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# Antioxidant and antibacterial activity of solid-liquid and enzyme-assisted extraction of phenolic compound from three species of tropical *Sargassum*

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**Abstract.** *Sargassum* has been well acknowledged for the potential natural product of its phlorotannins. Solid-liquid extraction (SLE) is the most common method used to extract them. However, this method has some drawbacks such as low yield and toxic. An alternative ecofriendly method has been proposed, i.e. enzyme-assisted extraction (EAE), proven to be more efficient. The aim is to compare the efficiency of SLE and EAE concerning their extraction yield, total phenolic content and antioxidant activity. *S. aquifolium*, *S. ilicifolium* and *S. polycystum* were extracted using water, methanol, methanol 50%, and ethanol 75% and enzymes (Viscozyme and Protamex). Total phenolic content (TPC) was analyzed by Folin-Ciocalteu and antioxidant activity via DPPH and FRAP analysis. This study implied that bioactivity of *Sargassum* extracted with enzymes is better compared to the one using organic solvents.

Keywords: Conventional extraction, alternative extraction, brown algae, bioactivities, polyphenols

## 1. Introduction

As an archipelagic country located in the tropical zone and the coral triangle, Indonesia has been extensively known for its remarkable diversity of marine organisms. One of them is marine seaweed. There are three groups of marine seaweed that can be found throughout Indonesian water, namely, green, red and brown seaweed. For decades, the green and red seaweed, particularly from the genera *Kappaphycus* and *Gracillaria*, have been cultivated for their carrageenan and agar [1–3].

Marine seaweed is also distinguished by its polysaccharides [4,5] but also by its biologically active compounds that have been studied and proven to have promising potential for the nutraceutical, pharmaceutical and cosmeceutical industries [6–10]. Hence, these marine plants have attracted a lot attention during the exploration of alternative natural products. The natural products derived from seaweed offer a sustainable resource with an infinite application.

Among the other three, brown seaweed from the class of Phaeophyceae is one of marine plants that has not been fully explored and exploited in Indonesia despite of their abundance and their remarkable potential [11–17]. A number of previous studies have reported that polysaccharides and bioactive compounds from the brown seaweed showed an encouraging potency as source of natural product with

their antimicrobial [18], antioxidant[11,19], antifouling [20,21], antiviral [22,23], anti-proliferative [24–26], anti-inflammatory [27], tyrosinase inhibition [28] activities and many other. This potency can be further applied in foods, medicines and cosmetics in order to reduce the application of chemical products or even to fully substitute them in the future [29–33].

There are five genera of brown seaweed widely distributed in Indonesian waters which four of them are the most species-rich, namely *Dictyota*, *Padina*, *Turbinaria* and *Sargassum* with 111 species in total [34]. These four genera have not been optimally exploited; however, *Sargassum* is the most extensively studied among them concerning its alginates and bioactive compound especially the phenolic compounds also known as phlorotannins. The phlorotannin in which are mostly present in the brown seaweed [35,36] are known for its strong antioxidant activity [37–43]. Nevertheless, it also shows promising effects against cancer, allergy, diabetes, inflammation and viral and microbial infection [36]. Basically, this compound is produced as one of the components composing the cell wall of brown seaweed [35]. However, it also serves as an induced defense system as phlorotannin has plastic responses to the environmental factors for examples nutrient availability, light, ultraviolet radiation, temperature, salinity and the intensity of herbivory [44–47].

Due to the various health beneficial performed by phlorotannin, various extractants are used in order to release this compound from the algal matrix. Solid-liquid extraction (SLE) is one of the most used methods in extracting the phlorotannin of brown seaweed [48–53]. This method relies on the organic solvents during the process such as hexane, ethyl acetate, ethanol, methanol, etc. [52]. The polysaccharides' complexity of marine seaweed often becomes the hindrance during the application of SLE. Thus, it reduces the extraction efficiency causing to the low yield of extraction with only 8% to 30% of the dry material [54]. It is crucial to apply an extraction method that could enhance the target compound, improve the bioactivity, time-saving, and favorable for human and environmental. These are the necessary requirements in order to generate a 'Green concepts' as in recent trend [55].

Enzyme-assisted extraction (EAE) has been widely mentioned as a potential alternative method since it offers an eco-friendly approach, an improvement in the quantity of target compound and an enhancement in the bioactivity [56]. The advance point of EAE compared to SLE method lies on its ability to degrade or disrupt the cell walls and membranes facilitating the release of target compound [57]. The EAE method has shown to improve the extraction yields and to enhance the recovery of bioactive compound in marine seaweed [56,58–62]. Nevertheless, this method has not been extensively applied in extracting the bioactive compound from Indonesian *Sargassum*.

Consequently, the objective of this study is to evaluate the efficiency of EAE in comparison to the SLE method in extracting bioactive compound of *Sargassum* sp., particularly phenolic compounds. Later, the phenolic content and the antioxidant activity of *Sargassum* extracts from both methods will be evaluated. In additional, tyrosinase and biofilm inhibition activity of the EAE extracts will also be analyzed in order to acknowledge another possible potential from this extracts. Finally, the enzymatic extracts of *Sargassum* will be characterized using the Fourier-Transform Infra-Red (FTIR) spectroscopy. This characterization will serve as a preliminary analysis to detect the presence of the phenolic compound, phlorotannins.

## 2. Material and Methods

### 2.1 Seaweed Collection

There were three different species of *Sargassum* collected for this study. *S. polycystum* was collected from Panjang Island, Jepara in October 2013 then *S. aquifolium* and *S. ilicifolium* were collected from Telukawur, Jepara in April 2014. Both of the locations are located in the northern region of Central Java, Indonesia.

The apical and median parts of *Sargassum* thalli were cut leaving the basal part still attached to the substrate for further regeneration, rinsed with seawater and placed in closed boxes. Samples were then transferred to the Central Laboratory of Research and Services Diponegoro University (CORES-DU). They were washed with tap water to remove the remaining epiphytes and other residual sands and

naturally dried away from the sunlight for seven days. After all samples had dried, they were all grinded and stored in a sealed plastic bag that was covered to avoid direct contact with the sunlight.

## 2.2 Solid-liquid extraction (SLE) of *Sargassum*

25 gr of algal dry material was diluted in 300 ml of four different solvents, i.e. water, methanol 50 % (v/v), pure methanol and ethanol 75 % (v/v). The filtered samples were then evaporated until dryness. The dried extract obtained was then added by 20 ml of H<sub>2</sub>O in order to get the crude extract. Finally, the crude extracts were frozen and lyophilized for further analysis.

## 2.3 Enzyme-assisted extraction (EAE) of *Sargassum*

19.5 gram of dry material was diluted in 300 ml of water, then 5 % (of dry material) commercial enzymes were added. Viscozyme® – a carbohydrase, and Protamex® – a protease, were chosen. These enzymes were provided by Novozymes®, manufactured in Denmark. Extraction without enzyme served as control, also called the aqueous extraction.

Sample solutions were incubated for 3 hours in 40 °C. After the filtration, the soluble samples were then frozen and lyophilized for further analysis. The residual materials were stored in -20 °C for TPC analysis to determine the insoluble phenolic compound that might not be digested by the enzymes.

## 2.4 Total Phenolic Content Analysis

Folin-Ciocalteu is a commonly used method for measuring the phenolic content. This assay is based on the reduction-oxidation (redox) reactions, which are usually considered to be relatively stoichiometric and on the redox potential of the phenolic hydroxyl group [63].

0.5 ml of samples or standard (Phloroglucinol) was added with 0.5 ml ethanol 95 %. 2.5 ml H<sub>2</sub>O was then introduced to the samples or standard solution followed by 0.25 ml of Folin-Ciocalteu 50 %. All samples were agitated and left to stand for 5 minutes. In the end, 0.5 ml of 5 % of Na<sub>2</sub>CO<sub>3</sub> (in 100 ml H<sub>2</sub>O) was added. Then the optical density was read against the blank prepared at 725 nm. Phloroglucinol was a standard for the calibration curve prepared in different concentrations ranged from 0 – 100 µg/ml [64–66].

## 2.5 Antioxidant Activity

2,2-diphenyl-1-picrylhydrazyl or DPPH is a stable free radical. DPPH assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. DPPH show a strong absorption maximum at 517 nm. This analysis is based on modified method of Yen and Chen (1995) and Chen et al. (2008).

A series of BHA, BHT and Ascorbic Acid solution – serves as standard – in different concentration is prepared (2 – 50 µg/ml). 0.25 mM of DPPH solution is introduced to 100 µL samples solution. Samples solution of *S. muticum* hydrolysates was made in different concentration by diluting the solution stock in methanol (0 – 1000 µg/mL). Before reading the optical density at 517 nm, all samples are incubated in 40 °C for 30 minutes. Then, the percentage of inhibition is calculated by following formula:

$$I (\%) = \left[ (A_c - A_s) / A_c \right] \times 100$$

$I (\%)$  = Inhibition (express in %)

$A_c$  = Absorbance of control

$A_s$  = Absorbance of samples

IC<sub>50</sub> of samples is determined based on the regression obtained from dose response curve. IC is defined at the concentration sufficient to obtain 50 % of a maximum scavenging capacity.

## 2.6 FRAP (Ferric Reducing Antioxidant Power)

This method is based on the reduction of a ferroin analogue, the complex of tripyridyltriazine Fe(TPTZ)<sup>3+</sup>, to the intensely bluish colour complex of Fe(TPTZ)<sup>2+</sup> by the presence of antioxidants in

acidic medium. Then, the results are obtained from the absorbance at 593 nm and expressed as  $\mu\text{M Fe}^{2+}$  equivalents (Benzie & Strain 1996; 1999)

The reagent of TPTZ consisted of 300 mM of acetate buffer with pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) and 20 mM  $\text{FeCl}_3 \cdot \text{H}_2\text{O}$  solution. 50  $\mu\text{L}$  of *S. muticum* hydrolysates was introduced in microplate mixed with 150  $\mu\text{L}$  reagent. After 15 minutes of incubation, the absorbance was read at 593 nm. The results were expressed in  $\mu\text{M}$  equivalent to  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  calculated from the calibration curve [69].

### 2.7 Antibacterial Activity

Agar diffusion method [70] was chosen as method for evaluating the antibacterial activity of *Sargassum*. One gram positive bacteria – *Bacillus subtilis* – and two gram negative bacteria – *Pseudomonas aeruginosa* and *Escherichia coli* – were chosen for this test. These bacteria were prepared in liquid nutrient broth media with bacterial density  $1.5 \times 10^8$  cfu/ml. Bacterial suspension was poured on to solidified agar media and incubated for 1 h in 37 °C.

5 mg/ml of SL extracts and hydrolysates were prepared in sterile physiological water. 20  $\mu\text{L}$  of tested samples solution – hydrolysates solution or positive control (Phosphomycin, Ampicillin and Streptomycin) – was introduced to sterile paper disc. All samples were made in triplicate. Later, the impregnated sterile paper discs were placed on the agar media in accordance with samples coding made in advance. The petri dishes were then incubated at 37 °C for 48 h. Zone of inhibition established on the agar media was then measured and expressed in cm.

### 2.8 Statistical Analysis

All results are expressed as mean  $\pm$  standard deviation (SD) with  $n = 3$ . As the design of this research was a factorial design with more than one independent variable than two ways analysis of variance (ANOVA) was chosen as statistical analysis using IBM SPSS Statistics 20. Further, the statistical difference between samples were determined via Tukey Post Hoc Test with significance level at 5% ( $p < 0.05$ ).

## 3. Results and Discussion

### 3.1 Comparison of SLE and EAE Extraction Yield, Total Phenolic Content, Antioxidant and Antibacterial activity of *Sargassum*

Two-ways analysis of variance was applied to determine whether there was a significant effect or not from several independent factors towards the dependant variable. In the case of *Sargassum* yield of extraction, total phenolic content and antioxidant activity – all were the dependant variables, different species and different type of extractants served as independent factors that would influence the dependant variables.

Statistical analysis of *Sargassum* extraction yield showed that there was a significant different between the three species extracted, i.e *S. aquifolium*, *S. ilicifolium* and *S. polycystum*. Different type of extractants used also gave a significant effect to the extraction yield. This result implied that the extraction yield of *Sargassum* showed an inter-individually significant effect.

As seen in Table 1, the EAE yielded higher dry material compared to the SLE. The order of samples with highest yield of enzymatic extraction was *S. polycystum* > *S. aquifolium* > *S. ilicifolium*. The highest extraction yield was shown by *S. polycystum* extracted with Protamex®,  $38.1 \pm 6.8$  % of dry material.

**Table 1.** Extraction yield of *Sargassum* (% of dry material) in different method of extraction (mean  $\pm$  standard deviation, n=3).

Samples	Water	MeOH	MeOH 50%	EtOH 75%	Viscozyme <sup>®</sup>	Protamex <sup>®</sup>
<i>S. aquifolium</i>	17.3 $\pm$ 2.2*	5.7 $\pm$ 1.8*	15.8 $\pm$ 1.5*	12.3 $\pm$ 0.4*	29.5 $\pm$ 5.4*	26.2 $\pm$ 5.8*
<i>S. ilicifolium</i>	14.7 $\pm$ 0.8*	3.1 $\pm$ 0.7*	7.3 $\pm$ 1.0*	7.5 $\pm$ 1.3*	27.9 $\pm$ 1.4*	21.4 $\pm$ 2.0*
<i>S. polycystum</i>	22.8 $\pm$ 1.1*	8.3 $\pm$ 3.2*	13.4 $\pm$ 1.9*	12.5 $\pm$ 1.9*	35.7 $\pm$ 2.6*	38.1 $\pm$ 6.8*

\*Significantly different at  $p < 0.05$ .

The total phenolic content of *Sargassum* is presented in Table 2 expressed in % of dry material. Viscozyme<sup>®</sup> extracted more phenolic content in *S. aquifolium* and *S. polycystum* with  $7.0 \pm 1.3$  and  $8.1 \pm 2.6$  % of dry material, respectively, compared to the organic solvents. In the contrary, the highest phenolic content of *S. ilicifolium* was obtained from the methanol  $7.5 \pm 2.4$  % of dry material. Apparently, the efficiency between Protamex and methanol 50% (v/v) in extracting the phenolic compound were the same in all *Sargassum*. As can be seen on Table 2, they only extracted between 3 up to 5 % of phenolic content.

The antiradical activity expressed in the value of IC<sub>50</sub> of *Sargassum* is presented in Table 3. *S. polycystum* only showed its antiradical activity when they were extracted with ethanol 75% and Protamex<sup>®</sup> and the best activity was shown by Protamex<sup>®</sup> with IC<sub>50</sub>  $1.9 \pm 1.1$  mg/mL. The weakest antiradical activity came from the methanolic extract of *S. aquifolium* with IC<sub>50</sub>  $23.8 \pm 9.0$  mg/mL. The three *Sargassum* extracted with Protamex<sup>®</sup> gave a better antiradical activity compared to the other extractants, it can be seen by their IC<sub>50</sub> that were lower as presented on Table 3. The 75% (v/v) ethanolic extracts of *S. aquifolium* and *S. ilicifolium* showed no antiradical activity. Meanwhile, the antiradical activity of all samples were still weaker compared to the standards, i.e BHA and BHT with IC<sub>50</sub>  $8 \times 10^{-3} \pm 0.5$  and  $12 \times 10^{-3} \pm 4.8$  mg/ml, respectively. The statistical analysis of two-ways ANOVA showed that there was a significant difference between the three species of *Sargassum* and between the types of extractant that affected the antiradical activity. The statistical analysis of two-ways ANOVA showed that there was a significant difference between the three species of *Sargassum* and between the types of extractant used. Based on its phenolic content, the order of samples with highest content was *S. polycystum* > *S. aquifolium* > *S. ilicifolium*. In addition, the efficiency order of extractants in extracting the phenolic compounds of *S. polycystum* was Viscozyme<sup>®</sup> > ethanol 75% (v/v) > Protamex<sup>®</sup> > methanol > methanol 50% (v/v) > water. As for *S. aquifolium*, the efficiency order was Viscozyme<sup>®</sup> > methanol > ethanol 75% (v/v) > methanol 50% (v/v) > Protamex<sup>®</sup> > water. Then, for *S. ilicifolium*, the efficiency order was methanol > ethanol 75% (v/v) > methanol 50% (v/v) > Protamex<sup>®</sup> > Viscozyme<sup>®</sup> > water.

Table 4 presents the reducing power activity also known as FRAP of *Sargassum* expressed by a concentration in  $\mu\text{M}$  equivalent with Fe<sup>2+</sup> ( $\mu\text{M}$  Eq Fe<sup>2+</sup>). Vitamin C was used as standard with reducing power  $58.1 \pm 0.7$   $\mu\text{M}$  Eq Fe<sup>2+</sup>. The reducing power activity that was almost near as the standard was the aqueous phase of *S. polycystum* with  $43.5 \pm 0.7$   $\mu\text{M}$  Eq Fe<sup>2+</sup>. In other word, this sample had the highest reducing power activity. Meanwhile, the lowest reducing power activity was shown by *S. ilicifolium* extracted with ethanol 75%,  $7.9 \pm 1.2$   $\mu\text{M}$  Eq Fe<sup>2+</sup>. In addition, it appears that Viscozyme<sup>®</sup> extracts of *S. aquifolium* and *S. polycystum* had better reducing power activity compared to methanol, methanol 50%, ethanol 75% (v/v) and even Protamex<sup>®</sup>. The statistical analysis of two-ways ANOVA showed that there was a significant difference between the three species of *Sargassum* and between the types of extractant that affected the reducing power activity.

As for the antibacterial activity of two methods of extraction, the tested bacteria were sensitive only to solid-liquid extraction (data not shown). In other words, the enzymatic extracts showed no sign of inhibition against the bacterial growth. It was assumed that the sugar contained in our enzymatic extracts might provoke the growth of bacteria instead of inhibiting them.

SLE has become the most used method during the exploration of marine bioactive compounds, in this case, phlorotannins. This method mostly relies on the polarity of the solvents used due to the

chemical nature of target compound [88]. In general, the process in SLE method can be categorized in three parts; (1) changing phase of the solute, in this case the target compounds, as it dissolve in the solvent, (2) its diffusion through the solvent in the pores of the solid to the outside of the particles, and (3) the transfer of the solute from the solution in contact with the particles to the main bulk of the solution [89]. The more dispersed the solute in the solid material, the more difficult the extraction will be and the extraction rate will fall. This is because the solvent will have to penetrate further in to the layer of solid material in order to reach the solute [89]. Furthermore, the efficiency of SLE highly depends on the particles size, temperature, and agitation during the process [90]. In the extraction of phenolic compounds, the storage time of dry materials and conditions, as well as the presence of interfering substances are other important factor to consider, influencing the extraction efficiency [91]. In marine seaweed, the main drawback during the extraction of phenolic compounds occurs due to the presence of complexes polysaccharides as the main component of seaweed's cell wall. Brown seaweed's phlorotannins are known to be incorporated in its cell wall [92] covalently bonded to the polysaccharides [35] and proteins [93]. Therefore, it requires a strong condition to degrade these bonds and extract the phlorotannins. The SLE method does not meet this requirement since it does not degrade such complex bonds since it works based on the solubility of target compound in to the solvents [89]. In addition, the polarity of target compound becomes the most important factor in choosing the solvent [94]. Methanol, ethanol, acetone, ethyl acetate, hexane [50,72,75,95,96] are some examples of organic solvents mostly used for phlorotannins extraction.

Phlorotannins, class of phenolic compounds synthesized only in brown seaweed [71], is one of the most studied bioactive compounds due to their wide range of bioactivities, showing a promising potential for the nutraceutical, pharmaceutical and cosmeceutical industries. As reported by previous studies, the increasing interest towards phlorotannins occurs as they have been for their antioxidant [72–77], antimicrobial [78–80], antitumor [81,82], antidiabetic [83], antiallergic [84], anti-inflammatory [85], and antiviral effects [51,86,87].

This study concerns about the possibility in applying alternative method that is not only environmentally friendly but also more efficient in extracting the phlorotannins. For that reason, enzyme-assisted extraction (EAE) is chosen as the alternative method. Additionally, the efficacy of SLE – as the conventional method, and EAE – as the alternative one, in extracting the phlorotannin from *Sargassum* were compared. For the SLE method, four different organic solvents were chosen, i.e water, methanol, methanol 50% (v/v), and ethanol 75% (v/v). Meanwhile, for the EAE method, there were two commercial enzymes, i.e. Viscozyme® – a carbohydrase, and Protamex® – a protease.

Based on the extraction yield of these two methods, the EAE yielded the dry algal material higher compared with the SLE (Table 1). As can be seen from this table, in three hours of extraction, the SLE extraction yield ranged from 3% to 15% of dry material. In the contrary to the SLE method, the extraction yield of EAE ranged from 21% to 38%. In overall, the average increase of extraction yield when using the Viscozyme® was around 62%. As for the Protamex®, the average increase of extraction was 58%. This result had shown the efficacy of EAE method in improving the extraction yield applied under the same circumstances as the SLE method. Interestingly, the efficiency of EAE in improving the extraction yield seems to be species-dependent. As can be seen on Table 1, Protamex® yielded more dry material in *S. polycystum* with 38% than Viscozyme®. In the contrary, the extraction yield of *S. aquifolium* and *S. ilicifolium* were higher in Viscozyme®, with 29% and 27%, respectively. Viscozyme® was also yielded more soluble material in *S. fulvellum* (24%), *S. horneri* (30%), *S. corneanum* (30%) and *S. thunbergii* (31%) than Protamex, with 17%, 27%, 21%, and 25%, respectively [97]. In *S. muticum*, the Viscozyme managed to extract 29% of soluble material [41,98]. It appears that the cellulose and the multicarbohydrase complex action of Viscozyme® were responsible for higher solid content in lyophilized extracts [98].



**Table 2.** Total phenolic content (TPC) of *Sargassum* (% of dry material) (mean  $\pm$  standard deviation, n=3).

Samples	Water	Methanol	Methanol 50 %	Ethanol 75%	Viscozyme®	Protamex®
<i>S. aquifolium</i>	1.7 $\pm$ 0.0*	6.3 $\pm$ 1.5*	3.7 $\pm$ 0.2*	5.9 $\pm$ 0.4*	7.0 $\pm$ 1.3*	3.5 $\pm$ 0.0*
<i>S. ilicifolium</i>	2.1 $\pm$ 0.0*	7.5 $\pm$ 2.4*	5.3 $\pm$ 0.3*	5.9 $\pm$ 0.4*	4.0 $\pm$ 0.1*	4.2 $\pm$ 0.2*
<i>S. polycystum</i>	1.4 $\pm$ 0.0*	4.8 $\pm$ 0.3*	4.0 $\pm$ 0.3*	5.8 $\pm$ 0.6*	8.1 $\pm$ 2.6*	5.2 $\pm$ 0.1*

\*Significantly different at p<0.05.

**Table 3.** Antioxidant activity of *Sargassum* expressed in IC50 (mg/ml) (mean  $\pm$  standard deviation, n=3).

Samples	DPPH (IC 50 mg/ml)					
	Water	Methanol	Methanol 50%	Ethanol 75%	Viscozyme®	Protamex®
<i>S. aquifolium</i>	14.6 $\pm$ 5.7	23.8 $\pm$ 9.0*	22.3 $\pm$ 8.0*	n.a	n.a	5.7 $\pm$ 7.9
<i>S. ilicifolium</i>	n.a	17.8 $\pm$ 9.4*	3.3 $\pm$ 1.7*	n.a	6.2 $\pm$ 3.4	2.9 $\pm$ 1.7
<i>S. polycystum</i>	n.a	n.a	n.a	5.2 $\pm$ 5.9	n.a	1.9 $\pm$ 1.1

\*Significantly different at p<0.05.

**Table 4.** Reducing activity of *Sargassum* expressed in  $\mu$ M Equivalent Fe<sup>2+</sup> (mean  $\pm$  standard deviation, n=3).

Samples	FRAP (Concentration Equivalent Fe <sup>2+</sup> ( $\mu$ M))					
	Water	Methanol	Methanol 50%	Ethanol 75%	Viscozyme®	Protamex®
<i>S. aquifolium</i>	14.9 $\pm$ 1.7*	12.6 $\pm$ 2.5*	22.4 $\pm$ 4.9*	14.4 $\pm$ 0.3*	33.2 $\pm$ 2.5*	17.0 $\pm$ 0.7*
<i>S. ilicifolium</i>	23.7 $\pm$ 1.6*	11.1 $\pm$ 0.8*	14.9 $\pm$ 0.8*	7.9 $\pm$ 1.24*	13.4 $\pm$ 2.2*	11.7 $\pm$ 1.2*
<i>S. polycystum</i>	43.5 $\pm$ 1.8*	12.8 $\pm$ 3.1*	16.9 $\pm$ 2.6*	13.8 $\pm$ 1.9*	27.7 $\pm$ 4.9*	15.9 $\pm$ 3.0*

\*Significantly different at p<0.05.

he EAE had also recovered more of phlorotannins represented by the total phenolic content. By using Viscozyme, the total phenolic content of *Sargassum* was 28% higher than using the organic solvents. Unfortunately, the phenolic content of Protamex® extracts of *Sargassum* were not in consistent with the Viscozyme® extracts. The Protamex did not seem to recover the phenolic content of *Sargassum* as they yielded lower content compared to the organic solvents, except for water. It implied that the Viscozyme® recovered more of phenolic compounds in *Sargassum* than the Protamex®. Phlorotannins are hydrophilic cell-wall-bound compounds [99] and despite of its soluble characteristic, compounds attached to the cell wall are not easily extracted using the typical extraction methods such as the SLE [56,61]. The application of Viscozyme®, as a carbohydrase, during the extraction allowed to facilitate the recovery of phenolic compound as this enzyme degrades the cell wall of seaweed mainly composed of polysaccharides. Viscozyme® has been extensively used for the recovery of phenolic compounds of terrestrial and marine plants as reported by many studies [41,65,100–102]. However, the efficiency of extraction relies on the optimum conditions applied. Selecting the appropriate hydrolytic enzymes is important to digest specific polymer bonds present in seaweed cell wall followed by the selection of suitable process conditions for maximum recovery of active compounds [56]. Combination of incubation time-temperature, pH, size of the interested molecules and agitation are mentioned to play a critical role during the process [14].

Phlorotannins have been reported to be responsible for many bioactivities, such as antioxidant, antimicrobial, antiviral and many more, as reported by previous studies [9,29,51,96]. The key of phlorotannins important bioactivities lies to its eight interconnected ring as they contain more hydroxyl groups than other tannins – hydrolysable tannin and condensed tannins [107,108]. As a consequence, they have greater antioxidant activity [107]. Hydrogen atom transfer is one of its mechanisms against the effects of unregulated production of free radicals [36]. As performed in this study, *Sargassum* extracts – extracted with SLE and EAE method- showed their capability in scavenging the free radicals with best activity exhibited by the Protamex® extracts in spite of Viscozyme® extracted more phenolics. It suggested the presence of other molecules like polysaccharides or proteins as shown by the FTIR since the current study used crude extracts instead of the purified. Besides free radical scavenging, our results showed that the enzymatic extracts of *Sargassum* had the ferric reducing ability – a conversion of Fe<sup>III</sup> to Fe<sup>II</sup>. In addition, it showed quite promising potential as skin whitening antifouling coating. Such potential was based on the tyrosinase and biofilm inhibition activities of *Sargassum* enzymatic extracts.

Antioxidant activity of *S. horneri*, *S. fullvelum*, *S. correanum*, and *S. thunbergii* have been reported by Heo et al [65]. *S. correanum* contained higher phenolics when it was extracted with Viscozyme® and higher free radical scavenging activity compared to the others. Another study also showed that Viscozyme® used to extract *S. horneri* had the ability to scavenge the free radicals until 95% of inhibition [109]. Sanchez-camargo et al. [41] stated that the total phenols and antioxidant activity of *S. muticum* extracted with Alcalase® and Viscozyme® were improved compared to control – aqueous extraction. Therefore, it is obvious that enzymes used have facilitated the release of active compounds and in the same time it enhances the bioactivities especially the antioxidant – best known activity for phlorotannins.

#### 4. Conclusion

A strong urge to explore marine bioactive compounds has brought green chemistry concept to the surface along the process. The principle of this concept is basically to apply a method that is environmentally friendly and favorable for human interest as well. In the exploration of marine bioactive compounds that can be further applied for the nutraceutical, pharmaceutical and cosmeceutical industries, the realization of this concept is crucial. Therefore, many studies have highlighted the possibilities of enzyme-assisted extraction as an alternative method that might replace the use of organic solvent to extract the active compounds.

This study has revealed that by using enzymes, the efficiency of the extraction increased compared to the solid-liquid extraction. This method not only improves the quality and quantity of extracts but it

also shows its promising potential by showing diverse bioactivities. Nevertheless, one should note that this study still works with crude extracts. Therefore, the interference of other compounds like polysaccharides and proteins should be considered. As a consequence, it is crucial to further evaluate the bioactivities of purified samples and characterize them.

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## 6. References

- [1] Sahat H J 2013 Rumpit Laut Indonesia *Minist. Trade* 1–20
- [2] Nafed K 2011 Warta Ekspor: Rumpit Laut dan Produk Turunannya *Minist. Trade* 1–20
- [3] Neish I C 2014 Social and economic dimensions of carrageenan seaweed farming in Indonesia *Social and economic dimensions of carrageenan seaweed farming* ed D Valderrama, C Junning, N Hishamunda and N Ridler (Rome: FAO) p 204
- [4] Wijesekara I, Pangestuti R and Kim S K 2011 Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae *Carbohydr. Polym.* **84** 14–21
- [5] Chanda S, Dave R, Kaneria M and Nagani K 2010 Seaweeds : A novel , untapped source of drugs from sea to combat Infectious diseases *Curr. Res. Technol. Educ. Top. Appl. Microbiol. Microb. Technol.* 473–80
- [6] Holdt S L and Kraan S 2011 Bioactive compounds in seaweed: Functional food applications and legislation *J. Appl. Phycol.* **23** 543–97
- [7] Gupta S and Abu-Ghannam N 2011 Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods *Innov. Food Sci. Emerg. Technol.* **12** 600–9
- [8] Michalak I and Chojnacka K 2015 Algae as production systems of bioactive compounds *Eng. Life Sci.* **15** 160–76
- [9] Li Y-X, Wijesekara I, Li Y and Kim S-K 2011 Phlorotannins as bioactive agents from brown algae *Process Biochem.* **46** 2219–24
- [10] Collins K, Fitzgerald G, Stanton C and Ross R 2016 Looking beyond the terrestrial: The potential of seaweed derived bioactives to treat non-communicable diseases *Mar. Drugs* **14** 60
- [11] Sinurat E and Marraskuranto E 2011 Fucoidan from brown seaweed and its bioactivity *Squalen* **7** 131–7
- [12] Mushollaeni W and Rusdiana E 2011 Characterization of sodium alginate from *Sargassum* sp., *Turbinaria* sp. and *Padina* sp. *J. Teknol dan Industri Pangan* **XXII** 26–32
- [13] Sivagnanam S, Yin S, Choi J, Park Y, Woo H and Chun B 2015 Biological Properties of Fucoxanthin in Oil Recovered from Two Brown Seaweeds Using Supercritical CO<sub>2</sub> Extraction *Mar. Drugs* **13** 3422–42
- [14] Wijesinghe W A J P and Jeon Y J 2012 Biological activities and potential industrial applications of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweeds: A review *Carbohydr. Polym.* **88** 13–20
- [15] Aulanni'am, Rosdiana A and Rahmah N L 2011 Potency of Brown Seaweed (*Sargassum duplicatum* Bory) Ethanol and Ethyl Acetic Fraction to Malondialdehyde Concentration Decreasing and Histomogical Retrival of IBD (Inflammatory Bowel Disease) Rat Small Intestinal Jejunum *J. Ilm. Kedokt.* **4** 57–64
- [16] Bachtiar S Y, Tjahjaningsih W and Sianita N 2012 Pengaruh ekstrak alga coklat (*Sargassum* sp.) terhadap bakteri *Eschericia coli* *J. Mar. Coast. Sci.* **1** 53–60
- [17] Pangestuti R and Wibowo S 2013 Prospects and health promoting effects of brown algal-derived natural pigments *Squalen* **8** 37–46
- [18] Manivannan K, Karthikai devi G, Anantharaman P and Balasubramanian T 2011 Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar *Asian Pac. J. Trop. Biomed.* **1** 114–20
- [19] Je J-Y, Park P-J, Kim E-K, Park J-S, Yoon H-D, Kim K-R and Ahn C-B 2009 Antioxidant activity of enzymatic extracts from the brown seaweed *Undaria pinnatifida* by electron spin resonance spectroscopy

- LWT - Food Sci. Technol.* **42** 874–8
- [20] Bazes A, Silkina A, Douzenel P, Faÿ F, Kervarec N, Morin D, Berge J P and Bourgoignon N 2009 Investigation of the antifouling constituents from the brown alga *Sargassum muticum* (Yendo) Fensholt *J. Appl. Phycol.* **21** 395–403
  - [21] Plouguerne E, Ioannou E, Georgantea P, Vagias C, Roussis V, Hellio C, Kraffe E and Stiger-Pouvreau V 2010 Anti-microfouling activity of lipidic metabolites from the invasive brown alga *Sargassum muticum* (Yendo) Fensholt *Mar. Biotechnol.* **12** 52–61
  - [22] Mandal P, Mateu C G, Chattopadhyay K, Pujol C A, Damonte E B and Ray B 2007 Structural Features and Antiviral Activity of Sulphated Fucans from the Brown Seaweed *Cystoseira Indica* *Antivir. Chem. Chemother.* **18** 153–62
  - [23] Thenmozhi K, Selvarani T, Prabhu B K, Clinic V and Nadu T 2013 Antiviral activity of aqueous extract from brown seaweed, *Sargassum illicifolium* (Turner) on three different types Herpes Simplex virus *Adv. Res. Pharm. Biol.* **3** 370–3
  - [24] Costa L S, Fidelis G P, Cordeiro S L, Oliveira R M, Sabry D A, Camara R B G, Nobre L T D B, Costa M S S P, Almeida-Lima J, Farias E H C, Leite E L and Rocha H A O 2010 Biological activities of sulfated polysaccharides from tropical seaweeds *Biomed. Pharmacother.* **64** 21–8
  - [25] Zorofchian Moghadamtousi S, Karimian H, Khanabdali R, Razavi M, Firoozinia M, Zandi K and Abdul Kadir H 2014 Anticancer and antitumor potential of fucoidan and fucoxanthin, two main metabolites isolated from brown algae *Sci. World J.* **2014** 10
  - [26] Costa L S, Fidelis G P, Telles C B S, Dantas-Santos N, Camara R B G, Cordeiro S L, Costa M S S P, Almeida-Lima J, Melo-Silveira R F, Oliveira R M, Albuquerque I R L, Andrade G P V and Rocha H A O 2011 Antioxidant and antiproliferative activities of heterofucans from the seaweed *Sargassum filipendula* *Mar. Drugs* **9** 952–66
  - [27] Ahn M J, Yoon K D, Kim C Y, Kim J H, Shin C G and Kim J 2006 Inhibitory activity on HIV-1 reverse transcriptase and integrase of a carmalol derivative from a brown Alga, *Ishige okamurae* *Phyther. Res.* **20** 711–3
  - [28] Yoon N Y, Eom T K, Kim M M and Kim S K 2009 Inhibitory effect of phlorotannins isolated from *Ecklonia cava* on mushroom tyrosinase activity and melanin formation in mouse B16F10 melanoma cells *J. Agric. Food Chem.* **57** 4124–9
  - [29] Balboa E M, Conde E, Moure A, Falqué E and Domínguez H 2013 In vitro antioxidant properties of crude extracts and compounds from brown algae *Food Chem.* **138** 1764–85
  - [30] Ale M T, Mikkelsen J D and Meyer A S 2011 Important determinants for fucoidan bioactivity: A critical review of structure-function relations and extraction methods for fucose-containing sulfated polysaccharides from brown seaweeds *Mar. Drugs* **9** 2106–30
  - [31] La Barre S, Potin P, Leblanc C and Delage L 2010 The halogenated metabolism of brown algae (phaeophyta), its biological importance and its environmental significance *Mar. Drugs* **8** 988–1010
  - [32] Maghin F, Ratti S and Corino C 2014 Biological Functions and Health Promoting Effects of Brown Seaweeds in Swine Nutrition *J. Dairy, Vet. Anim. Res. Biol.* **1** 1–3
  - [33] Liu L, Heinrich M, Myers S and Dworjanyan S A 2012 Towards a better understanding of medicinal uses of the brown seaweed *Sargassum* in Traditional Chinese Medicine: A phytochemical and pharmacological review *J. Ethnopharmacol.* **142** 591–619
  - [34] Atmadja W S and Prud'homme van Reine W F 2014 *Checklist of the Seaweed Species Biodiversity of Indonesia with their distribution and classification: green algae (Chlorophyta) and brown algae (Phaeophyceae, Ochrophyta)* (Jakarta: Coremap II-LIPI)
  - [35] Koivikko R 2008 *Brown algal phlorotannins: Improving and Applying Chemical Methods* (Turku: University of Turku)
  - [36] Kim S-K and Himaya S W A 2011 Medicinal effects of phlorotannins from marine brown algae. *Adv. Food Nutr. Res.* **64** 97–109
  - [37] Budhiyanti S A, Raharjo S, Marseno D W and Lelana I Y B 2012 Antioxidant activity of brown algae *Sargassum* species extract from the coastline of java island *Am. J. Agric. Biol. Sci.* **7** 337–46
  - [38] Prabowo A, Budhiyanti S A and Husni A 2013 *Sargassum* sp. extract as an antioxidant for fish oil emulsion system during storage at room temperature *JPB Perikan.* **8** 143–50
  - [39] Le Lann K, Jégou C and Stiger-Pouvreau V 2008 Effect of different conditioning treatments on total phenolic content and antioxidant activities in two Sargassacean species: Comparison of the frondose *Sargassum muticum* (Yendo) Fensholt and the cylindrical *Bifurcaria bifurcata* R. Ross *Phycol. Res.* **56** 238–45

- [40] Badrianathan S, Suneeva S C, Shiju T M, Girish Kumar C P and Pragasaam V 2011 Exploration of a novel hydroxyl radical scavenger from *Sargassum myriocystum* *J. Med. plants Res.* **5** (10) 1997–2005
- [41] Sanchez-Camargo A D P, Montero L, Stiger-Pouvreau V, Tanniou A, Cifuentes A, Herrero M and Ibanez E 2015 Considerations on the use of enzyme-assisted extraction in combination with pressurized liquids to recover bioactive compounds from algae *Food Chem.* **192** 67–74
- [42] Harnita A N I, Santosa I E, Martono S, Sudarsono, Widyarini S and Harren F J M 2013 Inhibition of lipid peroxidation induced by ultraviolet radiation by crude phlorotannins isolated from brown algae *Sargassum hystrix* v. *buxifolium* C. Agardh *Indones. J. Chem.* **13** 14–20
- [43] Nakai M, Kageyama N, Nakahara K and Miki W 2006 Phlorotannins as Radical Scavengers from the Extract of *Sargassum ringgoldianum* **8** 409–14
- [44] Jormalainen V and Honkanen T 2008 Macroalgal chemical defenses and their roles in structuring temperate marine communities *Algal Chemical Defence* ed C D Amsler (Berlin, Heidelberg: Springer Berlin Heidelberg) pp 57–89
- [45] Amsler C D and Fairhead V A 2005 *Incorporating Advances in Plant Pathology* vol 43 (Elsevier)
- [46] Jormalainen V, Honkanen T, Koivikko R and Eränen J 2003 Induction of phlorotannin production in a brown alga: defense or resource dynamics? *Oikos* **103** 640–50
- [47] Hemmi A, Mäkinen A, Jormalainen V and Honkanen T 2005 Responses of growth and phlorotannins in *Fucus vesiculosus* to nutrient enrichment and herbivory *Aquat. Ecol.* **39** 201–11
- [48] Nakamura T, Nagayama K, Uchida K and Tanaka R 1996 Antioxidant Activity of Phlorotannins Isolated from the Brown Alga *Eisenia bicyclis* **62** 923–6
- [49] Nagayama K, Shibata T, Fujimoto K, Honjo T and Nakamura T 2003 Algicidal effect of phlorotannins from the brown alga *Ecklonia kurome* on red tide microalgae *Aquaculture* **218** 601–11
- [50] Kim S M, Kang K, Jeon J-S, Jho E H, Kim C Y, Nho C W and Um B-H 2011 Isolation of phlorotannins from *Eisenia bicyclis* and their hepatoprotective effect against oxidative stress induced by tert-butyl hydroperoxide. *Appl. Biochem. Biotechnol.* **165** 1296–307
- [51] Kwon H-J J, Ryu Y B, Kim Y-M M, Song N, Kim C Y, Rho M-C C, Jeong J-H H, Cho K-O O, Lee W S and Park S-J J 2013 In vitro antiviral activity of phlorotannins isolated from *Ecklonia cava* against porcine epidemic diarrhea coronavirus infection and hemagglutination *Bioorg. Med. Chem.* **21** 4706–13
- [52] Michalak I and Chojnacka K 2014 Algal extracts: Technology and advances *Eng. Life Sci.* **14** 581–91
- [53] Heffernan N, Brunton N P, FitzGerald R J and Smyth T J 2015 Profiling of the molecular weight and structural isomer abundance of macroalgae-derived phlorotannins *Mar. Drugs* **13** 509–28
- [54] Robic A, Rondeau-Mouro C, Sassi J-F F, Lerat Y and Lahaye M 2009 Structure and interactions of ulvan in the cell wall of the marine green algae *Ulva rotundata* (Ulvales, Chlorophyceae) *Carbohydr. Polym.* **77** 206–16
- [55] Ibanez E, Herrero M, Mendiola J A and Castro-Puyana M 2012 Extraction and Characterization of Bioactive Compound with Health Benefits from Marine Sources: Macro and Micro Algae, Cyanobacteria and Invertebrates *Marine Bioactive Compounds: Sources, Characterization and Applications* vol 9781461412, ed M Hayes (Springer US) pp 55–98
- [56] Jeon Y J, Wijesinghe W A J P and Kim S K 2012 Enzyme-assisted Extraction and Recovery of Bioactive Components from Seaweeds *Handbook of Marine Macroalgae: Biotechnology and Applied Phycology* ed S-K Kim (John Wiley & Sons, Ltd) pp 221–6
- [57] Puri M, Sharma D and Barrow C J 2012 Enzyme-assisted extraction of bioactives from plants *Trends Biotechnol.* **30** 37–44
- [58] Kim K-N, Heo S-J, Song C B, Lee J, Heo M-S, Yeo I-K, Kang K A, Hyun J W and Jeon Y-J 2006 Protective effect of *Ecklonia cava* enzymatic extracts on hydrogen peroxide-induced cell damage *Process Biochem.* **41** 2393–401
- [59] Hahn T, Lang S, Ulber R and Muffler K 2012 Novel procedures for the extraction of fucoidan from brown algae *Process Biochem.* **47** 1691–8
- [60] Hardouin K, Bedoux G, Burlot A-S, Donnay-Moreno C, Bergé J-P, Nyvall-Collén P and Bourgougnon N 2016 Enzyme-assisted extraction (EAE) for the production of antiviral and antioxidant extracts from the green seaweed *Ulva armoricana* (Ulvales, Ulvophyceae) *Algal Res.* **16** 233–9
- [61] Wijesinghe W A J P and Jeon Y J 2012 Enzyme-assistant extraction (EAE) of bioactive components: A useful approach for recovery of industrially important metabolites from seaweeds: A review *Fitoterapia* **83** 6–12
- [62] Kadam S U, Tiwari B K and O'Donnell C P 2013 Application of novel extraction technologies for bioactives from marine algae *J. Agric. Food Chem.* **61** 4667–75

- [63] Hagerman A E and Butler L G 1989 Choosing appropriate methods and standards for assaying tannin. *J. Chem. Ecol.* **15** 1795–810
- [64] Wang T, Jónsdóttir R, Kristinsson H G, Hreggvidsson G O, Jónsson J Ó, Thorkelsson G and Ólafsdóttir G 2010 Enzyme-enhanced extraction of antioxidant ingredients from red algae *Palmaria palmata* LWT - *Food Sci. Technol.* **43** 1387–93
- [65] Heo S J, Park E J, Lee K W and Jeon Y J 2005 Antioxidant activities of enzymatic extracts from brown seaweeds *Bioresour. Technol.* **96** 1613–23
- [66] Chandler S F and Dodds J H 1983 The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasodine in callus cultures of *Solanum laciniatum* *Plant Cell Rep.* **2** 205–8
- [67] Benzie I F and Strain J J 1996 The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal. Biochem.* **239** 70–6
- [68] Benzie I F and Strain J J 1999 Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol.* **299** 15–27
- [69] Shahwar D, Raza M A, Bukhari S and Bukhari G 2012 Ferric reducing antioxidant power of essential oils extracted from *Eucalyptus* and *Curcuma* species *Asian Pac. J. Trop. Biomed.* **2**
- [70] Bauer A W, Kirby W M, Sherris J C and Turck M 1966 Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **45** 493–6
- [71] Stiger-Pouvreau V, Jégou C, Cérantola S, Guérard F and Le Lann K 2014 Phlorotannins in Sargassaceae Species from Brittany (France) Interesting Molecules for Ecophysiological and Valorization Purposes *Advances in Botanical Research: Sea Plants* ed N Bourgougnon (Oxford: Elsevier) pp 379–411
- [72] Girija K, Hemalatha A, Saranya C, Parthiban C, Anantharaman P and Girija K, Hemalatha A, Saranya C parthiban C, Anantharaman P 2013 Extraction and Isolation of Phlorotannins From Brown Seaweed *Turbinaria Ornata* (Turner) J. Agardh and Its Antioxidant Activity *Int. J. Bioassays* **9** 1185–9
- [73] Shin T, Ahn M, Hyun J W, Kim S H and Moon C 2014 Antioxidant marine algae phlorotannins and radioprotection: A review of experimental evidence *Acta Histochem.* **116** 669–74
- [74] Shibata T, Ishimaru K, Kawaguchi S, Yoshikawa H and Hama Y 2008 Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae *J. Appl. Phycol.* **20** 705–11
- [75] Sathya R, Kanaga N, Sankar P and Jeeva S 2013 Antioxidant properties of phlorotannins from brown seaweed *Cystoseira trinodis* (Forsskal) C. Agardh *Arab. J. Chem.*
- [76] Fujii Y, Tanaka R, Miyake H, Tamaru Y, Ueda M and Shibata T 2013 Evaluation for Antioxidative Properties of Phlorotannins Isolated from the Brown Alga *Eisenia bicyclis*, by the H-ORAC Method *Food Nutr. Sci.* **4** 78–82
- [77] Wijesekara I, Yoon N Y and Kim S K 2010 Phlorotannins from *Ecklonia cava* (Phaeophyceae): Biological activities and potential health benefits *BioFactors* **36** 408–14
- [78] Choi J S, Lee K, Lee B B, Kim Y C, Kim Y D, Hong Y K, Cho K K and Choi I S 2014 Antibacterial activity of the phlorotannins dieckol and phlorofucofuroeckol-a from *Ecklonia cava* against *Propionibacterium acnes* *Bot. Sci.* **92** 425–31
- [79] Eom S H, Kim Y M and Kim S K 2012 Antimicrobial effect of phlorotannins from marine brown algae *Food Chem. Toxicol.* **50** 3251–5
- [80] Lopes G, Sousa C, Silva L R, Pinto E, Andrade P B, Bernardo J, Mouga T and Valentão P 2012 Can phlorotannins purified extracts constitute a novel pharmacological alternative for microbial infections with associated inflammatory conditions? *PLoS One* **7**
- [81] Thomas N V and Kim S K 2011 Potential pharmacological applications of polyphenolic derivatives from marine brown algae *Environ. Toxicol. Pharmacol.* **32** 325–35
- [82] Yang Y I, Ahn J H, Choi Y S and Choi J H 2015 Brown algae phlorotannins enhance the tumoricidal effect of cisplatin and ameliorate cisplatin nephrotoxicity *Gynecol. Oncol.* **136** 355–64
- [83] Jung H A, Yoon N Y, Woo M H and Choi J S 2008 Inhibitory activities of extracts from several kinds of seaweeds and phlorotannins from the brown alga *Ecklonia stolonifera* on glucose-mediated protein damage and rat lens aldose reductase *Fish. Sci.* **74** 1363–5
- [84] Leyton A, Pezoa-Conte R, Barriga A, Buschmann A H, Mäki-Arvela P, Mikkola J P and Lienqueo M E 2016 Identification and efficient extraction method of phlorotannins from the brown seaweed *Macrocystis pyrifera* using an orthogonal experimental design *Algal Res.* **16** 201–8
- [85] Jung H A, Jin S E, Ahn B R, Lee C M and Choi J S 2013 Anti-inflammatory activity of edible brown alga *Eisenia bicyclis* and its constituents fucosterol and phlorotannins in LPS-stimulated RAW264.7 macrophages *Food Chem. Toxicol.* **59** 199–206

- [86] Kim E and Kwak J 2015 Antiviral phlorotannin from *Eisenia bicyclis* against human papilloma virus in vitro *Planta Med.* **81**PW\_22
- [87] Eom S, Moon S, Lee D, Kim H, Park K, Lee E, Kim T H, Chung Y, Lee M and Kim Y 2015 In vitro antiviral activity of dieckol and phlorofucofuroeckol-A isolated from edible brown alga *Eisenia bicyclis* against murine norovirus *Algae* **30** 241–6
- [88] Hans-Jörg Bart 2011 Extraction of natural products from plants – An introduction. Industrial scale natural products extraction. 1–26
- [89] Richardson J F, Harker J H and Backhurst J R 2002 *Coulson and Richardson's Chemical Engineering: Particle Technology and Separation Process*, vol. 5. (Oxford: Butterworth and Heinemann)
- [90] Richardson J F, Harker J H and Backhurst J R 2002 *Chemical Engineering: Particle Technology and Separation Processes* vol 2(London: Butterworth-Heinemann)
- [91] Naczki M and Shahidi F 2004 Extraction and analysis of phenolics in food *J. Chromatogr. A* **1054** 95–111
- [92] Schoenwaelder M E A and Clayton M N 1999 The presence of phenolic compounds in isolated cell walls of brown algae *Phycologia* **38** 161–6
- [93] Stern J L, Hagerman A E, Steinberg P D and Mason P K 1996 Phlorotannin-protein interactions *J. Chem. Ecol.* **22** 1877–99
- [94] Azmir J, Zaidul I S M, Rahman M M, Sharif K M, Mohamed A, Sahena F, Jahurul M H A, Ghafoor K, Norulaini N A N and Omar A K M 2013 Techniques for extraction of bioactive compounds from plant materials: A review *J. Food Eng.* **117** 426–36
- [95] Koivikko R, Loponen J, Pihlaja K and Jormalainen V 2007 High-performance liquid chromatographic analysis of phlorotannins from the brown alga *Fucus vesiculosus* *Phytochem. Anal.* **18** 326–32
- [96] Le Lann K, Surget G, Couteau C, Coiffard L, Cérantola S, Gaillard F, Larnicol M, Zubia M, Guérard F, Poupart N and Stiger-Pouvreau V 2016 Sunscreen, antioxidant, and bactericide capacities of phlorotannins from the brown macroalga *Halidrys siliquosa* *J. Appl. Phycol.*
- [97] Heo S J, Lee K W, Song C B and Jeon Y J 2003 Antioxidant Activity of Enzymatic Extracts from Brown Seaweeds *Algae* **18** 71–81
- [98] Rodrigues D, Sousa S, Silva A, Amorim M, Pereira L, Rocha-Santos T A P, Gomes A M P, Duarte A C and Freitas A C 2015 Impact of enzyme- and ultrasound-assisted extraction methods on biological properties of red, brown, and green seaweeds from the Central West Coast of Portugal *J. Agric. Food Chem.* **63** 3177–88
- [99] Koivikko R, Loponen J, Honkanen T and Jormalainen V 2005 Contents of soluble, cell-wall-bound and exuded phlorotannins in the brown alga *Fucus vesiculosus*, with implications on their ecological functions *J. Chem. Ecol.* **31** 195–212
- [100] Zheng H, Hwang I-W and Chung S-K 2009 Enhancing polyphenol extraction from unripe apples by carbohydrate-hydrolyzing enzymes. *J. Zhejiang Univ. Sci. B* **10** 912–9
- [101] Huynh N T, Smaghe G, Gonzales G B, Van Camp J and Raes K 2014 Enzyme-assisted extraction enhancing the phenolic release from cauliflower (*Brassica oleracea* L. var. *botrytis*) outer leaves *J. Agric. Food Chem.* **62** 7468–76
- [102] Charoensiddhi S, Franco C, Su P and Zhang W 2014 Improved antioxidant activities of brown seaweed *Ecklonia radiata* extracts prepared by microwave-assisted enzymatic extraction *J. Appl. Phycol.* **27** 2049–58
- [103] Lim S N, Cheung P C K, Ooi V E C and Ang P O 2002 Evaluation of antioxidative activity of extracts from a brown seaweed, *Sargassum siliquastrum* *J. Agric. Food Chem.* **50** 3862–6
- [104] Ganapathi K, Subramanian V and Mathan S 2013 Bioactive potentials of brown seaweeds, *Sargassum myriocystum* J. Argadh, *S. plagiaphyllum* C. Agardh and *S. ilicifolium* (Turner) J. Argadh *Int. Res. J. Pharm. Appl. Sci.* **3** 105–11
- [105] Kannan S 2014 full-textFT-IR and EDS analysis of the seaweeds *Sargassum wightii* *Gracilaria corticata* (red algae) *Int. J. Curr. Microbiol. Appl. Sci.* **3** 341–51
- [106] Gomez-Ordóñez E and Ruperez P 2011 FTIR-ATR spectroscopy as a tool for polysaccharide identification in edible brown and red seaweeds *Food Hydrocoll.* **25** 1514–20
- [107] Wang Y, Xu Z, Bach S J and McAllister T A 2009 Sensitivity of *Escherichia coli* to seaweed (*Ascophyllum nodosum*) phlorotannins and terrestrial tannins *Asian-Australasian J. Anim. Sci.* **22** 238–45
- [108] Munir N, Sharif N, Naz S and Farkhanda M 2013 Algae: A potent antioxidant source *Sky J. Microbiol. Res.* **1** 22–31
- [109] Park P-J, Fereidoon S and Jeon Y-J 2004 Antioxidant activities of extracts from an edible seaweed *Sargassum horneri* using ESR spectrometry *J. Food Lipids* **11** 15–27