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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®	Protamex®
<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® 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  $IC_{50}$  of *Sargassum* is presented in Table 3. *S. polycystum* only showed its antiradical activity when they were extracted with ethanol 75% and Protamex® and the best activity was shown by Protamex® with  $IC_{50}$   $1.9 \pm 1.1$  mg/mL. The weakest antiradical activity came from the methanolic extract of *S. aquifolium* with  $IC_{50}$   $23.8 \pm 9.0$  mg/mL. The three *Sargassum* extracted with Protamex® gave a better antiradical activity compared to the other extractants, it can be seen by their  $IC_{50}$  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  $IC_{50}$   $8 \times 10^{-3} \pm 0.5$  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® > ethanol 75% (v/v) > Protamex® > methanol > methanol 50% (v/v) > water. As for *S. aquifolium*, the efficiency order was Viscozyme® > methanol > ethanol 75% (v/v) > methanol 50% (v/v) > Protamex® > water. Then, for *S. ilicifolium*, the efficiency order was methanol > ethanol 75% (v/v) > methanol 50% (v/v) > Protamex® > Viscozyme® > water.

Table 4 presents the reducing power activity also known as FRAP of *Sargassum* expressed by a concentration in  $\mu$ M equivalent with  $Fe^{2+}$  ( $\mu$ M Eq  $Fe^{2+}$ ). Vitamin C was used as standard with reducing power  $58.1 \pm 0.7$   $\mu$ M Eq  $Fe^{2+}$ . The reducing power activity that was almost near as the standard was the aqueous phase of *S. polycystum* with  $43.5 \pm 0.7$   $\mu$ M Eq  $Fe^{2+}$ . 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%,  $7.9 \pm 1.2$   $\mu$ M Eq  $Fe^{2+}$ . In addition, it appears that Viscozyme® extracts of *S. aquifolium* and *S. polycystum* had better reducing power activity compared to methanol, methanol 50%, ethanol 75% (v/v) and even Protamex®. 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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