Antioxidant potential of ethanolic extract of *Sargassum polycystum* C.A. Agardh. naturally growing in Lange seashore

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Antioxidant Potential of Ethanolic Extract of *Sargassum polycystum* C.A. Agardh. Naturally Growing in Lange Seashore

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Abstract. This work aims to evaluate the antioxidant properties of an ethanolic extract of *Sargassum polycystum* C.A. Agardh. (EES) harvested from Lange coastal area (Aceh Besar). The bioactivity was estimated by conducting 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay followed by phenolic content measurement and infrared (IR) spectroscopy analysis to characterise the possible phytochemicals of the crude extract. The EES demonstrated a stronger free radical scavenging activity expressed in IC_{50} value of 2.178 ± 0.117 µg/mL compared to other studies in the same species. Furthermore, the phenolic-related component was confirmed based on its phenolic content of 18.572 ± 0.106 mg GAE/g and IR spectroscopy wavenumber of 3367.71 and 1624.06 cm^{-1} reflecting hydroxyl groups and aromatic rings, respectively. According to the finding, the brown algae from Lange could fairly be considered as a natural resource of antioxidants and phenolic bioactivities.

INTRODUCTION

Oxidative stress due to imbalance between oxidation agents and the antioxidant has been well recognised as the hallmark of some serious metabolic disorders such as diabetes mellitus, cardiovascular disease, Parkinson's disease, and many more. Overproduced prooxidants, including free radicals in the form of reactive oxygen species (ROS), reactive nitrogen species (RNS), and other related agents, could have detrimental effects on the functional and morphological characteristics of vital components in cellular systems related metabolisms. Moreover, animal studies show that an excessive number of such species has been associated with the downregulation of endogenous antioxidants such as glutathione (GSH) and superoxidase dismutase (SOD) [1, 2] that could eventually exacerbate the abnormalities. In this stage, the consumption of antioxidant-rich supplements has strongly been recommended.

Increased antioxidants intake could be achieved by consuming either synthetic supplements or a plant-based diet. Nonetheless, as no concern about safety risks of consuming antioxidants in natural sources has been raised, Department of Health and Human Services of the United States mentioned that the U.S. Government highly urges people to consume antioxidant-rich plants to meet their needs instead of replacing healthy diets with artificial supplements [3]. A group of phenolic compounds, secondary metabolites for the defence system, is the common bioactive found in natural products exhibiting neutralisation of oxidative species through the electron-donating effect of its hydroxyls with minimum to zero adverse effects [4]. Furthermore, both cellular and animal models also showed the protective properties on intracellular components demonstrated by the compounds against ROS through inhibition of protein oxidation and cellular GSH depletion [5].

Searching for alternative antioxidants based on natural products is important due to either some inconvenient events of artificial products or limited access to terrestrial resources. Marine resources have been well known to

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provide considerable nutrients that can be used not only to maintain our health system but also to combat numerous serious disorders biomarkers such as ROS. To exemplify, brown macroalgae species from genus *Sargassum* have been documented to possess a distinctive marine phenolic component, namely phlorotannin, in a higher percentage than two other seaweed families, red and green macroalgae [6]. Moreover, these species also comprise other bioactive phytochemicals, including sulphated polysaccharides, alginate, phytosterol, flavonoid, etc. Applications of the seaweed and the derived products in cosmetics, food and beverage supplements, medicines, and even food stocks could be linked to such diverse ingredients. In contrast, the potential of some species living in remote areas across the Indonesian archipelago has not been well revealed yet. For instance, there is a lack of scientific evidence of bioactivity and related chemicals of *Sargassum polycystum* found in Lange beach. However, European voyagers reported that Indies (former term before modern Indonesia) fishers collected the brown seaweeds for their dish in 1292 [7]. As a preliminary study is needed before going further, this work would address this gap.

Applying *S. polycystum*, a species of brown macroalgae readily spread in tropical regions, as the antioxidant source is highly relevant [8]. Even though it has been reported to consist of numerous bioactives such as fucoidan, alginate, phenolic derivatives, etc. associated with anticancer, antiinflammation, antiviral, and antibacterial [9, 10], the potential of the species widely living in the northern Sumatera (Indonesia) has not been fully estimated. For example, Lange coastline in Aceh Besar could be thought of as an ideal habitat for the seaweed to grow with temperature and salinity of 27.25–29.3 ^{o}C and 32–33.5 ppt, respectively. As previously reported, *S. polycystum* from this location has been revealed to contain a high level of ash, fibre, carbohydrate, protein, and lipid [11]. This may a good sign that the species could possess acceptable phenolic content to offer excellent free radicals inhibition as *S. polycystum* growth in Harbor beach (Tamil Nadu, India) was reported to have high contents of nutrition and phenolic as well as antibacterial activity [12]. Since there is no previous related study on the algae from Lange beach, this work was carried out to uncover the antioxidant potential and the phenolic content of the marine plant.

MATERIALS AND METHOD

Materials

Absolute ethanol (EtOH) for extraction was purchased from Chem Supply Australia. 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, Folin-Ciocalteau's reagent, Na_2CO_3, and gallic acid were supplied by Sigma-Aldrich (USA). All chemicals were in analytical grade.

Method

The brown algae were harvested from Lange beach (Aceh Besar, Indonesia) in July 2019 and taxonomically identified as *S. polycystum* C.A. Agardh. by Dr. Saida Rasnovi, M.Si. from Biology Laboratory of Universitas Syiah Kuala. The sample was rinsed to clean impurities with flowing water, followed by shade drying for a week and oven at 60 ^{o}C overnight. The dried seaweed was then grounded with a blender machine and kept in a 4 ^{o}C-fridge for further use.

Sample Extraction

The dried sample in powder (250 g) was soaked in EtOH (1 L) for 72 h at room temperature, followed by vacuum filtration. Finally, the filtrate was in vacuo concentrated using a rotary evaporator (Buchii, Germany) at 40 ^{o}C to give the dry ethanol extract of sargassum (EES, 10.5 g).

Antioxidant Assay

The assay followed Jiang, Li [13] with a minor modification. DPPH solution in EtOH (180 µL, 0.2 mM) was pipetted into the 96-well plate (Corning Incorporated, USA), followed by 20 µL of the ethanolic extracts and ascorbic acid in the concentration range of 1 to 10 µg/mL. The mixture was then incubated for 30 min at 30 ^{o}C prior to the absorbance measurement at 517 nm (Tecan Infinite M1000 PRO, Austria). The activity was calculated as:

$$\textbf{DPPH scavenging activity}\ (\%) = \left(\frac{\boldsymbol{A_0 - A_1}}{\boldsymbol{A_0}}\right) \times \mathbf{100}\%$$

A_0 and A_1 was the absorbance of the blank and the sample, respectively.

Phenolic Content Estimation

The Folin-Ciocalteau method was carried out to evaluate the phenolic level of the extract adapted from [14]. Briefly, the reagent dissolved in distilled water (1:10). First, 0.5 mL of *an* aqueous solution of EES *(100* µg/mL) was mixed with 7,5 mL distilled water and 0.5 mL of Folin-Ciocalteu reagent. The mixture was then homogenised, followed by the addition of 1.5 mL of 20 % Na_2CO_3 solution. Incubation for 120 min was applied towards the mixture, and the absorbance was observed using UV-Vis spectrophotometer (Dynamica Halo RB-10, UK) at 765 nm against a blank sample. The gallic acid standard equivalent concentration was used as the concentration of total phenolic. The phenolic content expressed in mg/g GAE (gallic acid equivalent) of dry weight extract.

FTIR (Fourier Transport Infrared) Analysis

The prominent functional groups were detected using KBR tables in FTIR spectrometer (Shimadzu, USA) in the wavenumber range of 4000–400 cm^{-1}.

Statistical Analysis

The data were in triplicate experiments' mean ± standard deviation (SD) (n = 3). A one-way analysis of variance (ANOVA) with Tukey's post hoc test was employed in GraphPad Prism 8 (USA), whereby the data were significant at $p < 0.05$.

RESULT AND DISCUSSION

Result

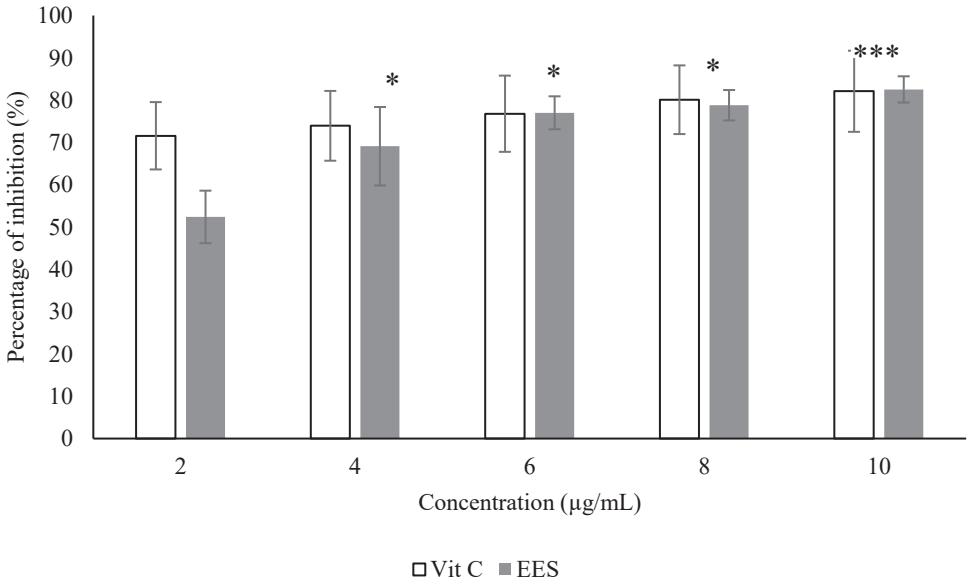

FIGURE 1. A bar chart representing DPPH scavenging activity of EES and vitamin C; $^{*}p = 0.031$, $^{**}p = 0.002$; $^{***}p = 0.0005$ (ANOVA, n = 3, Tukey)

EES showed concentration-dependent inhibitory effects towards DPPH free radicals scavenging, which was increased proportionally shown in **Fig 1**. At 2 µg/mL, it inhibited half activity of the free radicals while at the highest concentration, 10 µg/mL, more than 80% of activity was quantitatively neutralized. Compared to the positive control, it could be considered a fairly strong antioxidant as the activity was observed in line with the control. Therefore, EES could act as an antioxidant in terms of *in vitro* assay with DPPH.

The phenolic content evaluation of EES estimated 18.572 ± 0.105 mg GAE/g of phenolic-related compounds in terms of gallic acid equivalent. As summarised in **Table 1**, this finding seems to agree with those levels in the same species obtained from India [15], Indonesia [16], and Malaysia [17]. In other words, the sample in this work could be hypothesised to offer a normal level of phenolic phytochemicals. However, with regards to the IC_{50}, EES demonstrated the strongest potential compared to S. polycystum from other locations. It may indicate that other phytochemicals such as fucoidan, alginate, or non-polar compounds may contribute to EES's bioactivity. Further investigation is needed to confirm the hypothesis.

TABLE 1. Antioxidant activity and phenolic content of EES compared to other extracts of *S. polycystum* from related publications

No	Sample	Location sample	IC_{50} towards DPPH (µg/mL)	Phenolic content
1	EES	Lange beach (Indonesia)	2.178 ± 0.117	18.572 ± 0.105 mg GAE/g (this study)
2	EtOH extract	Sanur Beach (Indonesia)	-	0.038 ± 0.25 mg GAE/g [18]
3	MeOH extract	Southeast coast of Tamil Nadu (India)	214.59	18.88 ± 3.34 mg GAE/g [15]
4	EtOH extract	Dompu beach (Indonesia)	624.76	123.21 mg/mL [19]
5	MeOH extract	Teluk Kemang beach (Malaysia)	-	0.22 ± 0.2 mg GAE/g [20]
6	EtOH extract	Pok Tunggal Gunungkidul beach (Indonesia)	1,270 ± 0.01	1.18 ± 0.67 mg GAE/g [21]
7	EtOH extract	Panjang island (Indonesia)	5,200 ± 5.9	5.8 ± 0.6 % [22]
8	EtOAc extract	Jepara (Indonesia)	601	19 mg GAE/g [16]
9	EtOH extract	Semporna (Malaysia)	-	34.41 ± 0.01 mg GAE/g [17]

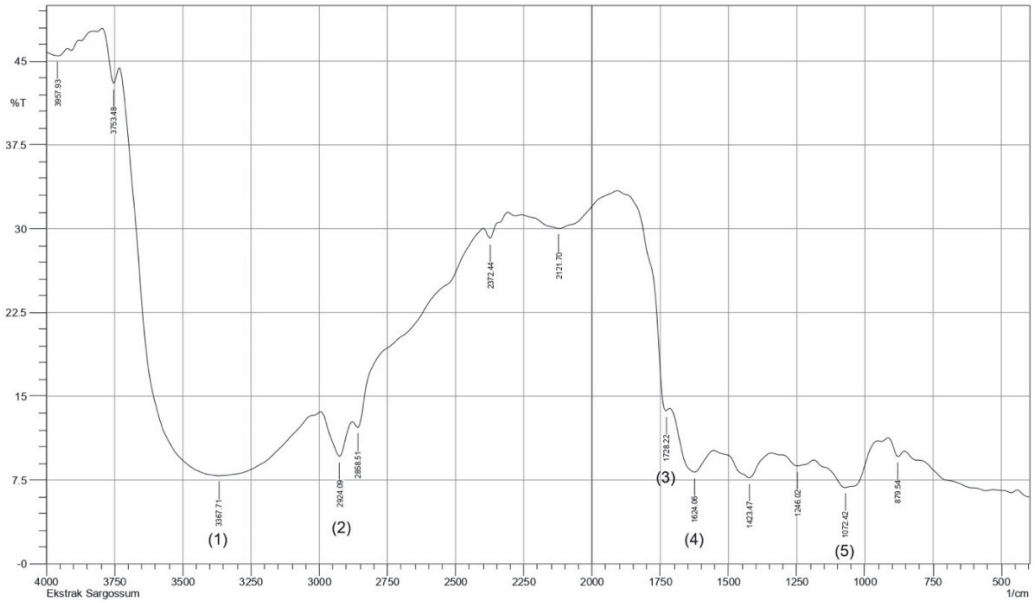

FIGURE 2. Annotated IR bands of typical functional groups for phenolic constituents in EES

To confirm the existing phenolic-related constituents in term of vibrational characteristics of the typical functional groups, an infrared spectroscopy analysis was undertaken towards the ethanol-soluble extract displayed in **Fig 2**. As listed in **Table 2**, the expected functional groups such as hydroxyl, aromatic ring, and alkoxy were detected in the range of their vibrational wavelength. With a strong intensity, hydroxyl was observed at the stretching point of 3367.71 cm^{-1}. A wavenumber of 1624.06 cm^{-1} could show another evidence of the existing aromatic ring, the unique component allowing us to distinguish a phenolic ring from other hydroxyl-containing compounds. In addition, a peak at 1072.42 cm^{-1} is most likely to represent the bonding between the carbon of aromatic and oxygen of hydroxyl.

TABLE 2. The list of identified bands in FTIR spectra of EES compared to references

No	Wavenumber (cm^{-1})	Type of vibration of functional group	Those reported for phlorotannins (cm^{-1}) [23-25]
1	3367.71	Stretching of -OH	~ 3250 (hydroxyl)
2	2924.09	Stretching of sp^3 C–H	~ 2900 (alkane)
3	1624.06	Stretching of aromatic C=C	1650–1450 (phenyl)
4	1072.42	Bending of C–O	1200–1050 (ether)
5	879.54	Bending of aromatic C–H	900–690 (substituted phenyl)

Discussion

By conducting a rapid and inexpensive method through DPPH evaluation, this study showed that the species could act as anti-free radicals. By the concentration of 6 µg/mL (**Fig 1**), the inhibition percentage of EES surpassed the value of the positive control. It would be an interesting phenomenon when a crude extract exerts at least a similar trend with the commercial product's. Phytochemicals in the extracts could play a great role in scavenging such readily reactive species. This simple method has been proven to guide researchers in gaining breakthroughs related to single active compounds and particular mechanisms from a cellular perspective. However, as DPPH scavenging test is an appropriate method for screening antioxidant potential of less polar group, confirming with other colorimetric approaches such as 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$)-based assay for more polar system is strongly recommended to validify this primary information. In addition, compared to previous documentations (**Table 1**), EES exhibited outstanding antioxidant property with a much lower value of IC_{50} despite the relatively normal phenolic content. Apart from phenolic compounds, ethanol as an upper polar group of organic solvent might extract a broad range of phytochemicals, including sterols, polysaccharides, fatty acids, and many more. This is the limitation of our current study to evaluate the other possibility of finding the appearance and contribution of the mentioned plant metabolites. The further work is to investigate those components and, eventually, to elucidate the major bioactive in the term of individual compounds.

Based on FTIR spectroscopy data in line with some relevant reports, EES could be considered the alternative source of marine phytophenols. It would be true that wavelength numbers from 3700 to 3200 cm^{-1} of -OH could come from any hydroxyl-containing compound, including the ethanol solvent used in the study. However, there are three data whereby we could take into account as an indication of phenol-related species. Firstly, a broad spectrum peaked at 3367.71 cm^{-1} might indicate the hydrogen bonding between hydroxyls of intermolecular. For the second, the fingerprint region of C–O may indicate that the hydroxyl is most likely attached to an organic skeleton. In other words, the observed OH might not represent water molecules. In addition, broad absorption between 1072 and 1246 could also correspond to halogenated phlorotannins[25].

Last but not least, the typical wavenumber of an aromatic ring would be a strong implication of the phenol ring further confirmed by a resonance in fingerprint area of 879.54 cm^{-1} for substituted phenyl group. Indeed, phloroglucinol derivatives have been uniquely reported to give vibration around 820 cm^{-1} [23]. Interestingly, a carbonyl spectrum detected as a low peak at 1728.22 cm^{-1} could also offer another possible appearance of flavonoid derivatives, modified phenolic-consisting groups, given that carbonyl vibrations are usually not present in naturally occurring phlorotannins.

CONCLUSION

This study documented the potent antioxidant activity of EES through the DPPH scavenging assay. The extracted inhibited the free radicals with much promising inhibition value of IC_{50} of 2.178 ± 0.117 µg/mL. The phenolic content of 18.572 ± 0.106 mg GAE/g showed that this extract indicated the species as a phenol-rich plant. Furthermore, FTIR-

based inspection confirmed the typical wavenumbers of hydroxyl, aromatic, and alkoxy groups that may represent the desired compounds.

Fractionation-based separation towards EES is needed to isolate the active compounds to gain a deeper insight into the findings. Multiple bioassays, including cell line-based studies, would be our further focus to evaluate the beneficial properties on the utilisation of this species from the site for human health.

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