Antioxidant and Anti-Arthritic Activities of Green Seaweed *Halimeda tuna* Methanolic Extract

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Abstract

Seaweed contains various bioactive compounds rich in pharmacological potentials, such as antioxidants and anti-arthritic. This study aims to determine the antioxidant and anti-arthritic activities of the methanolic extract of *Halimeda tuna*. *Halimeda tuna* samples were extracted using the maceration method and methanol. The methanol extract was tested for antioxidant (2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP)) and anti-arthritic activity using denatured bovine serum albumin (BSA). The methanol extract was also subjected to phytochemical and Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Research results revealed that the extract yield was 0.50 ± 0.06%. Phytochemical analysis showed that *H. tuna* methanolic extract contained flavonoids, steroids, and alkaloids. GC-MS analysis showed that *H. tuna* methanolic extract contained steroids, fatty alcohols, and fatty acids. The antioxidant activity tests revealed an IC_{50} value of 299.98 ± 121.15 ppm and a FRAP value of 200.02 ± 8.50 ηM/g, categorized as a weak antioxidant. Anti-arthritic activity of *H. tuna* methanolic extract showed an IC_{50} value of 877.29 ± 209.84 ppm. The anti-arthritic activity of *H. tuna* methanolic extract was lower than sodium diclofenac as a standard drug. The extract of *H. tuna* has antioxidant and anti-arthritic activities; hence, further exploration is needed as an alternative potential in the pharmacological field.

Keywords: *Halimeda tuna*; phytochemical; DPPH; FRAP; GC-MS

Introduction

Seaweed is a source of various biologically active compounds (Gomez et al., 2019), including antioxidants (Podungge et al., 2018; Gazali et al., 2018). The use of natural antioxidants is increasing due to public unrest regarding the harmful effects of synthetic drugs and the growing tendency to use natural ingredients in everyday life (Halliwell, 1996). Antioxidants are associated with the prevention of potential diseases, including rheumatoid arthritis. The pro-inflammatory signal expression can be modulated and possible protection against rheumatoid arthritis may be shown by substances taken from plants, specifically flavonoids, terpenes, quinones, catechins, alkaloids, anthocyanins, and anthoxanthins. These compounds are known to induce antioxidant effects (Khanna et al., 2007). Rheumatoid arthritis is a chronic autoimmune disease characterized by cartilage degradation, inflammation, synovial hyperplasia, and joint damage (Phull et al., 2017). There has been a significant inverse association between dietary total antioxidant capacity (DTAC) and the risk of rheumatoid arthritis (RA), suggesting that promoting a naturally elevated antioxidant capacity might help prevent the development of RA (Moradi et al., 2022). Elisha et al. (2016) reported that the free radical scavenging capacity of nine South African plants used traditionally to treat arthritis might be related to an immune-boosting potential with total phenolics and flavonoids. Arablou

et al. (2019) also reported that antioxidants play important roles in reducing inflammatory conditions and improving the function of antioxidant enzymes in patients with rheumatoid arthritis.

Halimeda sp. is known to contain bioactive compounds with pharmacological activities, such as anti-inflammatory (Ramalingam et al., 2022), antibacterial (Mariya & Ravindran, 2012), anticancer (Ahmad et al., 2014), antidiabetic (Gunathilaka et al., 2021), and antioxidants (Hendri & Delini, 2020). Gazali et al. (2019a) found that *H. macroloba* has a strong antioxidant ability. Furthermore, Gazali et al. (2019b) revealed that *H. opuntia* also exhibits antioxidant activity. *Halimeda* sp. is a green seaweed with a high abundance and ecological distribution along the coast of Lhok Bubon, West Aceh (Gazali, 2018). The distribution of the genus *Halimeda* is about 9% of the total seaweed vegetation in Aceh waters (Erniati et al., 2022). However, research and utilization of *H. tuna* species in the nutraceutical, health and cosmeceuticals industry remain limited.

Seaweeds reported to have anti-arthritic activity are *Ulva fasciata, U. linza, Corallina officinalis, Jania rubens,* and *Colpomenia sinuosa* which originate from the coast of Alexandria, Egypt (Shobier et al., 2023). Research on the antioxidant and anti-arthritic activity of green seaweed has been reported from the *Caulerpa racemosa* (Sumanya et al., 2015), while the antioxidant and anti-arthritic activity of *H. tuna* has not been widely studied. Therefore, this study aimed to determine the antioxidant and anti-arthritic activity of green seaweed *H. tuna* from Lhok Bubon Coast, West Aceh Regency, Aceh Province, Indonesia.

Material and Methods

Materials

H. tuna was collected from the Coast of Lhok Bubon, West Aceh, Indonesia. The chemicals used were bovine serum albumin (BSA) (Merck, USA), methanol (Merck, USA), phosphate buffer saline (Merck, USA), 2,2-diphenyl-1picrylhydrazil (DPPH) powder (Merck, USA), butylated hydroxytoluene (BHT) (Sigma-Aldrich, Germany), vitamin C (Merck, USA), tris pyridyl triazine (TPTZ) (Sigma-Aldrich, Germany), iron(III) chloride hexahydrate (Merck, USA), iron(II) sulfate heptahydrate (Merck, USA), sodium hydroxide (Merck, USA), ferric chloride (Merck, USA), ammonia (Merck, USA), chloroform (Merck, USA), sulfuric acid (Merck, USA), Dragendorff's reagent (Merck, USA), Mayer's reagent (Merck, USA), and Wagner's reagent (Merck, USA).

Sample Collection

The naturally grew samples were taken from the Pantai Lhok Bubon, West Aceh Regency, Aceh, Indonesia on 15th April 2021. The samples were washed thoroughly using running water before air-drying at 27 °C for four days. The dried samples were sent to Yogyakarta, Indonesia, in styrofoam boxes and stored in the freezer before use. Sample identification was conducted at the Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada, Indonesia.

Antioxidant Extraction

Extraction of *H. tuna* using methanol as solvent followed the procedures outlined by Ibrahim and Kebede (2020). Dried *H. tuna* (250 g) was soaked in 2,000 mL of methanol with a ratio of 1:8. The sample was macerated for 24 hours at 27 °C. The filtrate obtained was further filtered and evaporated using a rotary evaporator (Buchi Rotavapor R-100, Switzerland) with an ambient temperature of 40 °C. The concentrated extract obtained was then evaporated using nitrogen and a freeze-dryer (Alpha 1-4 LSCplus, Christ Germany). The extract was weighed and stored at 4°C.

Phytochemical Analysis

Phytochemical analysis was conducted to determine the content of secondary metabolites in the *H. tuna* extract. The analysis was conducted to examine the flavonoid, steroid-triterpenoid, saponin (Lubis et al., 2020), alkaloids, tannin (Widowati et al., 2021), and phenol hydroquinone contents (Manongko et al., 2020).

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity Assay

DPPH radical scavenging activity was performed by modifying the procedure proposed by Marraskuranto et al. (2021). Methanolic extract of *H. tuna*, BHT, and vitamin C was prepared using distilled water and allowed to homogenize. Furthermore, 0.76 mM DPPH solution was prepared by dissolving 3 mg of DPPH powder in 10 mL distilled water and stored at a temperature of 4 °C for 24 hours. The solution was further incubated at 27 °C for 30 minutes followed by absorbance reading using a UV-Vis spectrophotometer (Genesys 10S, Germany) at a wavelength of 517 nm. In addition, the antioxidant was presented in accordance with the inhibition percentage as follows.

$$\text{Inhibition activity (\%)} = \frac{(C-D)-(A-B)}{(C-D)} \times 100\%$$

where A is the sample (800 µL sample + 200 µL 0.76 mM DPPH), B is the control sample (800 µL sample + 200 µL distilled water), C is the negative control (800 µL distilled water + 200 µL 0.76 mM DPPH), and D is the blank (800 µL distilled water).

The percentage of inhibition was then generated by calculating the concentration log value and prohibition value using MS Excel. Then, a standard curve of the log concentration value was created and the line equation y=ax+b was determined. Finally, the IC_{50} value in ppm units was determined.

FRAP (Ferric Reducing Antioxidant Power) Assay

The FRAP assay was conducted referring to Clarke et al. (2013) and Marraskuranto et al. (2021), reducing Fe^{3+} to Fe^{2+} with a spectrophotometer (Genesys 10S, Germany) at a wavelength of 595 nm. The changes were analyzed to determine the formation of a blue color in the solution in the test sample. Furthermore, 0.775 g of sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$) was added to 4 mL of concentrated acetic acid dissolved in water to determine the acetate buffer solution with pH 3.6. The buffers were stored as stock solutions at a temperature of 4 °C with 0.15 g of TPTZ in 40 mM HCl dissolved in 50 mL of distilled water to obtain 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ). Furthermore, 0.828 mL of concentrated HCl was dissolved in 250 mL of distilled water and a TPTZ solution was stored at 4 °C for 24 hours to obtain 40 mM HCl. A solution of 20 mM $FeCl_3 \cdot 6H_2O$ was also prepared by dissolving 0.54 g of $FeCl_3 \cdot 6H_2O$ in 100 mL of distilled water for 24 hours at 4 °C before usage. A FRAP reagent was also prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ, 2.5 mL of $FeCl_3 \cdot 6H_2O$ solutions (10:1:1), and 100 mL of distilled water. Furthermore, the standard $FeSO_4 \cdot 7H_2O$ (10,000 ìM) solutions were produced by dissolving 2.78 g $FeSO_4 \cdot 7H_2O$ in 1,000 mL distilled water, before serially diluting to attain 50, 100, 150, 200, 250, and 300 ppm concentrations. This was followed by preparing sample solutions from *H. tuna* (1,000 ppm), BHT (20 ppm), and vitamin C (10 ppm). Furthermore, a 20 µL sample solution was added to 150 µL of FRAP reagent in a 96-well microplate and was measured the absorbance using a UV-VIS spectrophotometer (Genesys 10S, Germany) at a wavelength of 595 nm. The standard $FeSO_4$ solution was also given a similar treatment. Absorbance data were entered into the line equation until the FRAP value was obtained, expressed in µM/g.

In Vitro Anti-arthritic Assay using Denatured Bovine Serum Abumin (BSA)

In vitro anti-arthritic activity test refers to Sumanya et al. (2015) and Vaijayanthimala et al. (2019). The test solution was prepared using 0.5 mL of the test sample solution consisting of 0.45 mL of 5% BSA and 0.05 mL of *H. tuna* extract. Samples were made in a dilution series of 62.5, 125, 250, 500, and 1,000 ppm. Meanwhile, the control product was added with 0.45 mL of distilled water and 0.05 mL of diclofenac sodium. The dilution series were 62.5, 125, 250, 500, and 1,000 ppm. Then, a test control was made with 0.45 mL of 5% BSA and the addition of 0.05 mL of distilled water. Subsequently, the sample solutions, including the test solution, product control, and test control, were incubated in an oven for 20 minutes at 37 °C. After that, the sample solution was warmed for 3 minutes at 57 °C. After cooling, 2.5 mL of phosphate buffer solution pH 6.3 was added, and the absorbance was read using a UV-Vis spectrophotometer (Genesys 10S, Germany) at a wavelength of 416 nm. The absorbance value for each parameter was entered into the following formula

$$\% \text{ Inhibition} = 100 - \frac{(A)-(B)}{(C)} \times 100\%$$

Where A is the absorbance of the test solution, B is the absorbance of product control, and C is the absorbance of the test control.

The inhibition percentage obtained was generated by calculating the concentration log value and prohibition value using MS. Excel. Next, a standard curve of the log concentration value was created and the line equation y=ax+b was determined. Finally, the IC_{50} value in ppm units was determined.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Chemical compounds of *H. tuna* was identified using GC-MS QP2010S (Shimadzu, Japan), with an RTX-5MS 30 m long column and 0.25 mm in diameter. A total of 0.1 µL of the extract was injected in the column with the operating conditions of the injector temperature: 290 °C, oven temperature from 70 °C to 280 °C (4 °C/min), UHP grade Helium carrier gas with a flow rate of 0.5 mL/min at 290°C, methylated solution of methanol and BF_3, split ratio 193, and at a pressure of 13.7 kPa. Mass spectrum GC-MS was interpreted using the database of National Institute Standard and Technology (NIST), containing more than 62,000

patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained.

Data Analysis

Antioxidant and anti-arthritic activity data in the form of extract concentration versus percent inhibition were used to create a regression equation. Antioxidant and anti-arthritic activity was expressed in IC_{50}, obtained from the regression equation. The antioxidant activity data for the DPPH and FRAP methods were tested using ANOVA, while the anti-arthritic activity data was tested using the t-test (95% confidence interval). Data was analyzed using Microsoft Excel 2013 application and IBM Statistics SPSS 26.

Results and Discussion

Halimeda Tuna Methanolic Extract Yield

The amount of active substance produced in an extraction will affect the yield value. The yield value is critical to determine the amount of extract produced during extraction. The yield data is also closely related to the active compounds in a sample. The higher the yield, the more active compounds in the sample (Pawarti et al., 2023). The yield is generated by calculating the ratio between the initial and final weight of the *H. tuna* extract sample that has gone through the maceration, evaporation, and freeze-drying methods. The crude *H. tuna* methanolic extract yield is 0.50 ± 0.06%. The yield of the extract resulting from this research was lower than the ethanol extract of *Halimeda opuntia* (4.77%) (Nufus et al., 2017) but greater than the methanol extract of *Halimeda macroloba* (0.34%) (Muzaki et al., 2018). The differences are caused by the extraction method used, sample size, ratio of material and solvent, type of solvent, extraction time, extraction temperature, harvest age, and differences in the habitat of the samples used (Gazali et al., 2023).

Phytochemical Properties of H. Tuna Extract

Phytochemical assay was conducted to determine the content of secondary metabolites in the sample extract (Arsianti et al., 2016). The overall result of phytochemical testing can be seen in Table 1. Phytochemical tests showed that *H. tuna* extract contained secondary metabolites: flavonoids, steroids, and alkaloids. Steroids are classified as terpenoid compounds with antioxidant benefits and alkaloid compounds with pharmacological activities (Gunawan et al., 2016). Flavonoids are polyphenol compounds that can be used as antioxidants (Valaan & Raphael, 2016). The secondary metabolite compounds are flavonoids, containing primary and secondary antioxidants (Arinanti, 2018). The steroids are primary antioxidant (Hardiningtyas et al., 2014), and alkaloids are also categorized as primary antioxidant due to their working mechanism (Kartika et al., 2020). These results agree with a previous study by Gazali et al., (2023) reporting that the methanol extract and ethyl acetate fraction of *H. tuna* include bioactive substances such as phenol hydroquinone, alkaloids, steroids, and flavonoids. Husni et al., (2023) also reported that *H. tuna* crude extract contained flavonoid, steroid, and alkaloid. Ahsan et al., (2020) reported that *H. opuntia* extracts also contained phenolic compounds and steroids. Phytochemical test showed methanol extract of *H. gracilis* contains phenols and steroids (Basir et al., 2017). Chloroform, methanol, ethanol, acetone and water extracts of *H. gracilis* contain alkaloids, terpenoids, steroids, tannins, saponins, quinones, and glycosides (Suganya et al., 2019).

Table 1. Phytochemical properties of *H. tuna* extract

Phytochemical Properties	Result	Colour Indicator
Flavonoid	++	Yellow or Orange
Steroid	++	Blue or Green
Triterpenoid	-	Red or Purple
Saponin	-	Formed foam
Alkaloid test		
Dragendorff's	++	Orange Precipitate
Mayer	-	White Precipitate
Wagner	-	Brown Precipitate
Tannin	-	Blue – Black
Fenol hydroquinone	-	Reddish - orange

Notes: ++ = strongly detected; + = weakly detected; - = not detected

Antioxidant activity of Halimeda tuna methanolic extract

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

Based on the test results, the inhibition activity of DPPH free radicals by *H. tuna* methanolic extract, vitamin C, and BHT are shown in Table 2. The antioxidant activity of the DPPH method on the methanol extract of *H. tuna* with concentrations of 500, 1000, 2000, 3000, and 4000 ppm obtained percent inhibition of 64.69, 76.30, 77.42, 92.19, and 97.02, respectively. Table 2 illustrates that the greater the concentration of *H. tuna* methanolic extract, the higher the inhibition activity. Based on the inhibition activity of vitamin C towards DPPH free radicals, the higher the concentration tested, the higher the inhibition activity of vitamin C and BHT. Based on the research outcomes, the value of IC_{50} on vitamin C, BHT, and *H. tuna* methanolic extract is obtained and presented in Table 2.

Antioxidant activity can be classified based on the IC_{50} value (Hasim et al., 2019). The IC_{50} value is the effective concentration (ppm) of a sample to capture 50% of free radicals and is measured to evaluate the effectiveness of antioxidants (Molyneux, 2004). If the IC_{50} value is less than 50 ppm, the antioxidant activity is very strong. If the IC_{50} value is within the range of 50-100 ppm, it is assumed to be a strong antioxidant. Meanwhile, antioxidants are classified as moderate if the IC_{50} value is between 101 and 150 ppm. Meanwhile, antioxidants are considered weak if the IC_{50} value is between 151 and 200 ppm, and antioxidants are considered very weak if the IC_{50} is above 200 ppm (Salim et al., 2018). The results showed that the IC_{50} value of *H. tuna* methanolic extract was 299.98 ± 121.15 ppm, whereas, for BHT, it was 98.82 ± 21.60 ppm, and for vitamin C, it was 20.48 ± 1.64 ppm. Vitamin C has a lower IC_{50} value in this study compared to the research by Haryani et al. (2022), reporting the IC_{50} of 3.57 ppm. Meanwhile, BHT has a lower IC_{50} value than the one reported in a study by Mandrekar et al. (2019), which was 83.30 ppm.

The study by Basir et al. (2020) revealed that the IC_{50} value of *H. gracilis* is 470.49 ppm, while *H. macroloba* is 820.51 ppm. Similarly, Bharath et al. (2021) found that the IC_{50} of *Turbinaria ornate* is 70.62 ppm. In addition, the IC_{50} value of *Gracilaria Salicornia* is 12.81 ppm based on a study by Sanger et al. (2019). These credible studies underscore the importance of the antioxidant activity of different seaweed extracts, with *H. tuna* showing less significant activity compared to *H. gracilis* and *H. macroloba*, but falling short when compared to the brown seaweed extract *Turbinaria ornata* and red seaweed extract *Gracilaria salicornia*.

FRAP (Ferric Reducing Antioxidant)

FRAP method is an approach to assessing the activity of antioxidants based on the reduction of ion Fe^{3+} to Fe^{2+} forming *ferrous-tripyridyltriazin's* complex. The FRAP value is obtained by comparing changes in specific absorbances in the test reaction mixture with the content of Fe^{2+} ions at a particular concentration (Rodríguez-Bonilla et al., 2017). Based on the standard $FeSO_4$ curve, the equation $y = 0.0013x + 0.0573$ with $R^2 = 0.9673$ is obtained.

The $FeSO_4$ solution standard curve is assumed to measure the equivalent of the sample with the amount of Fe^{2+} from the reduction of Fe^{3+}. The solution of $FeSO_4$ was mixed with FRAP reagents, and the absorbance value was measured based on the environment of the sample used. The equivalence of Fe(II) as the concentration of the solution producing the same absorbance as the standard solution of $FeSO_4$ is expressed as antioxidant activity in the FRAP method.

Table 2. Inhibition activity of DPPH by *H. tuna* extract, vitamin C, and BHT

Sample	Concentration (ppm)	% Inhibition of DPPH	IC_{50} (ppm)
H. tuna extract	500	64.69 ± 9.34[a]	299.98 ± 121.15[c]
	1,000	76.30 ± 13.72[a]	
	2,000	77.42 ± 20.26[a]	
	3,000	92.19 ± 2.79[a]	
	4,000	97.02 ± 7.14[a]	
Vitamin C	5	29.59 ± 2.58[c]	20.48 ± 1.64[a]
	10	43.30 ± 1.84[b]	
	15	45.77 ± 1.04[ab]	
	20	48.25 ± 1.35[a]	
BHT	15	16.91 ± 1.10[b]	98.82 ± 21.60[b]
	20	17.72 ± 1.11[b]	
	25	18.89 ± 1.48[b]	
	30	32.38 ± 2.50[a]	

[a-c]The letter differences in the same column show a significant difference ($p<0.05$)

Table 3. Antioxidant activity of vitamin C, BHT, and *H. tuna* methanolic extract using the FRAP method

Sample	The concentration (ppm)	FRAP Value (µM/g)
Vitamin C	10	439.00 ± 19.13[a]
BHT	20	379.51 ± 13.26[b]
H. tuna	1,000	200.02 ± 8.50[c]

[a-c]The letter differences in the same column show a significant difference ($p<0.05$)

Table 4. The anti-arthritic activity of *H. tuna* methanolic extract and sodium diclofenac

Sample	Concentration (ppm)	% Inhibition	IC_{50} (ppm)
H. tuna	62.5	24.20±1.81[a]	877.29 ± 209.84[a]
	125	31.74±4.95[ab]	
	250	32.54±2.47[ab]	
	500	33.73±11.31[ab]	
	1,000	52.77±13.95[b]	
Sodium diclofenac	62.5	27.30±3.03[a]	262.07 ± 15.75[b]
	125	34.13±2.51[b]	
	250	46.98±3.19[c]	
	500	54.61±0.69[d]	
	1,000	82.33±1.84[e]	

[a-b]The letter differences in the same column show a significant difference ($p<0.05$)

According to the equation, the value of y increases by 0.0013 for every unit increase in x (the concentration of $FeSO_4$). The linear model explains a very high percentage of the variation in the measured y values based on variations in $FeSO_4$ concentration (x), as indicated by the R^2 value of 0.9673, demonstrating a extremely good fit. Table 3 shows a comparison of FRAP values in *H. tuna* samples, vitamin C, and BHT .

Based on antioxidant testing using the FRAP method on *H. tuna* extract, a value of 200.02 ± 8.50 ìM/g was obtained. The 10 ppm concentration of vitamin C and the 20 ppm concentration of BHT antioxidants obtained FRAP values of 439.00 ± 19.13 ìM/g and 379.51 ± 13.26 µM/g, respectively. This result shows the antioxidant activities of vitamin C and BHT are higher than that of *H. tuna* methanolic extract. According to Gazali et al., (2020), the green seaweed *Chaetomorpha antennina* has a FRAP value of 576.59 µM /g. Gazali et al., (2021) also reported that the green seaweed *Enteromorpha flexuosa* has a FRAP value of 474 µM/ g. Furthermore, Gazali et al., (2022) showed that the green seaweed *Caulerpa racemosa* from Aceh has a FRAP value of 568 ηM/g.

In vitro Anti-arthritic activity using denaturation BSA (Bovine Serum Albumin)

Table 4 presents the anti-arthritic activity test results of *H. tuna* methanolic extract and *so*dium diclofenac. Based on the results using the BSA denaturation method, the inhibition percentage of *H. tuna* methanolic extract with concentrations of 62.5, 125, 250, 500, and 1,000 ppm were 24.20, 31.74, 32.54, 33.73, and 52.77, respectively. The results show that the greater the concentration, the higher the inhibition percentage of the BSA denaturation process, indicating an anti-arthritic activity in *H. tuna* methanolic extract.

Sodium diclofenac is commonly used as a positive control because it is a commercial anti-arthritis drug of the NSAID group, and its ingredients are easily available (Abidin et al., 2019). In vitro anti-arthritic testing on sodium diclofenac was done by the BSA denaturation method at concentrations of 62.5; 125; 250; 500; and 1,000 ppm and showed an inhibitory value of 27.30, 34.13, 46.98, 54.61, and 82.32%, respectively, indicating the high anti-arthritic activity in sodium diclofenac.

Sodium diclofenac has a higher anti-arthritic activity (IC_{50} = 262.07 ± 15.75 ppm) when compared to *H. tuna* methanolic extract (IC_{50} = 877.29 ± 209.84 ppm). In vitro anti-arthritic research by Sumanya et al. (2015) found that *C. racemosa* had an inhibition activity of 49.33% at a concentration of 1,000 ppm. Meanwhile, *Chondrus crispus* can inhibit denaturation by 80.79% at a concentration of 200 µg/g (Alkhalaf, 2021). The percentage of inhibition of *Undaria pinnatifide* was 79.38% at a concentration of 100 µg/mg (Phull et al., 2017). Therefore, the anti-arthritic activity in *H. tuna* methanolic extract is greater than in *C. racemosa* methanolic extract.

One of the secondary metabolite compounds contained in *H. tuna* is flavonoids (Table 1). Aisyiyah et al. (2021) argued that flavonoids could regulate the transcription factor NF-kB in the expression of TNF-

a. The regulatory process inhibits the secretion of the proinflammatory cytokine TNF-a. The inhibition of lipoxygenase and the COX pathway prevents the accumulation of leukocytes, lowering the secretion of proinflammatory cytokines. Flavonoids selectively reduce the formation of proximal signal complex IL-1R, except in TNFR-1. In addition, flavonoids inhibit TACE, preventing the formation of the proinflammatory cytokine TNF-a through hydrogen bonds of proteins with negative bond energy affinity. Decreased proinflammatory cytokines TNF-a and IL-6 expression decreases the inflammatory potential in rheumatoid arthritis. As primary antioxidants, flavonoids can provide hydrogen ions to stabilize free radical ions (Hariyanto, 2017). Meanwhile, flavonoids act as secondary antioxidants by increasing the synthesis of the antioxidant enzyme endogenous superoxide dismutase (SOD), decreasing oxidative stress states in tissues with a stable ion state (Amarawati et al., 2019).

The tests conducted in this study revealed the correlation between anti-arthritic and antioxidant activity and the content of active compounds in *H. tuna* extract, with reasonably good results. However, the inhibition percentage of *H. tuna* is lower than that of the standard diclofenac sodium. In addition, it is necessary to carry out anti-arthritic testing *in vivo* to evaluate the biodistribution and stability of *H. tuna* extract against rheumatoid arthritis.

GC-MS analysis of Halimeda tuna methanolic extract

Identification of the compounds in the methanol extract of *H. tuna* was done using the GC-MS method to determine the compounds in the extract. Table 5 presents the identification results and biological activity of *H. tuna* extract compounds, potential antioxidant compounds, and anti-arthritic (anti-inflammation).

The compounds in *H. tuna* methanolic extract include steroids, fatty alcohol, and fatty acid. Stigmasta-5,22-dien-3-ol, trifluoroacetate, (3.beta., 22E)- (CAS) 24-Ethyl-3. Beta.-trifluoroacetoxy-5,22-cholestadiene are part of the steroid group. Meanwhile, 1-Hexadecanol, 3,7,11,15-tetramethyl- (CAS) dihydrophytol are classified as a fatty alcohol. However, Dodecanoic acid, 1a, 2, 5, 5a, 6, 9, 10, 10 a-octahydro -5a-hydroxy- 4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8a-methanocyclop enta [a] cyclopropa [e] cyclodecen -5 -yl ester, [1aR (1a.alpha., 2.alpha., 5.beta., 5a.beta., 8a.alpha., 9.alpha., 10a.alpha.)] or lauric acid; and Hexadecanoic acid, 2-(octadecyloxy) ethyl ester (CAS) 2-Octadecyloxy-1-O-hexadecanoylethanol are considered as fatty acid. Steroids act as antioxidants by absorbing free radicals

Table 5. Identified compounds and their biological activities from *H. tuna* methanolic extract

Peak	RT	Area (%)	SI	Compounds	Group	Activity
1	12.308	32.340	19	*Stigmasta-5,22-dien-3-ol, trifluoroacetate, (3. beta.,22E)-(CAS) 24-Ethyl-3. Beta. -Trifluoroacetoxy-5,22-Cholestadiene*	Steroid	Antioxidant, antimicrobial, anticancer, inhibition of chemo-carcinogens (NCBI, 2022)
4	19.683	0.010	61	*1-Hexadecanol, 3,7,11,15-tetramethyl- (CAS) Dihydrophytol*	*Fatty alcohol*	Antioxidant and Antimicrobial (NCBI, 2022)
6	20.883	6.780	34	*Dodecanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8a-methanocyclop enta[a]cyclopropa[e]cyclodece n-5-yl ester, [1aR-(1a. alpha.,2. alpha.,5. beta.,5a. beta.,8a. alpha.,9. alpha., 10a.alpha.)]* or l*auric acid*	*Fatty acid*	Anti-inflammation and Antimicrobial (NCBI, 2022)
7	21.065	20.730	70	*Hexadecanoic acid, 2-(octadecyloxy) ethyl ester (CAS) 2-Octadecyloxy-1-O-hexadecanoylethanol*	*Fatty acid*	Antioxidant, Anti-inflammation, Antivirus (NCBI, 2022)

and exhibit anti-inflammatory, anti-chemo-carcinogens, and anticancer properties (Loori et al., 2021). According to Sofyan et al. (2017), fatty acids are beneficial for preventing atherosclerosis, thrombosis, and arthritis. Additionally, they function as antioxidants by reducing free radicals and exhibit anticancer properties (Kim et al., 2010).

Based on Nazarudin et al. (2020), *H. macroloba* contains stigmast-5-en-3-ol (RT 30.028 dan % area 1.24) compounds, hexadecanoic acid methyl ester (RT 20.545 dan % area 6.40), 1-dodecanol (RT 22.334 dan % area 1.17), and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (RT 23.601 dan % area 1.91). Thus, it can be assumed that the *Halimeda* genus contains compounds from similar groups.

Conclusion

The methanolic extract of *H. tuna* from Lhok Bubon Coast, West Aceh Regency, Aceh Province, Indonesia has antioxidant activity, as evidended its inhibition of DPPH (IC_{50} 299.98 ± 121.15 ppm) and FRAP (200.02 ± 8.50 ìM/g), categorizing it as a weak antioxidant. *Halimeda tuna* methanolic extract also exhibits anti-arthritic activity (IC_{50} 877.29 ± 209.84 ppm), although its activity is lower than that of the standard drug sodium diclofenac (IC_{50} 262.07 ± 15.75 ppm). GC-MS analysis showed that *H. tuna* methanolic extract contained steroids, fatty alcohols, and fatty acids. These findings suggest a promising potential for the *H. tuna* extract in the field of natural products and drug discovery.

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Supplementary Materials

Supplementary materials is not available for this article

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