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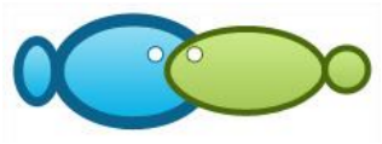
Submission date: 28-Jul-2022 10:17AM (UTC+0700)
Submission ID: 1876042690
File name: C-5-IW-Antibact-AACL.pdf (538.94K)
Word count: 4375
Character count: 24831



Antibacterial activity and proximate analysis of *Sargassum* extracts as cosmetic additives in a moisturizer cream

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Abstract. Indonesia is known for its marine biodiversity, including the richness of its brown seaweed, *Sargassum*. This genus has attracted many attention as it produces active compounds showing potential for the food, pharmacology and cosmetic industries. In this study, a mixture of *S. duplicatum, S. echinocarpum* and *S. polycystum* extracts was applied as an additive in a moisturizer cream serving as an antibacterial agent. Proximate analysis was conducted to evaluate the chemical composition in *Sargassum* spp. There were 5 moisturizer creams prepared: A (standard), B (without antibacterial agent), C (with antibacterial agent), D (with *Sargassum* extracts and antibacterial agent) and E (with *Sargassum* extracts but without antibacterial agent). Antibacterial analyses showed that cream E had the best antibacterial activity in this study. It indicates that the crude extract of *Sargassum* added in the cream could inhibit the development of bacteria for a longer period of time. Bioactive compounds contained in *S. duplicatum, S. echinocarpum, S. polycystum* are steroids, quinones, flavonoids and alkaloids. Saponins were only found in *S. duplicatum.* The five cosmetic creams presented adequate odor and color. These results indicate that *Sargassum* shows a promising potential as a cosmetic additive that could replace commercial antibacterial agents.

Key Words: antibacterial agent, brown seaweed, cosmetic application, *Sargassum*.

Introduction. The equatorial location, a relatively benign climate and vast areas of coastal reefs among about 17500 islands are some advantages of Indonesia that make it the an important area for marine biodiversity. Tropical seaweeds generally grow best between ten degrees latitude north and south of the equator, and the Indonesian seacoast is within this zone (Neish et al 2015). Therefore, Indonesia has a great diversity of marine seaweeds. Brown seaweeds (Phaeophyceae) are particularly abundant with four major genera: *Hydroclathrus, Padina, Sargassum,* and *Turbinaria*, widely distributed in Indonesian waters. *Sargassum* can be found in waters throughout the year, though it is mostly abundant between November to February, depending on the species (Rao et al 2014). Higher nutrient inputs are affecting the growth of *Sargassum* near the equator, with variable volumes of algae landing on the beaches of different countries (Dominican Republic, Guadeloupe, Bahamas, Puerto Rico, Indonesia), depending on water current, weather and wind conditions (Engelen et al 2008). In addition, *Sargassum* species are also acknowledged for their secondary metabolites in response to ecological pressures for space, deterrence of predation and the ability to successfully reproduce (Fairhead et al 2005; Schwartz et al 2016). *Sargassum* has been used traditionally in food and in folk medicine for treatment of skin-related disorders (eczema, scabies and psoriasis), renal dysfunction, heart ailments, lung diseases, ulcer and also to promote bile secretion (Liu et al 2012).

Among the arsenal of molecules produced by *Sargassum*, phenolic compounds can be accumulated in high levels, up to nearly 20% dry weight in Fucales (Koivikko et al

2005). Phenolic compounds constitute a class of molecules separated into phloroglucinols (mono-, di-, tri-, tetra- and oligomeric) and phlorotannins. Halogenated monomeric phenolic compounds are occasionally found in brown algae, as well as in a few red algae. Phlorotannins have various putative roles (Gomez & Huovinen 2010; Stiger-Pouvreau et al 2014). Published reports show that the production of phenolic compounds by marine algae is usually associated with a chemical defence and have been proved to be involved in various protection mechanisms, such as against grazing, pathogen attacks, epiphytes, fouling organisms and UV damages (Amsler & Fairhead 2006). Phenolic compounds of *Sargassum* have been extensively reported as strong antioxidants and antibacterial agents (Rattaya et al 2014; Widowati et al 2014; Puspita et al 2017). Because of its potential, *Sargassum* might be an excellent candidate for the cosmetic industry. This study aims to evaluate the antibacterial activity of a mixture of three species of *Sargassum* and its potential application as an additive in a cosmetic moisturizer cream. The phytochemical constituents of *Sargassum* were also determined.

Material and Method

Preparation of algal material. *Sargassum* was sampled in July 2017, from Jepara waters and was collected by cutting the thallus approximately 5 cm above the holdfast. Algae material was quickly cleaned with seawater and was kept in a closed box to prevent direct contact with sunlight. Later, the algae material was washed with tap water to remove the remaining sand and epiphytes. Algae material was naturally dried for five to seven days. To maintain the quality of the target compound, it is crucial to prevent any direct contact with sunlight. Thus, algae were dried under the shade. After drying, they were cut to small pieces with scissors and were grinded by using a multi-use home blender.

Algal extracts. The extraction method was based on Tanniou et al (2015). 25 g of dry algal material were diluted in 300 mL of ethanol/water solution, 50/50. The filtered (Whatman Filter Paper Ø15 cm) samples were then evaporated until dryness after which, the dried extract obtained was added to 20 mL of H_2O in order to get the crude extract.

Chemical composition analysis of Sargassum extracts. Chemical compositions of three species of *Sargassum* were analyzed including water content, carbohydrates, acid detergent fiber (ADF), cellulose and hemicelluloses, crude fat, ash, fiber and protein content. The methods used to analyze the chemical compositions in *Sargassum* extracts were based on the AOAC methods (2000).

Preparation of the moisturizer cream. The method to prepare the moisturizer cream was based on the method used in the Laboratoire de Biotechnologie et Chimie Marines, Université de Bretagne-Sud, France. The moisturizer creams were made based on the standard formula consisting of four phases: A, B, C and D. Phase A was made of 72.9% water and 0.5% satiaxane CX2QD; phase B consists of emulium kappa 7%, cetiol CC 6%, cotton seed oil 2%, coviox T50 0.2%, tegosoft M 2%, vegetal glycerin 2%, tropical fruit seed oil 1%; phase C was RoRe Fluid 55 2%; phase D consisted of softouch CC 6058 1%, provitamin B5 2%, bactecar 125S 1.25%, dye agent and aromatic extract 0.15%.

Three categories of moisturizer cream were prepared in order to evaluate the mixture of the extracts of three species of *Sargassum* as an antibacterial agent compared with bactecar 125S a commercial antibacterial agent, as follows:

1. Standard formula as a control (Moisturizer cream A);
2. Experimental formulas without and with the commercial antibacterial agent:
 - standard formula without antibacterial agent (Moisturizer cream B);
 - standard formula with antibacterial agent (Moisturizer cream C);
3. Formula with, without the commercial antibacterial agent and with *Sargassum* extracts:

- standard formula with *Sargassum* extracts and commercial antibacterial agent (Moisturizer cream D);
- standard formula with *Sargassum* extracts, but without the commercial antibacterial agent (Moisturizer cream E).

Odor and color tests were performed for the moisturizer creams, by sight and smell observations. Further, after a year of storage at room temperature, all moisturizer creams were tested for their antibacterial activity in order to acknowledge the effectiveness of *Sargassum* extracts as a substitute of the commercial antibacterial agent.

Antibacterial activity of Sargassum mixed extracts in moisturizer creams. The agar diffusion method (Bauer et al 1966) was selected as the method for evaluating the antibacterial activity of *Sargassum* extracts mixed in the moisturizer creams. Bacterial cultures of *Staphylococcus epidermidis* were prepared in a liquid nutrient broth media with a bacterial density $1.5x10^8$ cfu mL^{-1}. Bacterial suspension was poured on to solidified agar media and incubated for 1 h at 37°C.

Sargassum spp. extracts presented a final concentration of 1 mg mL^{-1} in the moisturizer cream. The preparation was made in sterile physiological water. The concentrated cream was then diluted in sterile freshwater to obtain a dilution concentration of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. Twenty µL of solution samples were introduced in sterile paper discs. All samples were triplicated. 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. The zone of inhibition established on the agar media was then measured and expressed in cm. The antibacterial activity was represented by following formula:

Antibacterial activity = Diameter of inhibition zone (cm) – 0.6 cm (the diameter of the paper disc)

Statistical analysis. The data obtained in this study was based on the mean values from triplicate and presented as mean value ± standard of deviation, with n=3. Analysis of variance (ANOVA) was performed to analyze if significant differences were caused by the independent variables ($P<0.05$) by using the IBM SPSS Statistics 23 software. If there was any significant difference observed ($P<0.05$), then Tukey's post-hoc test was used to evaluate where the differences appeared.

Results and Discussion

Phytochemical constituents of Sargassum. Crude extract of *S. echinocarpum*, *S. polycystum* and *S. duplicatum* contained 46%, 44% and 45% carbohydrates (Table 1). As for their cellulose and hemicelluloses contents, it ranged from 25% to 30% and from 8% to 11%, respectively. Acid detergent fiber (ADF), which was the least digestible fiber portion in *Sargassum*, totaled up to 18% of dry extract. In the crude extracts of *S. echinocarpum*, *S. polycystum* and *S. duplicatum,* crude protein represents 7%, crude fat 9%, ash 14%, fiber 5% and lignin 0.5%.

Table 1

Phytochemical constituents of *Sargassum* spp.

No	*Content*		*Sargassum echinocarpum*	*Sargassum polycystum*	*Sargassum duplicatum*
1	Water	%	19.28	21.8	18.67
2	Ash	%	13.62	12.96	14.25
3	Crude Protein	%	6.43	6.8	7.15
4	Crude Fat	%	9.05	9.27	9.52
5	Acid Detergent Fiber	%	18.23	15.2	13.28
6	Fiber	%	4.78	4.68	4.55
7	Cellulose	%	30.24	28.83	25.56
8	Hemicelullose	%	11.23	8.67	9.1
9	Lignin	%	0.46	0.35	0.4
10	Carbohydrate	%	46.84	44.49	45.86

Antibacterial activity of moisturizer creams. Results demonstrated that the dilutions of 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} did not present positive reactions towards *S. epidermidis*. Therefore, these four dilutions were excluded from further analysis. Focus was given specifically to the three first dilutions of moisturizer creams, 10^{-1}, 10^{-2} and 10^{-3}.

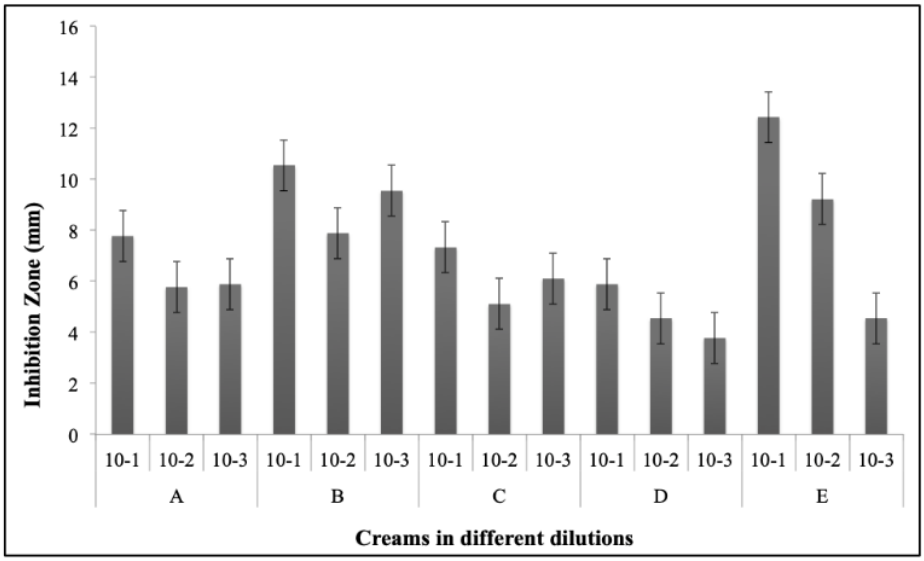

Figure 1. Inhibition zone (mm) of the 5 moisturizer creams in three different dilutions. A - moisturizer cream with standard formula; B - moisturizer cream without antibacterial agent; C - moisturizer cream with antibacterial agent; D - moisturizer cream with *Sargassum* mixed extracts and commercial antibacterial agent; E - moisturizer cream with *Sargassum* mixed extracts, but without commercial antibacterial agent.

The media showed no bacterial development in all tested creams 24 hours after incubation. The same case happened with the cream dilutions. However, after 48 hours from incubation, the media distributed with cream dilutions began to show different proportions of bacterial development. The indication of bacterial development was the white spots appearing beneath the agar media. Some cream dilutions showed small bacterial development, nearly invisible unless examined thoroughly. On the other hand, some creams showed obvious round-shaped bacterial development. Opinions related to those bacterial developments tended to refer to the contamination of the media caused

by the unwrapping process during observation. Furthermore, slightly warm room temperatures occurred due to direct morning sunlight heating the room even with window curtains closed. This condition could have led to the emerging of water dew under the Petri dish lid, causing the media to get humid. Details are presented in Table 2.

After knowing that the 24 hours incubation showed no bacterial development even though it appeared after 48 hours, further tests will be performed. Based on the results obtained after the 24-hour incubation, indicating that the creams were not contaminated, the next step conducted will be the antibacterial activity. This test will reveal whether or not the creams still possess an antibacterial activity after being stored for one year, especially the one containing the *Sargassum* mixed extract (Table 2).

Table 2

Observation of antibacterial activity from 5 moisturizer creams after 24 and 48 hours

No	*Samples*	*Observation (hours)*	
		24	48
1	Cream A	There were no bacterial developments in any of the Petri dishes; the media remained transparent.	Dilution of 10^{-1} did not show any bacterial development beneath the media. 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions showed different levels of bacterial development beneath the media.
2	Cream B	There were no bacterial developments in any of the Petri dishes; the media remained transparent.	Dilution of 10^{-1} did not show any bacterial development beneath the media. 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions showed different levels of bacterial development beneath the media.
3	Cream C	There were no bacterial developments in any of the Petri dishes; the media remained transparent.	For Cream C, dilution of 10^{-7} did not show any bacterial development beneath the media. As for 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-1} dilutions showed different levels of bacterial development beneath the media.
4	Cream D	There were no bacterial developments in any of the Petri dishes; the media remained transparent.	For Cream D, 10^{-6} did not show any bacterial development beneath the media. Meanwhile, for 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-1} dilutions showed different levels of bacterial development beneath the media.
5	Cream E	There were no bacterial developments in any of the Petri dishes; the media remained transparent.	All dilutions showed bacterial development.

Based on the statistic analysis, as presented in Table 3, moisturizer cream E had the best antibacterial activity followed by cream B, C, D, and A. Cream A showed the weakest antibacterial activity.

Table 3

Two-way Anova of *Sargassum* moisturizer cream prepared in different dilutions, significant difference at P<0.05

Source of variation	*df*	*F*	*P*	*Tukey P<0.05*
Cream	4	2.227	0.07	$A^{a} < D^{ab} < C^{ab} < B^{ab} < E^{b}$
Concentration	2	3.857	0.024	$10^{-2a} < 10^{-3ab} < 10^{-1b}$
Cream*Concentration	8	3.701	0.001	

Note: the same superscript letters show no significant differences.

Sargassum have been reported to contain 60% polysaccharides (Marinho-Soriano et al 2006; Haque et al 2009), 6-13% crude protein (Balboa et al 2013), 5% lipids (van Ginneken et al 2011), and 30% minerals (Balboa et al 2016). Cellulose of brown algae makes up the main structural skeleton (Kloareg & Quatrano 1988) and its content ranges from 5.7 to 14% (Kloareg & Quatrano 1988; Park et al 2000). The phytochemical composition determined in this study was in accordance with the range observed in previous studies.

Active compounds contribute to the antibacterial activity of moisturizer creams. They are usually polyphenolic compounds. Polyphenol in brown algae, mainly from the phlorotannins group, presents strong antioxidant and antibacterial activities (Wang et al 2009; Puspita et al 2015). Moisturizer cream E (cream with *Sargassum* mixed extract but, without commercial antibacterial agent) was the one with the best antibacterial activity out of the tested creams. The effectiveness of cream E in inhibiting the growth of bacteria might be due to the presence of the *Sargassum* mixed extract.

Polyphenols exhibit highly active antioxidative properties and are also involved in photoprotection mechanisms, particularly to counteract the cytotoxic effects of UV radiation. Zaragoza et al (2008) demonstrated some interesting activities like preventive and curative activities against atheroms of a hydro alcoholic extract from *Fucus.* Several antibacterial and antifungal (Nagayama et al 2002; Lopes et al 2012), antilarval and antialgal activities (Ragan & Glombitza 1986; Bhadury & Wright 2004; Hellio et al 2004), as well as UV-protection (Connan 2004; Le Lann et al 2008) were observed for phlorotannins isolated from macroalgae.

As polymers of phloroglucinol, phlorotannins of brown seaweeds exhibit strong antioxidant activities (Shibata et al 2008; Wang et al 2012). Phlorotannins possess superior antioxidant properties compared with catechin, a-tocopherol and ascorbic acid (Shibata et al 2008). The high antioxidative capacity of phenolic compounds from brown algae make them substantial candidates as natural products for the cosmetic industry. Moreover, the bioavailability of *Sargassum* as a source of phenolic compounds brings particular interest in studying this genus, because it has not been optimally explored.

Conclusions. The cream cosmetics with *Sargassum* extracts presented good texture, odor and color. These results indicate that *Sargassum* spp. show a promising potential as a cosmetic additive that might replace commercial antibacterial agents. Extracts from this species could serve as preservatives. This effect might be due to the antibacterial compounds contained in the extracts. This preliminary study has demonstrated other possible benefits of *Sargassum* spp. extracts in moisturizer creams. Nevertheless, further studies need to be performed with more detailed analysis related to the feasibility of this species to be commercially used in the cosmetic industry.

Acknowledgements. The authors would like to acknowledge Diponegoro University, Semarang, Indonesia, for its financial support through the International Publication Research (RPI) 2018 (Contract number:474-83/UN7.P4.3/PP/2018). Furthermore, authors extend their gratitude to the technical assistance performed by all parties involved during this research.

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Received: 17 January 2019. Accepted: 4 August 2019. Published online: 30 October 2019.
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How to cite this article:
Widowati I., Suprijanto J., Trianto A., Puspita M., Bedoux G., Bourgougnon N., 2019 Antibacterial activity and proximate analysis of *Sargassum* extracts as cosmetic additives in a moisturizer cream. AACL Bioflux 12(5):1961-1969.

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