Preliminary Study of Sonication Time for Extracting Natural Dye and Antioxidant from *Sargassum duplicatum*

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ABSTRACT

Sargassum duplicatum is a brown seaweed containing various chemical compounds with potential applications as a natural dye and antioxidant. These compounds include alginate, protein, vitamin C, tannins, iodine, phenols, and pigments such as chlorophyll a, chlorophyll c, fucoxanthin, xanthophyll, and carotene. Therefore, this study aimed to determine the effect of varying extraction times on the color and antioxidant quality of *Sargassum duplicatum* extract. One of the methods used to extract *Sargassum duplicatum* is ultrasonication, which involves using ultrasonic waves to break down the cell walls of the material and facilitate efficient compound extraction. The extraction was carried out at 15 (A), 30 (B), and 45 minutes (C), with three repetitions for each treatment. The assessed parameters included yield calculation, total carotenoids, antioxidant activity, and color intensity. Parametric data were analyzed using ANOVA and Tukey HSD follow-up tests. The extraction process used an 80% ethanol solvent at 40 °C and a frequency of 42 kHz. This study employed an experimental laboratory approach using a completely randomized design (CRD) with different extraction times. The results showed significant differences (p<5%) in yield value, total carotenoids, antioxidant activity, and color intensity of *Sargassum duplicatum* extract at different extraction times. The best results were obtained at 45 minutes, yielding 86.66%, with 9.62% total carotenoids, an IC_{50} value of 1241.02 ppm, and lightness (L*), redness (a*), and yellowness (b*) values of 4.44, -3.46, and 37.90, respectively.

Keywords: Ultrasonic waves, carotenoids, extraction time, *Sargassum duplicatum*, bioactive compounds

INTRODUCTION

Sargassum sp. is an outstanding example of brown seaweed thriving abundantly in Indonesian waters. These waters are estimated to host about 15 species, of which 12 have been recognized and identified. This marine plant persists throughout the year, enduring both rainy and dry seasons. Besides producing alginates, it also contains several pigments that serve as natural dyes, including carotenes, fucoxanthin, chlorophyll a, and chlorophyll c. Furthermore, it functions as an antioxidant, effectively inhibiting damage caused by free radicals. Phenolic compounds such as phlorotannins found in *Sargassum* sp. play a crucial role as a source of antioxidants. This was highlighted by Nursid et al. (2013), who emphasized that brown seaweed contained carotenoids, laminarin, alginates, fucoidan, phlorotannins, and phenolic compounds, which served as antioxidants.

Persistent use of synthetic dye in food products can lead to adverse effects on health. Certain individuals even misuse Rhodamine B and Methanyl Yellow textile dyes as food colorants, prompting the Indonesian Ministry of Health to prohibit their use in food items

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with Regulation Number 239/Menkes/Per/V/85. Consequently, a safe alternative source of natural dye for consumption becomes imperative. Commonly used natural dyes include pandan leaves, turmeric, and various fruit extracts. *Sargassum* sp. contains carotenoids that give yellow, orange, and red colors to food items. Among the different carotenoid types are α-carotene, β-carotene, astaxanthin, lycopene, lutein, zeaxanthin, β-cryptoxanthin, and fucoxanthin (Takaichi, 2013; Wrolstad and Culver, 2012; Amaya, 2016).

Numerous factors can influence the characteristics and biological activities of extracts derived from *Sargassum* sp. One highly influential factor is the extraction method. Therefore, an appropriate method is required for the extraction of *Sargassum* sp., as it can affect the outcomes, composition, structure, and integrity of desired bioactive compounds. Factors to be considered in the selection of an extraction method pertain to its advantages and disadvantages in terms of cost, efficiency, and time required (Liu et al., 2019).

The extraction of a substance can be carried out conventionally through maceration or by using modern techniques such as Ultrasonic-assisted Extraction (UAE), also called ultrasonication. Both methods have their respective advantages and disadvantages. Maceration is advantageous due to its simplicity, ease of use, and cost-effectiveness (Ginting, 2013). However, its disadvantages include prolonged extraction time and the presence of organic solvents in the resulting yield (Putra et al., 2014). The advantages of UAE include enhanced extraction yield, shorter extraction time, operation at low temperatures, and reduced solvent consumption (Dey & Rathod, 2013). According to Rostagno & Prado (2013), the yield obtained using this method was higher compared to conventional methods. The disadvantage of UAE is its substantial energy and cost requirements (Wang & Weller, 2006).

Ultrasonication can be used to extract natural materials by reducing solvent usage and extraction time, resulting in higher yield. Koo et al. (2012) stated that the extract yield of natural pigments, including carotenoids, using UAE was three times higher than conventional extraction methods. However, according to Savitri (2017), the highest yield and antioxidant activity were obtained from *Sargassum* sp. using maceration for 15 minutes. Raguraman et al. (2018) also asserted that maceration for 15 minutes was optimal for extracting fucoidan from *Padina tetrastromatica*.

Sargassum duplicatum can be used as a natural dye and antioxidant, with ultrasonication proving to be an effective and efficient extraction method. Therefore, the purpose of this study was to investigate the effect of ultrasonication extraction time on the color and antioxidant quality of *Sargassum duplicatum* extract.

METHODS

This study employed an experimental laboratory method, and data were quantitatively analyzed using Completely Randomized Design (CRD). The extraction of *Sargassum duplicatum* was carried out using UAE at varying times of 15, 30, and 45 minutes. The data obtained included quantitative measurements such as yield, total carotenoids, antioxidant activity, and color intensity.

Materials

The primary materials used in this study were *Sargassum duplicatum* harvested at 45 days of age from Blebak Beach (Mlonggo, Jepara), 80% ethanol, and filter paper obtained from Indrasari Chemical Store (Semarang, Indonesia). The chemicals used included p.a. methanol (Merck), p.a. n-hexane (Sigma), distilled water, Folin-Ciocalteu phenol (Merck), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma).

Sample Preparation

Fresh *Sargassum duplicatum* samples obtained from Blebak Beach, Mlonggo, Jepara were placed in black plastic bags and stored in a cool box. The samples were then washed with flowing water until clean and air-dried for seven days. Subsequently, the dried samples were processed into a coarse powder with a particle size of 0.1-1 mm using a blender.

Sargassum duplicatum Extraction using Ultrasonic-assisted Extraction (UAE)

The extraction of *Sargassum duplicatum* was performed using a Krisbow DSA50-GL2-2.5L ultrasonication chamber. A total of 90 g *Sargassum duplicatum* powder was extracted with 900 mL of 80% ethanol solvent (1:10 ratio) using the sonicator for 15, 30, and 45 minutes at 40 °C and a frequency of 42 kHz. The obtained extract was then filtered using Whatman No. 42 filter paper.

Analysis of *Sargassum duplicatum* Extract

The analysis of *Sargassum duplicatum* extract included yield calculation (AOAC, 2005), total carotenoids analysis (Porim 1995), antioxidant activity analysis using the DPPH method (Dhinakaran and Geetha 2015), and color intensity analysis (AOAC, 2005).

Statistical Analysis

This study employed CRD with three treatments and three replications. The observed factor was the extraction time of *Sargassum duplicatum* using the ultrasonication method, namely 15, 30, and 45 minutes. Parametric data analysis involved calculating

yield, total carotenoids, antioxidant activity, and color intensity. Additionally, the collected data underwent normality and homogeneity tests. At *p* values greater than 0.05, an Analysis of Variance (ANOVA) test was conducted to determine the treatment effect. The existence of a treatment effect was indicated when F-count exceeded F-table at a 5% significance level. This confirmed that ANOVA requirements were fulfilled and a Tukey's Honestly Significant Difference (HSD) test could be performed to identify differences between treatments.

RESULTS AND DISCUSSION

Yield

Table 1. The mean yield calculation results of *Sargassum duplicatum* extract

No.	Treatment	Yield (%)
1.	A (15 minutes)	49.99 ± 5.55[a]
2.	B (30 minutes)	75.92 ± 3.2[b]
3.	C (45 minutes)	86.66 ± 2.22[c]

Note: Data represent the mean results of three repetitions ± standard deviation. Different lowercase letters show significant differences (*p* <5%)

Table 1 shows the yield results of *Sargassum duplicatum* extract. Yield represents the total weight of all secondary metabolite compounds that can be extracted from a sample or plant (Sari and Triyasmono, 2017). It is calculated by comparing the final weight of *Sargassum duplicatum* extract with the initial weight, then multiplying by 100%. The results showed that the obtained yield values for each treatment were significantly different. The highest yield value was achieved in sample C at 86.66%, followed by B and A at 75.92% and 49.99%, respectively. These indicated higher values compared to those of Pratista et al. (2017), where yield values ranging from 1.28 to 2.21% were obtained using the maceration method

The high yield values are attributed to the cavitation process induced by ultrasonic waves during extraction. This cavitation process aids in breaking down the cell walls of the material, enhancing solvent penetration, and promoting mass transfer, resulting in rapid cell breakdown. The cavitation process during extraction also facilitates solvent osmosis into the plant cell walls (Nuraisah, 2010).

Based on the results, extraction time affected the yield outcomes of the extract. The highest yield was obtained after a 45-minute extraction due to the sufficient time allowed for the solvent to permeate the cell walls and extract the components or compounds contained within the material. A longer extraction time produced a higher yield since extended contact time between the material and the solvent enhanced the penetration of compounds from the material into the solvent (Sholihah et al., 2017).

Total Carotenoids

Table 2. Mean total carotenoids of *Sargassum duplicatum* extract

No.	Treatment	Carotenoids (%)
1.	A (15 minutes)	0.71 ± 0.76[a]
2.	B (30 minutes)	5.92 ± 0.24[b]
3.	C (45 minutes)	9.62 ± 0.18[c]

Note: Data represent the mean results of three repetitions ± standard deviation. The lowercase letters indicate significant differences (*p*<5%)

Table 2 shows the total carotenoids in *Sargassum duplicatum* extract. Carotenoids are pigments that serve as precursors to vitamin A and antioxidants that protect the body from free radicals (Maleta et al., 2018). The results showed the significant effect of different extraction times on the total carotenoid content of *Sargassum duplicatum*. The highest total carotenoids value of 9.62% was achieved with a 45-minute extraction and the lowest value of 0.71% was obtained with a 15-minute extraction. Similarly, a 30-minute extraction yielded a total carotenoid of 5.92%. These results showed higher values compared to Savitri et al. (2017), where the total carotenoids ranged from 0.08% to 0.23% for *Sargassum duplicatum* extract obtained through maceration. The elevated total carotenoids are attributed to ultrasonication, a more effective extraction method than maceration. This highlights UAE as an alternative non-thermal extraction method offering better efficiency, speed, solvent reduction, and resulting in purer extracts and higher yield compared to conventional methods. This method has been successfully applied to food component extraction such as aroma, pigments, and antibacterial agents (Vinatoru, 2001).

The total carotenoids in *Sargassum duplicatum* extract can be affected by the extraction time. Based on Table 2, an increase in extraction time leads to an increase in total carotenoid yield. According to Wuryantoro & Susanto (2014), a longer extraction time provides more opportunities for contact between the material and the solvent, facilitating enhanced extraction of bioactive components until saturation is achieved.

Several factors enhance carotenoid extraction using the UAE method. These factors include particle size, solvent type, solvent-to-material ratio, temperature, extraction time, acoustic intensity, sample height (in liquid form), and the cycle of ultrasonic wave exposure (Wijngaard et al., 2012).

Antioxidant Activity (IC_{50})

Table 3. Antioxidant Activity (IC_{50}) of *Sargassum duplicatum* Extract

No.	Treatment	IC_{50} (ppm)
1.	A (15 minutes)	2207.28 ± 62.88[a]
2.	B (30 minutes)	2210.34 ± 86.38[a]
3.	C (45 minutes)	1241.02 ± 7.60[b]

Note: The data represent the mean results of three repetitions ± standard deviation, with the different lowercase letters indicating significant differences (p<5%)

Table 3 shows the values of antioxidant activity in *Sargassum duplicatum* extract. An antioxidant is a compound that functions to inhibit free radicals. The antioxidant activity of a substance can be assessed by its IC_{50}, with a lower IC_{50} indicating higher antioxidant activity (Erviani et al., 2016). Table 3 shows that there is no significant difference in antioxidant activity between samples A and B. However, sample C significantly differed from A and B. Based on these results, the highest antioxidant activity value was achieved with an extraction time of 45 minutes and an IC_{50} of 1241.02 ppm. The IC_{50} for A and B were 2207.28 ppm and 2210.34 ppm, respectively.

The antioxidant activity of *Sargassum duplicatum* extract in this study was classified as very weak due to its IC_{50} exceeding 500 ppm. A compound is considered to have very strong, strong, moderate, weak, or very weak antioxidant activity based on its IC_{50} falling below 50 ppm, between 50-100 ppm, 100-150 ppm, 150-500 ppm, or above 500 ppm. The observed weak antioxidant activity was likely due to the tested sample being a crude extract, possibly hindered by the presence of other compounds, such as salts, minerals, and nutrients (Husni et al., 2014).

Extraction time can influence the antioxidant activity of *Sargassum duplicatum* extract. An extraction time of 45 minutes exhibits higher antioxidant activity compared to 15 and 30 minutes. This can be attributed to maximal extraction of antioxidant compounds such as carotenoids during the 45 minutes extraction. According to Almey et al. (2010), a longer extraction time led to an increased presence of antioxidant compounds in an extract, resulting in higher activity.

Color Intensity (L*a*b*)

Table 4. Analysis results of the color intensity in *Sargassum duplicatum* extract

No.	Treatment	L*	a*	b*
1.	A (15 minutes)	29.56 ± 0.99[a]	4.40 ± 0.32[a]	13.79 ± 0.33[a]
2.	B (30 minutes)	5.04 ± 0.13[b]	-3.17 ± 0.34[b]	37.31 ± 0.25[b]
3.	C (45 minutes)	4.44 ± 0.27[b]	-3.46 ± 0.22[b]	37.90 ± 0.09[b]

Note: Data represent the mean results of three repetitions ± standard deviation. The different lowercase letters indicate significant differences (p<5%)

Lightness Value (L*)

The lightness value (L*) indicates the color intensity on a scale of 0 to 100, with higher values suggesting brighter colors and lower values indicating darker colors. Table 4 shows significant differences in L* between samples A, B, and C. However, there was no significant difference in L* value between samples B and C.

The mean L* values for *Sargassum duplicatum* extract from A, B, and C were 29.56, 5.04, and 4.44, respectively. The lowest L* value was obtained from C at 4.44, while the highest was obtained from A at 29.56. These results differed from Pratista et al. (2017), which reported an L* of 4.41 for *Sargassum duplicatum* extract.

The results suggested that different extraction times influenced the L* value of *Sargassum duplicatum* extract. The decrease in L* occurred due to the increasing presence of pigments, such as carotenoids, which darkened the color of the extracted material. Khuluq et al. (2017) stated that high pigment content in the extracted material affected its L* value. This was observed in sample A, which had the highest L* value compared to samples B and C. This difference could be attributed to the longer time required to extract compounds, particularly carotenoids, from *Sargassum duplicatum*. The similar L* values of samples B and C suggested that the difference was not significant, possibly due to the majority of compounds already extracted from *Sargassum duplicatum*.

Redness Value (a*)

The redness value (a*) indicates the color level from green to red within a range of -100 to +100. A higher a* indicates that the color of *Sargassum duplicatum* extract is leaning toward red, while a lower value suggests greenish. Table 4 shows significant differences in a* between A, B, and C. However, there was no significant difference in a* between B and C.

The mean a* values for *Sargassum duplicatum* extract from A, B, and C were 4.40, -3.17, and -3.46, respectively. The lowest a* was obtained from sample

C at -3.46, while the highest was from A at 4.40. The results from samples B and C aligned with those of Savitri et al. (2017), who reported a mean a* value of -3.00 for *Sargassum polycystum* extract using different solvents. However, this value significantly differs from a* value in this study for A at 4.40. This discrepancy is likely due to the 15-minute extraction time not allowing for the complete extraction of compounds from the material. The resulting color corresponds to the number of pigments present in the extract (Satriyanto et al., 2012).

The results indicate that different extraction times can affect a* value of *Sargassum duplicatum* extract, with a longer extraction time leading to a lower a* value. This is evident from the highest a* value obtained from A at 4.40 and the lowest value from C at -3.46. As more pigments were extracted, particularly chlorophyll from *Sargassum duplicatum*, the a* value decreased. According to Aryanti et al. (2016), a* value is related to the solubility of chlorophyll pigments. Lower chlorophyll content leads to a higher a* value, resulting in a redder color, while higher chlorophyll content leads to a greener color with a lower a* value, indicating a tendency towards green.

Yellowness Value (b*)

The yellowness value (b*) indicates the color spectrum between blue and yellow within a range of -100 to +100. A higher b* value suggests a tendency towards yellowish color, while a lower value indicates a tendency towards bluish color. Table 4 shows significant differences in b* between A, B, and C. However, no significant difference exists between B and C.

The mean b* values for *Sargassum duplicatum* extract from A, B, and C were 13.79, 37.31, and 37.90, respectively. The highest b* value was obtained from C at 37.90, while the lowest value was from A at 13.79. The B and C values were similar to those of Novientari et al. (2017), who reported a value of 38.66 for *Sargassum polycystum* extract using 95% acetone as the solvent. However, this value significantly differed from the b* value of A in this study.

The results indicated that different extraction times affected the b* value of *Sargassum duplicatum* extract, where longer extraction times resulted in higher b* values. This is evident from the 45 and 15-minute extractions, which produced the highest and lowest b* values of 37.90 and 13.79, respectively. As more carotenoid compounds from *Sargassum duplicatum* were extracted, the resulting b* value increased. According to Satriyanto et al. (2012), the b* value is related to the total carotenoids. Lower total carotenoids lead to a lower b* value, while higher total carotenoids result in a higher b* value, indicating a tendency towards yellow or even reddish colors.

CONCLUSION

In conclusion, varying extraction times influenced the yield, total carotenoids, antioxidant activity, and color intensity of *Sargassum duplicatum* extract. A 15-minute extraction time yielded total carotenoids of 0.71% and an IC_{50} of 2207.28 ppm. Extending the time to 30 and 45 minutes resulted in total carotenoids of 5.92 and 9.62% and IC_{50} values of 2210.34 and 1241.02 ppm, respectively. Among the various extraction times, the most effective for *Sargassum duplicatum* extraction using UAE was 45 minutes. This extraction time produced the highest value for yield at 86.66%, total carotenoids at 9.62%, antioxidant activity (IC_{50}) at 1241.02 ppm, as well as color intensity at 4.44, -3.46, and 37.90.

CONFLICT OF INTEREST

The authors declare that the results are original and have not been published before, hence, no conflicts of interest to report.

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