [58], and can be used to treat pellagra-induced diarrhea or dermatitis [59]. Considering the diverse medicinal properties of 4-hydroxybenzoic acid, naringin, and naringenin, the analysis of these phenolic compounds in different solvent extracts of different parts of Asian kelp might be useful for the identification of beneficial compounds in Asian kelp.

The analysis of phenolic compounds in the different solvent extracts of the different parts of Asian kelp resulted in the identification of three types of phenolic acid: 4-hydroxybenzoic acid, naringenin, and naringin. The highest 4-hydroxybenzoic acid (µg/g) content was obtained in the UPSP hot water extract (9.96 ± 0.07 µg/g), followed by the methanolic extracted UPB (8.88 ± 0.06 µg/g), whereas the lowest content was achieved in the UP ethanolic extract (1.40 ± 0.03 µg/g) (Table 5). Interestingly, 4-hydroxybenzoic acid was not detected in all the UPS extracts as well as in the UPSP ethanolic and methanolic extracts. Farvin and Jacobsen [60] analyzed the phenolic compounds in several brown algae species. Their results showed that 4-hydroxybenzoic acid was not detected in the water and ethanolic extracts of all the Fucus species examined (F. vesiculosus, F. serratus, F. distichus, and F. spiralis). Moreover, the water extracts of Laminaria digitata and Saccharina latissimi, which belong to the same order as Asian kelp, had a hydroxybenzoic acid content of 0.3 and 2.7 mg/g, respectively [60]; however, in the present study, the UPSP water extract showed the highest 4-hydroxybenzoic acid content. These findings indicate that phenolic content in Asian kelp extracts differ based on the extraction solvent and seaweed part used.

Table 5. Phenolic compound content (µg/g) of different parts of Undaria pinnatifida.

<table>
<thead>
<tr>
<th>Seaweed Part</th>
<th>4-Hydroxy Benzoic acid</th>
<th>Naringenin</th>
<th>Naringin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95% Ethanol</td>
<td>Methanol</td>
<td>Water</td>
</tr>
<tr>
<td>UP</td>
<td>1.40 ± 0.03 b</td>
<td>2.30 ± 0.03 b</td>
<td>3.11 ± 0.04 b</td>
</tr>
<tr>
<td>UPB</td>
<td>2.64 ± 0.08 a</td>
<td>8.88 ± 0.06 a</td>
<td>3.02 ± 0.05 c</td>
</tr>
<tr>
<td>UPS</td>
<td>ND</td>
<td>ND</td>
<td>10.30 ± 0.12 a</td>
</tr>
<tr>
<td>UPSP</td>
<td>ND</td>
<td>ND</td>
<td>9.96 ± 0.07 a</td>
</tr>
</tbody>
</table>

UP: whole U. pinnatifida, UPB: U. pinnatifida blade, UPS: U. pinnatifida stipe, UPSP: U. pinnatifida sporophylls. ND: not detected. The different letters (a–d) indicate a significant difference (p < 0.05).

The highest naringenin content (µg/g) was detected in the UPS ethanolic extract, whereas the lowest was found in the UPB ethanolic extract. The naringenin content in the ethanolic extracts of all parts of the Asian kelp ranged from 10.30–0.15 µg/g (Table 5); however, naringenin was not detected in the methanolic and hot water extracts of all parts of the Asian kelp. Lou et al. [61] analyzed the naringin content in different solvent extracts of Citrus mitis. Their results showed that a 50–80% ethanolic extract showed a higher naringenin content than a hot water extract. In addition, it has been reported that naringin is more soluble in ethanol, which leads to a higher naringenin content in ethanolic extracts than in the hot water extracts [61]. They also found that the highest naringin and naringenin content was found in the ethanolic extract. A similar result was obtained in the present study, in which naringin and naringenin were detected only in the ethanolic extract, and not found in the hot water extract. These results indicate that ethanolic extraction is a suitable method for the extraction of naringin and naringenin from Asian kelp.

The naringin content was significantly higher in the ethanolic extracts than in the other solvent extracts of all parts of the Asian kelp. Among the ethanolic extracts, the UPS
extract had the highest naringin content (2860.03 ± 11.61 µg/g), followed by the UPSP (393.29 ± 6.37 µg/g), UP (379.03 ± 7.72 µg/g), and UPB (236.26 ± 9.62 µg/g) extracts. Remarkably, naringin was not detected in the methanolic and hot water extracts of all parts of the Asian kelp. These results suggest that the naringin content of Asian kelp may vary according to the extraction solvent used and the season. The reason for this difference in the phenolic composition of each extract might be that the chemical constituents of Asian kelp are affected by the harvest time and cultivation region.

2.10. Metabolic Profiling of Different Parts of Asian Kelp

Figure 3 shows the PCA and PLS-DA plot between the total polyphenol content, total flavonoid content, DPPH radical scavenging activity, ABTS radical scavenging activity, FRAP value, SOD activity, CAT activity, APX activity, collagenase inhibitory activity, elastase inhibitory activity, tyrosinase inhibitory activity, and phenolic content of the different parts of the Asian kelp extracts. The PCA plot showed a clear separation of the identified compounds that differed between the different parts, along with the two main components (a 41.9% and 30% variance) (Figure 3A). The most significant metabolites of PC1 in the kelp extracts were the collagenase inhibitory activity and the eigenvector value which was −0.30908, whereas the ABTS, APX, CAT, SOD activities, and FRAP values, were 0.41315, 0.38154, 0.37766, 0.37192, and 0.30207, respectively. These activities determined the separation among the different parts of the Asian kelp. The PLS-DA component analysis also showed a clear separation between the different parts of the Asian kelp, with a 24.9% and 19.9% variance (Figure 3B). This supports the results of the PCA. In addition, the total flavonoid content, elastase inhibitory activity, DPPH activity, and CAT activity were identified as discriminating metabolites in different parts of the Asian kelp (VIP > 1) (Figure S1).

A significantly high positive correlation was shown between the DPPH scavenging activity and total phenolics (r = 0.93328, p < 0.000) as well as between the ABTS activity and CAT activity (r = 0.82008, p < 0.001) (Figure 3 and Figure S2). In particular, there was a positive correlation (r = 0.57178−0.1756) between the total polyphenol content and SOD, tyrosinase, collagenase, and elastase activity, whereas all others showed a negative correlation. Among them, the relationship between the CAT activity and FRAP value (r = 0.81756, p < 0.001) showed a positive correlation. A strong negative correlation was found between the total polyphenolic content and collagenase inhibitory activity (r = −0.96374, p < 0.000), which indicates that the collagenase inhibition activity of all the extracts was lower than the polyphenolic content. This means that collagenase inhibition activity is slightly affected by polyphenolic content. Moreover, there was a negative correlation between the total polyphenol content and DPPH scavenging activity of the extracts, whereas a positive correlation to SOD activity was shown. Nowak et al. [62] reported that DPPH scavenging activity reflects the total antioxidant potential of an extract, not its polyphenol content. They also revealed no correlation between the total polyphenol content and ABTS scavenging activity because the total polyphenol content is highly correlated with the antioxidant capacity; however, the present study showed strong positive correlations between the polyphenol content and tyrosinase, collagenase, elastase, and SOD activities of all the Asian kelp extracts, suggesting that the antiaging and antioxidant activities of all the Asian kelp extracts are very closely related to the polyphenol content, which reflects the antiaging and antioxidant activities of Asian kelp. This result is consistent with the finding of Fung et al. [63] who reported that this effect was due to the antioxidant activity of polyphenol compounds.
were separated, washed three times in distilled water to remove the salt, and dried at 65 °C.

The oxidant are metabolites to tracts, reflects nol Figure pounds. who revealed phenolic content. however, This effect was detected from different parts of Undaria pinnatifida. The specimens were stored at the Marine Bio Research Center, Chosun University and the accession number is MBRC-CU-202006. Different parts of the seaweed, namely, the whole Undaria pinnatifida (UP), U. pinnatifida blade (UPB), U. pinnatifida stipe (UPS), and U. pinnatifida sporophylls (UPSP), were separated, washed three times in distilled water to remove the salt, and dried at 65 °C for 3 days. The dried samples were then crushed into powder with a plant crusher (USC, Seoul, Korea). After that, an extraction was performed with different solvents.

![Figure 3](image-url)

Figure 3. Score and loading plot of (A) PCA model and (B) PLS-DA model acquired from the metabolites detected from different parts of Undaria pinnatifida. Note: ABTS, ABTS radical scavenging activity; APX, ascorbate peroxidase activity; CA, collagenase inhibitory activities; CAT, catalase activity; DPPH, DPPH radical scavenging activity; EA, elastase inhibitory activities; FRAP value, ferric reducing antioxidant power value; SOD, superoxide dismutase activity; TA, tyrosinase inhibitory activity; TFC, total flavonoid content; and TPC, total polyphenol content.

3. Materials and Methods

3.1. Collection of Seaweed

The *U. pinnatifida* seaweed was collected from Wando, Korea in June 2020. The collected samples were identified by Dr. Lee WJ, Research Associate at Honam National Institutes of Biological Resources, Republic of Korea. The specimens were stored at the Marine Bio Research Center, Chosun University and the accession number is MBRC-CU-202006. Different parts of the seaweed, namely, the whole *U. pinnatifida* (UP), *U. pinnatifida* blade (UPB), *U. pinnatifida* stipe (UPS), and *U. pinnatifida* sporophylls (UPSP), were separated, washed three times in distilled water to remove the salt, and dried at 65 °C for 3 days. The dried samples were then crushed into powder with a plant crusher (USC, Seoul, Korea). After that, an extraction was performed with different solvents.
3.2. Extraction Method for Asian Kelp Samples

Different parts of Asian kelp (UP, UPB, UPS, and UPSP) were soaked into ~50 times volume of distilled water and kept at 37 °C for 1 h. Next, hot water extraction was carried out at 80 °C for 3 h. Then, 95% ethanolic and methanolic extractions were carried out by adding 10 times the volume of ethanol and methanol to the Asian kelp powders, followed by incubation for 24 h at 25 °C in a shaker with shaking at 120 rpm. In the hot water extraction, the extract was filtered through Whatman No. 2 paper and concentrated at 37 °C by using a rotary evaporator (VV2000; Heidoiph, Schwabach, Germany). The ethanolic and methanol extracts were centrifuged at 3000 rpm for 20 min at 4 °C and then concentrated at 38 °C, and the remaining solvent was removed using nitrogen gas. After extraction, the samples were kept at 4 °C until further analysis. The yield (%) of the sample was calculated using the following formula described by Chan et al. [64]:

\[ \text{Yield (%) } = (W_1/W_2) \times 100 \]

\[ W_1 = \text{the weight of the dried extract} \]
\[ W_2 = \text{the weight of the freeze-dried samples} \]

3.3. Determination of Total Polyphenol Content

The total polyphenol content was determined according to the protocol described by Folin and Denis [65], with a slight modification. To the different parts of Asian kelp extracted with different solvents (10 µg/µL), 80 µL of Folin–Denis Reagent was added and kept at room temperature for 3 min, then 80 µL of 10% sodium carbonate (Sigma Aldrich Co., St. Louis, MO, USA) was added to the mixture and incubated at room temperature for 1 h, with the absorbance measured at 760 nm. The total polyphenols content of the different solvent extracts of the different parts of Asian kelp was quantified by preparing a standard calibration curve using tannic acid (Sigma Aldrich Co., St. Louis, MO, USA).

3.4. Determination of Total Flavonoids

The total flavonoid content was determined according to the protocol described by Moreno et al. [66], with a slight modification. Briefly, 10 µg/µL of the different solvent extracts of the different parts of Asian kelp was mixed with 5 µL of 10% aluminum nitrate and 5 µL of 1 M potassium acetate, followed by the addition of 235 µL of each extraction solvent. Next, the mixture was incubated at 37 °C for 40 min, and the absorbance was measured at 415 nm. The total flavonoid content of the extract was measured from a standard calibration curve using quercetin (Sigma Aldrich Co., St. Louis, MO, USA).

3.5. DPPH Radical Scavenging Assay

A 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay of the Asian kelp extracts was performed using the method of Blois [67]. In brief, 0.02 mL of the different solvent extracts of the different parts of Asian kelp was mixed with 0.98 mL of 150 µM DPPH solution (Sigma Aldrich Co., St. Louis, MO, USA) and then incubated in the dark for 30 min. The DPPH scavenging activity was measured at 517 nm. Synthetic antioxidant butylated hydroxytoluene (BHT) (Sigma Aldrich Co., St. Louis, MO, USA) was used as a positive control. The DPPH radical scavenging activity percentage was measured by the following equation:

\[ \text{DPPH scavenging activity (%) } = \frac{(A_0 - A_1)}{A_0} \times 100 \]

where \( A_0 \) is the absorbance of the control, and \( A_1 \) is the absorbance of the extractives/standard. Next, the % inhibition was plotted against the concentration, and the IC\(_50\) was calculated using the plotted graph. The experiment was repeated three times for each concentration.
3.6. ABTS Radical Scavenging Activity

The 2,2’-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging activity of the Asian kelp extracts was measured according to the following procedure. An ABTS radical cation solution was prepared by adding 7 mM ABTS solution to a 140 mM potassium persulfate stock solution. The mixture was then incubated in the dark for 16 h at 37 °C. Next, 10 µg/µL of the different solvent extracts of the different parts of Asian kelp was mixed with 200 µL of ABTS solution and incubated at 37 °C for 3 min. The absorbance was measured at 734 nm. The percentage of the free radical scavenging activity was calculated using the following formula:

\[
ABTS + \text{scavenging effect} (%) = \left( \frac{AB - AA}{AB} \right) \times 100
\]

where AB is the absorbance of ABTS radical + methanol, and AA is the absorbance of ABTS radical + sample extract/standard. Ascorbic acid (Sigma Aldrich Co., St. Louis, MO, USA) was used as a standard substance.

3.7. FRAP Assay

A ferric reducing antioxidant power (FRAP) assay was performed according to the protocol described by Nowak et al. [62], with a slight modification. The FRAP reagent was freshly prepared by mixing 300 mM sodium acetate (pH 3.6), 20 mM FeCl₃, and 10 mM TPTA dissolved in 40 mM HCl at a ratio of 10:1:1. In brief, 10 µL of the different solvent extracts of the different parts of Asian kelp was mixed with 150 µL of the FRAP solution and incubated for 30 min at 37 °C. Ascorbic acid (Sigma Aldrich Co., St. Louis, MO, USA) was used as a positive control. The mixture was incubated for 30 min at 37 °C, and the absorbance was measured at 593 nm. The FRAP activity of the sample was calculated using the following formula:

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

where A = Absorbance value of the sample

B = Absorbance value of the control

3.8. Measurement of Antioxidant Enzyme Activity

To the different solvent extracts of the different parts of Asian kelp, a 50 mM potassium phosphate (pH 7.0), 1% Triton X-100, and 1% PVP-40 solution were added at a ratio of 4:1. The mixture was incubated at 4 °C for 20 min and then centrifuged at 12,000 rpm for 15 min. Next, the supernatant was separated and used for measuring the antioxidant enzymatic activity using a BSA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA).

The superoxide dismutase (SOD) activity of the Asian kelp extracts was analyzed based on the reduction of nitroblue tetrazolium (NBT) using a superoxide anion generated by the reaction of xanthine and xanthine oxidase, as described by Beauchamp and Fridovich [68]. To the different solvent extracts of the different parts of Asian kelp, a 50 mM potassium phosphate (pH 7.0), 3 mM EDTA, 3 mM xanthine, and 0.75 mM NBT solution was added, and the mixture was incubated at 25 °C for 10 min, followed by the addition of 0.3 unit/mL xanthine oxidase. The reduction of the NBT was measured at 560 nm using a UV spectrophotometer. The SOD activity was calculated according to the following formula:

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

where A = Absorbance value of the sample

B = Absorbance value of the control

The catalase (CAT) activity was measured according to the method of Aebi [69], with a slight modification. To 20 µL of the different solvent extracts of the different parts of...
Asian kelp, 0.2 mL of 50 mM potassium phosphate (pH 7.0) and 10 mM hydrogen peroxide (H$_2$O$_2$) solution were added, and the absorbance was measured at 240 nm every 30 s for 2 min. The CAT activity was determined from a decrease in the substrate of H$_2$O$_2$ at a 240 nm absorbance (1 unit of CAT activity is defined as the amount of enzyme that breaks down in 1 µmol H$_2$O$_2$ per minute under assay conditions). Here, 43.6 is the extinction coefficient of H$_2$O$_2$ at 240 nm, and the CAT activity was calculated according to the following formula:

\[
\text{CAT activity (Unit/mg)} = \frac{(A - B)}{43.6} \times \frac{\text{total volume}}{\text{sample volume}} \times \frac{\text{sample concentration (mg/mL)}}{}
\]

A = Absorbance value of the sample
B = Absorbance value of the control

Ascorbate peroxidase (APX) is an important enzyme that removes H$_2$O$_2$ along with CAT. The APX activity was measured according to the method of Nakano and Asada [70]. The APX activity was determined from a decrease in H$_2$O$_2$ by oxidizing ascorbate. Briefly, 20 µL of the different solvent extracts of the different parts of Asian kelp was mixed with 50 mM potassium phosphate (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA, and 0.2 mL of 0.1 mM H$_2$O$_2$ solution, and the mixture was incubated at 37 ºC for 5 min. The absorbance of the mixed solution was measured at 290 nm. The extinction coefficient of the ascorbate at 290 nm was measured similarly to the CAT activity. The APX activity was calculated according to the following formula:

\[
\text{APX activity (Unit/mg)} = \frac{(A - B)}{2.8} \times \frac{\text{total volume}}{\text{sample volume}} \times \frac{\text{sample concentration (mg/mL)}}{}
\]

A = Absorbance value of the sample
B = Absorbance value of the control

3.9. Measurement of Antiaging Activity
3.9.1. Collagenase Inhibition Assay

The collagenase inhibitory activity was measured according to the protocol described by Wünsch and Heidrich [71], with a slight modification. First, 50 µL of the different solvent extracts of the different parts of Asian kelp (1 mg/mL) was mixed with 12.5 µL of 0.1 M Tris-HCl (pH 7.5), 4 mM CaCl$_2$, and 75 µL of 0.5 mg/mL collagenase, and the mixture was incubated at 37 ºC for 20 min. The reaction was terminated by adding 0.25 mL of 6% citric acid. Next, the mixture was centrifuged, and 0.2 mL of the supernatant was transferred into a 96-well plate, to which 750 µL of ethyl acetate was added. The absorbance was measured at 320 nm. The collagenase inhibitory activity was calculated according to the following formula:

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

A = Absorbance value of the sample
AB: Absorbance value of the enzyme-free group in the sample-added section
C: Absorbance value of the control
CB: Absorbance value of the enzyme-free group in the control

3.9.2. Elastase Inhibition Assay

The elastase inhibitory activity was determined using the method of Cannell et al. [72], with a slight modification. Briefly, 50 µL of the different solvent extracts of the different parts of Asian kelp (1 µg/µL) was mixed with 50 µL of 0.5 mg/mL N-succinyl-(Ala)$_3$-p-nitroanilide and incubated at 37 ºC for 5 min. After incubation, the absorbance of the samples was measured at 410 nm by a UV/VIS spectrophotometer. As a positive control,
50 µL of 0.2 unit/mL porcine pancreas elastase was prepared using 50 mM Tris-HCl (pH 8.6) and incubated at 37 °C for 5 min. Next, the absorbance of the samples was measured at 410 nm. The elastase inhibitory activity was calculated according to the following formula:

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

A: Absorbance value of the control and enzyme-added groups
B: Absorbance value of the sample and enzyme-added part
C: Absorbance value of the enzyme-free group after sample addition

3.10. Tyrosinase Inhibition Assay

The tyrosinase inhibitory activity was measured according to the protocol described by Yagi et al. [73], with a slight modification. In brief, 20 µL of the different solvent extracts of the different parts of Asian kelp (1 µg/µL) was mixed with 70 µL of 1.5 mM 3,4-dihydroxy-L-phenylalanine (L-DOPA) and a 0.1 M potassium phosphate buffer (pH 6.8). The mixture was then gently mixed and incubated at 37 °C for 5 min, followed by the addition of 50 µL of 500 U/mL mushroom tyrosinase. The mixture was again incubated at 37 °C for 5 min, and then the absorbance was measured at 490 nm. The tyrosinase inhibitory activity was calculated according to the following formula:

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

A: Absorbance value of the control and enzyme-added groups
B: Absorbance value of sample and enzyme-added part
C: Absorbance value of the enzyme-free group after sample addition

3.11. Analysis of Phenolic Compound Content

An LC-MS/MS mass spectrometer (AB SCIEX 4000 Q-Trap; Shimadzu LC 20A System) was used for the quantitative analysis of phenolic compounds in the Asian kelp extracts. A total of three individual phenolic compounds, namely, 4-hydroxy benzoic acid, naringenin, and naringin, were analyzed. First, 20 mg of the different solvent extracts of the different parts of Asian kelp was mixed with 1 mL of each extraction solvent (acetonitrile and methanol), and the upper layer of the mixture was diluted up to 40 times with distilled water. Next, the samples were filter sterilized. The phenolic compounds were separated using a Gemini C18 column (3 µm, 110 A, 50 × 2.0 mm), with an injection volume of 10 µL/mL. All the standards were purchased from Sigma-Aldrich Co., Ltd., St. Louis, MO, USA, and the purity of each standard was as follows: 4-hydroxybenzoic acid (≥99%), Naringenin (≥99.5%), and Naringin (≥95%).

3.12. Statistical Analysis

All experiments were repeated three times, and the data were expressed as mean and error values. The statistical analysis was conducted using IBM SPSS Statistics (version 25; SPSS Inc., Chicago, USA). An ANOVA followed by a Duncan’s multiple range test was performed to analyze the significance of the differences between the data at the level of \( p < 0.05 \). The principal component analysis (PCA), partial least-squares discriminant analysis (PLS-DA), correlation analysis, and the variable importance in projection (VIP) were analyzed by MetaboAnalyst 5.0 (http://www.metaboanalyst.ca/, accessed on 24 May 2022).
4. Conclusions

In this study, the antioxidant, antiaging activities, and phenolic compounds of the UP, UPB, UPS, and UPSP were measured after extraction with different solvents. Among the various Asian kelp extracts, the UPB methanolic extract had the highest polyphenol and flavonoid content, and the UPB ethanolic extract had significantly higher DPPH scavenging activity (IC$_{50}$ 12.73 mg/mL). In addition, the activity of the antioxidant enzymes SOD, CAT, and APX, was higher in the UPB methanolic extract than in the other extracts. To evaluate the antiaging effect of the extracts, the collagenase and elastase inhibitory activities were measured, and the results showed that the UPB methanolic extract showed the highest collagenase (68.9%) and elastase (31.0%) inhibitory activities. In the tyrosinase inhibition assay, which represents the skin whitening activity, the UPB methanolic extract showed the highest activity (36.0%). Among the phenolic compounds analyzed, 4-hydroxybenzoic acid showed the highest content in the UPSP hot water extract, whereas naringenin and naringin were found only in the ethanolic extract. The PCA and PLS-DA analysis showed a strong positive correlation between the polyphenol content and tyrosinase, collagenase, elastase, and the SOD activities of all parts of the Asian kelp extracts had strong antiaging and antioxidant activities that was closely related to the polyphenol content. Based on these results, we conclude that the UPB extracts of Asian kelp can be used in the development of functional materials. This result might be helpful for exploring new applications of Asian kelp in the future.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jmse10091193/s1, Figure S1. The main components separating the different parts of the Asian kelp based on the VIP in PLS-DA, Figure S2. Correlation matrix of metabolites identified in different parts of Asian Kelp.

Author Contributions: S.Y.L. and C.S.K. conceived the study; H.-H.L., J.-S.K., J.-H.J., S.M.P. and R.S. performed the experiments and analyzed the data; H.-H.L., R.S. and S.Y.L. writing—original draft; R.S., S.Y.L. and C.S.K. writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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

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