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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT POTENTIAL OF EXTRACTS ETHANOL MACROALGAE *Padina australis* FROM TEGAL MAS ISLAND LAMPUNG

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ABSTRACT

In the seas of Lampung, a kind of brown macroalgae known as *Padina australis* is commonly seen.. *Padina australis* is widely used in the food industry as a gelling agent, emulsifier, thickener, stabilizer and also in the medical industry. The content of phytochemical compounds that can be developed as phytopharmaceutical agents is one of them as antioxidants. The aim of this study was to analyze the phytochemical content and potential antioxidant activity of the macroalgae *Padina australis*. The method used was extraction by maceration in 70% ethanol as a solvent. The phytochemical test was performed qualitatively by a color reagent test. The FTIR test was performed to analyze the functional groups of the active ingredients, while the antioxidant test used the DPPH (1,1-diphenyl-2-picrylhydrazil) method. The results obtained show that the ethanol extract of *Padina australis* contains chemical compounds such as saponins, steroids, tannins, alkaloids, and flavonoids. *Padina australis* extract shows its potential as an alternative source of natural antioxidant compounds because it is able to reduce free radicals with an IC_{50} value of 407.96 ppm, which is classified as very weak antioxidant activity. Further research needs to be done to obtain other concentration doses so as to get the best antioxidant activity results that can be developed clinically for health products.

Keywords: antioxidant, DPPH, phytochemical, macroalgae, *Padina australis*

INTRODUCTION

A large radical is an unstable molecule that has one free electron. This molecule seeks electron pairs from other molecules to achieve its stability. Sources of free radicals include sunlight, air pollution, cigarette smoke, chemicals in food, drugs, beauty products and are also formed naturally during metabolism and radiation stress (Nurkhasanah et al., 2023). Free radicals can damage body

cells, especially DNA and proteins (Liu, 2022). Oxidative stress caused by free radicals can be prevented by consuming antioxidants. Antioxidants can be found in a variety of foods and beverages. Antioxidants work by capturing and neutralizing free radicals, thereby increasing the body's defenses (Martemucci et al., 2022).

Antioxidants are divided into two types, namely natural and synthetic. Natural antioxidants are found in many plants such as fruits, grains, and vegetables, including flavonoid derivatives, phenols, ascorbic acid and coumarins (Dalimartha, 1999). Synthetic antioxidants are made in the food industry with a variety of raw materials and technologies. The price is relatively cheaper compared to natural antioxidants. Consuming enough antioxidants can prevent degenerative disease and boost the immune system. Therefore, optimal intake of antioxidants is very important to maintain. The development of natural products as a source of antioxidants, such as macroalgae is very necessary to overcome free radicals.

Macroalgae have habitats in tropical waters such as Indonesia and tidal areas always get continuous exposure to sunlight or ultraviolet radiation. Ray radiation UV has enough energy to encourage excessive formation of free radicals causing oxidative stress (Takshak & Agrawal, 2019). Secondary metabolites contained in macroalgae have important pharmacological effects such as antioxidant, antibacterial, antiviral, and toxic (Hidayah, 2016).

Macroalgae *P. australis* is a large-sized species of marine algae of the Division *Phaeophyta* with a wide transparent brown sheet shape scattered in shallow to deep sea waters (Fadel et al., 2022) and is found widely in the coastal waters of Indonesia. Brown algae *P australis* Often used as a gelling agent, thickener, emulsifier, and stabilizer in the food industry, as well as anticancer and anti-obesity in the drug industry, due to the presence of hydrocolloid compounds such as fucosanthine and alginate *(Pesang* et al., 2020). Based on phytochemical research by Haryani et al., (2015) extract *P. australis* contains active compounds flavonoids, alkaloids, tannins, saponins and phenol hydroquinone. The structural components of phenolic compounds in brown macroalgae can also act as protection against UV exposure, biotic and abiotic environmental factors, and have beneficial therapeutic properties. It's the Magic et al., (2017) It also reported that phenol hydroquinone, triterpenoids, alkaloids, flavonoids, saponins, and tannins were found in *P. australis* has an IC_{50} value amounting to 87,028 ppm in order for it to serve as a powerful antioxidant source.

Previous research has proven that *P. australis* macroalgae have the potential to be an antioxidant agent. In addition, exploration of the potential antioxidant activity of *non-commercial P. australis* in the waters of Tegal Mas Island, Lampung has not been widely studied. Efforts to optimize the use of Indonesia's marine natural resources are by examining the potential antioxidant activity of several marine macroalgae found in Lampung so that they can be used as traditional medicine ingredients for various disease treatments. So further pharmacological studies are needed to identify the active constituents of plant extracts in this case brown macroalgae *P. australis* originating from the waters of Tegal Mas Island, Lampung which can be responsible for antioxidant activity.

METHOD

Tools and Materials

The tools in this study are ovens, rotary evaporators, blenders, beakers, 60 mesh filters, analytical balances, erlenmeyers, funnels, test tube racks and test tubes, vial bottles, micro-pipettes and micro-tips, drip pipettes, spatulas, Shimadzu 1800 UV-Vis Spectrophotometer, FTIR Agilent Carry 630 Spectrophotometer.

The materials in this study are *Padina australis macroalgae*, 70% ethanol, iron(III) chloride ($FeCl_3$), glacial acetic acid (CH_3COOH), chloroform, mercury(II) chloride ($HgCl_2$), potassium iodide (KI), concentrated hydrochloric acid (HCl), sulfuric acid (H_2SO_4), Mg powder, DPPH (1,1-diphenyl-2-picrylhydrazil), and ascorbic acid.

Procedure

Macroalgae Sampling and Preparation

Brown macroalgae samples *P. australis* taken from the coast of Tegal Mas Island, Teluk Pandan District, Pesawaran Regency, Lampung. Samples were taken directly within 10 m of the shore and carried out when the sea water was receding (Saputra, 2023). The sample is then sorted and washed using running water and then dried to be further mashed into fine simplisia using a blender. The sample is filtered by 60 sieves *Mesh*, the simplisia powder obtained is weighed and stored at room temperature.

Macroalgae Extraction

The extraction method uses maceration by immersing 300 g of macroalgae samples in a 70% ethanol solvent with a ratio of 1:10. In order for the entire surface of the simplisia to mix well, macroalgae samples are occasionally stirred during the maceration process. The maceration process is carried out in as much as 3 x 24 hours. After that, the sample is filtered with filter paper to obtain maceration filtrate. This extraction method refers to research Marlis (2008) that has been modified. The resulting filtrate is concentrated using *rotary evaporator* temperature 40°C until a thick paste is formed with the aim that the ethanol content is lost and macroalgae ethanol extract *P. australis* become more durable. Next, the extract is stored in vial bottles and labeled.

Identification of macroalgae phytochemical compounds

Kandungan senyawa bioaktif ekstrak ethanol macroalgae *P. australis* analyzed by conducting phytochemical screening tests. Test methods used based on research Tasmin *et al*., (2014) Includes examination of flavonoid compounds, alkaloids, taniins, saponins, steroids and terpenoids as follows:

1. **Water Saponin**

A total of 0.5 mL of macroalgae sample was inserted into a test tube and followed by the addition of 5 mL of aquadest, then shaken until foamy and added 1 drop of HCl 2 N. The resulting foam, indicating a positive sample containing saponins.

2. **Test Steroids and Terpenoids**

A total of 0.5 mL of sample was transferred to a test tube and dissolved in 0.5 mL of glacial acetic acid and concentrated H_2SO_4. If there is a brownish or violet ring in the solution, this indicates the presence of terpenoids in the sample. However, blue, purple, or bluish-green colors will appear, so it is indicated that there are steroids in the sample.

3. **Tanin Test**

Test tannin compounds by adding 1 mL sample and $FeCl_3$ as much as 3 drops in a test tube. The tannin content will be indicated by the formation of a bluish-black solution.

4. **Alkaloid Test**

A total of 0.5 mL of macroalgae sample extract was mixed with 5 drops of chloroform in a test tube followed by the addition of 5 drops of Meyer's reagent. Meyer's reagent is prepared by dissolving 1 g of KI in an aqueous of 20 mL plus 0.271 g $HgCl_2$ until dissolved. If the solution changes color to white-brown, it indicates the presence of alkaloids.

5. Flavonoid Test

A total of 0.5 mL of macroalgae sample extract mixed with 0.5 g of Magnesium (Mg) powder and 5 mL of concentrated HCl dripped slowly. If flavonoids are present, then the solution will form foam with a red or yellow color.

Compound Analysis using FTIR (*Fourier-Transform Infrared Spectroscopy*)

Functional groups were identified using FTIR spectrophotometer instruments at wavelengths between 4000-400 cm^{-1} by preparing 2 mg ethanol extract of *P. australis* macroalgae into cuvettes and mixed with KBr powder then crushed using mortar until smooth.

Antioxidant Activity Test with DPPH Method

A total of 50 mg of thick extract *of P. australis* was dissolved in 50 mL ethanol and homogenized, so that a standard solution of 1000 ppm was obtained. The standard solution is then made a concentration series of 50 ppm, 75 ppm, 100 ppm, 125 ppm, and 150 ppm. For dilution of the standard extract solution, 1000 ppm stock solutions of 0.25 mL, 0.375 mL, 0.5 mL, 0.625 mL, and 0.75 mL were taken. The final volume of the dilution solution is made up to 5 mL using a measuring flask. The dilution solution is then transferred into a test tube. The positive control in this study used ascorbic acid with concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm.

Measurement of antioxidant activity was done by taking 1 mL of each sample concentration, then mixing it with 1 mL DPPH (0.4 mM) in a test tube and adding absolute methanol until it reached 5 mL, then homogenized and left for 30 minutes. The sample is stored by covering the entire surface of the tube using aluminum foil, then measure the maximum wavelength absorption of 516 nm using a UV-Vis spectrophotometer.

Data Analysis

Data on antioxidant activity are expressed in the form of percentage inhibition calculated using the calculation formula according to Seisler, (2017):

$$\%\ inhibition\ antioxidan = \frac{Abs.control - Abs.handling}{Abs.control}\ x\ 100\%$$

Curve analysis and regression equations are performed between sample concentration (x) and percentage inhibition (y) using the regression formula y = ax + b to calculate the IC50 value of the extract. The value of y in this formula is 50, because IC_{50} is a concentration that can reduce 50% of DPPH free radicals, and the value of x indicates IC_{50}.

RESULTS AND DISCUSSION

Analysis of Phytochemical Content of Ethanol Extract of Macroalga *P. Australis*

The content of secondary metabolite compounds of *P. australis* extract qualitatively showed positive results on the presence of alkaloid compounds, flavonoids, saponins, tannins, steroids, and terpenoids as seen in Table 1.

Table 1. Phytochemical test results of P. *australis macroalgae ethanol extract*

Types of Qualitative Tests	Phytochemical Test Results	Information
Alkaloid	+ + +	Strong Positive
Flavonoid	+ + +	Strong Positive
Saponins	+ + +	Strong Positive
Tannins	+ + +	Strong Positive
Steroids	+ + +	Strong Positive
Terpenoid	-	Negative

These results are in accordance with the study Nurrahman et al., (2020) that is *P. australis* contains flavonoid compounds, terpenoids, steroids, saponins, tannins and, alkaloids. However, negative results in this study were seen in terpenoid compounds, likely caused by sun exposure before the extraction process. Sun exposure may result in damage to secondary metabolite compounds in sample extracts (Lantah et al., 2017). Extract macroalgae *P. australis* Proven to contain saponins, based on positive phytochemical test results. This is indicated by the formation of a stable foam after shaking for less than 10 minutes. The formation of foam is caused by interactions between chemical compounds in the sample, and the nature of saponins that are easily soluble in water. When shaken, the saponins disperse and form air bubbles, resulting in a stable foam. The presence of hydroxyl and carbon groups in the structure of organic saponins further strengthens its ability to dissolve in water and produce foam (Baud et al., 2014).

In this study, alkaloid bioactive compounds were identified through the use of Meyer's reagents (Setyowati et al., 2014). Flavonoids were tested by adding 5 mL of concentrated HCl drop by drop and magnesium metal to the extract sample to produce a red-brown color. High flavonoid compounds found in pigments *Thallus algae* and *phycoerythrin* (Mantiri et al., 2021). Esctrak Ethanol *P. australis* showed positive results containing tannins with the effect of adding a 1% $FeCl_3$ solution that changed the color to bluish-black (Halimu et al., 2017)**.** Tannins have various biological activities such as antidiarrheal, antioxidant, antibacterial, and astringent (Up to et al.,

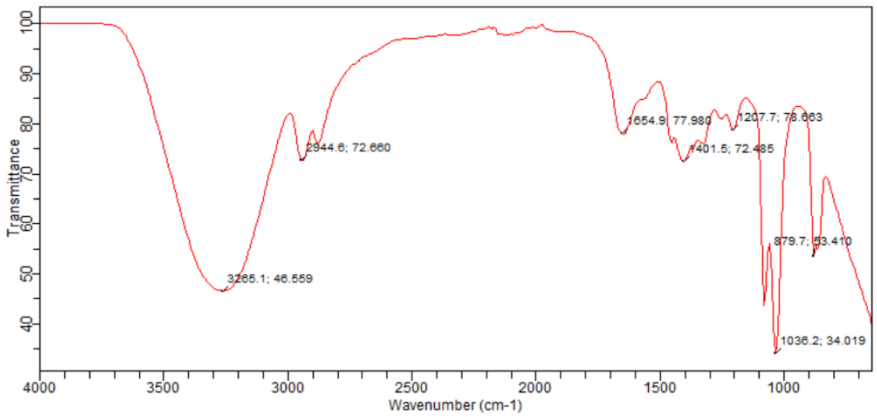
2015). The antibacterial activity of tannins is related to their ability to disrupt the process of protein transport in cells, inactivate enzymes and cell adhesin in microbes (Ngajow et al., 2013).

The phytochemical compounds contained in *P. australis* are an integral part of the self-defense system of the marine environment full of pathogenic bacteria. The marine alga *P. australis* seeks to develop effective self-defense strategies to counter pathogenic organisms and ensure their survival in the ocean. One way this is done is through the production of active phytochemical compounds known as secondary metabolite compounds (Simanjuntak, 1995).

Secondary metabolites in marine algae have an important role in the self-defense mechanism of marine organisms. This shows its potential as a source of medicinal materials for various diseases in humans. Recent research has proven that the active compounds in algae have various important biological activities, such as antibacterial, antioxidant, antifungal, antiviral, neuromodulatory, NAPDPH-dependent lipid peroxidation, anti-inflammatory, and anticancer activity (Lutfiyanti et al., 2012; Ariani, 2015; Handayani & Zuhrotun, 2017). In addition, carotenoid pigments are contained in *Padina australis* (as researched by Wirast et al., (2023)) proven to have antioxidant properties and able to slow premature skin aging by inhibiting the activity of collagenase enzymes.

Functional Group Identification Using FTIR (*Fourier-Transform Infrared Spectroscopy*)

The FT-IR Agilent Carry 630 spectrophotometer, which can demonstrate functional group absorption in the wavenumber range of 400 to 4000 cm^{-1}, was used to conduct the test, as shown in Figure 1. This method provides a clear picture of the chemical components contained in macroalgae ethanol extracts *P. australis* through infrared energy absorption analysis (Devi & Battu, 2019).



Picture 1. Spectral FTIR extrak ethanol macroalga *P. australis*

Based on the spectrum in Figure 1, absorption shows peaks at wavelengths 3265.1 cm^{-1}; 2944.6 cm^{-1}; 1654.9 cm^{-1}; 1401.5 cm^{-1}; 1207.7 cm^{-1}; 1036.2 cm^{-1}; and 879.7 cm^{-1}. The results of identification of functional groups *of P. australis* ethanol extract with FTIR are described in Table 2.

Table 2. FTIR yield data of P. australis macroalgae ethanol extract

Obtained Wavenumber value (cm^{-1})	Pustaka (cm^{-1})	Gugus Fungsi	Information
3265,1	3200-3600	O-H	Alcohol
	3100-3500	N-H	Amina
2944,6	2850-2970	C-H	Alkana
1654,9	1610-1680	C=C	Alkena
	1640-1550	N-H	Amine and Amide
	1640-1700	C=O	Amida
1401,5	1340-1470	C-H	Alkana / –CH3 bending
1207,7	1000-1300	C-O	Eter
1036,2			Acham Karbaksilat
879,7	675-995	C-H	Alkena
	690-900	C-H	aromatic rings

(Source: Skoog *et al*., 2016; Socrates, 1994)

FTIR spectral data on extracts *P. australis* Suspected to contain saponin compounds at a peak absorption of 3295.1 cm^{-1} indicating an O-H group (Siregar et al., 2022)**.** Based on the results of the study Sahribulan & Pagarra (2022), that at a wavelength of 3424.39 cm^{-1} there are O-H groups that indicate the presence of phenol and flavonoid compounds with free OH groups. In addition, the C-O bending vibration of alcohol in the number range of 1000 - 1300 cm^{-1}, especially at absorption of 1252.4 cm^{-1}, 1207,7 cm^{-1}, and 1036.2 cm^{-1}, also corroborating the findings. Amine group strain vibration bands (N–H) appear in absorption regions 3100 – 3500 cm^{-1}, this was reported by Khairuddin et al., (2018) that the N-H functional group is characteristic of alkaloid compounds. Salisbury & Ross (1995) said flavonoids always contain a hydroxyl group (-OH). FTIR spectrum interpretation data show that tannin compounds contain C–H groups at a wavelength of 2937.1 cm^{-1} and 2944.6 cm^{-1}, as well as C=O groups 1690–1760 cm^{-1} (Presented, 2023)**.** FTIR results of macroalgae extract *P. australis* indicates a number of polar compounds of the flavonoid group, alkaloids, steroids, tannins, and saponins.

Antioxidant Activity of P. australis *Macroalgae Ethanol Extract*

Tests of antioxidant activity of *P. australis* ethanol extract were conducted at concentrations of 50, 75, 100, 125, 150 ppm with three repeats. Table 3 illustrates how antioxidant activity is measured in terms of percent inhibition or inhibition of DPPH free radicals.

Table 3. Antioxidant activity and IC50 value

Sample	Concentration (ppm)	Installment-installment Absorbansi	Inhibition (%)	IC50 (ppm)
Padina australis	50	0,697	1,37	407,96
	75	0,680	3,87	
	100	0,655	7,36	
	125	0,624	11,74	
	150	0,604	14,57	
Ascorbic acid	2	0,105	85,10	16,895
	4	0,090	87,32	
	6	0,062	91,23	
	8	0,029	95,95	
	10	0,012	98,35	

IC_{50} (*inhibition concentration*) is a parameter used to interpret the test results with the DPPH method of sample solution concentration that is able to dampen 50% of DPPH free radicals (Molyneux, 2004). In particular, the antioxidant activity of test samples against DPPH can be categorized based on IC_{50} values as follows; IC_{50} worth < 50 ppm is a very strong antioxidant category, IC_{50} 50-100 ppm is a strong antioxidant; IC_{50} 100-150 ppm, moderate antioxidant; IC_{50} 151-200 ppm, weak antioxidant; and IC_{50} > 200 ppm showed very weak activity (Salim, 2018).

According to test results, P. australis ethanol extract exhibited antioxidant activity with an IC_{50} value of 407.96 ppm, which is considered a very weak antioxidant (IC_{50} > 200 ppm). With an IC_{50} value of 16.89 ppm, ascorbic acid, a positive control, exhibits very significant antioxidant activity against DPPH. Ascorbic acid is a secondary antioxidant that can capture free radicals and prevent chain reactions from occurring. Kurniawan (2017) reported weak antioxidant activity in ethanol extracts *P. australis* with IC_{50} of 182.76 ppm while the ethanol extract cream research *P. australis* also showed very weak antioxidants with an IC_{50} of 399,316 ppm.

Antioxidants have the ability to stop the oxidation process, prevent or delay cell damage caused by free radicals (Pham-huy et al., 2008) or unstable molecules produced in the body in response to biological, metabolic, environmental, or other factors. Antioxidants can inhibit oxidative stress, by capturing reactive oxygen species (ROS), preventing and repairing DNA

damage, and modifying signal transduction pathways and gene expression, functional natural product components can boost the natural antioxidant defense system (Nosrati et al., 2017).

The penring factors that contribute to antioxidant activity are closely related to the rich variety and abundance of chemical compounds that have been identified such as phenols, flavonoids, alkaloids, saponins, antrachinons, phenylpropanoids and terpenoids that are known sources of radicals *scavengers*. Flavonoid compounds are the main phenol group compounds that act as antioxidants for the prevention of cancer, and are found in many leaves, flowers, fruits and plant stems (Hudaifah et al., 2020).

CONCLUSION

The ethanol extract of *P. australis* macroalgae is categorized as having extremely poor antioxidant activity, with an IC_{50} value of 407.96 ppm (IC_{50} > 200 ppm). Important factors contributing to antioxidant activity are closely related to the content of chemical compounds that have been identified in *P. australis extracts* such as saponins, steroids, tannins, alkaloids, and flavonoids that are known to be sources of free radical antidote. P. *australis* extract has shown its potential as an alternative source of natural antioxidant compounds and further research needs to be done to find other concentration doses to achieve optimal antioxidant activity results and can be clinically developed for health products that are safe for humans.

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BIBLIOGRAPHY

Baud, G. S., Sangi, M. S., & Koleangan, H. S. J. (2014). Analisis Senyawa Metabolit Sekunder dan Uji Toksisitas Ekstrak Etanol Batang Tanaman Patah Tulang (Euphorbia tirucalli L.) dengan Metode Brine Shrimp Lethality Test (BSLT). *Jurnal Ilmiah Sains*, *14*(2), 106–112.

Dalimartha, S. (1999). *Atlas Indonesian Medicinal Plants* (1st ed.). Trubus Agriwidya.

Devi, D. R., & Battu, G. R. (2019). Qualitative Phytochemical Screening and FTIR Spectroscopic Analysis Of Grewia Tilifolia (Vahl) Leaf Extracts. *International Journal of Current Pharmaceutical Research*, *11*(4), 100–107.

Fadel, A. H., Abubakar, Y., Salim, F. D., Abubakar, S., Susanto, A., & Sunarti. (2022). Pertumbuhan Rumput Laut Padina australis di Pesisir Pulau Toduku Desa Dehe Kecamatan Jailolo Selatan Kabupaten Halmahera Barat. *Jurnal Agribisnis Perikanan*, *15*(2), 783–790.

Hadi, F., W, D. A., & Cahyani, F. (2015). Uji Toksisitas Tanin dari Kulit Manggis (Garcinia mangostana L .) terhadap Sel Fibroblas BHK - 21. *Conservative Dentistry Journal*, *5*(1), 6–11.

Halimu, R. B., S.Sulistijowati, R., & Mile, L. (2017). Identifikasi Kandungan Tanin pada Sonneratia Alba. *Jurnal Ilmiah Perikanan Dan Kelautan*, *5*(4), 93–97.

Handayani, N. K., & Zuhrotun, A. (2017). Padina australis dan Potensinya sebagai Obat Herbal Antikanker, Antibakteri, dan Antioksidan. *Farmaka*, *18*(2).

Haryani, T. S., Sari, B. L., & Triastinurmiatiningsih. (2015). Efektivitas Ekstrak Padina australis Sebagai Antibakteri Escherichia coli Penyebab Diare. *Fitofarmaka: Jurnal Ilmiah Farmasi*, *4*(2), 1–9.

Hidayah, N. (2016). Pemanfaatan Senyawa Metabolit Sekunder Tanaman (Tanin dan Saponin) dalam Mengurangi Emisi Metan Ternak Ruminansia. *Jurnal Sain Peternakan Indonesia*, *11*(2), 89–98.

Hudaifah, I., Mutamimah, D., & Utami, A. U. (2020). Komponen Bioaktif dari Euchema cottonii, Ulva lactuca, Halimeda opuntia, dan Padina australis. *Jurnal Ilmu Perikanan Dan Kelautan*, *2*(2), 63–70.

Khairuddin, Taebe, B., Risna, & Rahim, A. (2018). Isolasi dan Karakterisasi Senyawa Alkaloid Ekstrak Metanol Klika Faloak (Sterculia populifolia). *Ad-Dawaa' Journal Pharmceutical Sciences*, *1*(2), 62–70.

Kumari, S., Manohar, S., Kumari, P., Krishnan, V., Maheshwari, C., Narwal, S., Gupta, O. P., Gowda, V. T., Bansal, N., & Dahuja, A. (2023). The Role of Major Phenolics in Apple to Total Antioxidant Capacity. In A. Küden (Ed.), *Apple Cultivation - Recent Advances*. Çukurova University.

Kurniawan, A. (2017). *Skrining Fitokimia dan Uji Aktivitas Antioksidan Ekstrak Alga Coklat Padina australis*. Universitas Brawijaya.

Lantah, P. L., Montolalu, L. A. D. Y., & Reo, A. R. (2017). Kandungan Fitokimia dan Aktivitas Antioksidan Ekstrak Metanol Rumput Laut Kappaphycus alvarezii. *Jurnal Media Teknologi Hasil Perikanan*, *5*(3), 73–79. https://doi.org/10.35800/mthp.5.3.2017.16785

Liu, K. (2022). Free Radicals : Functions, Types and its Source. *Oxidants and Antioxidants in Medical Science*, *11*(2), 1–2.

Lu, J., Bao, J., Chen, X., Huang, M., & Wang, Y. (2012). Alkaloids Isolated from Natural Herbs as the Anticancer Agents. *Evidence-Based Complementary and Alternative Medicine*, *2012*, 1–12. https://doi.org/10.1155/2012/485042

Maharany, F., Nurjanah, Suwandi, R., Anwar, E., & Hidayat, T. (2017). Kandungan Senyawa Bioaktif Rumput Laut Padina australis dan Eucheuma cottonii sebagai Bahan Baku Krim Tabir Surya. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *20*(1), 10–17.

Mantiri, D. M. H., Kepel, R. C., Boneka, F. B., & Sumilat, D. A. (2021). Phytochemical Screening, Antioxidant and Antibacterial Tests on Red Algae, Halymenia durvillaei , and Phycoerythrin Pigments. *AACL Bioflux*, *14*(6), 3358–3365.

Marlis, A. (2008). *Isolasi Oligosakarida Ubi Jalar (Ipomoea batatas L.) dan Pengaruh Pengolahan terhadap Potensi Prebiotiknya*. Institut Pertanian Bogor.

Martemucci, G., Costagliola, C., Mariano, M., Luca, D., Napolitano, P., & D'Alessandro, A. G. (2022). Free Radical Properties, Source and Targets , Antioxidant Consumption and Health. *Oxygen*, *2*, 48–78.

Molyneux, P. (2004). The use of the stable free radical diphenylpicryl- hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, *26*(2), 211–219.

Ngajow, M., Abidjulu, J., & Kamu, V. S. (2013). Pengaruh Antibakteri Ekstrak Kulit Batang Matoa (Pometia pinnata) terhadap Bakteri Staphylococcus aureus secara In vitro. *Jurnal MIPA*, *2*(2), 128–132.

Nosrati, N., Bakovic, M., & Paliyath, G. (2017). Molecular Mechanisms and Pathways as Targets for Cancer Prevention and Progression with Dietary Compounds. *International Journal of Molecular Sciences*, *18*(2050). https://doi.org/10.3390/ijms18102050

Nurkhasanah, Bachri, M. S., & Yuliani, S. (2023). *Antioksidan dan Stres Oksidatif* (1st ed.). UAD Press.

Nurrahman, N. W. D., Sudjarwo, G. W., & Putra, O. N. (2020). Skrining fitokimia metabolit sekunder alga cokelat (Padina australis) dari Kepulauan Poteran Madura. *Journal of Pharmaceutical Care Anwar Medika*, *2*(2), 60–69.

Pesang, M. D., Ngginak, J., Kase, A. G. O., & Bisilissin, C. L. B. (2020). Komposisi Pigmen pada Ulva sp ., Padina australis dan Hypnea sp . dari Pantai Tablolong Provinsi Nusa Tenggara Timur. *Jurnal Kelautan Tropis*, *23*(2), 225–233.

Pham-huy, L. A., He, H., & Pham-huy, C. (2008). Free Radicals , Antioxidants in Disease and Health. *International Journal of Biomedical Science*, *4*(2), 89–96.

Salim, R. (2018). Uji Aktivitas Antioksidan Infusa Daun Ungu Dengan Metoda DPPH (1,1-diphenil- 2-picrylhidrazil). *Jurnal Katalisator*, *3*(2), 153–161.

Salisbury, F. B., & Ross, C. W. (1995). *Fisiologi Tumbuhan Jilid 2*. ITB.

Saputra, Y. D. (2023). *Aktivitas antikanker ekstrak etanol Sargassum duplicatum, Padina australis, dan taurin sebagai supresor gen p21 terhadap sel kanker serviks (HeLa)*. Universitas Lampung.

Seisler, H. W. (2017). *Near-Infrared Spectra, Interpretation. Encyclopedia of Spectroscopy and Spectrometry* (Third edit).

Setyowati, W. A. E., Ariani, S. R. D., Ashadi, Mulyani, B., & Rahmawati, C. P. (2014). Skrining Fitokimia dan Identifikasi Komponen Utama Ekstrak Metanol Kulit Durian (Durio zibethinus Murr) Varietas Petruk. *Seminar Nasional Kimia Dan Pendidikan Kimia VI*, 271–280.

Siregar, M. A., Santri, J. A., Aksani2, D., Maas, A., & Nurudin, M. (2022). Analysis of FTIR Spectroscopic Data to Observe Hydrophilic and Hydrophobic Levels of Peat in Hemic and

Sapric Maturity. *IOP Conference Series: Earth and Environmental Science*, *1025*. https://doi.org/10.1088/1755-1315/1025/1/012026

Skoog, D. A., Holler, F. J., & Crouch, S. R. (2016). *Principles of Instrumental Analysis* (7th ed.). Cengage Learning.

Socrates, G. (1994). *Infrared Characteristic Group Frequencies Tables and Charts Second edition*. The University of West London.

Sunardi. (2023). Analisis Gugus Fungsi dan Penentuan Kadar Total Fenol Ekstrak Kulit Buah Naga Merah dan Putih. *Jurnal Redoks: Jurnal Pendidikan Kimia Dan Ilmu Kimia*, *6*(1), 8–18.

Takshak, S., & Agrawal, S. B. (2019). Defense potential of secondary metabolites in medicinal plants under UV-B stress. *Journal of Photochemistry & Photobiology, B: Biology*. https://doi.org/10.1016/j.jphotobiol.2019.02.002

Tasmin, N., Erwin, & Kusuma, I. W. (2014). Isolasi, identifikasi dan uji toksisitas senyawa Flavonoid fraksi kloroform dari daun terap (Artocarpus odoratissimus blanco). *Jurnal Kimia Mulawarman*, *12*(1), 45–53.

Wirasti, Slamet, & Rahmasari, K. S. (2023). Studi Pencegahan Penuaan Dini Kulit dari Ekstrak Rumput Laut Coklat (Padina Australis) Hauck Melalui Penghambatan Aktivitas Enzim Kolagenase. *CERATA Jurnal Ilmu Farmasi*, *14*(2), 89–96.