Characteristic and bioactive potential of brewed Sargassum sp. with the additional bay leaf (Syzygium polyanthum)

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Abstract. Brewed Sargassum sp. is a product of dried seaweed that is packaged in tea bags and then brewed. The natural fishy odor from seaweed itself makes consumer acceptance of this brewed drink relatively low. The purpose of this study was to determine the effect of the addition of bay leaves (Syzygium polyanthum) and to determine consumer acceptance using hedonic test for Sargassum sp. Samples were obtained from UD Seaweed Mandiri, Wonosari, Yogyakarta. The research method used is experimental laboratories with a completely randomized design (CRD) model. The treatment given was in the form of different concentrations of bay leaves, namely 0%, 10%, 20%, and 30%. Parameters observed were water content, ash content, image processing, hedonic, phytochemical, antioxidant activity, and bioactive components. Parametric data were analyzed using analysis of variance ANOVA and Honest Significant Difference to determine differences between treatments, while non-parametric testing included hedonic tests consisting of aroma and taste tests. The results showed that the different concentrations of bay leaves gave a significantly different effect on the hedonic parameters. Brewed Sargassum sp. with a concentration of 30% had the highest score on the hedonic test, namely 7.71 < µ < 8.11 which was included as favored by the panelists. The addition of bay leaves with a concentration of 30% resulted in an IC₅₀ value of 13.6251 ppm. Phytochemical screening which was carried out qualitatively showed positive results for the parameters of flavonoids, phenols, tannins, and saponins. The total flavonoid content of 30% concentration is 302,333 mg QE/g and produces six compounds with bioactive components. The highest compound is pentane at 69.12%. Based on the test of bioactive components in the brewed Sargassum sp. there is a pentane compound which has the potential as an antimicrobial and anti-inflammatory.

1. Introduction
Seaweed is known to have dominant polysaccharides, protein, a little fat, and ash in the form of sodium and potassium salts, vitamins A, B1, B2, B6, B12, and C and is rich in bioactive compounds, namely beta-carotene, chlorophyll pigments, and minerals [1]. Sargassum sp. is one of the potential sources of brown seaweed for functional food that can be used for health because it contains chemical compounds that have biological activity or bioactive substances. Biologically active compounds are secondary metabolites which include alkaloids, flavonoids, terpenoids, tannins and saponins as a source of natural antioxidants [2]. Sargassum sp.
contains a lot of fat, protein, vitamins and minerals. In addition, *Sargassum* sp. have bioactive compounds such as flavonoids, triterpenoids, polyphenols, chlorophyll.

The problem of brewed seaweed is usually the fishy aroma that consumers don't like. The source of the fishy aroma comes from trimethylamine compounds, fatty acids, ammonia and fatty acid oxidation. In addition, the percentage of antioxidant activity in all treatments was still categorized as weak [3]. One way to reduce the fishy smell and increase the bioactive compounds steeped in *Sargassum* sp. is to provide additional ingredients that have bioactive content such as bay leaves.

Bay leaves are quite effective and economical to be used as additives, because of their abundance and relatively affordable prices. In addition, bay leaf (*Syzygium polyanthum*) contains chemical compounds such as tannins, essential oils and flavonoids. Bay leaves contain flavonoids, triterpenes, tannins, polyphenols, and alkaloids as well as essential oils. Flavonoids are polyphenolic compounds that are useful as anti-inflammatory, antitumor, protecting blood vessels, antioxidants, antidiabetic, anticarcinogenic and as the body's defense system [4]. This shows that bay leaves have the potential to be used as drinks such as *Sargassum* sp.

Seaweed brewed explains that seaweed brewed is a product of seaweed in the form of small flakes and is brown or green in color with or without the addition of other ingredients and packaged in bags with or without ropes or adhesives for dyeing or brewing. Tea drinks and seaweed brews have the same principle, which is made from fresh raw materials that are dried and then brewed with hot water [5].

Brewed *Sargassum* sp. is a functional product whose process is very simple. The manufacturing process is similar to the extraction of fucoidan bioactive compounds from brown seaweed. Even the main components of brown seaweed such as alginate can also be extracted during brewing with hot water. This study aims to determine the characteristics and bioactive compounds of *Sargassum* sp. with the addition of bay leaf (*Syzygium polyanthum*).

2. Material and methods
The main ingredients used to make *Sargassum* sp. namely *Sargassum* sp. and bay leaf. *Sargassum* sp. obtained from UD Rumput Laut Mandiri, Wonosari, Yogyakarta, while bay leaves were obtained from Damar Market, Banyumanik, Semarang. Condition of *Sargassum* sp. in a dry state on the beach where there is dirt stuck to it, while the bay leaves are fresh. The main equipment used is a UV-VIS spectrophotometer.

2.1. Making infusion of *Sargassum* sp.
Samples of *Sargassum* sp. and bay leaves cleaned and washed to remove dirt that sticks. Then, *Sargassum* sp. soaked in hot water (blanching) at a temperature of 100°C for 1 minute. The next process, *Sargassum* sp. and bay leaves were dried by the wind dry method. The temperature used was ±27°C for 5-7 days until *Sargassum* sp. and bay leaves turn dark in color. Then, *Sargassum* sp. and bay leaves were blended and the samples were stored at 4°C before use. Each sample was tested for moisture content and ash content [6].

2.2. The process of making *Sargassum* sp.
The flakes of infusion of seaweed are put into an empty dipping bag. Making brewed dips, each bag is 3 g and sealed. The brewed dip is put into 100 mL of boiling water and left for six minutes. During the immersion, stirring is done 2-3 times while being raised and lowered into the boiling water. Next, the brewed dip is removed from the solution and then filled to 100 mL [7].
2.3. Hedonic test
Hedonic test parameters include color, aroma, and taste. This test uses 30 semi-trained panelists. The panelists gave an assessment in the form of a score on the hedonic test form steeped in *Sargassum* sp. Sensory scale information with a value of 9 = very much like; 7 = like; 5 = dislike; 3 = very dislike [8].

2.4. Image processing
a. Retrieve image data
Data retrieval was carried out in a box as a place to take digital images from *Sargassum* sp. This box is designed not translucent with a size of 24 cm x 21 cm x 39 cm made of alphaboard. The box used is equipped with 4 white 5-watt LED lights that are turned on during shooting, serving as light sources when taking sample pictures. At the top of the box there is a hole for placing the camera as a digital image capture tool. Brewed *Sargassum* sp. which has been prepared is then placed in a box that has been provided which has added lights and cameras.

b. Convert image from RGB, HSV, and L*a*b*
In the colorgrab application, the results will appear immediately consisting of RGB, HSV, and L*a*b*. The RGB color model (Red, Green, blue) is a color model that is widely used in the technology world, one of which is like a monitor screen. In addition to the RGB model, there is also an HSV (Hue, Saturation, Value) color model. Hue is a measure of the wavelength contained in the dominant color received by vision, saturation is a measure of the amount of white light mixed in the hue. Value is an attribute that states the amount of light received by the eye regardless of color [9].

Sampling of the RGB color model in this study used the same points starting from the control treatment image without the addition of bay leaves to the image of adding bay leaves with a concentration of 30%. Taking at the same point is done so that the data obtained is homogeneous and aims to avoid bias in the data. The results of the sampling are then converted into RGB, HSV and L*a*b* color models.

2.5. Extraction
A total of 100 g of *Sargassum* sp. The extraction process was carried out using the maceration method in methanol: (1 : 1) for 48 hours [10]. After that it was filtered and dried with a Rotary Vacuum Evaporator (EYELA N-1100). The maceration process was carried out once. Then, the dried extract was weighed and stored in the refrigerator.

2.6. Phytochemical screening test
Qualitative test of chemical content in 96% ethanol extract steeped in *Sargassum* sp. carried out with chemical reagents to identify the class of tannins, saponins, flavonoids, and phenols. The method used as in [11] is as follows:

2.6.1. Tannin test
A small amount of the extract sample was added to 10 mL of distilled water and then brought to a boil. Add a few drops of FeCl$_3$. The presence of a brownish green or bluish black color indicates a tannin compound.

2.6.2. Saponin test (foam test)
Saponins can be detected by the foam test in hot water. Stable foam will continue to be seen for 5 minutes and does not disappear when the addition of 1 drop of 2 N HCl indicates the presence of saponin compounds.
2.6.3. Phenol test
A number of samples were extracted with 20 ml of 70% ethanol. The resulting solution was taken as much as 1 ml and then added 2 drops of 5% FeCl3 solution. Positive compound reactions are indicated by the formation of a green or blue green color.

2.6.4. Flavonoid test
A number of samples were added with 0.1 mg magnesium powder and 0.4 ml of amyl alcohol (a mixture of 37% hydrochloric acid and 95% ethanol with the same volume) and 4 ml of alcohol and then the mixture was homogenized. A positive reaction is indicated by the formation of a red, yellow or orange color on the amyl alcohol layer.

2.7. Total flavonoid content test
The method used refers to the method of [12], using AlCl3 reagent. A total of 0.5 ml of extracts of with a concentration of 1000 ppm were pipetted into a test tube, added 1.5 ml of methanol, 0.1 ml of 10% AlCl3, 0.1 ml of 1 M CH3COOK and 2.8 ml of distilled water. The solution was homogenized and incubated for 30 minutes. The absorbance of the solution was measured with a UV-Vis spectrophotometer at a wavelength of 415 nm. Absorbance measurements were repeated 3 times. Quercetin was used as a standard with a concentration series of 50 ppm, 100 ppm, 150 ppm and 200 ppm. The quercetin calibration curve was used to determine the total flavonoid content contained in the sample through a regression equation and expressed in units of mg quercetin equivalent/g extract (mg GAE/g extract) with the calculation formula:

\[
C = \frac{C_1 \times V \times FP}{m}
\]

2.8. Antioxidant activity test
Testing the antioxidant activity of Sargassum sp. carried out using the DPPH method according to [13], the test was carried out by making a sample extract of 30% concentration of 10 g. The prepared 10 g sample was then mashed and then dissolved in 100 ml of hot water. The samples were then macerated for 3 days. The sample solution was taken as much as 200 L and then added 1000 L of 0.05 ppm DPPH. The blank solution was made without using a sample by adding only 200 L of ethanol solution and 1000 L of DPPH. All samples were centrifuged and then incubated for 30 minutes in a dark room. The absorbance value of the sample was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. The absorbance value of the DPPH solution was also measured and determined based on the IC50 (The half maximal inhibitory concentration) value. Then, the percentage of inhibition was plotted on the y-axis and x-axis as the concentration of the dissolved extract, to obtain a linear regression equation \(y=a+bx\). The IC50 value was determined as the concentration of the extract solution required to scavenge DPPH free radicals by 50%. The test was carried out with three repetitions and the measurement results were expressed with a standard deviation. The percentage of sample inhibition was calculated using the equation:

\[
\text{Percentage of Inhibition (\%)} = \frac{\text{Absorbance blank} - \text{absorbance of sample}}{\text{Absorbance blank}} \times 100\%
\]

3. Result and discussion

3.1. Moisture content of brewed Sargassum sp.
Based on data from Table 1, the value of water content in brewed Sargassum sp. no significant effect with the addition of bay leaves. The brewed value of Sargassum sp. with the addition of bay leaves at
concentrations of 0%, 10%, 20%, and 30% respectively 5.44%, 5.61%, 4.42%, and 2.1%. The results of the analysis of water content of *Sargassum* sp. obtained based on the 2019 seaweed brewed SNI standard still meets the standard (maximum 10%). Moisture content is a very important factor in spoilage and shelf life of food products. The moisture content of dry matter should be below 10% to prevent enzymatic processes and microbial growth. This is because in general dry matter is stored for a long time, if an enzymatic process occurs it will change the chemical composition stored in it. The results of water content are influenced by several factors including the drying process of *Sargassum* sp. Uneven drying process and fluctuating temperature changes can affect the moisture content. The longer the drying time, the lower the water content in a food ingredient.

| Treatment | Water content |
|-----------|---------------|
| 0%        | 5.44 ± 0.15a  |
| 10%       | 5.61 ± 0.45a  |
| 20%       | 4.42 ± 0.21a  |
| 30%       | 2.1 ± 0.14a   |

Description:
- The data is the average result of 3 repetitions ± standard deviation.
- Data followed by the same superscript letter in the same column showed no significant difference between treatments (Sig. > 0.05).

The higher drying temperature can damage the content or active compounds in seaweed. In addition, a long drying time can also damage the content of the seaweed produced [14].

### 3.2. Ash content of brewed *Sargassum* sp.

Based on the data in Table 2, the value of ash content in brewed *Sargassum* sp. no significant effect with the addition of bay leaves. The brewed value of *Sargassum* sp. with the addition of bay leaves at concentrations of 0%, 10%, 20%, and 30% that is 7.49%, 6.39%, 5.68%, and 5.96%. *Sargassum* sp. is one of the brown algae that contains quite high minerals such as Na, Ca, K, Cl, Mg, Fe and S. High ash content can reduce the quality of *Sargassum* sp. The higher the ash content, the higher the inorganic content. Based on research results [15], the highest ash content of brown algae tea was obtained from samples soaked in the control treatment of 11.53%. The resulting ash content comes from the salt and minerals attached to the seaweed. The high ash content of *Sargassum* sp. due to differences in the habitat and environment of *Sargassum* sp. Other than that, soaking treatment with hot water before making brewed *Sargassum* sp. also has an effect, because in the state of immersion with hot water many inorganic minerals contained in seaweed dissolve in the immersion. Stew products contain a large number of essential elements that are needed by the body such as Ca, Mg, Mn, Fe and essential microelements Na, Zn, Rb, Br, Cr, Cs, La, Sc and Co with varying concentrations depending on the type of brewing [16].

| Treatment | Ash Level |
|-----------|-----------|
| 0%        | 7.49 ± 1.29a |
| 10%       | 6.39 ± 0.98a |
| 20%       | 5.68 ± 1.26a |
| 30%       | 5.96 ± 0.30a |

Description:
- The data is the average result of 3 repetitions ± standard deviation.
- Data followed by the same superscript letter in the same column showed no significant difference between treatments (Sig. > 0.05).
The percentage of dissolved elements in the brewed water of each element is different, with an average range between 27.89 - 68.94%. This may be influenced by the nature or physiological structure of the plant, other binding mineral components, the initial concentration of the element in tea, the pH of the water, and the solubility of the element in hot water.

3.3. Image processing brewed Sargassum sp.

The brewed water condition was measured using a color grab application to measure the L* (Lightness), a* (redness), and b* (yellowness) scales. Color grab is a color picker application that can show the color of the photos taken. The app also displays hues, color names, RGB HEX, RGB decimal, and HSV. The state of brewed water for color testing is also tested according to the color intensity of L*, a* and b*.

| Treatment | L*        | a*        | b*         |
|-----------|-----------|-----------|------------|
| 0%        | 51.27 ± 7.66a | 20.13 ± 3.41a | 54.30 ± 12.7a |
| 10%       | 59.17 ± 3.38a | 20.67 ± 2.64a | 57.67 ± 10.7a |
| 20%       | 59.87 ± 5.45b | 21.73 ± 3.25b | 64.30 ± 4.44b |
| 30%       | 61.77 ± 2.65a | 22.30 ± 2.71a | 66.57 ± 1.46a |

Table 3. Image processing results of Sargassum sp.

Description:
- The data is the average result of 3 repetitions ± standard deviation.
- Data followed by the same superscript letter in the same column showed no significant difference between treatments (Sig. > 0.05).

3.3.1. L value

The results of the L* value in brewed Sargassum sp. were not significantly different with the addition of bay leaves. The result of image processing L* shows a positive value, which means the L* notation shows a bright color. The resulting L* value belongs to the bright brightness level. The value of L* is influenced by several factors, one of which is the brewing time and temperature. The longer the brewed of Sargassum sp. soaked, compounds in brewed will be extracted, one of which is tannin compounds. Tannins can cause the brewed color to become darker. According to [17], tannins can cause the color of the brewed to be darker so that the higher the tannin content in the material, the darker the resulting brew.

3.3.2. a* value

The results of the brewed value of Sargassum sp. were not significantly different with the addition of bay leaf concentration. The result of image processing a* shows a positive value, meaning that the notation shows a red color. The value of the level of redness is related to the total levels of carotenoids, as well as other pigments contained in the brewed of Sargassum sp. The darker the resulting color means the more pigments it contains. The higher the total carotenoid content, the more yellow or red the color will produce. According to [18], that the constituent pigments in brown marine algae Sargassum sp. derived from the chlorophyll group and its derivatives as well as polar (xanthophyll) and non-polar (carotene) carotenoids. The increase or decrease in the intensity of the red color is due to the influence of the amount of pigment extracted in the material.

3.3.3. b* value

The results of the b* value in brewed Sargassum sp. were not significantly different from the addition of bay leaves. The result of image processing b* shows a positive value, meaning that the b* notation shows a yellow color. The yellow color produced by brewed water also comes from theaflavin compounds which are the result of the degradation of tannin compounds. In addition, beta carotene is an organic pigment of yellow, orange or red orange color that can occur naturally in photosynthetic plants, algae, some types of fungi and bacteria. One of the yellow pigments derived from carotene...
contained in Sargassum sp. namely fucoxanthin. According to [19], fucoxanthin is the main pigment of the carotenoid group found in brown seaweed and is estimated to account for more than 10% of the total natural carotenoid production from other biota.

3.4. Sensory assessment
Sensory assessment is a test of psychological reactions in the form of responses by a group of people called panelists. Panelists are tasked with assessing the quality of materials based on subjective assessments. Some sensory assessments that need to be done are descriptive tests and hedonic tests. This study uses a hedonic sensory test and a score test to determine the most preferred treatment by the panelists. The results of the analysis are presented in Table 4.

| Treatment  | Color       | Scent      | Taste      | Trust Lapse |
|------------|-------------|------------|------------|-------------|
| 0% (Kontrol) | 6.80 ± 1.21 | 3.67 ± 0.96a | 4.47 ± 0.90a | 4.89 <µ< 5.29 |
| 10%        | 7.13 ± 1.28 | 4.90 ± 1.38b | 6.00 ± 1.14b | 5.70 <µ< 6.04 |
| 20%        | 7.53 ± 0.90 | 6.73 ± 1.55c | 7.13 ± 1.38c | 6.84 <µ< 7.44 |
| 30%        | 7.60 ± 0.93 | 7.80 ± 1.45d | 8.33 ± 1.09d | 3.4 <µ< 8.11 |

Description:
- The data is the mean ± standard deviation.
- Data followed by different superscript letters in the same column showed a significant difference between treatments (Sig. <0.05).

3.4.1. Color
The results of the average value of the color parameter showed that the lowest average in the control treatment was 6.8 while the 10% treatment was 7.13. The average value of the 20% concentration treatment was 7.53 and the 30% concentration was 7.60. Based on these results, it can be concluded that the brewed color of Sargassum sp. what the panelists like is the brownish yellow color typical of tea products. According to [20], the highest color of Mahkota Dewa tea was treated with the addition of stevia leaves with a concentration of 2 g. This is because bright colors and not too dark (reddish yellow) are generally preferred by panelists. While the treatment using the addition of stevia leaves with a high concentration, produces a very dark tea color (brown).

3.4.2. Flavor
The results of the average value of the aroma parameter ranged from 4.47 to 8.33. The highest average value with 30% concentration treatment and the lowest value was found in the control treatment. The highest value indicates that the aroma parameter is highly favored by the panelists and the lowest value indicates that the aroma is not favored by the panelists. Based on these results, it can be concluded that the addition of bay leaf concentration can reduce the fishy aroma caused by Sargassum sp. The results of research by [21], stated that the level of consumer acceptance of the aroma of instant drinks decreased along with the addition of Sargassum polycystum extract due to the distinctive aroma of fishy seaweed. Reinforced by [22], which states that another fishy odor occurs in fish caused by the high protein content of fish. Reduced freshness of fish mainly comes from ammonia, trimethylamine, volatile fatty acids and the products of fatty acid oxidation. One way that is usually done to reduce the fishy smell of fish is to marinate the lime juice for a long time. Lime juice is quite effective in reducing the fishy smell of fish because it contains citric acid and ascorbic acid, both acids can react with TMA to form bimetal ammonium, so the fishy smell can be reduced.

3.4.3. Taste
Based on the test results, it can be seen that the average taste value ranges from 3.67 to 7.8. The highest value was found in the addition of 30% bay leaf concentration and the lowest value was found in the control treatment. Brewed Sargassum sp. it has a strong seaweed specific taste and is neither
bitter nor sour. In the control treatment, the taste was too astringent and pungent due to the fishy taste of Sargassum sp. so it is not liked by the panelists. The 10% concentration treatment had a slightly astringent taste because of the fishy taste of Sargassum sp. which is still present in the tea. At treatment concentrations of 20%, and 30% have a taste that is not so apt so that it is liked by the panelists. According to [23], The taste arises due to chemical stimuli that can be received by the taste buds or tongue. The mangosteen rind tea has a slightly bitter and astringent taste. The astringent and bitter taste was not liked by some of the panelists. However, some panelists considered tea as herbal medicine, so based on the results obtained, the S4 treatment produced a more dominant (preferred) taste because the resulting taste was not too bitter.

3.5. Phytochemical screening of brewed Sargassum sp.

The results of the phytochemical screening analysis of the steeped extract of brewed Sargassum sp. at concentrations of 0% and 30% can be seen in Table 5. Phytochemical screening aims to provide information on the types of chemical compounds contained in plants and can provide physiological effects.

| No | Identification Compound | Parameter | Sample Name | Results |
|----|-------------------------|-----------|-------------|---------|
| 1. | **Flavonoids** | Orange, Brick Red, Pink, Dark Red | Brewed Sargassum sp. 0% | (+) Positive |
|    |                        |           | Brewed Sargassum sp. 30% | (+) Positive |
| 2. | **Phenol**             | Dark Chocolate, Dark Blue | Brewed Sargassum sp. 0% | (+) Positive |
|    |                        |           | Brewed Sargassum sp. 30% | (+) Positive |
| 3. | **Tannins**            | Dark Chocolate, Dark Blue | Brewed Sargassum sp. 0% | (+) Positive |
|    |                        |           | Brewed Sargassum sp. 30% | (+) Positive |
| 4. | **Saponins**           | Permanent Foam | Brewed Sargassum sp. 0% | (+) Positive |
|    |                        |           | Brewed Sargassum sp. 30% | (+) Positive |

Phytochemical screening assays followed the procedure of [24]. The results obtained for the phytochemical screening test at concentrations of 0% and 30% in brewed Sargassum sp. The results were positive for flavonoids, phenols, tannins, and saponins.

3.5.1. Flavonoids

Phytochemical screening tests on flavonoid parameters were carried out qualitatively and quantitatively. At concentrations of 0% and 30%, qualitative phytochemical screening tests obtained positive results, which means that Sargassum sp. containing active compounds is indicated by the presence of an orange color. The presence of flavonoid phytochemical compounds indicated that brewed Sargassum sp. potential as an antioxidant.

This analysis aims to determine the total flavonoid content in brewed Sargassum sp. obtained from the standard curve equation. The concentration of gallic acid on the absorbance data resulted in the standard curve line equation \( y = 0.0039x + 0.0044 \) with \( R^2 = 0.9866 \). From this equation, the total
flavonoid concentration of 0% was 161.1282 mg Q/g. The result of total flavonoid content at 30% concentration was 302.3333 mg Q/g.

Total levels of flavonoids in brewed Sargassum sp. concentration of 30% greater than the total flavonoid concentration of 0% concentration or control. This shows that the addition of bay leaf concentration can increase the total flavonoid in Sargassum sp. [25], bay leaves contain secondary metabolites, namely flavonoids, alkaloids, saponins, tannins, phenolics and triterpenoids. The higher the value of flavonoids, the greater the ability of antioxidants to ward off free radicals. In addition, the selection of bay leaves in the processing also affects the flavonoid content. The older the plant, the more bioactive compounds it contains. This increase in bioactive compounds is due to the process of synthesis of bioactive compounds which increases when plants are exposed to direct light. Compounds of the flavonoid group can increase due to the influence of light. This is confirmed by [26], the age difference of bay leaves can affect the flavonoid compounds. The older the age of the plant, the more the bioactive compounds contained in it accumulate. This increase in bioactive compounds is due to the process of synthesis of bioactive compounds which increases when plants are exposed to direct light. Compounds of the flavonoid group can increase due to the influence of light. This increase in bioactive compounds is due to the process of synthesis of bioactive compounds which increases when plants are exposed to direct light. Compounds of the flavonoid group can increase due to the influence of light. The addition of bay leaf concentration can increase the role of flavonoids because flavonoids function as antihypertensive, anti-inflammatory, antioxidant and help reduce pain analgesics. Bay leaves contain secondary metabolites that have many pharmacological activities in overcoming various diseases. Therefore, the synergistic effect between secondary metabolite compounds causes pharmacological effects. Bay leaves contain high levels of tannins and flavonoids, making it possible to make herbal teas for people with hypertension. [27], that bay leaf extract dissolved in water solvent tested on male mice in vivo was able to reduce the average blood pressure.

3.5.2. Phenol

Phytochemical screening tests on phenol parameters were carried out qualitatively. Brewed Sargassum sp. had positive results containing phenolic compounds at concentrations of 0% and 30%. This is indicated by the presence of a blackish brown color. Based on the SNI for seaweed brewed, the polyphenol content is at least 10%. Research conducted by [28], resulted in the phenol content in samples of Sargassum sp. by 1.81%. In addition, there is a study conducted by [29], the phenol content of tea made from young leaves has an average value of 0.372%, while tea from old leaves has an average value of 0.405%. Based on research conducted by [30], regarding avocado leaf herbal tea, the results showed that the total phenol of tea from young avocado leaves was lower than tea from old avocado leaves. According to [31], during plant growth, different amounts of secondary metabolites and bioactive compounds are synthesized, influenced by leaf morphology and leaf age. Phenolic compounds are the largest group of compounds that act as natural antioxidants in plants. Phenolic compounds have one (phenol) or more (polyphenol) phenol rings, namely hydroxy groups attached to aromatic rings so that they are easily oxidized by donating hydrogen atoms to free radicals. Its ability to form stable phenoxy radicals in oxidation reactions causes. The phenol parameter is directly proportional to the results of the antioxidant test where Sargassum sp. contains phenols which can be used as antioxidants. The addition of bay leaves at a concentration of 30% is the best concentration with an IC50 value of 13.6251 ppm. This shows that the higher the total phenol content, the higher the antioxidant activity or vice versa, the higher the antioxidant activity, the more total phenol content contained. This is in accordance with [32], that the higher the phenolic content, the greater the antioxidant activity.
3.5.3. Tannins
In the phytochemical screening test, the tannin parameters were carried out qualitatively. Positive results containing tannins at concentrations of 0% and 30% are indicated by the presence of a blackish brown or blackish blue color. [33], the type of phenolic component that is commonly found in brown seaweed is phlorotannin, which ranges from 0.74–5.06%. Sargassum sp. qualitatively contains flavonoids, saponins, tannins, and terpenoids in almost the same amount. In addition, the addition of bay leaf concentration also helps increase the tannins in the Sargassum sp. [34], an analysis of tannin compounds in bay leaves is positive for tannins. Tannins are divided into two groups and each group gives a different color reaction to 1% FeCl₃. The hydrolyzed tannin group will produce a blue-black color while the condensed tannin group will produce a green-black color. At the time of addition, it is estimated that 1% FeCl₃ reacts with one of the hydroxyl groups present in the tannin compound. The result of the reaction that can cause color if it is positive contains tannins. FeCl₃ 1% reagent is widely used to identify phenolic compounds including tannins. In brewed Sargassum sp. There is hydrolysis of tannins because the results of the observations produce a blue-black color.

The content of tannins in tea can be used as a quality guide, because tannins provide flavor stability. The astringent taste in tea drinks is caused by tannin compounds. In addition to taste, tannins also affect the color and aroma of tea drinks. Factors that can affect tannins are the drying process. The drying process was able to reduce the bioactive content of a material. The faster drying will cause the tannin compounds to be slightly damaged and vice versa. [35], bioactive compounds such as tannins, flavonoids, polyphenols, and saponins are compounds that are not heat resistant and at temperatures > 60°C can undergo structural changes and produce low extracts. In addition, the temperature and duration of brewing also affect the presence of tannin compounds. In the research of [36], which states that in the process of brewing green tea at a temperature of 70°C for 5 minutes, the highest tannin content is 4.783% and the lowest is brewing for 15 minutes, namely 1.825%. Water that is too hot will cause an imbalance of components, so that the relative levels decrease drastically. In addition to temperature and brewing time, the value of the tannin content of tea products is also influenced by the level of fineness of the powder. The level of fineness of the tea powder affects the yield of tannin levels. The finer the tea powder, the lower the tannin content.

3.5.4. Saponins
Phytochemical screening of saponin parameters resulted in permanent foam at concentrations of 0% and 30%, which means positive for saponin compounds. The biologically active compounds are secondary metabolites which include alkaloids, flavonoids, terpenoids, tannins and saponins. Saponins are complex glycosides consisting of a condensation product of a sugar with an organic hydroxyl compound. The structure of saponins causes saponins to act like soap or detergent so that saponins are called natural surfactants. The name saponin is taken from its main nature, namely "sapo" in Latin which means soap. In a study conducted by [37], the results of saponin testing on Sargassum sp. ie 1.06%. Apart from being an antioxidant, saponin compounds also have the ability to be used as an antibacterial. Research by [38], that the results of the saponin test on the extract of Sargassum sp. with methanol solution of 3.50%. Based on the results of the study, the highest phytochemical content in the extract of Sargassum sp. namely saponins. This is because saponins and methanol have the same polarity so that saponins are easily soluble when using methanol as a solvent. This is confirmed by [39], the most appropriate saponins extracted from plants with 70-95% ethanol or methanol as a solvent. The high and low content of phytochemical compounds in brewed Sargassum sp. also depends on the solvent used for the extraction process.

Saponins are distinguished based on their hydrolysis results, namely carbohydrates and sapogenins, while sapogenins are divided into two groups, namely steroidal saponins and triterpenoid saponins. Saponins have a characteristic form of foam, so when reacted with water and shaken it will form a foam that can last a long time. The addition of bay leaf concentration can help increase saponin compounds because bay leaves also contain bioactive compounds such as flavonoids, phenols, saponins and essential oils. Saponins have glycosyls that function as polar groups and steroid
triterpenoid groups as nonpolar groups. Compounds that have polar and non-polar groups are surface active so that when shaken with water, saponins can form micelles. In the micelle structure, the polar groups are facing outwards while the nonpolar groups are facing inwards. It is this state that looks like foam, because it is in brewed *Sargassum* sp. positive contains saponin.

### 3.6. Antioxidant activity

The results of antioxidant activity using ABTS and DPPH (IC$_{50}$) methods in brewed *Sargassum* sp. with the addition of bay leaf concentration is presented in Table 6.

| Treatment | % inhibisi |
|-----------|------------|
| 0% (Kontrol) | 3,08 |
| 10% | 67,82 |
| 20% | 95,73 |
| 30% | 98,76 |

The results of the research on the antioxidant activity of brewed *Sargassum* sp. 0% treatment had 3.08 % inhibition. The 10% and 20% treatments respectively had the % inhibition value of 67.82 and 95.73. At a concentration of 30% it has a % inhibition value of 98.76 so that the concentration of 30% is continued by testing with DPPH followed by IC$_{50}$ to determine the antioxidant value. Based on the IC$_{50}$ value, the treatment concentration of 30% is included in the very strong antioxidant with a value of 13.6251 ppm. Where, bay leaves also contain compounds that can be used as antioxidants. This shows that the addition of bay leaf concentration can increase antioxidant activity and is classified as a very strong antioxidant activity. Based on research conducted by [40], that bay leaf filtrate has antioxidant activity of 81.18%, and ginger filtrate of 66.88%, so it can be stated that bay leaf filtrate has high free radical inhibitory activity compared to filtrate. ginger. The antioxidant value of *Sargassum* sp. directly proportional to the phenol content. The phenolic compounds present in brown seaweed are the most effective antioxidants. Florotanin present in brown seaweed is known to be a major source of phenolics. However, there are several factors that can affect the antioxidant value, one of which is the heating process. The heating process that is too long can reduce the bioactivity of the active ingredients of a product, this can also occur when drying tea, antioxidant activity can be reduced due to the oxidation process in polyphenols. In addition, in this study, the process of immersion in hot water (blanching) was carried out. This is intended to improve the characteristics of brewed *Sargassum* sp. but if the immersion is too long will reduce the antioxidant value. According to [41], that a longer soaking time can reduce antioxidant activity. In general, heating treatment of the material will reduce the antioxidant activity contained therein, this is because heating can damage the phenolic and flavonoid compounds contained in the material.

### 3.7. Bioactive components of *Sargassum* sp.

The results of the bioactive components of the steeped extract of *Sargassum* sp. Using GC-MS, 6 compounds were detected, with a retention time range of 1.764 – 17.489 minutes. Screening of the chemical profile of *Sargassum* sp. dominated by pentane compounds (69.12%). The pentane compound in the brewed extract of *Sargassum* sp. potential as antimicrobial and anti-inflammatory. According to the research of [42], that pentane compounds have low oral toxicity and have high antimicrobial abilities for activity against bacteria, fungi and viruses. In addition, another study conducted by [43], produced pentane compounds which are natural compounds identified from Labisia pumila extract as antimicrobial and anti-inflammatory. The results of chemical profile screening of R. apiculata leaf extract with GC-MS can be seen in Table 7.
Table 7. Screening of the chemical profile of *Sargassum* sp. with GC-MS

| No | Compound Name              | Formula   | Retention Time (Minutes) | Area(%) |
|----|----------------------------|-----------|--------------------------|---------|
| 1  | Pentane                    | C₅H₁₂     | 1,764                    | 69.12   |
| 2  | 2-Undecanone               | C₁₁H₂₂O   | 10,749                   | 3.80    |
| 3  | Nerolidol                  | C₁₅H₂₆O   | 14,279                   | 2.25    |
| 4  | Biphenyl                   | C₁₂H₁₀    | 14,854                   | 8.50    |
| 5  | Methylisoeugenol           | C₁₁H₁₄O₂  | 16,502                   | 13.08   |
| 6  | 1-Phenanthenecarboxylic acid | C₁₅H₁₀O₂ | 17,489                   | 3.26    |

4. Conclusion

The conclusions from this research are comparison of control treatment with the addition of bay leaves significantly affected the a* value of image processing. The difference in the concentration of bay leaves did not significantly affect the parameters of water content and ash content. The addition of bay leaves can increase flavonoid levels at a concentration of 30% and can increase antioxidant activity with a proven treatment of 30% with a value of 13,6251 ppm and produce six compounds with pentane dominant compounds that can be used as antimicrobials and anti-inflammatory agents. Consumer acceptance of brewed *Sargassum* sp. with the addition of 30% bay leaf (*Syzygium polyanthum*) treatment with the highest score of 7.71 < μ < 8.11 which was also favored by the panelists.

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