

Potentiality of Using Spreading *Sargassum* Species from Indonesia as an Interesting Source of Antibacterial and Radical Scavenging Compounds: A Preliminary Study

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ABSTRACT

As an archipelagic country with 95,181 km long coastline, Indonesia has great potential as the producer of seaweeds. The diverse phyla of marine macroalgae (red, brown and green seaweeds) are known to produce molecules which are attractive for diverse industries. Applications of algal products range from simple biomass production for food, feed and fuels to valuable products such as sugar polymers, cosmetics, pharmaceuticals, pigments, and food supplements. Seaweeds also have the potential to be used as a source of new bioactive for human, animal or plant health, as well as a source of new synthons and biocatalysts in sustainable chemistry (Bourgougnon and Stiger-Pouvreau, 2011). In this paper, among species of economic value we focus on brown seaweeds belonging to family Sargassaceae and genus *Sargassum* spreading along Indonesian coasts. Members of this genus are especially abundant in tropical and subtropical regions (Zemke-White and Ohno, 1999). The purpose of this study is to analyze the antibacterial and antioxidant activity of three species of *Sargassum*, i.e. *S. echinocarpum*, *S. duplicatum* and *S. polycystum*. Both polar and non-polar extracts have been prepared from those three species. *In vitro* antibacterial activities of extracts were evaluated against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*. Results indicated all the three species tested showed an antibacterial activity. The most effective antibacterial activity against *S. aerous* was from *S. echinocarpum* with ethyl acetate, inhibition zone 1.13 ± 0.25 mm; *S. duplicatum* with N-Hexane was most effective against *E. coli*, 1.20 ± 0.28 mm.

Key words: Antibacterial, *Sargassum*, *Staphylococcus aureus*, *Escherichia coli*.

INTRODUCTION

Coral reef benthic ecosystems from Indonesia are particularly rich in algal biodiversity and constitute a reserve of species of considerable economic, social and ecologic potential. As an archipelagic country with 81,000 km long coastline, Indonesia has great potential as the producer of seaweeds. Since their introduction to the southern Philippines and Indonesia, seaweed farming has spread rapidly. The most common types of seaweed farmed in South East Asia belong to red genera, with *Kappaphycus* (mainly species *K. alvarezii*) and *Eucheuma* (mainly species *E. denticulatum*). *K. alvarezii* major component of seaweed exports from Indonesia contributed 78% of Indonesian seaweed production in 1991. In 2010, the country contributed with 0.91 million T of the world's seaweed production. Seaweed farming in Indonesia is an export-oriented activity designed

to produce hydrocolloids carrageenan or agar-agar for the international market.

The diverse phyla of marine macro algae (red, brown and green seaweeds) are known to produce molecules which are attractive for diverse industries. Applications of algal products range from simple biomass production for food, feed and fuels to valuable products such as sugar polymers, cosmetics, pharmaceuticals, pigments, and food supplements. Seaweeds also have the potential to be used as a source of new bioactive for human, animal or plant health, as well as a source of new synthons and biocatalysts in sustainable chemistry (Bourgougnon and Stiger-Pouvreau, 2011).

In this study, among species of economic value we focus on brown seaweeds belonging to family Sargassaceae and genus *Sargassum* spreading along Indonesian coasts. Members of this genus are especially abundant in tropical and subtropical regions (Zemke-White and Ohno, 1999).

Moreover, this genus exhibits an important species-richness (340 species currently accepted taxonomically) especially in the Indo-Pacific basin. Species in this region have been used traditionally in folk medicine for treatment of skin-related disorders (i.e. Eczema, scabies and psoriasis), renal dysfunction, heart ailments, lung diseases, ulcer, and also to promote secretion of bile. Members of this genus are especially abundant in tropical and subtropical regions. The purpose of this study is to analyze the antibacterial and antioxidant activity of three species of *Sargassum*, i.e. *S. echinocarpum*, *S. duplicatum* and *S. polycystum*.

MATERIAL AND METHOD

Seaweeds collection

Seaweeds were collected by hand using snorkeling (5 m depth) and preserved on ice until further processing. Numbers of samples were sampled between August and December 2011 at various sites, along the Java coasts (Jepara, Teluk Awur, central Java), in Indonesia. All species tested are deposited in the herbarium of the Laboratory of Microbiology, Department of Marine Science, Faculty of Fisheries and Marine Science, University Semarang.

After collecting, the samples were rinsed with sterile seawater to remove associated debris and salt. Epiphytes were removed from the algae. The surface micro flora was removed by washing the algal samples for ten minutes with 30% ethanol (Hellio *et al.*, 2000).

The cleaned material was then surface dried by pressing it briefly between sheets of paper towelling and air dried in the shade at 30 °C during 24 hours.

Preparation of algal extracts

One part of the dried algae was suspended by stirring in distilled water (50 g L⁻¹ of dried weight) with an Ultra-turrax (2 hours) at 4 °C. After centrifugation (30 min, 3,000 g, 4 °C) and filtration (Whatman cat n° 1822 047), the supernatant was lyophilised and we obtained then the aqueous extract (Extract A). For organic extracts, the dried algae were suspended by stirring in ethanol 95 °C (200 g in 300 mL) with an Ultra-turrax (2 hours) at 4 °C. After centrifugation (30 min, 3000 g, 4°C), the resultant pellet was re-extracted five times in the same way. The alcoholic extracts were combined and evaporated under vacuum at low temperature (<40 °C). Distilled water (100 mL) was then added and partitioned with methylene chloride (4 x 100 mL). The aqueous phases were collected, lyophilised, re-suspended in absolute ethanol (100 mL), filtered and concentrated under vacuum at low temperature (Extract B). The organic phases were collected, dried during 24 hours under Na₂SO₄, filtered and concentrated under vacuum at low temperature (Extract C). These three phases were stored at -40 °C before use (Hellio *et al.*, 2000).

Evaluation of antibacterial activities

In vitro antibacterial activities of extracts were evaluated

against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*. All species of bacteria were grown on nutrient plates. All the stock agar cultures were stored at 4 °C.

Determination of antioxidant activities

The determination of the activity of DPPH was performed according to the protocol described by Molyneux (2003). Control or blank was prepared by reacting 0.2 mL of methanol with 3.8 mL of DPPH. Then, the solution was allowed to stand for 30 minutes in the dark. Finally, the absorbance of this solution was measured at a wavelength of 516 nm using a spectrophotometer Shimadzu UV-VIS 1601. It corresponded to the maximum absorbance A₀.

The determination of the antioxidant activity of the algae extract in methanol and extracted with ethyl acetate was carried out by preparing solutions of the two different samples at concentrations 20, 40, 60, 80 and 100 ppm. Then, 0.2 mL of each solution was mixed with 3.8 mL of DPPH to 50 microns. After 30 minutes, the absorbance at 515 nm of solutions is measured. For comparison, the same is made from a solution of quercetin at concentrations of 20, 40, 60, 80 and 100 ppm. The percentage reduction in the percentage of DPPH or anti-radical activity is calculated using the following equation:

$$\text{DPPH (\%)} = \{(A_0 - AP) / A_0\} \times 100 \%$$

where A₀ is the absorbance of the blank and AP is the absorbance of the sample.

The extract concentration providing 50% of the activity of free radical scavenging (EC₅₀) was calculated from the graph of percentage reductions depending on the concentration.

Phytochemical screening of algal extract

The method used in this experiment was the addition of certain reagents giving a positive reaction if the extract belongs to the chemical class Wanted (Harborne, 1973). This analysis determined the presence or absence of different compounds alkaloids, saponins, flavonoids, tannins, steroids and terpenoids.

RESULT AND DISCUSSION

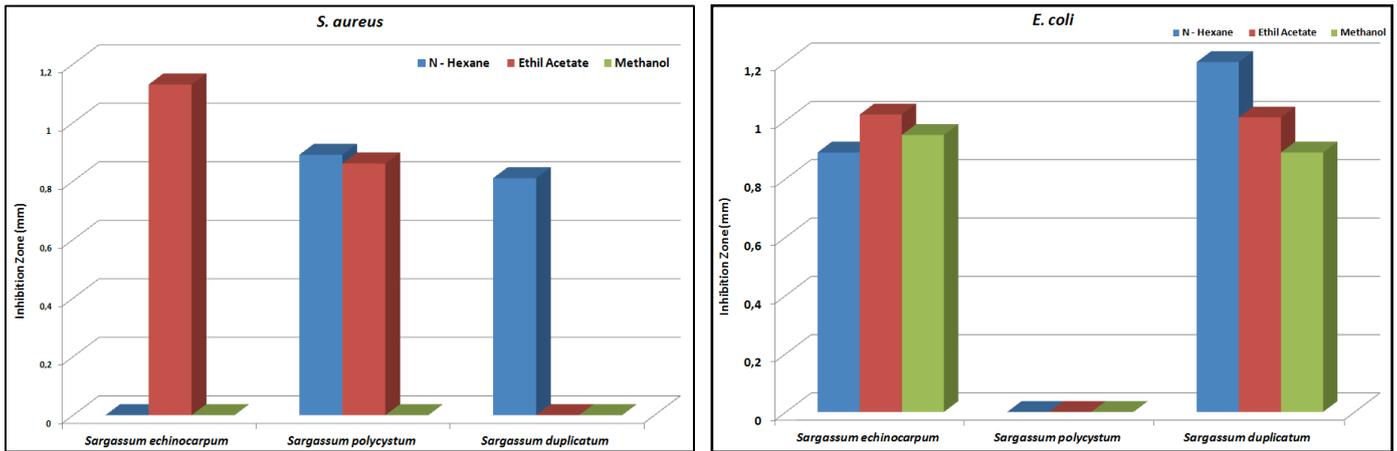
Antibacterial Activity

Antibacterial activities from the three *Sargassum* extracts in three different solvents were measured *S. aureus* and *E. coli*. Results of the inhibition zone shown by the extracts were represented in the following table 1.

Based on above table, the most effective extract against *S. aerous* was *S. echinocarpum* with ethyl acetate (1.13 ± 0.25 mm). Then, against *E. coli*, *S. duplicatum* with n-hexane was the most affective (1.20 ± 0.28 mm). Results indicated that *S. aerous* seemed to be resistant towards the three *Sargassum* extracted in methanol (Fig. 1). Furthermore, results also implied that *S. polycystum* either in n-hexane, ethyl acetate or methanol was not efficacious against the *E. coli* (Fig. 2).

Table 1. Antibacterial activity of *Sargassum* against *S. aureus* and *E. coli*

IV. Seaweed	V. Solvent		
	N – Hexane	Ethyl Acetate	Methanol
<i>Staphylococcus aerous</i>			
<i>Sargassum echinocarpum</i>	0.00 ± 0.00	1.13 ± 0.25	0.00 ± 0.00
<i>Sargassum polycystum</i>	0.89 ± 0.13	0.86 ± 0.17	0.00 ± 0.00
<i>Sargassum duplicatum</i>	0.81 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
<i>Escherichia coli</i>			
<i>Sargassum echinocarpum</i>	0.89 ± 0.12	1.02 ± 0.02	0.95 ± 0.21
<i>Sargassum polycystum</i>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>Sargassum duplicatum</i>	1.20 ± 0.28	1.01 ± 0.11	0.89 ± 0.13



(1)

(2)

Figure (1) Antibacterial activity of *Sargassum* against *S. aureus* (2) Antibacterial activity of *Sargassum* against *E. coli*.

Antioxidant Activity

Table 2. Percentage (%) of discoloration of crude extract of *Sargassum duplicatum* and *S. echinocarpum*

Species	Solvent	Concentration (ppm)	Antioxidant activity (% of discoloration)
<i>S. duplicatum</i>	n-hexane	100	1.723
		300	5.014
		600	8.181
		900	12.549
<i>S. echinocarpum</i>	ethyl acetate	100	0.098
		300	0.248
		600	15.253
		900	41.370

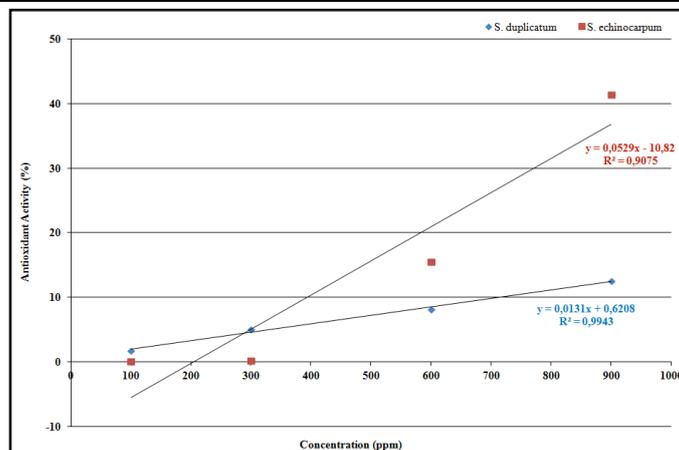


Figure 3. IC 50 of two species of *Sargassum*; *S. duplicatum* (red) and *S. echinocarpum* (blue).

Table 3. Test of antioxidant activity of *Sargassum*

Species	Solvent	Percentage of Inhibition				EC50 (ppm)
		100 ppm	300 ppm	600 ppm	900 ppm	
<i>S. duplicatum</i>	n-hexane	1.723	5.014	8.181	12.549	3,798.4
<i>S. echinocarpum</i>	ethyl acetate	0.098	0.248	15.253	41.370	1,170

Results showed that crude extract of *Sargassum* in hexane and ethyl acetate could inhibit free radical. The higher concentration of *Sargassum extract* generates higher antioxidant activity. However, the antioxidant activity of maximum concentration applied in this test had not reached 50% inhibition (Table 2).

From figure 3, it implied that the IC 50 of *S. echinocarpum* is better than *S. duplicatum*. *S. duplicatum* could inhibit the free radical at concentration 3,798.4 ppm; meanwhile the *S. echinocarpum* could inhibit at 1,170 ppm. The color changement of extract from violet to yellow indicated an inhibition of free radical DPPH by crude extract. This changement of color showed that crude extract has a potentiality of antioxidant even in a high concentration (Table 3).

Phytochemical of algal extract

In the n-hexane extract of *S. duplicatum*, the analysis revealed the presence of bioactive compounds such quinones, steroids, flavonoids and alkaloids. While in ethyl-acetate and methanol extract, the tests revealed the presence of steroids, saponinquinones, alkaloid and flavonoids.

The n-hexane, ethyl acetate and methanol extract of *S. echinocarpum* contained steroid, quinone and flavonoid. In addition, alkaloid was presence in the ethyl acetate and methanol extract of *S. echinocarpum*.

Steroid and alkaloid were revealed in all extract of *S. polycystum*, while flavonoid seemed to be presence merely in the ethyl acetate and methanol extract of *S. polycystum*. Then quinone was the only substance existed in the methanol extract. Phenol was absence in all extract of *Sargassum*.

Discussion

In the present study, three species of *Sargassum* were examined for their antibacterial against Multi Drug Resistance bacteria, i.e. *S. aureus* and *E. coli*; and antioxidant activity. The choice of solvents was based on solvents having indices of increasing polarity. Methanol is a polar solvent, N-hexane is a nonpolar solvent and ethyl acetate is a polar solvent. Solvent has a greater affinity with the chemical species of the same polarity. Indeed, the chemical species are soluble polarity around them while chemical species of different polarities are poorly soluble them. The photochemical study shows that depending on the nature of the solvent used, the composition of the sample changes. Indeed, methanol can extract the polar substances and ethyl acetate as the semi-polar substances. Here, the high concentration of green extracts shows that the extraction with methanol and ethyl acetate are most effective. It is possible that the low polar and polar substances contained in the algae are present in large quantities. The extracts and active constituents of various algae have been shown to have antibacterial activity *in vitro* against Gram-positive and Gram-negative bacteria; some examples are given in Table 1. Then,

the extract of *Sargassum* having the best antibacterial activity was further tested for their capability to inhibit the free radical in antioxidant activity. They were *S. duplicatum* in hexane and *S. echinocarpum* in ethyl acetate. It indicated that these two seaweeds contained potential antioxidant compound. The phytochemical test revealed the absence of phenol in all three *Sargassum*. Therefore, the potential antibacterial and antioxidant activity presented in this study was probably due to other compounds instead of phenol.

The production of antibacterial activity was considered to be an indicator of the capacity of the seaweeds to synthesize bioactive secondary metabolites (del Val, 2001). There are numerous reports of compounds derived from macroalgae with a broad range of biological activities, such as antibacterial (Nair et al., 2007), antivirals (Richards et al., 1978) antitumorals (Espeche et al., 1984), anticoagulant (Athukorala et al., 2006) and antifouling (Marechal et al., 2004). Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae (Newman et al., 2003). Also, considering their great taxonomic diversity, investigations related to the search of new biologically active compounds from algae can be seen as an almost unlimited field.

CONCLUSION

This study revealed the potentiality of different *Sargassum* species as new antibacterial and antioxidant agent. Phenol seemed to be absence in all three *Sargassum* species. Instead, other bioactive compounds such as alkaloid, flavonoid, etc appeared which might be responsible for the antimicrobial activity possessed by *Sargassum*. However, further investigation considering the characterization of antimicrobial compound of *Sargassum* needs to be conducted. Therefore, we can identify which compound is actively potential as pharmacological agents for human interest.

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