Antioxidant activity of phlorotannins from brown algae

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Abstract: The antioxidant activity of the phlorotannins extracted from five marine algae species (Saccharina latissima, Alaria esculenta, Laminaria digitata, Fucus vesiculosus and Ascophyllum nodosum) was studied. Three phlorotannin groups, including soluble, membrane-bound, and extracted membrane-bound phlorotannins obtained by two solvent extraction methods were investigated for their DPPH radical scavenging activity. F. vesiculosus and A. nodosum showed the highest phlorotannin yield (14.83 mg-extract/g-algae and 12.80 mg-extract/g-algae, respectively) among the five algae species. Their soluble phlorophannin (SP), membrane-bound phlorotannin (MP) and extracted membrane-bound phlorotannin (eMP) extracts all showed equal or greater DPPH radical scavenging activity than the commercial antioxidants of butylated hydroxytoluene and ascorbic acid. The antioxidant potential that combines phlorotannin yield and antioxidant activity of the MP extracts of F. vesiculosus and A. nodosum (5890 mL/g and 5278 mL/g algae, respectively) were higher than those of SP and eMP, suggesting that the MPs of F. vesiculosus and A. nodosum had great potential to be used as antioxidants. Different extraction methods also showed significantly different effects on the antioxidant activity of the phlorotannin extracts.

Keywords: brown algae, phlorotannin, antioxidant activity, antioxidant, bioseparation, polyphenol, solvent extraction methods

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1 Introduction

Reactive Oxygen Species (ROS) are the major causes of oxidative damage and related diseases such as atherosclerosis, rheumatoid arthritis, muscular dystrophy, cataracts, some neurological disorders and some types of cancer as well as aging. ROS are a class of highly reactive molecules formed during aerobic life[1]. Normal levels of ROS may be essential for many cellular functions such as killing phagocytes, bacterial ingestion and redox regulation of signal transduction. However, overproduction of ROS in living organisms can cause harm to DNA, cell membrane, proteins and consequently induce degeneration, destruction and toxicity of various molecules in cells[2].

Natural antioxidants in terrestrial plants and their applications in food preservation and nutraceuticals have been studied in numerous cases. Some synthetic antioxidants like butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone and propyl gallate have been applied in food, cosmetic and drug products[3]. However, the side effects and toxicity of these synthetic antioxidants have been questioned and researchers are looking for natural antioxidants that can be safely used in food and medicine[4]. Polyphenols are the most abundant dietary antioxidants by scavenging free radicals and inhibition of the generation of ROS during cell metabolism[5]. Phlorotannins in brown algae are prominent natural antioxidants that may replace synthetic antioxidants. Some phlorotannins isolated from edible brown algae have shown stronger antioxidant activity than commercial antioxidants[5,6].
Phlorotannins are the polyphenolic compounds that are only found in marine brown macroalgae. They consist of polymers of phloroglucinol (1,3,5-tryhydroxybenzene) units that are formed in the acetate-melazone pathway as secondary metabolites. They are highly hydrophilic compounds with a wide range of molecular sizes from 400 Da to 400 000 Da and occur in variable contents (0.5%-20%) in brown algae. They have been reported to have pharmaceutical activities such as antibacterial, antioxidant, antifungal, anti-HIV, anti-diabetes, anti-inflammatory and anti-allergic functions. Phlorotannins can be divided into two groups, soluble phlorotannins (SPs) and membrane-bound phlorotannins (MPs), according to their location in brown algae cells. SPs are stored in cell organelles, physodes, which are round or elliptical, highly mobile, vesicle-like, strongly refractive bodies in the cytoplasm of brown algae cells. MPs are believed to transform into components of cell walls when physodes fuse with cell membrane and the phlorotannins are secreted into the cell wall, complexing finally with alginic acid.

To the best knowledge of the authors, the antioxidant activity of MP and eMP extracts has not been studied. Radical scavenging activity of the SP has been reported in some articles but the five algae species, Saccharina latisima (kelp), Alaria esculenta (alaria), Laminaria digitata (digitata), Fucus vesiculosus (bladderwrack) and Ascophyllum nodosum (rockweed) have not been fully investigated. Furthermore, the effects of different extraction processes on the antioxidant activity of the phlorotannin extracts were not fully studied. Thus the objectives of this study were to evaluate the antioxidant activity of soluble and membrane-bound phlorotannins of S. latisima, A. esculenta, L. digitata, F. vesiculosus and A. nodosum, and examine the influence of two commonly used solvent extraction methods on the antioxidant activity of the phlorotannin extracts.

2 Materials and methods

2.1 Materials

Five brown algae S. latisima (kelp), A. esculenta (alaria), L. digitata (digitata), F. vesiculosus (bladderwrack) and A. nodosum (rockweed) were obtained from Maine Coast Sea Vegetables (Franklin, ME, USA), all in dried powder except for S. latisima which was in dried whole leaf. The samples were sealed in airtight bags and had a greenish brown color. The whole leaf of S. latisima was ground and sieved with a 1-mm sieve before extraction. All samples were sealed and stored under −20°C until experiments.

2.2 Methods

2.2.1 Phlorotannin extraction and sample preparation

The SP was extracted with two solvent extraction methods commonly found in the literature. The extraction processes are shown in Figures 1 and 2. In the first extraction method (designated as Method 1), methanol (MeOH), chloroform (CHCl₃), deionized water and ethyl acetate (EtOAc) were applied in sequence. In the second extraction method (designated as Method 2), MeOH, dichloromethane (CH₂Cl₂), EtOAc, and n-butanol were used. The EtOAc fraction in both extraction methods contained phlorotannins and was analyzed for TPC and DPPH radical scavenging activity. Algae species with high phlorotannin content and antioxidant activity by Method 1 were also extracted by Method 2.

Algae powder residue from solvent extraction was extracted with the method of Budhiyanti et al. to obtain the MP. Firstly, 200 mg algae residue after SP extraction was dissolved in 8 mL 1 M NaOH, stirred for 2 h, and concentrated under 2400 g for 5 min. Then, the supernatant was neutralized with phosphoric acid and its MP content was tested.
Figure 2 Second extraction method for SP extraction

The eMP extracts were obtained by extracting the MP extracts with a modified version of the first extraction method, in which ethyl ether was applied in place of ethyl acetate\[15\]. The liquid fractions containing phlorotannins obtained by these processes were evaporated with rotary evaporator and dried in vacuum under 60°C for 24 h to obtain the dried extracts of phlorotannins. The mass of dried extracts were measured to evaluate the yields of phlorotannin extracts. All samples were freshly prepared and tested within 24 h. All chemicals and organic solvents were of analytical grade and purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2.2 DPPH radical scavenging activity test

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals are stable organic nitrogen radicals bearing a deep purple color\[16\]. They may be neutralized by either direct reduction via single electron transfer or by radical quenching via hydrogen atom transfer. During reactions, color of the solution changes from purple to yellow when the antioxidants eliminate the DPPH radicals\[17\]. The DPPH assay has been widely used due to its stability, simplicity, rapidity and reproducibility. The other reason for applying DPPH test is that both phlorotannins and DPPH radicals are easily dissolved in methanol, which is the reaction condition for DPPH assay. The most widely used parameter evaluating the property of scavenging DPPH radicals is the IC\(_{50}\) value, which is the phlorotannin amount that can eliminate half of given DPPH radicals\[18,19\]. The extracts with lower IC\(_{50}\) value indicate stronger antioxidant activity.

DPPH free radical scavenging activity was measured using the method of Cox et al.\[20\] with minor modifications. A 1 mg/mL phlorotannin solution was made by dissolving dried phlorotannin extracts in deionized water. Then the solution was diluted with deionized water by 10, 100, 1000 and 10 000 times to make a series of phlorotannin samples with various concentrations. A 152 µM DPPH radical solution was made by dissolving DPPH radicals in methanol. Then 0.1 mL phlorotannin sample was added to 0.1 mL of 152 µM DPPH radical solution. The reaction mixtures were incubated in the dark for 30 min at room temperature, and the optical density (OD) was measured at 517 nm using a BioTek 96-well microplate reader (Winooski, VT, USA). The DPPH test was performed in triplicate and the result was expressed as half maximum inhibitory concentration (IC\(_{50}\)) value (mg-extract/mL-water), which was the phlorotannin concentration whose radical scavenging capacity was 50%. The ability to scavenge the DPPH radical was calculated with the following equations:

\[
\text{Scavenging capacity} = 1 - \frac{A_{\text{sample}} - A_{\text{sampleblank}}}{A_{\text{control}}} \quad (1)
\]

where, \(A_{\text{control}}\) is the OD of the DPPH solution; \(A_{\text{sample}}\) is the OD of DPPH solution with sample; \(A_{\text{sampleblank}}\) is the OD value of the sample only. The DPPH radicals were purchased from Sigma-Aldrich CO. LLC. (St. Louis, MO, USA). Commercial antioxidants BHT and ascorbic acid were purchased from Fisher Scientific and their antioxidant activities were also measured for comparison purpose.

2.2.3 Total phlorotannin content test

The total phlorotannin content (TPC) was determined according to a modified version of Folin-Ciocalteu method, using phloroglucinol (PHG) as the standard\[20\]. Samples were diluted taking into account the range of the standard curve. A 0.04 mL aliquot of the sample was mixed in a 1.5 mL centrifugation tube with 0.4 mL 1 N
Folin-Ciocalteu reagent and 0.8 mL 20% Na₂CO₃. After standing for 3 min, the sample was incubated in the dark at room temperature for 45 min and centrifuged at 1600 g for 8 min. Optical density (OD) of the supernatant was measured at 730 nm using a BioTek 96-well microplate reader (Winooski, VT, USA). TPC test was performed in triplicate and the result was expressed as mg phloroglucinol equivalent per gram algae (mg PHG/g algae) using the following calibration equation: 

\[ Y = 2.3356X - 0.0544 \]  
\[(r^2=0.996)\], where Y is the OD at 730 nm and X is the concentration of phloroglucinol as the standard (mg/mL). Folin-Ciocalteu phenolic reagent and phloroglucinol were obtained from Sigma-Aldrich CO. LLC. All other chemicals and organic solvents were obtained from Fisher Scientific.

2.2.4 Statistical analysis

The results of the present study were expressed as mean ± standard deviation. Statistical analysis was performed by one-way ANOVA and Tukey test with SAS (Cary, NC, USA). A p-value of 0.05 or less was considered significant.

3 Results and discussion

3.1 DPPH scavenging activity of SP

The DPPH scavenging activity, expressed as IC₅₀ value, is shown in Table 1. *A. nodosum* and *F. vesiculosus* had the strongest DPPH scavenging activity among the SP extracts of the five algae species by Method 1. Meanwhile, significant differences in antioxidant activity were observed between the two extraction methods for *A. nodosum* and *F. vesiculosus*. Method 1 was better on *F. vesiculosus* while Method 2 was better on *A. nodosum* in terms of antioxidant activity of SP. It indicated that extraction process had effects on the antioxidant activity of the extracts obtained, which agreed with the results of Turkmen et al., who reported that the antioxidant activity of black tea extracts were dependent on the solvent used and length of the extraction processes. The IC₅₀ value of *S. latissima* (0.62 ± 0.06 mg/mL) was significantly lower than *L. digitata* (0.74 ± 0.10 mg/mL). It suggested that the SP extract of *S. litissima* was a better antioxidant than *L. digitata*. However, Cox et al. reported that the IC₅₀ value of SP from *L. digitata* was much lower than *S. latissima*. A study investigating the DPPH radical scavenging activity of extracts from brown algae *Sargassum marginatum, Padina tetrastomatica* and *Turbinaria conoides*, used a method that was similar to the present study. The IC₅₀ values reported in their study were higher than those of *A. nodosum* and *F. vesiculosus* investigated in the present study, which suggested that *A. nodosum* and *F. vesiculosus* could be better sources of antioxidants than *S. marginatum, P. tetrastomatica* and *T. conoides*. The DPPH radical scavenging activity of SP extracts from the two algae species were equal to or better than BHT (0.051 ± 0.0005 mg/mL) and ascorbic acid (0.0063 ± 0.0002 mg/mL).

Note: The letters a, b, c, d indicate significant difference among the SP of the five algae species obtained by Method 1 (a>b>c>d); Letters p and q indicate significant difference between the SP of *Ascophyllum nodosum* obtained by the two extraction methods (p>q); Letters r and s indicate significant difference between the SP of *Fucus vesiculosus* obtained from the two extraction methods (r>s). A p-value of 0.05 or less was considered to indicate significant difference.

The correlation between TPC and IC₅₀ values of the SP extracts obtained by the first extraction method is shown in Figure 3. A strong correlation coefficient (r² = 0.98) was observed among the four algae species *A. nodosum, F. vesiculosus, S. latissima* and *L. digitata*, suggesting the algae species with higher SP content also had stronger antioxidant activities. Interestingly, when *A. esculenta* was considered in this correlation, the coefficient decreased to r² = 0.61. It indicated that some nonphenolic compounds, e.g. polysaccharides, with strong antioxidant activity were probably in the SP extract of *A. esculenta* thus further identification and antioxidant activity test is needed. The correlation between TPC and the antioxidant activity of extracts from both macroalgae and terrestrial plants were reported in the literature. High correlation coefficient (r² = 0.99) was found between TPC and DPPH scavenging activity between SPs of Iceland seaweeds. Total phenols of wild berry fruits grown in southeast Serbia were found to...
correlate negatively with the IC$_{50}$ values\cite{23}. Similar results were also reported in the enzyme-assisted extract from grape residues\cite{24}. These studies suggested that polyphenols were the major antioxidants in macroalgae and terrestrial plants.

The yields of SP extracts are shown in Table 2. The highest yield, 26.34±3.54 mg-extract/g-algae, was obtained in $A$. nodosum by Method 2. However, the yield of the same algae by Method 1 was significantly lower at 12.80±1.37 mg-extract/g-algae. Interestingly, $F$. vesiculosus showed no differences in the yield between the two extraction methods. The yields of the other three algae $A$. esculenta, $S$. latissima and $L$. digitata, were significantly lower than $A$. nodosum and $F$. vesiculosus. Budhiyanti et al.\cite{4} reported that the solvents used had an impact on the yield of phenolic compounds from marine macroalgae. They also found that the yield of $S$. Latissima was low because of the high content of alginate in the extracts.

Table 2  Yield and antioxidant potential of SP extracts by both extraction methods

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Algae species</th>
<th>Yield /mg-extract (g-algae)$^{-1}$</th>
<th>Antioxidant Potential /mL-DPPH solution (g-algae)$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ascophyllum nodosum</td>
<td>12.80±1.37</td>
<td>1777.78±190.31s</td>
</tr>
<tr>
<td></td>
<td>Fucus vesiculosus</td>
<td>14.83±3.67</td>
<td>3903.51±996.04R</td>
</tr>
<tr>
<td>Method 1</td>
<td>A. esculenta</td>
<td>4.10±0.46</td>
<td>45.56±5.09R</td>
</tr>
<tr>
<td></td>
<td>Laminaria digitata</td>
<td>1.78±0.16</td>
<td>2.41±0.22R</td>
</tr>
<tr>
<td></td>
<td>Saccharina latissima</td>
<td>1.83±0.15</td>
<td>2.96±0.25L</td>
</tr>
<tr>
<td>Method 2</td>
<td>Ascophyllum nodosum</td>
<td>26.34±3.54</td>
<td>4180.42±561.96R</td>
</tr>
<tr>
<td></td>
<td>Fucus vesiculosus</td>
<td>15.25±1.13</td>
<td>1980.52±147.08L</td>
</tr>
</tbody>
</table>

Note: The letters a, b, c and d indicate significant differences among SP yields in the order of a>b>c>d while r, s and t indicate significant differences among the AP in the order of r>s>t (p<0.05).

The Antiradical Power (ARP), which was 1/IC$_{50}$, was used in the literature as the parameter evaluating antioxidant activity instead of IC$_{50}$. Wang et al.\cite{4} found that the ARP of $A$. nodosum and $F$. vesiculosus were higher than that of $A$. esculenta, $L$. digitata and $S$. latissima. However, ARP does not reflect the amount of extracts from the source material. In order to evaluate the potential of the brown algae extracts as natural antioxidant sources, a new parameter named Antioxidant Potential (AP), which considered both the yield and antioxidant activity of the extracts was applied to evaluate the potential of brown algae extracts as natural antioxidants.

$$AP = \text{yield of extracts} \times \frac{1}{\text{IC}_{50}} \quad (2)$$

As shown in Table 2, the SP extracts of $F$. vesiculosus by Method 1 and $A$. nodosum by Method 2 had the highest AP values of 3903.51 ± 996 mL/g and 4180.42 ± 562 mL/g algae, respectively. The AP values of $A$. nodosum and $F$. vesiculosus were much higher than $A$. esculenta, $S$. latissima and $L$. digitata, which indicated their greater potential of usage as natural antioxidant sources.

3.2 DPPH scavenging activity of MP and cMP

The DPPH scavenging activities of the MP extracts of $A$. nodosum and $F$. vesiculosus that had the strongest antioxidant activities in their SP extracts are shown in Figure 4. The IC$_{50}$ values of MP extracts from $A$. nodosum and $F$. vesiculosus by Method 1 were lower than Method 2. The lowest IC$_{50}$ value, which was 0.0047±0.0002 mg/mL, was observed from $F$. vesiculosus by Method 1. However, the largest IC$_{50}$ value, 0.0092±0.0003 mg/mL, was also observed in the same algae by Method 2. It indicated that extraction methods had significant effects on the antioxidant activity of MP extracts. Budhiyanti et al.\cite{8} reported that the IC$_{50}$ values for scavenging DPPH radicals of membrane bound phlorotannin extracts of $Sargassum hystrix$ were between 0.27 mg/mL and 3.98 mg/mL, which were larger than BHT. This result indicated that $F$. vesiculosus and $A$. nodosum might be better sources of membrane bound phlorotannin sources than $S$. hystrix. The algae powder residue of $Sargassum microcraanthum$ was treated with methanol and chloroform and the extracts showed better DPPH radical scavenging activity than BHT\cite{25}. These two studies suggested that the algae residue after solvent
extraction can be good sources of phlorotannins and antioxidants.

Unlike the DPPH scavenging activity, yields of MP extracts were not very different from each other except for F. vesiculosus, whose yield by Method 1 was greater than by Method 2. Similar result was also observed in its eMP yields, with the eMP yield by Method 1 much greater than Method 2. These results suggested that Method 1 was better than Method 2 in terms of phlorotannin yields from F. vesiculosus. Surprisingly, the yields of MP and eMP extracts from A. nodosum were not significantly different between the two extraction methods, suggesting that extraction processes had no effects on the yields of MP and eMP of A. nodosum. For every algae and both extraction methods, the AP value of the eMP was lower than that of the MP extract, indicating better potential of MP than eMP as natural antioxidants.

### Table 3  Yield and antioxidant potential of MP and eMP extracts

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Algae species</th>
<th>Yield of MP (mg·extract·g-algae⁻¹)</th>
<th>Antioxidant potential of MP (mg·DPPH solution·g-algae⁻¹)</th>
<th>Yield of eMP (mg·extract·g-algae⁻¹)</th>
<th>Antioxidant Potential of eMP (mg·DPPH solution·g-algae⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method 1</td>
<td>A. nodosum</td>
<td>26.39±0.24ab</td>
<td>5278.03±47.82o</td>
<td>7.68±0.50</td>
<td>1969.53±128.73y</td>
</tr>
<tr>
<td></td>
<td>F. vesiculosus</td>
<td>27.68±1.71a</td>
<td>5890.13±364.36o</td>
<td>24.11±1.56</td>
<td>4382.64±283.22x</td>
</tr>
<tr>
<td>Method 2</td>
<td>A. nodosum</td>
<td>26.03±0.74ab</td>
<td>3772.91±107.80p</td>
<td>7.30±0.48</td>
<td>2212.21±144.59y</td>
</tr>
<tr>
<td></td>
<td>F. vesiculosus</td>
<td>25.45±0.73b</td>
<td>2766.02±79.03q</td>
<td>13.63±0.74</td>
<td>1391.23±75.69y</td>
</tr>
</tbody>
</table>

Note: Different groups of letters, a, b, c, d, o, p, q, r, s, t and x, y, z indicate significant difference (p<0.05) among yield of MP, AP value of MP, yield of eMP, and AP value of eMP, respectively, in the order of a>b>c>d>e>f. Lower IC₅₀ values mean higher antioxidant activity.

#### 3.3 Comparison of the antioxidant activity among SP, MP and eMP

The antioxidant activities of the three phlorotannin groups, SP, MP and eMP, are presented in Table 4. In A. nodosum, the eMP extracts showed the strongest antioxidant activity while the SP extracts of F. vesiculosus were the strongest antioxidants by both extraction methods. It has been demonstrated in a previous study that high molecular weight phlorotannins had more potent antioxidant activities than the monomers[24]. Thus phlorotannins with higher degree of polymerization and higher molecular weight might be in the SP of F. vesiculosus and eMP of A. nodosum. It was reported that the membrane bound phlorotannin extracts
of *S. hystrix* showed stronger DPPH scavenging activities than the cytoplasmic extracts and was probably caused by high molecular weight components in the membrane bound extracts\(^8\). Surprisingly, the antioxidant activity of MP was stronger than eMP for *F. vesiculosus*, indicating that the purification process for obtaining eMP might have removed non-phenolic compounds with strong radical scavenging activities.

Table 4 Comparison of IC\(_{50}\) values among the three phlorotannin groups SP, MP and eMP obtained from the same extraction method and algae species

<table>
<thead>
<tr>
<th>Phlorotannin group</th>
<th>IC(_{50}) of Method 1</th>
<th>IC(_{50}) of Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. nodosum</em></td>
<td><em>F. vesiculosus</em></td>
</tr>
<tr>
<td>SP</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td>MP</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>eMP</td>
<td>*</td>
<td>***</td>
</tr>
</tbody>
</table>

Note: Different numbers of ‘*’ indicate the significant difference *(p<0.05)* among the SP, MP, and eMP groups in a column. Fewer ‘*’ indicates smaller IC50 (stronger antioxidant activity) in the column. Comparisons were made among SP, MP, and eMP obtained from one algae by one extraction method.

Table 5 shows the AP values of the three phlorotannin groups. MP extracts were the highest among the three phlorotannin groups except for *A. nodosum* by Method 2. From this table it can be concluded that the MP was the phlorotannin group with the best potential to be applied as natural antioxidants. Compared with Table 4 it can be found that the phlorotannin group with strong antioxidant property may not be the best source of natural antioxidants when the yield of that phlorotannin is taken into consideration.

Table 5 Comparison of AP values among three phlorotannin groups SP, MP and eMP obtained from the same extraction method and algae species

<table>
<thead>
<tr>
<th>Phlorotannin group</th>
<th>AP of Method 1</th>
<th>AP of Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. nodosum</em></td>
<td><em>F. vesiculosus</em></td>
</tr>
<tr>
<td>SP</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>MP</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>eMP</td>
<td>**</td>
<td>*</td>
</tr>
</tbody>
</table>

Note: Different numbers of ‘*’ indicate the significant difference *(p<0.05)* among the SP, MP, and eMP groups in a column. The AP value with more ‘*’ indicates greater AP in the column. Comparisons were made among SP, MP, and eMP obtained from one algae by one extraction method.

4 Conclusions

The SP extracts of *A. nodosum* and *F. vesiculosus* showed better antioxidant activity than that of *S. latissima*, *A. esculenta*, and *L. digitata*, indicating that there existed significant differences in the phlototannin contents among different algae species. The MP extracts showed the highest antioxidant potential among the three phlorotannin groups of SP, MP and eMP, which suggested that obtaining phlorotannins from leftover residue after SP extraction could be worthwhile for isolation of natural antioxidants. The antioxidant activity and yield of phlorotannins were also found to be affected by the solvent extraction methods used. For the two extraction methods studied here, Method 1 was much simpler and less time and solvent consuming, therefore, Method 1 should be considered as the preferred method in commercialization, unless the resultant antioxidant activity and phlorotannins yield from Method 1 are much lower than from Method 2.

Acknowledgements

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[References]


