

## The Potential Secondary Metabolites of Macroalgae *Sargassum polycystum* C. Agardh (1824) from the Coast of West Aceh as Raw Material of Body Scrub

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**ABSTRACT** *Sargassum polycystum* is one brown seaweed with a rich alginate source. The previous study showed that *S. polycystum* could be used as a cosmeceutical raw material because of its natural attribute and safe. *S. polycystum* always grows in many coastal of Indonesia. This study aims to characterise brown seaweed (*S. polycystum*) as the raw material of the body scrub. The research method includes the extraction, phytochemical assay, antioxidant assay, and total phenolic content-the phytochemical screening-detected alkaloid and steroid compounds in *S. polycystum* extract. The antioxidant activity by using the CUPRAC method showed the ethanol extract was  $53.55 \pm 1.07 \mu\text{M/g}$  and FRAP was  $201.95 \pm 6.33 \mu\text{M/g}$  with total phenolic content was  $177.647 \pm 21.39 \text{ Mg GAE/g}$ . We compared the antioxidant capacity of the body scrub by adding brown seaweed of *S. polycystum* 7% (S7) used the CUPRAC method was  $34.23 \pm 0.33 \mu\text{M/g}$ , and FRAP was  $124.05 \pm 1.87 \mu\text{M/g}$  with total phenolic content was  $416.31 \pm 78.44 \text{ Mg GAE/g}$ . Kruskal Wallis analysis showed that adding brown seaweed *S. polycystum* with different formulas influenced the panellist's preference for smearing ability, odor, texture, colour, and consistency. According to the Mean rank obtained, the level of preferences of the panellists was gained from product S7 namely body scrub with the addition of brown seaweed *S. polycystum* as much as 7%. It is indicated that the brown seaweed *S. polycystum* possesses body scrub raw material that is prospective in the cosmeceutical industry.

**Keywords:** Body scrub; brown seaweed; raw material; *S. polycystum*

### INTRODUCTION

Indonesia The marine environment consists of various organisms, such as algae, molluscs, sponges, corals, and tunicates. Oceans are called the "Lung of the Earth" due to Cyanobacteria and other algae living there, contributing up to 80% of the atmospheric oxygen we use to breathe. Algae are almost ubiquitous among microscopic and macroscopic species, found in every wet environment on land, freshwater, or oceans (Lewin & Andersen, 2019). Fucoxanthin, chlorophylls a and c, carotenoid pigments, reserve substances, oils, and polysaccharides (such as laminarin) are discovered in the brown algae (Vidotti & Rollemberg, 2004; Barsanti & Gualtieri, 2014). Today, cosmeceuticals attract the attention of industries and consumers. It is not yet officially legal, but the industry uses this term involving a product with cosmetics and pharmaceuticals benefits (Vermeer et al., 1996; Brandt et al., 2011). Natural cosmetics are continuously in demand for their benefit. They offer to replace the dangerous chemicals in traditional cosmetics products. Thus, the cosmetic industry is rapidly growing to satisfy the demands.

Some of the primary natural ingredients in cosmetics are

seaweeds, one of the wealthiest marine sources of vitamins, minerals, amino acids, antioxidants, and essential fatty acids. Seaweeds are unique with their bioavailable ingredients. Its active, nutrient-rich compounds can be absorbed easily by the skin and body. Due to their bioavailable nature, including reducing redness and blemishes, brightening, hydrating, re-mineralising, reducing sun damage and firming skin (Thomas & Kim, 2013; Pereira, 2018; Osea Malibu, 2020).

One of the promising seaweed species for the cosmeceutical industry is *Sargassum*. The genus *Sargassum*, a type of brown algae, is tropical and subtropical brown seaweed in subtidal and intertidal areas, with 150 species (Olabarria et al., 2009). Seawater current and substrate type (i.e., rocky shores) contribute to the distribution and population structure of the *Sargassum* (Ang, 1986; Ateweberhan et al., 2005). Various types of substrate in Aceh coasts, Indonesia, especially West Aceh coasts, have abundant resources of *Sargassum* (Gazali & Nurdin, 2017). Studies have revealed the seaweed diversity in Aceh, distributed along the coastlines (Gazali et al., 2019; Gazali et al., 2019; Gazali et al., 2020; Gazali et al., 2021). However, the exploration for

cosmetic prospects like body scrubs remains limited.

Studies related to the *Sargassum* genus have shown the high antioxidant potential in vitro (Lim et al., 2002; Santoso et al., 2004; Kim et al., 2005; Park et al., 2005; Cho et al., 2007; Zubia et al., 2007; Zubia et al., 2008; Budhiyanti et al., 2011; Gazali et al., 2018) that was utilised in body scrub products by the cosmeceutical industry. Antioxidant compounds are very fascinated in the pharmaceutical industry. Antioxidants can be added in preparing cosmetic ingredients because they possess compound activities against free radicals (Chermahini et al., 2011). Recently, the increasing application of natural antioxidants from terrestrial plants in cosmetic raw materials consists of polyphenols and terpenes compounds. Polyphenols have -OH groups attached to the benzene ring. The antioxidant activity is determined by the number and the position of -OH groups on the benzene ring. Phenolic groups also inhibit lipid peroxidation against free radicals. Flavonoids and stilbenes are the largest groups of polyphenols, and the terpene groups are carotenoids that serve as singlet oxygen quenchers (Pouillot et al., 2011). Latifah et al. (2021) reported that the *Sargassum* sp with the addition of turmeric rhizome powder in the body scrub raw material possesses intense antioxidant activity. The antioxidant compound that has an important role is the phenolic group in the *Sargassum* sp plant that enhances the functional value of body scrub, such as antioxidant activity.

The body scrub consists of rough material to remove dead skin cells (Alam, 2009). Its primary ingredients are equal to those used in cleansing creams with extra ingredients, i.e., abrasive, coarse grains to remove dead skin cells (Ulfa et al., 2016). Body scrub ingredients may originate from synthetic and natural ingredients. Natural ingredients include apricot seeds, walnuts, and almonds, while synthetic ones are polyethylene and oxidised polyethylene (Ertel, 2006). People have recently preferred cleansing products with natural ingredients because they are safer and rarely irritate. One of the potential brown seaweed from seawater is *S. polycystum*. This study aimed to characterise brown seaweed *S. polycystum* in the raw material of body scrub.

## MATERIALS AND METHODS

### Samples collection and formulation

Samples of brown seaweed *S. polycystum*, dried algae, were gathered from the coast of Lhok Geulumpang, Aceh Jaya, Indonesia. Fresh *S. polycystum* was washed, chopped and crushed using a blender. The dried *S. polycystum* was prepared using the same fresh *S. polycystum*.

### Chemicals and instruments

The chemicals used were 4,6-tri (2-pyridyl)-s-triazine (TPTZ), Dragendorff's reagent, mercuric chloride, ascorbic acid, potassium iodide bought from Sigma-Aldrich. The ethanol pa (Merck), methanol pa (Merck), ethyl acetate pa (Merck), n-hexane pa (Merck), hydrochloric acid (HCl), sulfuric acid ( $H_2SO_4$ ), chloroform, ammonia, glacial acetic acid, sodium hydroxide (NaOH),  $CuCl_2 \cdot 2H_2O$  (Merck),  $FeCl_3 \cdot 6H_2O$  (Merck), Follin-Ciocalteu, 2- deoxyribose and  $H_2O_2$  (30%, v/v) were from Merck and potassium

peroxodisulfate. This study used the ultraviolet-visible spectrophotometer of Shimadzu, Pharmaspec-1700, as the instrument.

### The proximate analysis

The chemical compositions were examined by proximate analysis using the AOAC. They included the water, ash, carbohydrate, protein, and fatty acid content (AOAC, 2005).

### Extraction of *S. polycystum*

The simplicity of *S. polycystum* (300 g) was extracted using three solvents (ethanol, ethyl acetate, and n-hexane). The maceration was done with a ratio of 1:3, with three solvents soaked and filtered by filter paper. A vacuum rotary evaporator was used to evaporate the solvents. The extracts determined antioxidant activity.

### Phytochemical analysis

The qualitative analysis of the phytochemical compound from brown seaweed *S. polycystum* extract can be identified by observing the extract's colour changes added with several chemical compounds. The initial phytochemical analysis of different extracts was undertaken based on Khandelwal, Wallis, and Harborne (Harborne, 1999; Wallis, 2005; Khandelwal, 2007). The phytochemical compounds include phenolic, flavonoid, tannin, saponin, alkaloid, and terpene.

### Antioxidant activity of FRAP (ferric reducing antioxidant power)

The FRAP antioxidant activity of *S. polycystum* extract refers to Kumar et al. (2012) with little modification. The reagent preparations of FRAP consisted of acetate buffer 300 mM (8 mL  $CH_3COONa$  and 92 mL  $CH_3COOH$ ) pH 3,6 10 mM TPTZ solution (2,4,6-tripyridyl-striazine) in 40 mM HCl and  $FeCl_3 \cdot 6H_2O$  20 mM. The freshly prepared solution was mixed with 3.5 mL acetate buffer, 3.5 mL TPTZ dan 3.5 mL of  $FeCl_3 \cdot 6H_2O$ . Absorbance measurement used samples of 50  $\mu$ L, 600  $\mu$ L aquadest, and 3000  $\mu$ L of FRAP reagent. The sample mix and FRAP reagent were homogenised using vortex before being incubated using a water bath for 30 minutes (37 °C). Absorbance was measured in wavelength with Trolox solution with various concentrations.

### Antioxidant activity of CUPRAC (cupric reducing antioxidant capacity)

Antioxidant activity with CUPRAC Assay according to Apak et al. (2008) with modification. *S. polycystum* crude extract (0.3 mL) were diluted with ethanol 99.9% with with 1 mL  $CuCl_2 \cdot 2H_2O$  0.01 M, 1 mL neucoprine 0.0075 M, 1 mL of ammonium acetate buffer pH 7 1 M and 0.8 mL aquadest. The sample mix and this reagent were homogenised using a vortex, followed by incubation at the temperature room (30 minutes). Absorbance was measured with a wavelength of 450 nm. The calibration curve was made using a Trolox solution with various concentrations.

### Determination of total phenolic content

The Follin-Ciocalteu method refers to Chandini et al. (2008) to examine the total phenolic content (TPC). Firstly, 2 mL of 2% sodium carbonate was put into the solution 100  $\mu$ L aliquot, adding 100  $\mu$ L of 50% Folin-Ciocalteu's phenol reagents after 2 min. The mixtures

were put for 30 min at dark ambient temperature, and the absorbances were measured at 720 nm. The total of phenolic compounds was generated following a phloroglucinol standard curve by plotting concentration ( $\text{mg m}^{-1}$ ) and absorbance (nm), with regression equation of  $y = 3.910x + 0.002$ ,  $R^2 = 0.99$ , where  $x =$  concentration and  $y =$  absorbance. The phenolic content was expressed as g of Gallic Acid Equivalent (GAE) per 100 g of the dry extract. This analysis was made in three replications for each extract.

#### Formulation of body scrub

The body scrub was made in three formulas, 1, 2, and 3, using dried *S. polycystum*. In the preparation stage, dried *S. polycystum* was chopped and crushed to a pulp using a blender. The seaweed pulp was then dried using a freeze dryer. The body scrub was formulated by mixing water bases 4, 5, and 6 using dried *S. polycystum*. The body scrub was formulated by mixing water-base (propylene glycol, glycerine, aquadest) and oil base (cetyl alcohol, stearic acid, DEA, perfume) at 70 °C and 80 °C, then the water base and oil base were mixed to form a cream. Afterwards, the mixture was added with 3% of *S. polycystum* konjac flour and a preservative (methylparaben or chitosan). The body scrub was then analysed.

#### Body scrub characteristic

Body scrub characteristic containing *S. polycystum* and konjac flour includes mineral content, pH, and hedonic test. A hedonic test was carried out on 30 panellists. Parameters of the hedonic test include colour, aroma, consistency, texture and appearance.

#### Statistical analysis

The three replicates' mean and standard deviation (SD) presented the experimental results. Linear regression analysis was also performed. Hedonic test by Kruskal Wallis Test. Analysis was conducted using SPSS. The result that showed the differences are continued with the Duncan Test.

## RESULTS AND DISCUSSION

#### Chemical composition

Seaweed extract has a high nutritional value, benefiting human nutrients, such as a protein with all essential amino acids, minerals, and vitamins. Also, they comprise bioactive secondary metabolites and many other compounds beneficial for health (Wong et al., 2000; Zvyagintseva et al., 2005; Alves de Sousa et al., 2007; Cardozo et al., 2007; Artan et al., 2008; Choi et al., 2009; Cho et al., 2009). The chemical composition of brown algae *S. polycystum* is in Table 1.

Table 1. The chemical composition of dried *S. polycystum*.

The components	Content (%)
Water content	32.70
Ash content	23.13
Protein	6.72
Fatty acid	2.00

The chemical composition of *S. polycystum* can be seen in Table 1. The result showed that the water content of *S. polycystum* was obtained at 32.70%. The water content in

seaweed is always influenced by drying processing. Osman et al. (2011) revealed that the brown seaweed's water content from the red sea, namely *S. subrepandum*, was 13.02%, and *P. tetrasromatica* was 13.37%. Ash content of *S. polycystum* was 23.13%. This value indicated that the *S. polycystum* has high ash content. The variation of ash content can be related to the amount of an organic compound and salts. Vijay et al. (2017) reported that brown seaweed contained high ashes content (45.04%), followed by red seaweed (28.79%) and green seaweed (14.10%). The fatty acid of *S. polycystum* has a low value, namely 2.00%. Garcia et al. (2016) stated that seaweed species that grow in tropical areas possess lower fatty acids than those from subtropics areas. The protein content from *S. polycystum* was 6.72%. The protein of brown seaweed is lower (5-15%) than in red seaweed and green seaweed. The analysis of crude fibre from the sample of *S. polycystum* was 10.48%. The value is still lower than the crude fibre of other seaweed. Generally, crude fibre was 30-40% dried weight (Ate et al., 2017). Crude fibre is a carbohydrate component that can be hydrolysed using intestine enzymes.

#### Phytochemical result

The phytochemical analysis result of *S. polycystum* extract is presented in Table 2. The *S. polycystum* extract possesses alkaloid and steroids compounds, according to the results. Alkaloids are a chemical compound group diluted in organic solvents and primarily found in the extract that used polar solvents. Alkaloid possesses the potential as an antioxidant source because it is a polar compound and is also extracted in polar solvents (Sa'adah & Nurhasnawati, 2015). Mooradian (1993) reported that steroid compounds are an index of antioxidant properties of these compounds.

Table 2. The bioactive compound of dried *S. polycystum*.

Phytochemical properties	<i>S. polycystum</i>	Information
Alkaloid		
-Dragendroff	+	Orange/yellow
-Wagner	+	Orange/yellow
-Mayer	-	-
Tannin	-	-
Flavonoids	-	-
Saponin	-	-
Phenol Hydroquinone	-	-
Triterpenoid	-	-
Steroids	+	Blue-green

Note: Detected (+); Not Detected (-).

Seaweed is rich in secondary metabolites, including alkaloids, phenols, flavonoids, saponins, steroids, and related active compounds, with high medicinal values and is widely used by pharmaceutical industries. Some latest global studies have revealed the phytochemistry of seaweed (Selvin & Lipton, 2004; Fayaz et al., 2005; Somepalli et al., 2007; Adaikalaraj et al., 2011).

#### Antioxidant capacity and total phenolic content

According to the FRAP method, the antioxidant activity value is  $201.95 \pm 6.33 \mu\text{M/g}$ . This result showed that the



*S. polycystum* extract has the potential as an antioxidant agent for body scrub products. Diachanty et al. (2017) reported that the *S. polycystum* extract from Seribu Island has 105.357  $\mu\text{mol}$  trolox/g extracts. The value of FRAP antioxidant capacity is higher than *S. polycystum* from Seribu Island, Jakarta. The FRAP is a determination method of antioxidant activity to determine the antioxidant ability for decreasing  $\text{Fe}^{3+}$  in  $\text{Fe}^{3+}$ -TPTZ complex to be  $\text{Fe}^{2+}$ -TPTZ with donor their electron. The previous study shows that *S. tenerrimum* possesses 4.076,54  $\mu\text{mol}$  trolox/g (Guru et al., 2015) and *P. tetrastomatica* 1,168 mg ascorbic acid/g (Megha & Anjali, 2013).

**Table 3.** Antioxidant capacity and total phenolic content of *S. polycystum*.

Sample	Total phenolic content mg GAE/g	CUPRAC ( $\mu\text{M/g}$ )	FRAP ( $\mu\text{M/g}$ )
<i>S. polycystum</i>	177.647 $\pm$ 21.39	53.55 $\pm$ 1.07	201.95 $\pm$ 6.33

The CUPRAC antioxidant activities of *S. Polycystum* were 53.55 $\pm$ 1.07  $\mu\text{M/g}$  showing that *S. Polycystum* extract has more phenolic compounds than other crude extracts. Fadhilah (2018) reported that the content of ethanol extract from brown seaweed, *Sargassum* sp showed relatively antioxidant solid activity with  $\text{IC}_{50}$  of 56.60 ppm. Subsequently, the best herbal body scrub formula is with the addition of 1% carrageenan. Body scrub produced had a pH value, viscosity, and scatter following the standard.

Apak et al. (2008) argued that the CUPRAC method is more common for measuring the antioxidant capacity of the phenolic compound. Therefore, this *S. Polycystum* crude extract was predicted to have more phenolic compounds. The CUPRAC method was based on Cu (II) – Cu (I) reduction by antioxidants due to neocuproine. The antioxidant activity was investigated using a CUPRAC assay with a routine compound. It was created from several concentration ((10 $\mu\text{g/mL}$ , 15  $\mu\text{g/mL}$ , 20  $\mu\text{g/mL}$ , 25  $\mu\text{g/mL}$  dan 30  $\mu\text{g/mL}$ ) before adding  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  0,01 M solution, ethanol neocuproine 0.0075 and acetate ammonium buffer pH 7 1M. Antioxidant activity, the capacity of CUPRAC reduction, was based on sample absorbance decreasing to control absorbance (Apake., 2008). Nevertheless, the FRAP method showed the potential antioxidant capacity (201.95 $\pm$ 6.33  $\mu\text{M/g}$ ) to be the raw material of body scrub.

#### Characteristic of body scrub

The body scrub was made into three different formulas that are formula 1 (S3) (body scrub with added dried *S. polycystum* 3%), formula 2 (S5) (body scrub with added *S. polycystum* 5%) and formula 3 (S7) (body scrub with added *S. polycystum* 7%). To identify the sensory of body scrub product of brown seaweed *S. polycystum*, we have involved panellists in giving appraisals on body scrub products. The panellists conducted the appraisal of body

scrub with the addition of brown seaweed *S. polycystum* 3% (S3), 5% (S5), and 7% (S7). The organoleptic test, including the smearing ability, aroma, texture, colour, and consistency parameters, is displayed in Table 4.

The Kruskal Wallis analysis showed that differences in body scrub formula significantly affected panellist acceptance of body scrub smearing ability ( $p > 0.05$ ) see Table 4. The average value of the panellists' preference for smearing ability has ranged from 2.70 to 3.32 (neutral-prefer). Duncan's other test results show that formula 1 (S3) was not significantly different from

formula 2 (S5) except for formula 3 (S7). Panellists prefer more than the smearing ability of body scrub with dried *S. polycystum* in formula 3, adding *S. polycystum* 7%. The average value of the panellists' preference for aroma ranged from 2.67 to 3.70 (instead prefer). This showed that the panellists liked the aroma. The result of Kruskal Wallis analysis showed that differences in body scrub formula did not have a significant effect on panellist acceptance of the aroma of body scrub ( $p > 0.05$ ) except the formula 3 (S7) see Table 4. The aroma of that panellist was caused by the addition of perfume to the body scrub.

The results of Kruskal Wallis analysis show that differences in body scrub formulas significantly affect panellist acceptance of the texture of body scrub ( $p > 0.05$ ) see Table 4. The average value of panelists' preferences for body scrub textures ranges from 2.77-to 3.53 (neutral-prefer). Duncan's further analysis showed that formula 1 was not significantly different from formula 2 but significantly different from formula 3. Formula 3 (S7) found the most preferred texture, with a value of 3.53. panellist's assessment showed that most panellists liked the texture of body scrub with dried *S. polycystum*. The colour test showed that differences in body scrub formula significantly affected panellist acceptance of body scrub colour ( $p > 0.05$ ). Duncan's test results showed that the panellist's preference for the colour of formula 1 was not significantly different except for formula 3 (S7). The most preferred colour was formula 3 (S7), with a value of 3.87. The colour of the body scrub is affected by *S. polycystum* seaweed, which has brown. Inconsistency parameter showed that panellist preferences value of all formulas ranges from 3.37-3.60 (instead prefer). Duncan's further test showed that all formula inconsistencies were not significantly different. Several body scrubs have additional ingredients like konjac flour, which contains

**Table 4.** Hedonic value of body scrub of *S. polycystum*.

Formulas	Smearing ability	Aroma	Texture	Color	Consistency
S3 (Formula 1)	2.70 <sup>ab</sup>	2.67 <sup>a</sup>	2.77 <sup>a</sup>	2.50 <sup>a</sup>	3.40 <sup>abc</sup>
S5 (Formula 2)	2.53 <sup>a</sup>	2.73 <sup>a</sup>	2.73 <sup>a</sup>	2.77 <sup>ab</sup>	3.37 <sup>ab</sup>
S7 (Formula 3)	3.32 <sup>bc</sup>	3.70 <sup>cd</sup>	3.53 <sup>b</sup>	3.87 <sup>d</sup>	3.60 <sup>bc</sup>

Note: different superscript letters indicate significant differences ( $P < 0.05$ ).

high glucomannan. Wigoeno *et al.* (2013) reported that adding konjac flour to the formula results in a buttercream consistency. Body scrub becomes thicker after adding konjac flour. Besides adding konjac flour, chitosan added to the formula can provide a thick cream consistency of body scrub.

The hedonic value showed the highest value. With the addition of brown seaweed *S. polycystum* with different formulas, Kruskal Wallis influenced the panellist's level of preference for smearing ability, aroma, texture, colour, and consistency parameters. According to the Mean rank obtained, the level of preferences of the panellists was gained from product S7 namely body scrub with the addition of brown seaweed *S. polycystum* as much as 7%. The pH value of body scrub with the addition of *S. polycystum* 7% (S7) is  $6.56 \pm 0.02$ . Body scrub with the addition of *S. polycystum* 7% (S7) has a stable emulsion that has not occurred during the separation of the oil and water phase in the product, emulsion stability of body scrub (Figure 1). The results were emphasised antioxidant activity that explained previously.



Figure 1. The emulsion stability of body scrub with the addition of *S. polycystum* 7%.

Sampebarra (2016) believed that the higher the consistency and the better the product's texture, the higher the smearing ability. The consistency and thickness of the body scrub in this study were promising, making it convenient and easy to put evenly on the skin's surface.

## CONCLUSIONS

We concluded that the extract of brown seaweed *S. polycystum* has a potential antioxidant agent used as the raw material of body scrub products with relatively strong antioxidant activity. The difference in body scrub formula significantly affected its smearing ability, colour, consistency, and texture. Based on the hedonic assessment result, the best body scrub formula was a body scrub using 7% of dried *S. polycystum*.

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## AUTHORS' CONTRIBUTIONS

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