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# Antioxidant Activity and Tyrosinase of Brown Seaweed Extract Using Ultrasonic and Magnetic Stirrer Extraction Methods

***Miranticha Abubakar[1], Iriani Setyaningsih[1], Asep Bayu[2], and Desniar[1]***

*[1]Department of Aquatic Product Technology, Faculty of Fisheries and Marine Science, Bogor Agricultural Institute, Bogor, Indonesia.*

*[2]Research Center of Vaccine and Drugs, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta-Bogor KM 46 Cibinong, Bogor, West Java 16911, Indonesia.*

**Abstract**

Seaweed holds significant potential in various industries such as cosmetics, food, textiles, and pharmaceuticals, with brown seaweed being one of the most prominent. Brown seaweed has been a source of bioactive compounds and human consumption for centuries due to its potential active compound content, making it viable for development in both industrial and research fields. This study aims to investigate the potential bioactive components of brown seaweed (*Sargassum* sp., *Turbinaria* sp., *Padina* sp.) as antioxidants and tyrosinase inhibitors using ultrasonic and magnetic stirrer extraction methods. The research consisted of two stages: sample preparation, followed by ultrasonic and magnetic stirrer extraction. The results showed that the brown seaweed extract contained bioactive components with significant total phenolic content, indicating potential as antioxidants and tyrosinase inhibitors. The extract yield from the best combination of *Sargassum* sp. extract using the ultrasonic method, reaching 20,30±0,2%. The total phenolic content of *Sargassum* sp. extract, obtained using ultrasonic extraction methods, was the highest, reaching 50.98 ±0.3 mg GAE/g respectively. Qualitative phytochemical tests revealed the presence of alkaloids, flavonoids, hydroquinone phenols, tannins, steroids, tannins, and triterpenoids. Antioxidant activity, as measured by ABTS and DPPH radical scavenging assays, correlated with the total phenolic content. The *Sargassum* sp. extract obtained through ultrasonic extraction showed $IC_{50}$ values of 47.94 ±0.5 µg/mL and 90.02 ±0.1 µg/mL, respectively. The best tyrosinase inhibition activity was observed in *Sargassum* sp. extract obtained through ultrasonic extraction, with an $IC_{50}$ inhibition value of 650.4 ±0.1 µg/mL using the L-DOPA substrate.

**Keywords:** Brown seaweed, Antioxidant, Tyrosinase, Total phenol

**Full length article** **Corresponding Author*, e-mail: *mirantichaabubakar@apps.ipb.ac.id* Doi # https://doi.org/10.62877/18-IJCBS-24-26-20-18

## 1. Introduction

Seaweed or marine macro algae is a natural resource with significant potential in industries such as cosmetics, food, textiles, and pharmaceuticals [1]. The quality and potential of seaweed make it highly sought after by various countries worldwide, particularly brown seaweed. Brown seaweed has been a source of active compounds for human consumption, both for food and non-food purposes, for centuries due to its active compound content Andika *et al.* [2]. The high content of bioactive compounds exhibits various biological activities such as antioxidant, tyrosinase enzyme activity (anti-aging), anti-inflammatory, antiviral, anticancer, and antibacterial effects. Abundant potential of bioactive compounds, frequently encountered in research, includes alkaloids, saponins, flavonoids, hydroquinone phenols, tannins, steroids, and triterpenoids, which have substantial potential in various fields [3]. Active compounds possess various pharmacological properties, including antidiabetic, anticancer, antioxidant, and tyrosinase inhibitory activities [4]. Brown seaweed, second-largest group, is a source of active compounds and demonstrates various biological activities. Species such as *Sargassum* sp., *Padina* sp., and *Turbinaria* sp. exhibit the ability to inhibit oxidative damage and the tyrosinase enzyme, making them highly potential agents for antioxidants and skin-brightening applications in cosmetics and pharmaceuticals [5].

In recent years, brown seaweed has been globally consumed due to its rich bioactive potential. It has garnered significant attention for its active compound content, including phenols, polyphenols, flavonoids, fucoidan, phlorotannins, and other bioactive compounds such as alkaloids, triterpenoids, steroids, saponins, and tannins, as

secondary metabolites with potential antioxidant and tyrosinase activity [6]. Antioxidants are compounds that can inhibit the oxidation of free radicals and function to prevent degenerative diseases associated with anti-aging. Natural antioxidants play an important role in combating oxidative stress related to degenerative diseases, including cancer, cardiovascular diseases, diabetes, Alzheimer's disease, and the aging process [7]. Antioxidants in brown seaweed, such as phenolic and flavonoid compounds, along with other bioactive compounds, have been proven to play various important roles, including inhibiting oxidative damage caused by free radicals [8]. Tyrosinase inhibitors are compounds capable of inhibiting the melanin biosynthesis process, representing a novel approach for skin brightening and anti-aging treatments [8]. The mechanism of tyrosinase enzyme inhibition involves antioxidant activity and tyrosinase inhibition, making it an effective method for inhibiting melanin formation [9]. Melanin, a dark pigment produced by melanocytes in basal epidermis, is primary pigment and plays a key role in protecting humans from ultraviolet radiation.

Excessive melanin accumulation can lead to hyperpigmentation disorders such as freckles, age spots, chloasma, and melanoma [10]. Tyrosinase inhibition has been the subject of several studies, using inhibitors as additives in cosmetics and medicinal products, particularly for treating hyperpigmentation. Extraction is the process of drawing out active components from raw materials. Ultrasonic-assisted extraction has been proven to be an economical and environmentally friendly technology suitable for extracting bioactive compounds due to its low equipment and energy costs [11]. The magnetic stirrer method is a more conventional mechanical stirring method that operates efficiently without the need for additional chemicals. Different extraction methods can influence the extraction of bioactive compounds from raw materials, thereby affecting the antioxidant properties and tyrosinase inhibition abilities of the sample quality. Therefore, this study was conducted to examine the potential of brown macro algae as an effective source of antioxidants and tyrosinase inhibitors, which could replace synthetic compounds through the use of ultrasonic and magnetic stirrer extraction methods. The study aims to determine the effects of extraction methods (ultrasonic and magnetic stirrer) and types of brown seaweed (*Sargassum* sp., *Turbinaria* sp., *Padina* sp.) On the active components related to antioxidant activity and tyrosinase activity.

## 2. Materials and Methods

### *2.1 Materials*

The primary materials used in this study were brown seaweeds *Sargassum* sp., *Padina* sp., and *Turbinaria* sp., obtained from the waters of Sukabumi, West Java. The extraction materials included deionized water, 60% ethanol, and analytical materials such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich), 3,4-Dihydroxy-L-phenylalanine (L-DOPA) (Sigma-Aldrich), ethanol p.a. (Merck), Folin-Ciocalteu reagent (2N) p.a., Magnesium, amyl alcohol, 1 N NaOH, dragendoorff, wagner, mayer sulfurid acid, kojid acid ’and sodium carbonate (Merck). The equipment utilized comprised an analytical balance, a Philips HR 211 blender, a centrifuge (Hitachi Model Part R12A6904357D0), a spectrophotometer (UV-Vis 2500 (Shimadzu), a Branson Ultrasonic Cleaner M2800-E 2.8L, a magnetic stirrer, Buchi Rotary Evaporator R-300, micropipettes, and a beaker.

### *2.2 Methods*

#### *2.2.1 Moisture Content*

The analysis of moisture content began with the drying of a porcelain dish in an oven at 105°C for one hour, followed by weighing. Five grams of the seaweed sample were placed in the pre-weighed porcelain dish. The sample was then dried in an oven at 100-105°C for three hours until a constant weight was achieved. The weighing was conducted after the dish, along with sample, placed in a desiccator for approximately 30 minutes and left to cool before being weighed again [13]. Percentage of moisture content calculated using formula:

$$Moisture\ content\ (\%) = \frac{(B - C)}{(B - A)} \times 100\%$$

#### *2.2.2 Preparation and Extraction*

Seaweed samples collected by diving directly into coastal waters of Sukabumi, West Java, Indonesia. The seaweed species used included *Sargassum* sp., *Padina* sp., and *Turbinaria* sp., which were cleaned and washed with fresh water to remove salt content. Subsequently, fresh seaweed samples were cut into pieces and dried using an oven at 35°C. Dried samples then ground using a blender to obtain a powdered form, which stored in a sealed container. Extraction of active ingredients conducted using several methods referenced in extraction of active ingredients was conducted using several methods referenced in [13-14]. A total of 100 g of seaweed powder from each sample of *Sargassum* sp., *Padina* sp., and *Turbinaria* sp. was placed in 60% ethanol solvent at a ratio of 1:10 g/mL and extracted with the aid of ultrasonic at 30°C for 60 minutes. The filtrate was then cooled at 4°C and centrifuged at 3,230 rpm for 25 minutes. Resulting liquid was slowly separated and evaporated at 35°C to remove the solvent. For comparison, extraction also performed using a maceration method with magnetic stirrer agitation for 2x 24 hours at a cool temperature. Obtained liquid separated and evaporated in same manner as previous method, resulting in a thick, paste-like, and dry extract.

#### *2.2.3 Total Phenol*

The analysis of total phenolics was conducted by [15]. A sample amount of 1 mg/mL was weighed, then 0.5 mL of the sample was taken and reacted with 2.5 mL of 10% (v/v) Folin-Ciocalteu reagent added to a clean test tube, followed by the addition of 2 mL of 7.5% (b/v) Na2CO3. The solution was then thoroughly mixed using a vortex and incubated in a covered space for 1 hour. The resulting blue color change was measured for absorbance at a wavelength of 765 nm using a UV-Vis spectrophotometer. Gallic acid was used as the standard, with varying concentrations.

#### *2.2.4 Phytochemical Analysis*

Qualitative analysis was conducted to determine the bioactive components contained in the seaweed. Phytochemical bioactive component tests were performed qualitatively by [65], including tests for alkaloids, hydroquinone phenols, flavonoids, triterpenoids, steroids/triterpenoids, tannins, and saponins.

#### *2.2.5 Antioxidant Activity ABTS and DPPH*

Antioxidant analysis was conducted using the ABTS (2, 2-Azino-bis (3-ethylbenzothiazoline - 6 - sulfonicacid) method according to [16] with modifications. The ABTS stock solution was prepared by mixing 7.2 mM ABTS solution with

2.6 mM potassium persulfate solution (1:1 v/v). This mixture was incubated for 16 hours in a covered room at room temperature. Afterward, 2 mL of ABTS stock solution was added to 90 mL of ethanol, and its absorbance was measured to reach 0.8-0.7 at a wavelength of 734 nm. Subsequently, 1 mL of sample solution reacted with ABTS stock solution, vortexed, and incubated for 10 minutes in a dark room at room temperature. Antioxidant activity expressed as percentage of inhibition and interaction between sample concentration and percentage inhibition to determine $IC_{50}$ value. The DPPH (1, 1-diphenyl-2-picrylhydrazyl) method performed according to [17] with modifications. Extract sample solution prepared at a parent concentration of 2000 ppm, diluted to various concentrations of 1000, 800, 600, 400, and 200 ppm. DPPH solution prepared by dissolving DPPH crystals in ethanol at a concentration of 0.15 mM at room temperature and in dark. Then, 1 mL of test or control solution reacted with 1 mL of 0.05 mM DPPH solution in a test tube. Mixture incubated for 30 minutes, and its absorbance measured at a wavelength of 517 nm. Antioxidant activity expressed as percentage of inhibition calculated using formula (blank Abs – sample abs)/blank abs to further deduce remaining percentage of DPPH (% DPPH). Final result is expressed as $IC_{50}$ (µg/mL).

#### *2.2.6. Tyrosinase Activity*

The analysis of tyrosinase inhibition activity was conducted using methods [18-19] with modifications. A total of 30 µL of the enzyme (Sigma, 333 units/mL) and 70 µL of the sample (extracts from *Sargassum* sp., *Padina* sp., and *Turbinaria* sp.) were tested. Extract concentrations varied up to 10,000, 5,000, 2,500, 1,250, 625, 312, 152, 625, 312.5, 157.25, 500, 250, 125, and 1,250 µg/mL in a 96-well microplate. To this, 110 µL of L-DOPA substrate and 80 µL of potassium phosphate buffer solution (50 mM, pH 6.5) were added. The mixture was shaken for 60 seconds and incubated for 30 minutes at room temperature. Afterward, absorbance was measured using a microplate reader at a wavelength of 510 nm. To determine percentage inhibition and half-maximal inhibitory concentration ($IC_{50}$). The percentage inhibition of tyrosinase enzyme activity was calculated using the formula % inhibition (blank abs-abs control) (abs sample- abs control sample) x 100%. Final result is expressed as $IC_{50}$ (µg/mL) the concentration of the sample solution needed to inhibit 50%.

#### *2.2.7. Data Analysis*

The experimental design used the Analysis of Variance (ANOVA) was performed to determine the moisture content of brown seaweed types at a 95% confidence interval ($\alpha$=0.05); if significant effects were observed ($p<0.05$), subsequent testing was conducted using Duncan's test. The completely randomized factorial design with two factors, namely types of brown seaweed and extraction methods, was used to determine best extract based on total phenol analysis, antioxidant, and tyrosinase activity. Qualitative descriptive data analysis is used to present phytochemical analysis. The software used for data analysis were Microsoft Excel 2019 and Statistical Product and Service Solutions (SPSS) 25.

## 3. Results and Discussion

### *3.1 Results*

#### *3.1.1 Moisture Content*

The moisture content analysis was conducted to determine the percentage of water content in the seaweed samples. The prepared and dehydrated brown seaweed samples were analyzed for moisture content. The results of the moisture content in the seaweed can be seen in Table 1.

#### *3.1.2. Brown Seaweed Extract Yield*

The technique used for the separation of bioactive compounds was through extraction processes using solvents according to their polarity. The yield of the brown seaweed extracts can be seen in Figure 1.

#### *3.1.3. Total Phenol Content of Brown Seaweed Extracts*

The results of the total phenol content measurements from all the extracts obtained are presented in Table 2.

#### *3.1.4. Bioactive Compounds of Extracts Brown Seaweed*

Qualitative descriptive results indicate that the brown seaweed extracts treated with ultrasonic and magnetic stirrer methods were identified to contain active compounds. Phytochemical results of brown seaweed extracts obtained by ultrasonic and magnetic stirrer methods presented in Table 3.

#### *3.1.5 Antioxidant Activity of Brown Seaweed Extracts*

Extracts of *Sargassum* sp., *Padina* sp., and *Turbinaria* sp. demonstrated antioxidant activity with 50% inhibition in ABTS and DPPH radical scavenging assays. The results of the antioxidant activity of brown macroalgae extracts are displayed in Figures 2 and 3.

#### *3.1.6. Tyrosinase Activity of Brown Seaweed Extracts*

The tyrosinase inhibition activity was tested using kojic acid as a positive control and comparative standard. Brown seaweed extracts from *Sargassum* sp., *Padina* sp., and *Turbinaria* sp. were analyzed using L-DOPA as a substrate to observe tyrosinase inhibition reactivity. Kojic acid, serving as a positive control and comparator in the tyrosinase activity tests, demonstrated enzyme inhibition activity, achieving an $IC_{50}$ value of 34.047 ppm and a determination coefficient ($R^2$) of 0.99. The $_{IC50}$ inhibition results for the tyrosinase enzyme from the brown seaweed extracts are shown in Figure 4.

### *3.2 Discussion*

The dried samples of brown seaweed, *Sargassum* sp., *Padina* sp., and *Turbinaria* sp., were analyzed for moisture content. Table 1 shows that the moisture content in the samples of *Sargassum* sp., *Padina* sp., and *Turbinaria* sp. ranged from 21%, 13%, and 23%, respectively. The Analysis of Variance (ANOVA) revealed that the moisture contents of *Sargassum* sp. and *Turbinaria* sp. were not significantly different, while *Padina* sp. showed significantly different values ($p<0.05$). This difference in moisture content may be due to the distinct characteristics of the samples; *Sargassum* sp. and *Turbinaria* sp. are suspected to have hard and thick thallus structures compared to *Padina* sp., which has a thinner thallus structure. These characteristics affect water absorption processes of each brown macroalgae. However, *Turbinaria* sp. had highest moisture content, followed by *Sargassum* sp., and *Padina* sp. had the lowest. According to [20], the moisture content reported for *Sargassum* sp. 26.25±0.36, *Padina* sp. 24.37±0.30, and *Turbinaria* sp. 30.58±0.07%. Moisture content for *S. hystrix var. fluitans* was 13.33% and *T. decurrens* 8.66% [21], while *P. tetrastomatica* was 16.40% [22]. Based on these data, three types of brown seaweed have relatively high moisture content.

Variability in moisture percentage is speculated to be due to differences in habitat, which affects moisture content produced. This is in line with [23] which states that differences in species characteristics, cell structure, nutrient content, and chemical composition influence moisture content. Furthermore, moisture content is affected by various environmental factors, including storage conditions, temperature, and relative humidity (RH). This aligns with. [24], who noted that variations in moisture content are also influenced by these environmental factors. Moisture content has a significant impact on the presence and stability of secondary metabolites. Moreover, the moisture content of seawater can also affect the efficiency of the seaweed extraction process. Seaweed with optimal moisture content facilitates the solvent's ability to extract active compounds. According to [25], drying time also leads to lower moisture content in a material. The longer a material is exposed to heat, the lower its moisture content becomes due to temperature effects. Extraction is a technique used for the separation of bioactive compounds.

Figure 1 illustrates that the type of brown seaweed, extraction method, and interaction between the type of brown seaweed and extraction method significantly influenced yield of extracts ($p<0.05$). Most optimal interaction observed in combination of *Sargassum* sp. extract with ultrasonic method. Overall, highest yield among three types of brown seaweed was from *Sargassum* sp. extracted using ultrasonic methods, achieving a yield of 20.30±0.17%, followed by *Turbinaria* sp. at 14.51±0.02%. However, the yield from *Padina* sp. treated with both ultrasonic and magnetic stirrer methods was the lowest. Nonetheless, the ultrasonic-treated *Turbinaria* sp. extract showed higher yields compared to the *Sargassum* sp. extract treated with a magnetic stirrer. The varying yields of extracts could indicate differences in the chemical components of macroalgae extracted in polar solvent ethanol. In this context, higher yields indicate a greater presence of polar active components extracted. Differences in macroalgae types and extraction methods were found to significantly influence extract yields. This suggests an interaction between type of brown seaweed and the extraction method regarding yield values. Ultrasonic extraction treatments achieved yields above 10% and tended to be higher compared to those using magnetic stirrer methods. According to [1], ultrasonic assisted extraction (UAE) is a technique that produces higher levels of extraction and effectively yields bioactive components, resulting in significant biological activity. Yields using an ultrasonic assisted extraction (UAE) tend to be higher compared to conventional extraction methods [24]. According to Lee [26] also noted that extractions of *Ecklonia cava* extract ethanol (brown seaweed) using ultrasonic resulted in higher percentages (28.33% and 30.67%) when conducted for 6 and 12 hours at 30°C. According to research reported the [65] ethanol extract of *Sargassum plagyophyllum* yielded an percentage of 0.31±0.06%. Additionally, [67] reported that the ethanol extract of *Padina sp.*, resulted in a yield of 21.16 %. The ethanol extract of *Turbinaria sp.* yielded between 4.4% and 6% [48].

The higher the yield of an active substance, the better the quality of the bioactive components and biological activity in the seaweed. Extract yield is also influenced by the polarity of the solvent, temperature, and extraction time. Ethanol, being a polar solvent, can attract bioactive compounds. Use of 60% ethanol in this study also affected extract yield. According to [27], use of a mixture of water and ethanol, as well as pure ethanol, results in relatively high extract yields, but no significant differences observed, with each solvent Differences in extract yields from a given material influenced by extraction method, sample size, ratio of material to solvent, type of solvent, extraction time, extraction temperature, harvest age, and habitat differences, as stated by [28].

Total phenol test used gallic acid as standard. Total phenol content calculated based on regression equation of gallic acid as standard, $Y=0.038x+47.55$, with a determination coefficient ($R^2$) of 0.9985. Resulting linear regression equation had a good correlation coefficient. Measurement of gallic acid standard showed an increase in absorbance values as concentration increased. Gallic acid in total phenol test is used to identify accurate and consistent results and to provide insights into potential health benefits of phenolic compounds present in samples. Table 2 shows that type of brown seaweed, extraction method, and that interaction between type of brown seaweed and extraction method had a significant effect influenced the total phenol contet ($p<0.05$). Best interaction was observed in combination of *Sargassum* sp. extract with ultrasonic method. Overall, *Sargassum* sp. extracts had highest total phenol content under ultrasonic and magnetic stirrer extraction treatments compared to *Padina* sp. and *Turbinaria* sp. This result is consistent with findings of [21]; [22], who reported that *Sargassum* sp. extracts had highest total phenol content, at 30.34 mg GAE/g, and *Sargassum vulgare* at 10.13±0.166 mg GAE/g.

According to previous researchers [24], also reported that *Sargassum siliquosum* had a relatively high total phenolic content of 30.34 mg GAE/g, and *Sargassum vulgare* had 10.13±0.166 mg GAE/g. Besides [23] further stated that extraction using ethanol as a solvent and assisted by ultrasonics showed that *Sargassum wigtii* had significantly higher phenolic content, at 19.27±0.05 mg GAE/g. The research [27]; [12] reported found ethanol extracts of *Padina* sp. and *Turbinaria* sp. had lower total phenolic content, ranging from 1.59 mg GAE/g to 0.86 mg GAE/g. These results indicate that type of seaweed influences the total phenolic content. Matanjun [29] also stated that differences in total phenolic content in seaweed are due to several factors, such as species, geographic location, season, and physiological conditions. The total phenolic content of brown seaweed generally ranges from 20-30%, as noted by [28]. Variation in total phenolic content in *Sargassum* sp., *Padina* sp., and *Turbinaria* sp. is caused by differences in seaweed species, extraction methods, and variations in chemical composition and biological properties, which impact the amount of bioactive components produced.

The increase in total phenolic content correlates with yield percentage, as higher yields indicate more phenolic content and bioactive components extracted from the seaweed. A higher total phenolic content generally corresponds to greater antioxidant biological activity. Differences in total phenolic content obtained from brown seaweed can also be linked to ecological factors, such as environmental variables including seasonal variations, salinity, irradiation, as well as type of solvent used, and extraction methods. Kumar. [1] reported that ultrasonic extraction is an effective technique for extracting bioactive components and results in significant biological activity. Additionally, solvent concentration used in this study, specifically 60% ethanol, is polar, which is believed to influence total phenolic content. Phenolic compounds are

predominantly polar, necessitating the use of polar solvents for their extraction. that phenolic compounds are largely polar. Polar solvents, such as methanol and ethanol, are particularly effective because they can attract and extract substantial amounts of phenolic compounds [30]. In general, polar solvents like ethanol effective extraction agents for phenolic compounds in brown seaweed. Phytochemical analysis conducted to indicate several classes of bioactive compounds, including flavonoids, steroids, triterpenoids, tannins, saponins, hydroquinone phenols, and alkaloids [31].

Qualitative phytochemical results of brown seaweed identified presence of bioactive components (Table 3). Qualitative screening of phytochemical tests on *Sargassum* sp., *Padina* sp., and *Turbinaria* sp. under ultrasonic treatment identified presence of alkaloids (Wagner and Dragendorff), hydroquinone phenols, flavonoids, tannins, steroids, and triterpenoids, although saponins not detected (negative). Screening under magnetic stirrer treatment detected flavonoids, hydroquinone phenols, steroids, triterpenoids, tannins, and alkaloids, but some compounds were not detected in Sargassum sp. and *Padina* sp., such as saponins and tannins, while *Turbinaria* sp. was negative for triterpenoids and saponins. Overall, it indicates ultrasonic treatment was most effective in extracting various bioactive compounds from three types of brown macroalgae, such as alkaloids, phenols, flavonoids, tannins, steroids, and triterpenoids, while magnetic stirrer method was less efficient in extracting certain compounds or there were differences in compound content depending on species of brown macroalgae.Based on observations from phytochemical testing, presence of alkaloids positively indicated by color changes using Dragendorff's reagent, forming an orange to reddish-orange precipitate, and Wagner's reagent marked by a brown precipitate; alkaloids not detected with Mayer's reagent.

Hydroquinone phenol compounds showed a positive result, indicated by a blackish-blue color change. Flavonoids were characterized by a strong reddish-orange layer. The presence of steroids and triterpenoids was indicated by the formation of a greenish color, and a positive result for tannins was marked by a blackish-blue color. The ultrasonic and magnetic stirrer extraction methods, as well as the type of brown seaweed, showed varied results for bioactive components. This indicates that differences in extraction methods and types of brown seaweed influence bioactive compound components. Gazali [28] stated that the extraction method and solvent are decisive in determining quantity and type of compounds detected from the testing. Variations in bioactive components are also suspected to be due to environmental factors and water conditions affecting content of bioactive compounds. Several studies have reported that brown seaweed contains bioactive compounds. Erniati Syahrial, E, Imanullah and Y. Andika, [2] reported that extracts from *Sargassum* sp. contained bioactive compounds such as flavonoid steroids, tannins, saponins, and hydroquinone phenols but were negative for triterpenoids. *Sargassum* sp. as containing positive results for alkaloids, tannins, quinones, and flavonoids but negative for steroids/triterpenoids [32]. Rushdi et al. [33] found that *Padina* sp. extract detected bioactive compounds such as alkaloids, flavonoids, saponins, steroids, and triterpenoids but was negative for phenolic quinones.

In contrast [24] reported that extracts from *Padina* sp. only contained compounds such as saponins, phenol quinones, and steroids, with negative results for alkaloids, flavonoids, and tannins. Alharbi *et al*. [8] also reported that ethanol extracts from *Turbinaria* sp. contained bioactive compounds like alkaloids, flavonoids, saponins, steroids, and phenol quinonen [34]. added that *Turbinaria decurrens* was detected with compounds such as flavonoids, tannins, and hydroquinone phenols, but negative for steroids and triterpenoids. Based on these results, the three types of brown seaweed have varying contents of bioactive compounds. Bioactive components also offer numerous health benefits. Phenolic compounds are chemical structures with hydroxyl groups capable of donating hydrogen atoms to free radicals, making them potential antioxidants as noted by Vo Dinh et al. [35]. Alkaloids function as antioxidants, antibiotics, and anti-inflammatories, which can reduce pain, improve blood flow, and restore stamina [36]. Phenol quinone is a secondary metabolite widely used in pharmaceutical treatment of malaria and tumors.

This compound has been identified as a potential source of anti-inflammatory, antibacterial, and antioxidant properties by Publishing [37]. Quinone is a compound extensively utilized in pharmaceuticals for treating malaria, as reported by [38]. Flavonoids have identified as a source of antioxidants, free radical scavengers, and agents for antileukemia, vasodilators, and antibacterial applications according to Prasain *et al.* [39]. Tannins have biological properties in medicine as antiseptics, antimicrobials, and antioxidants Rahman et al. [40]. Triterpenoids can enhance psychological and physiological functions in humans and are useful in prevention and therapy of various diseases, including cancer, possessing antimicrobial, antifungal, antiparasitic, antiviral, and anti-inflammatory properties Dembitsky [41]. Steroids play a crucial role in medical health and can treat a variety of conditions such as asthma, eczema, lupus, and hypogonadism disorders [42]. Steroids also have anti-inflammatory properties used in treatments like rheumatoid arthritis and are useful in monitoring medications that inhibit steroidogenic enzymes or detect disease recurrence such as tumor activity and chemotherapy [43]. Bioactive components like phenolic alkaloids, flavonoids, steroids/triterpenoids, and hydroquinone phenols biologically provide health benefits.

Antioxidant substances in brown seaweed such as phenolics and flavonoids, along with other bioactive compounds, have proven to play a crucial role in inhibiting oxidation processes. ABTS (2, 2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (1, 1-diphenyl-2-picrylhydrazyl) are widely used in research as free radicals to investigate compounds in neutralizing free radicals. Figures 2 and 3 show that the type of brown seaweed, extraction method, and that the interaction between the type of brown seaweed and the extraction method had a significant effect influenced the antioxidant activity radical ABTS and DPPH ($p<0.05$). The most optimal interaction was observed in combination of *Sargassum* sp. extract with ultrasonic method. $IC_{50}$ inhibition results of *Sargassum* sp., *Padina* sp., and *Turbinaria* sp. extracts under ultrasonic and magnetic stirrer treatments, demonstrating significant differences in antioxidant activity. In this context, *Sargassum* sp. extracts showed best antioxidant activity among both extraction methods, resulting in $IC_{50}$ inhibition values of 47.94±0.5µg/mL, 99.02±0.9µg/mL, 90.02±0.1µg/mL, and 119.19±0.9µg/mL. Molyneux [44] reported strength of antioxidant activity based on $IC_{50}$ values, categorizing them as

very strong, strong, moderate, and weak if they are respectively less than 50 μg/mL, 50-100 μg/mL, 101-150 μg/mL, and 150-200 μg/mL.

The lower the $IC_{50}$ value, more effective a compound is at reducing free radicals. Conversely, a higher $IC_{50}$ value indicates lower antioxidant activity. Antioxidant activity of *Sargassum* sp. extracts demonstrated $IC_{50}$ values with better reactivity levels compared to antioxidant activities of *Padina* sp. and *Turbinaria* sp. extracts. However, the antioxidant activity of *Sargassum* sp. using the ABTS method showed the best $IC_{50}$ inhibition values in reducing free radicals. This indicates that ABTS tends to be more responsive to chemical interactions or processes faster than the DPPH radical. According to Vijayabaskar *et al.* [45], the ABTS radical reaction involves electron transfer, making it faster than the DPPH radical. Thanigaivel *et al.*[46] stated that ABTS scavenging activity of ethanol extracts from brown algae is far more effective than those using water as a solvent. Overall, the antioxidant activity of the three types of brown seaweed identified *Sargassum* sp. as the best source of antioxidants, with its activity strength ranging from very strong to strong in ultrasonic treatments, while magnetic stirrer treatments ranked as strong to moderate. *Padina* sp. exhibited moderate to weak antioxidant activity, whereas *Turbinaria* sp. showed strong to moderate antioxidant activity.

The higher seaweed extract's capability to scavenge radicals, stronger its antioxidant activity. Variation in antioxidant activity is believed to be due to differences in species, extraction methods, and environmental factors. Total phenolic content is thought to influence quality of seaweed antioxidants, as phenolic compounds play a crucial role in preventing oxidative damagehigher total phenolic content, higher antioxidant activity in a sample. This aligns with findings of [47], who reported that an increase in total phenolic content leads to higher antioxidant activity. Addition of ABTS radical solution to brown seaweed extracts from *Sargassum* sp., *Padina* sp., and *Turbinaria* sp. caused the color to change from blue-green (ABTS) to paler or colorless. This indicates antioxidant compounds in brown seaweed extracts donate electrons to ABTS radical, reducing it to a non-radical, colorless ABTS. Similarly, addition of DPPH solution to brown seaweed extracts resulted in a color change from purple to pale yellow. This occurs when free radicals react with antioxidant compounds, transforming DPPH into a non-radical and harmless form. Working mechanism suggests that ability of antioxidant compounds to neutralize free radicals is measured based on observed color change and quantitatively. In this study, measurement of ABTS and DPPH radical antioxidant activities in *Sargassum* sp. extracts correlated positively with phenolic compound content.

Arguelles [21] reported that the antioxidant activity of brown seaweed *Sargassum polycytum* measured using the ABTS radical had an $IC_{50}$ value of 18.50 μg/mL, capable of inhibiting free radical oxidation. In contrast, Sedjati [48]; Gazalin [28] reported that the DPPH $IC_{50}$ values for *Sargassum* sp. were 62.272 μg/mL and 239.51 μg/mL, respectively. *Padina tetrastromatica* showed an $IC_{50}$ of 29.13±2.31 μg/mL measured by the ABTS method followed by DPPH at 32.39±3.11 μg/mL according to Naveen et al. [49]. The antioxidant activity of *Turbinaria* sp. on the ABTS radical showed an $IC_{50}$ inhibition of 3.15 μg/mL, categorized as very strong antioxidant activity [50]. Ethanol extract of *Turbinaria* sp. showed antioxidant activity against the DPPH radical with an $IC_{50}$ of 0.30±0.02 μg/mL [47]. The variation in antioxidant extraction from seaweed is generally influenced by several factors such as type of solvent, solvent concentration, solvent polarity, seaweed species, time, extraction technique, environmental temperature, and water salinity. According to Lu et al. [51], tyrosinase is a multi-copper oxidative enzyme crucial in melanin synthesis to prevent excessive production of melanin. Tyrosinase catalyzes three reactions in melanin synthesis: the hydroxylation of tyrosine to 3, 4-dihydroxyphenylalanine (DOPA), the oxidation of L-DOPA to dopaquinone, and reaction of 5, 6-dihydroxyindole to melanochrome.

Reactions involving tyrosine and DOPA substrates occur at the same active site on tyrosinase [19]. The use of kojic acid in this study as a positive control shows more effective tyrosinase activity in diphenolase reaction by inhibiting the oxidation of L-DOPA to dopaquinone. According to [52], kojic acid is known to have inhibitory activity against tyrosinase and is more effective in monophenolase reactions. High inhibitory activity of kojic acid on tyrosinase is influenced by period (time) and storage conditions before testing. Kojic acid is an antioxidant used in industry and has been described as an alternative skin-lightening agent Hashemi & Emami [53]. Kojic acid is also non-toxic in acute, chronic, reproductive, and genotoxicity studies. Ability of brown seaweed extract to inhibit tyrosinase activity can be measured by $IC_{50}$ value. Figure 4 shows that differences in types of brown seaweed, extraction methods, and the interaction between extraction methods and sample types have a significant influence ($p<0.05$) on tyrosinase activity. The best interaction was observed in the combination of *Sargassum* sp. extract with the ultrasonic method. Overall, the measurement results of tyrosinase activity from the brown macroalgae extract of *Sargassum* sp. showed the best activity, as indicated by the $IC_{50}$ values of 650.4±1.1 μg/mL and 777.32±1.2 μg/mL on the diphenolase substrate L-DOPA.

Dolarassa, Nurjanah, Anwar, and Hidayat [54] reported that the methanol extract of *Sargassum plagyophyllum* exhibited tyrosinase activity of 1769.339 μg/mL on diphenolase, which is categorized as very weak. Additionally, extracts of *Padina sp.* and *Turbinaria sp.* extracted using ultrasonic and magnetic stirrer methods yielded IC50 values of approximately 1260±1.2 μg/mL, 1077±1.5 μg/mL, 898.2±1.2 μg/mL, and 812.2±1.2 μg/mL, respectively. In contrast, the $IC_{50}$ values of kojic acid are smaller compared to those of the brown seaweed extract. The lower the IC50 value, the stronger the inhibitory effect of a compound against oxidation. Kamakshi [55] reported however, the use of kojic acid as a lightening agent can cause allergies in human skin. The Curto *et al.* [9] reported that a substance is considered to have very strong, strong, moderate, and weak tyrosinase inhibitory activity, respectively, if the $IC_{50}$ values are <25 μg/mL, 50-100 μg/mL, 100-200 μg/mL, and >200 μg/mL in the very weak category. The ability to inhibit the tyrosinase enzyme are also influenced by the content of active components in extract. The phytochemical screening tests on extracts of *Sargassum sp.*, *Padina sp.*, and the *Turbinaria sp.* have shown positive results for the flavonoid compounds.

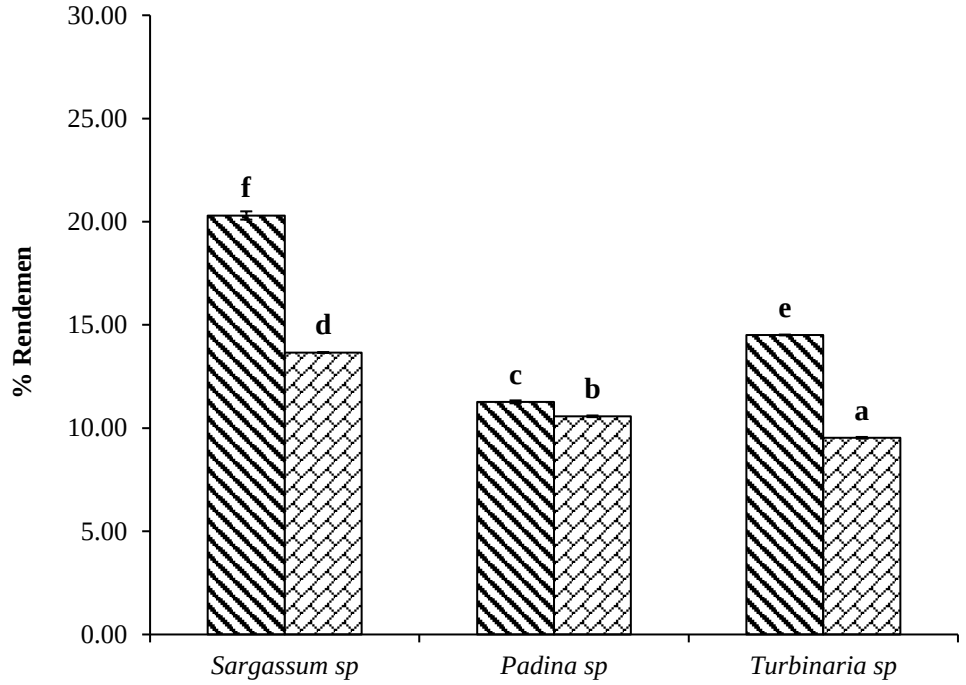


**Figure 1:** The interaction between brown seaweed species and extraction methods on Yield of Extracts. Numbers followed by *superscript* letters (a-f) indicate statistically significant differences ($p<0.05$). Note: (Ultrasonic); (*Magnetic stirrer)*

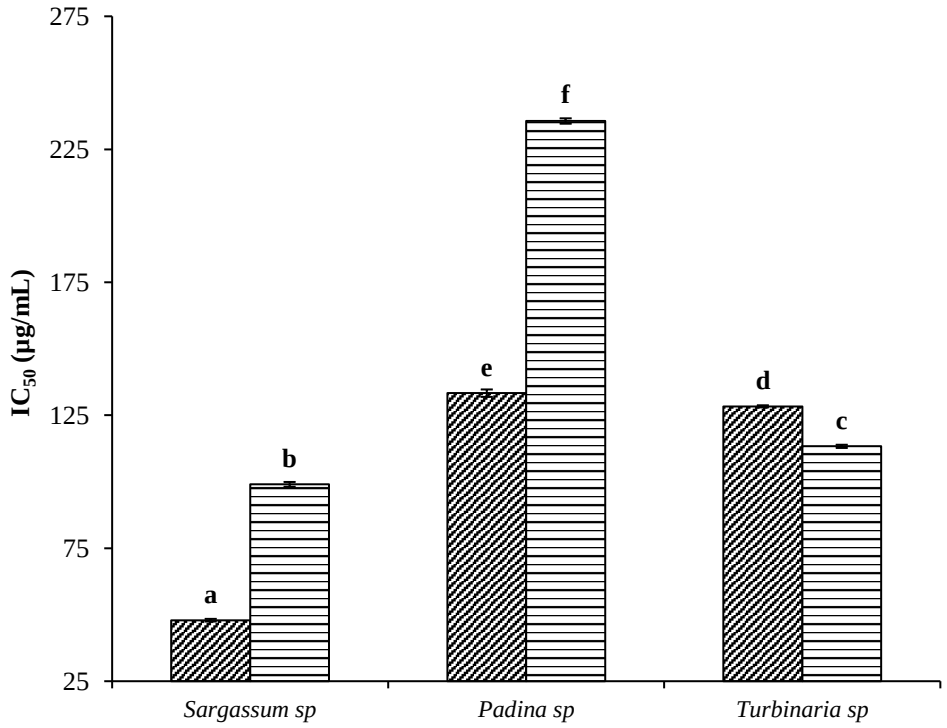


**Figure 2:** The interaction between brown seaweed species and extraction methods on the $IC_{50}$ Antioxidant Inhibition Values for ABTS. Numbers and different superscript letters (a-f) indicate statistically significant differences ($p<0.05$). Note: (Ultrasonic); (*Magnetic stirrer)*

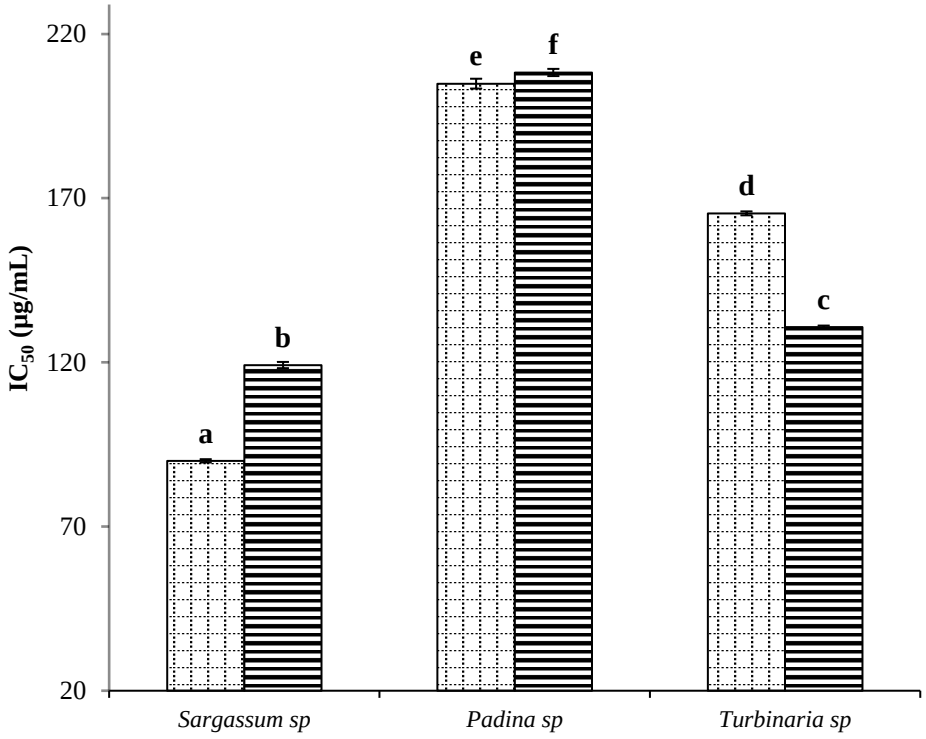


**Figure 3.** The interaction between brown seaweed species and extraction methods on the $IC_{50}$ Antioxidant Inhibition Values for DPPH. Numbers and different superscript letters (a-f) indicate statistically significant differences ($p<0.05$). Note: ⊡ (Ultrasonic); ⊟ (*Magnetic stirrer*)

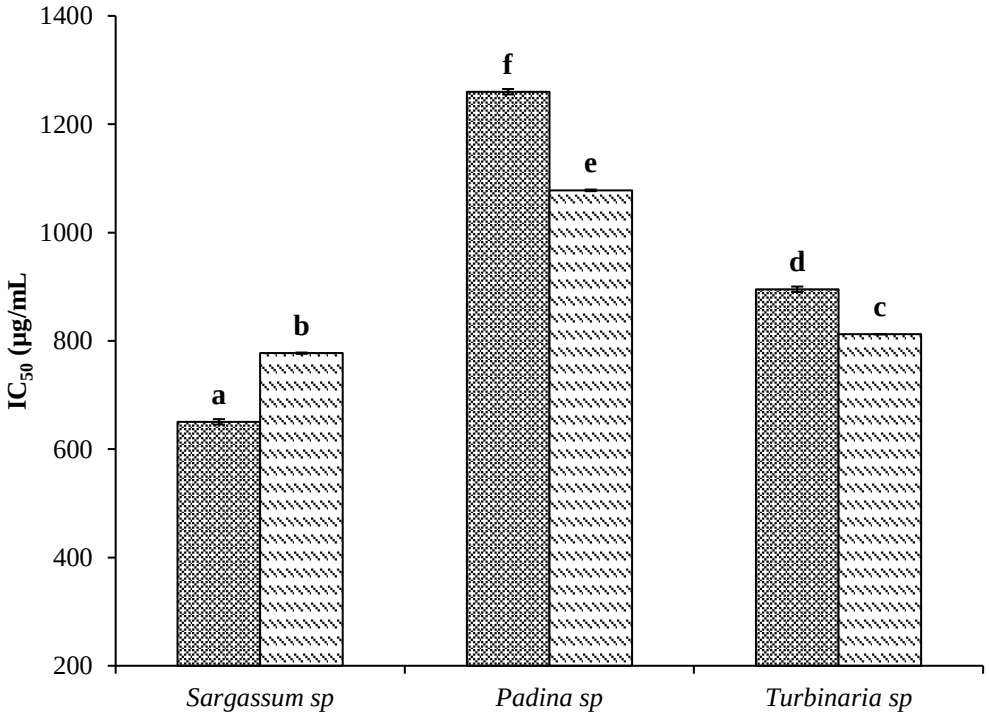


**Figure 4.** The interaction between brown seaweed species and extraction methods on the $IC_{50}$ Inhibition of Tyrosinase Enzyme by L-DOPA. Numbers and different superscript letters (a-f) indicate statistically significant differences ($p<0.05$).
Note: ▨ (Ultrasonic);▨(Magnetic stirrer)

**Table 1.** Moisture Content of Brown Seaweed

| Sample | Moisture Content (%) |
|---|---|
| *Sargassum* sp. | 21.73±0.1[b] |
| *Padina* sp. | 13.31±0.6[a] |
| *Turbinaria* sp. | 23.61±0.5[b] |

Note: Different *superscript* letters (a-b) indicate statistically significant differences ($p<0.05$)

**Table 2.** Total Phenol Content of Brown Macroalgae Extracts

| Sample | Total Phenol Content (mg GAE/gr) | |
|---|---|---|
| | Methods | |
| | Ultrasonic | Magnetic Stirrer |
| *Sargassum* sp. | 50.84±0.3[f] | 43.87±0.4[e] |
| *Padina* sp. | 30.89±0.4[b] | 28.92±0.5[a] |
| *Turbinaria* sp. | 40.19±0.4[d] | 36.82±0.4[c] |

Note: Different superscript letters (a-f) indicate statistically significant differences ($p<0.05$)

**Table 3.** Phytochemical Results of Brown Macroalgae Extracts by Ultrasonic and Magnetic Stirrer Methods

| Parameter | *Sargassum* sp. | | *Padina* sp. | | *Turbinaria* sp. | |
|---|---|---|---|---|---|---|
| | Ultrasonic | Magnetic Stirrer | Ultrasonic | Magnetic Stirrer | Ultrasonic | Magnetic Stirrer |
| Alkaloids: | | | | | | |
| - Wagner | + | + | + | - | + | + |
| - Mayer | - | - | - | - | - | - |
| - Dragendorff | + | + | + | + | + | + |
| Hydroquinone Phenols | - | + | - | + | + | - |
| Flavonoids | + | + | + | + | + | + |
| Saponins | - | - | - | - | - | - |
| Tannins | + | - | + | - | + | + |
| Steroids | + | + | + | + | + | - |
| Triterpenoids | + | + | - | + | - | - |

Chang [56] stated that flavonoids play a significant role in tyrosinase activity due to presence of phenol groups and a pyrene ring. Structure of flavonoids is principally suitable as a substrate and is able to compete for binding at the active site of tyrosinase (Cu atom), thereby inhibiting tyrosinase. Bonding of flavonoids with copper and their antioxidant effects play a role in inhibiting action of tyrosinase enzyme. Charissa [57] reported that skin depigmentation ability of flavonoids occurs by directly inhibiting tyrosinase activity during melanogenesis process. Binding of flavonoids to copper, as well as their antioxidant effects, contributes to inhibiting the function of tyrosinase enzymes. This difference activity tyrosinase is presumed because brown macroalgae extracts of *Sargassum* sp., *Padina* sp., and *Turbinaria* sp., extracted by ultrasonic and magnetic stirrer. De Morais *et al.* [58] suggested varying capabilities of extracts to inhibit tyrosinase result from differences in bioactive compounds contained within extracts, which vary by species, extraction method, type of solvent, substrate, temperature, and extract purity. According to [59], active components in a material are influenced by age, environmental conditions, and growth location, where nutrient and mineral content can differ significantly. Additionally, kojic acid is an inhibitor known for its strong inhibitory effect and stability in cosmetic products. Brown seaweed species such as *Sargassum* sp., *Padina* sp., and *Turbinaria* sp. have also been reported by several studies to possess potential tyrosinase inhibitory capabilities, which can be utilized as skin-lightening agents. One study found that *Padina australis* inhibits tyrosinase activity with an $IC_{50}$ of 32.0 µg/mL [21]. *Turbinaria conoides* reported with an $IC_{50}$ of 188.85 µg/mL [21]. *E. cottonii* exhibited an $IC_{50}$ of 2631.648 µg/mL on a diphenolase substrate. *Sargassum muticum* had $IC_{50}$ values ranging from 21.6-32.8 µg/mL Zeng *et al.* [60]. These results demonstrate that different seaweed species have varying tyrosinase inhibition properties. Differences in tyrosinase inhibition characteristics of seaweed extracts also influenced by environmental factors such as seasonal variations and habitat characteristics, as well as physiological factors as age, harvesting conditions, and strain differences among seaweed species. Balboa *et al.* [61] reported that brown seaweed contains phlorotannins, which are oligomers or polymers of phloroglucinol (1, 3, 5-trihydroxybenzene) linked by aryl (fucol), ether (phloretol, hydroxyphloretol, fuhalol) bonds, both (fucophloretol), or with dibenzodioxin (eckol and carmalol). Tyrosinase is a multi-copper oxidative enzyme essential in melanin synthesis. Melanin is dark pigment produced by skin cells in deepest layer of epidermis Phloroglucinol derivatives from Ecklonia stolonifera have reported to inhibit tyrosinase by capturing free radicals produced during catalytic cycle of enzyme [18].

## 4. Conclusion

The brown seaweed extract from *Sargassum* sp., obtained using ultrasonic extraction method, produced the highest yield percentage and total phenolic content compared to extracts of other brown macro algae species such as *Padina* sp. and *Turbinaria* sp. This extract also contains bioactive components including alkaloids, flavonoids, tannins, steroids, and triterpenoids, and demonstrates significant antioxidant activity and tyrosinase enzyme inhibition. Therefore, it has

potential to be used as an antioxidant material to prevent premature aging, protect against oxidative damage, and serve as a skin-lightening agent due to its tyrosinase inhibitory activity. It is recommended that further research be conducted to analyse the active compound components of seaweed using different species and extraction methods, to expand potential of seaweed as a biomaterial in industry.

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