Secondary Metabolites Content of Seaweed (*Sargassum sp.*) Based on the Different Drying Methods

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ABSTRACT
Sargassum sp. is a non-consumable and underutilized seaweed that is considered waste, although it has the potential to provide secondary metabolites to ruminants. The purpose of this study was to see how different drying processes affected the secondary metabolite content of the seaweed *Sargassum sp* seaweed. Gunungkidul Yogyakarta, Indonesia, provided the *Sargassum sp* seaweed. They were rinsed with seawater after being collected to remove any unwanted materials. Sun-drying (drying for three days from 7:00 a.m. to 2:00 p.m.), oven-drying (at 55°C for four days), and freeze-dryer-drying were the three methods used to dry Sargassum sp. (at -20°C for 3 days from 07.30 to 3.30 pm). The seaweed was then pulverized using a Willey mill, and the secondary metabolite content was determined using the UV-vis Spectrophotometer technique. Using SPSS, the data were analyzed using a one-way analysis of variance (ANOVA). The sun-drying, oven, and freeze-drying treatments of the seaweed Sargassum sp. had no significant influence ($P>0.05$) on total alkaloids and saponins but did have a significant effect ($P<0.05$) on flavonoids, tannins, and total phenols. Sun-drying and oven-drying have nearly identical secondary metabolite concentrations, while both are lower than freeze-dryer drying.

Keywords: *Alkaloid, tannin, saponin, phenolic, flavonoid*

1. INTRODUCTION

Secondary metabolite compounds are chemical compounds found in plants which have, bioactivity ability to protect these plants from pests or environmental disturbances. Secondary metabolites are chemical compounds modified from primary metabolites, including genetic material, medicinal substances, and biotoxins [1]. In everyday life, secondary metabolite compounds are used as coloring agents, poisons, and food aromas as well as medicines such as antiparasitic, antifungal, and antiviral agents. Secondary plant metabolite compounds include flavonoids, tannins, terpenoids, saponins, phenols, and alkaloids [2,3]. Secondary metabolite compounds that are originated from both plants on land and seaweed are beneficial for ruminants, as mineral sources. The advantage of seaweed is not only used as a source of secondary metabolites for livestock but also as a source of macro and micro-organic minerals, which is 10-20 times better than plants on land [4].

Seaweed contains various natural bioactive sources of secondary metabolites which are very potential in the field of animal husbandry, especially for ruminants. Seaweed secondary metabolites include alkaloids, flavonoids, terpenoids, and tannins, as well as saponins [3]. In addition, seaweed is a source of natural additives to substitute antibiotics in animals [5] Secondary metabolite compounds affect, antiparasitic in ruminant livestock production systems [6] Tannin compounds are used as anthelmintics because they can inhibit egg hatching and motility of nematode parasite larvae in the gastrointestinal of small ruminants [7] Tannins and saponins reduce methane gas production, in ruminants. Methane gas production in ruminants will lose 2-12% of

energy in feed ingredients which should be useful for livestock production [8] Phenols are useful for suppressing the amount of unsaturated fatty acids and volatile compounds in ruminant milk production [9] Flavonoids have anti-microbial effects.

The use of secondary metabolites of seaweed by ruminants is dependent on processing, particularly post-harvest processing. Fresh seaweed is made up of 75-85% water and 15-25% organic and mineral components. Sargassum sp. is perishable after a few days of harvesting in its fresh state. Because of the high-water content in the seaweed Sargassum sp. after collection, drying is required before determining the presence of secondary metabolites. The goal of drying is to limit water activity and microbiological development while preserving quality and reducing storage volume. [11]. Low water content is ideal for preventing bacteria and fungi from growing and causing harm to the material during storage [12].

Drying seaweed is very important because if it is fresh, it does not produce gel during processing [13]

After harvesting the seaweed, it was dried, sun-dried, oven-dried, and freeze-dried. The proximate composition, macro, and micro mineral content, and vitamin C of *Sargassum* hemyphyllum were examined in a study done in Tung Ping Chau, Northeast Hong Kong, using sun-drying, oven, and cold dryer methods [13]. Milledge and Harvey [14] reported that various countries on the Caribbean, Gulf of Mexico, and West African coasts sun-dried *Sargassum* sp. seaweed to test the amount of phenol as an anti-cancer agent in humans. Masdugi *et al*. [15] studied the effects of sun, oven, and wind drying on the phenol content of Sargassum polycystin from Jepara, Central Java. The results showed that wind drying had a lower phenol content than oven drying and sun-drying. Lalopua *et al*. [16] sun-dried red seaweed from Wael village, West Seram Regency, Maluku Province for 3 days on phenol content, yielding 15.93 percent in hexane extract. However, no research has been done to compare the secondary metabolites content of *Sargassum* sp. found in Sepanjang Beach Gunungkidul, Yogyakarta using the sun-drying method, oven, or freeze dryer. This study was designed to provide information on the number of secondary metabolites in seaweed *Sargassum* sp. from Sepanjang Beach, Gunungkidul Regency, Yogyakarta, Indonesia, using the methods of sun-drying, 55ºC oven, and freeze dryer drying at -20ºC.

2. MATERIALS AND METHODS

2.1. Drying Methods

Sargassum sp. was collected from Sepanjang Beach in Yogyakarta's Gunungkidul Regency. The seaweed was cleaned of dirt and other undesired things when it was collected. *Sargassum* sp. drying methods were split into three categories. The first way was to sun-dry it for three days, from 7:00 a.m. to 2:00 p.m. The second process involved four days of oven- drying at 55°C. The third approach was freeze dryer drying at -20ºC for three days, with each day starting at 7:00 a.m. and ending at 3.30 p.m. The seaweed *Sargassum* sp. was dried, then processed using a willy mill with a 1 mm filter diameter, and finally evaluated using the UV-vis Spectrophotometer method.

2.2. Phenolic Content

The reference method of [17] was used to calculate the total phenolic content. A 0.5 g dried *Sargassum* sp. the sample was homogenized after being disseminated in 10 mL distilled water. The dispersion was then centrifuged for 10 minutes at 3000 rpm. The supernatant was then removed and put into a tube, along with 5 mL water. After that, 1.5 mL of 10 percent Folin-Ciocalteu solution and 1.5 mL sodium carbonate 6 percent (w/v) were added to 0.6 mL of the resultant solution. After that, the sample was incubated for 60 minutes at room temperature. A UV-VIS spectrophotometer with a wavelength of 725 nm was used to measure the absorbance. A standard solution of gallic acid was utilized at five different concentrations: 5, 10, 15, 20, and 25 ppm. The following equation is used to calculate total phenolic content in gallic acid equivalents, where C, V, Df, and G are the approximate phenol concentration, sample volume, dilution factor, and sample weight, respectively.

Total phenolic content

$$\left(mg\ \frac{GAE}{100\ g}\right) = \frac{C\ x\ V\ x\ Df}{G}$$

2.3. Total Flavonoid Content

To assess total flavonoid concentration, Chang *et al*. [18] used the colorimetric aluminium chloride ($AlCl_3$) procedure. A 0.5 g sample was dissolved in 10 ml ethanol and homogenized for 2 minutes using a vortex. After that, the solution was centrifuged. A total of 0.6 mL of supernatant was combined with 1.5 mL of 95% ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1.0 M potassium acetate, and 2.8 mL of water. After that, the mixture was left to sit at room temperature for around 30 minutes. A UV-VIS spectrophotometer was used to measure the absorbance of the reaction mixture at a wavelength of 415 nm. The total flavonoid content was determined using a standard calibration curve based on quercetin. The approximate flavonoid concentration, sample volume, dilution factor, and sample weight were calculated using the following equation, where C, V, Df,

and G are the approximate flavonoid concentration, sample volume, dilution factor, and sample weight, respectively.

Total flavonoid content $\left(mg\ \frac{QE}{100\ g}\right) = \frac{C\ x\ V\ x\ Df}{G}$

2.4. Saponin Content

Harborne [19] described a double extraction gravimetric method for determining the saponin content of the samples. In a flask, a measured weight (5 g) of powdered materials was combined with 50 ml of a 20% aqueous ethanol solution. The mixture was cooled in a water bath for 90 minutes at 55oC with occasional agitation; it was then filtered through Whatman filter paper (No 42). The residue was extracted with 50 mL of 20% ethanol, and the combined extract was reduced to about 40 mL at 90°C, then transferred to a separating funnel with 40 mL of diethyl ether and forcefully shaken. The aqueous layer was periodically partitioned and re-extracted until the color of the aqueous layer became clear. With 60 mL of regular butanol, the saponins were extracted. In a pre-weighed evaporation dish, the mixed extracts were rinsed with a 5 percent aqueous sodium chloride (NaCl) solution and evaporated to dryness. It was dried in an oven at 60 degrees Celsius and then reweighed after cooling in a desiccator. To get an average, the operation was repeated twice. Saponin content was calculated as a percentage of the original sample using a different method**.**

Percentage of saponin = [(W2-W1)/weight sample] x 100%

2.5. Tannin Content

The tannin content was assessed using the method of Broadhurst *et al.* [21], with a minor change in that catechin was used as a reference ingredient. 400 mL of the extract is combined with 3 mL of vanillin (4 percent in methanol) and 1.5 mL of strong hydrochloric acid. The absorbance was measured at 500 nm after 15 minutes of incubation. g E. Catechin was used to represent the condensed tannin.

2.6. Alkaloids Content

The reference method of Sreevidya and Mehrotra [21] was used to determine the total alkaloids content. The calibration curve for bismuth nitrate is being prepared. 5 mL of 3 percent thiourea solution was mixed with 1 mL of 2 mg/mL bismuth nitrate solution. At 440-460 nm, the absorbance was measured. In a 10 mL flask, 5 mL of 3 percent thiourea solution was added to each of five different bismuth nitrate concentrations.

Each 5 mL of papaverine solution (pH 2-2.5) was reacted with 2 mL of dragendorff reagent. The precipitate was centrifuged and cleaned in 96 percent ethanol before being used. To make the blackish-brown chemical, the precipitate was mixed with 2 mL disodium disulfide and centrifuged. The precipitate was dissolved in 2 mL concentrated nitric acid, diluted with aqua dest in 10 mL in a volumetric flask, and then 1 mL of solution was added. The absorbance of a blank containing nitric acid and thiourea was measured at the maximum wavelength. By multiplying the absorbance with the factor and using a suitable dilution factor, the amount of bismuth was estimated. The calibration curve, which is a constant at varying concentrations, yields the factor. Recovery, quantification, and detection limits were all determined based on the findings. The total alkaloids content of a 5 mL extract was determined using the same method as papaverine.

2.7. Statistical Analysis

The data were analyzed using SPSS (Windows version of SPSS, release SPSS [22]) and one-way analysis of variance (ANOVA). The significance level was set to $P<0.05$. Duncan multiple range tests [23] were used to examine the difference between treatment means.

3. RESULTS AND DISCUSSION

The sun-drying method, the 55°C oven, and the -20°C freeze drier had no significant effect ($P>0.05$) on total alkaloids and saponins but had a significant effect ($P<0.05$) on alkaloids, tannins, and total phenols shown in Table 1.

The alkaloid content of the freeze dryer -20ºC drying method is higher than that of sun and oven drying. In comparison to freezing dryer drying, alkaloids were reduced by 280 mg/kg (18.91%) in oven drying and 94.48 mg/kg (6.38%) in sun drying.

The saponin content of freeze dryer drying treatment -20oC was higher than sun drying and oven drying, compared to freeze dryer drying, there was a decrease in saponins 125.15 mg/kg in oven drying and 210.88 mg/kg in sun drying. Based on the Duncan multiple range test (DMRT) for flavonoid content, the total phenol of freeze dryer drying was higher ($P<0.05$) than those of sun drying and oven drying, while sun-drying and oven drying were not significantly different ($P>0.05$). DMRT test results freeze dryer drying tannin content was higher ($P<0.05$) than oven drying but did not differ ($P>0.05$) with sun drying. Compared to freeze dryer drying, there was a 27.05% reduction in oven drying and 28.69% sun drying.

Table 1. Sargassum sp. Metabolite secondary content Different drying processes are used.

Parameters	Sun-dried	Oven-dried	Freeze-dried
Alkaloids (mg/kg)	432,99± 33,16[a]	518,69± 33,15[a]	643,87± 236,75[a]
Saponins (mg/kg)	1386,02± 183,56[a]	1200,50± 54,80[a]	1480,16± 292, 67[a]
Phenolic % (b/b)	6,61± 0,77[a]	6,80± 0,39[a]	9,43± 0,32[b]
Flavonoids (mg/kg)	2393,43± 1046,69[a]	4474,38± 444,97[a]	7233,03± 1795,41[b]
Tannins % (b/b)	0,89,53± 0,24[ab]	0,77± 0,04[a]	1,22± 019[b]

[a,b,c]Superscript of different within rows without common superscript differ at P<0.05

The low content of secondary metabolites in sun drying and oven drying due to drying Sargassum sp. At a maximum temperature of 55oC, oxidation and degradation have started so that the content of secondary metabolites begins to decline. Prathapan et al. [24] Sandra and Chong [25] found that high drying temperatures stimulate oxidation and degradation of secondary metabolites such as phenols and flavonoids so that their content begins to decrease. The process of decreasing phenol and flavonoid compounds with drying temperature is due to the loss of antioxidant compounds from plant biomass due to oxidation of fat components and the protective layer of plant tissue [26] Even though the tannin content was heated to high temperatures, the decrease was not up to 60%. Anbreen [27] reported that heating tannins by autoclaving at 125oC for 30 minutes decreased 1.32 grams / 100 g to 0.54 grams / 100 g or decreased 59.09%.

The results of this study showed that the flavonoid content in the oven drying method was 4.47 mg /100 g higher than the results of the research reported by Fauziah et al. [28] namely 1.98 mg/100 g; Sun-drying was lower, namely 2.39 vs 5.05 mg / 100g; while the higher phenolic content was oven drying 6.80 Vs 2.48% while sun drying was 6.61 Vs 3.00%. The difference is due to differences in the location and method before drying. This research seaweed was taken at Sepanjang Beach Gunungkidul Yogyakarta Indonesia, The sample soon after collection was washed with seawater to remove sand and dirt then dried on oven drying at 55oC for 4 days and sun drying for 3 days, while Fauziah et al., (28) the seaweed was taken from Talango Sumenep, Madura Island, East Java, Indonesia, where after collection the seaweed washed with tap water then cut and boiled at 85oC for 2 minutes and then dried in the oven-drying at 60oC and sun- drying until the weight is constant.

4. CONCLUSION

The content of secondary metabolites in sun-drying and oven-drying was not different but lower than that of the freeze dryer. Sun drying was the chosen method for practical aspects and economic reasons to determine the secondary metabolite content of *Sargassum* sp.

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