Trop J Nat Prod Res, December 2022; 6(12):2025-2028

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Identification of Active Antioxidant Compounds with Neuro-protective Effects in Kora-kora Coastal Macro-algae

Finny Warouw[1]*, Christi Mambo[1], Junita M. Pertiwi[1], Veibe Warouw[2], Robert A. Bara[2]

[1]*Medical Faculty, Sam Ratulangi University, Jl. Kampus Unsrat Bahu, Manado 95115, North Sulawesi, Indonesia*
[2]*Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Unsrat Bahu, Manado 95115, North Sulawesi, Indonesia*

ARTICLE INFO

Article history:
Received 11 November 2022
Revised 20 December 2022
Accepted 21 December 2022
Published online 01 January 2023

Copyright: © 2022 Warouw *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Padina australis Hauck and *Turbinaria ornata* (Turner) J. Agardh are two of the most common macroalgae species encountered in the Kora-kora beach coastline area. These species produced active compounds as a result of extreme environmental conditions, which could be useful in medicine. In macroalgae, these naturally occurring active substances that may have neuroprotective effects are widely distributed. This study was aimed at identifying the active antioxidant compounds with neuroprotective potential in the macroalgae collected from the Kora-kora coastal area. *Padina australis* and *Turbinaria ornate* were collected, prepared, and extracted with 96% ethanol. The antioxidant activity of the macroalgae extracts was determined using the DPPH (1,1-diphenyl-2-picrylhydrazil) technique. More so, phytochemical screening of the extracts was performed. The results indicated that the two macroalgae have high antioxidant activity. *Padina australis* and *Turbinaria ornata* had IC_{50} values of 16.63 and 43.22 ppm, respectively. The phytochemical analysis of these two macroalgae revealed the presence of secondary metabolites, such as alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, and phenolics. The findings of this study suggest that both macroalgae may have neuroprotective properties.

Keywords: Antioxidant, Neuro-protective properties, *Padina australis*, *Turbinaria ornate*.

Introduction

Macroalgae is a type of flora in the marine environment that plays an important role in the ecosystem. This species exhibits such biological activity in response to the production of different complex organic, primary, and secondary metabolites due to diverse and extreme environments.[1] People have been shown to benefit from macroalgal active compounds in their daily lives. An antioxidant, in particular, can neutralize free radicals and stop the oxidation process that occurs within cells, preventing cellular damage in the body.[2] Active compounds found in macroalgae that can function as natural antioxidants are phenol, fucoxanthin pigment, polysaccharide sulphate, phycobilin, and vitamins. These active compounds found in macroalgae can act as neuroprotectors.[3] Neuroprotective active compounds are required to protect neurons in the human brain from potential harm, such as that caused by numerous neurological diseases, especially neurodegenerative ones. This neuroprotective property is achieved due to the compounds' potential as cholinesterase inhibitors, beta-secretase inhibitors, and substances to prevent glutamate and beta-amyloid proteins that may cause neurotoxicity in neurons.[4]
According to the research conducted by Syad in 2016, macroalgae have active compounds that are protective of the human brain. This finding is further supported by a 2019 study by Olasehinde *et al*. that found Alzheimer's disease can benefit from the neuroprotective properties of macroalgal active chemicals. Additionally, it showed that macroalgae contain a variety of active substances, including glycoprotein, pigment, lipid, and polysaccharide, which can protect neurons from degenerative diseases.[5] Neurodegenerative diseases are a group of nervous system diseases that occur in the later stage of life, and are caused by progressive neuronal cell dysfunction and cellular death. Cellular dysfunction and death will eventually result in cognitive system dysfunction and movement disorders. The neurodegenerative process in neurons is characterized by aberrant protein aggregation, the development of intracellular and extracellular depositions, and the loss of normal neuronal cell function. The excessive production of reactive oxygen species (ROS) and direct inflammation that results in hemostatic disruption in the central nervous system is the pathophysiomechanics of cellular degeneration.[3] According to epidemiological data, Alzheimer's disease is the most prevalent neurological condition. Typical symptoms of Alzheimer's disease include decreased levels of acetylcholine, increased beta-amyloid aggregation, phosphorylated Tau protein, and glutamate, all of which are toxic to neurons. These events will eventually cause decreased cholinergic and elevated oxidative stress, which will further accelerate the neurodegenerative process in the brain. The most common symptoms are memory loss and cognitive dysfunction, which have a significant impact on daily living. Therefore, pharmaceutical intervention with natural antioxidant compounds is required to prevent the increasing nature of neurodegenerative diseases in society.[3] The East Lembean region's Kora-kora beach is home to a diverse array of marine life, including macroalgae. There is a need to investigate the potential benefit of macroalgae with a neuroprotective effect in the coastal area using antioxidant activity tests and active compound identification.
The present study was therefore conducted to identify the active antioxidant compounds with a neuroprotective effect in the macroalgae obtained in the Kora-kora coastal area.

Materials and Methods

Source and preparation of samples
Macroalgae were obtained from the Kora-kora coastal area of Indonesia in July 2022. They were cleaned with fresh water to remove dirt, sand, silt, and adhering organisms. Running water (freshwater)

*Corresponding author. E mail: finnywarouw@unsrat.ac.id
Tel: +62-81243679016

Citation: Warouw F, Mambo C, Pertiwi JM, Warouw V, Bara RA. Identification of Active Antioxidant Compounds with Neuro-protective Effects in Kora-kora Coastal Macro-algae. Trop J Nat Prod Res. 2022; 6(12):2025-2028. http://www.doi.org/10.26538/tjnpr/v6i12.21

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

© 2022 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

was utilized for the rinsing (3-4 times) to remove the surface salt content. After draining the clean sample and weighing the wet weight, the clean macroalgae were chopped into 5 cm pieces. In addition to increasing the contact area during the maceration process, this procedure was done to make storage and sample handling more practicable. The samples were dried in an oven at 400 ^{o}C, after which the dry weight was weighed again. The algae samples were ground up using a blender.[5]

Preparation of extracts

The ground algal samples were sieved, and 10 g each were weighed. They were then placed in a closed vessel, and 150 ml of 96% ethanol was poured until the simplicia was completely submerged. Soaking (maceration) was carried out for 3 days while stirring 3-4 hours per day, then filtered. The filtrate thus obtained was collected. The simplicia powder was then re-soaked (re-macerated) for 2 days before being filtered once again using a funnel and the same quantity as before. The filtrate that resulted was combined. The recovered filtrate was concentrated using a rotary evaporator at 55 ^{o}C and again in the oven at 400 ^{o}C until a thick extract was obtained. The extract obtained was stored in a freezer at -20 ^{o}C, and the weight was determined using the formula :[5]

We = Wv2 - Wv1

Where We is the extract weight (grams), Wv1 is the empty vial weight (grams), and Wv2 is the vial weight after the extract has been filled (grams).

Determination of the antioxidant activity of the macroalgae extracts

In preparing a 0.1 mM DPPH (1,1-diphenyl-2-picrylhydrazil) solution, 0.39432 grams of DPPH powder (BM 394.32) were dissolved in 10 ml of methanol p.a. Using a micropipette, 100 µl of DPPH (0.1M) solution was obtained and transferred gradually to a 100 ml volumetric flask to produce a 0.1 mM DPPH solution. To determine the maximum DPPH wavelength, 2 mL of the 0.1 mM DPPH solution was placed into a test tube, and 2 ml of methanol p.a. were added, vortexed until the solution was homogenous, and then poured into a cuvette. The wavelength was measured between 400 and 800 nm with a UV-Vis spectrophotometer, with a maximum wavelength of 517 nm. In preparing the blank solution, 2 mL of a 0.15 mM DPPH solution was added along with 2 mL of methanol in a test tube. The solution was vortexed to ensure homogeneity before being incubated in the dark for 30 minutes. The wavelength of 517 nm was then used to measure the absorption. To prepare 1000 ppm of the main extract solution, 50 mg of the sample was diluted with methanol p.a. and poured into a 50 ml volumetric flask. The volume was then adjusted with methanol p.a. until the indicator was reached. Each concentrated test solution was diluted to a volume of 2 mL and added to 2 mL (0.15 mM) of DPPH in a test tube. Then, the solution was vortexed to ensure homogeneity before being incubated for 30 minutes in a dark room. The wavelength of 517 nm was then used to measure absorption on a UV-Vis spectrophotometer. The radical prophylactic activity was expressed as an inhibition percentage, which was calculated with the following formula:

$$\%\ \text{DPPH inhibition} = \frac{Control\ absorption - Test\ solution\ absorption}{Control\ absorption} \times 100\%$$

The x- and y-axes of the linear regression equation were used to plot the sample concentration and inhibition percentage separately. This equation was used to determine the value of IC_{50} from each sample, with a y-axis value of 50 and an x-axis value that was obtained as the IC_{50} value.[6]

Phytochemical screening of the macroalgae extracts

Phytochemical screening of the macroalgae extracts for the presence of alkaloids, phenol hydroquinone, flavonoids, tannins, saponins, steroids, and triterpenoids was performed using the method of Harborne.[7,8]

Results and Discussion

Algal sampling in the coastal waters of Kora-kora was carried out using the exploration survey technique. Furthermore, identification was done using identification guides from Field guide and atlas of the seaweed resources.[9] The two species of macroalgae that were obtained were *Padina australis* Hauck (Figure 1) and *Turbinaria ornata* (Turner) J. Agardh (Figure 2). *Padina australis* and *Turbinaria ornata* are groups of brown seaweed (Phaeophyceae) with habitats in tropical oceans that can produce active compounds in the form of secondary metabolites that are better as a defense system against ultraviolet radiation from the sun. This macroalga has the highest antioxidant activity, owing to the presence of phenol as its primary antioxidant component.[7] *Padina australis* is a brown macroalga of the division Phaeophyta. This macroalga has a fan-shaped thallus with thin sheet segments. The thallus is 5-8 cm high and 3 cm wide. *Padina australis* is yellowish-brown and consists of flabellate lobes. It has a double curved line (concentric hair lines). Calcification occurs on the surface of the leaves. This brown macroalga has a small holdfast and lives on sandy substrates and dead coral in tidal areas.[8,9]

Turbinaria ornata also shares common characteristics with other *Turbinaria* species, such as a brown thallus color and a body shape resembling a tree or bush. The shape of the thallus is like a cylindrical stem, upright, and rough, and there are traces of branching. Holdfasts are small disks with radial roots and branches rotating around the main stem. The shape of the blade is like a trumpet, amethyst, or funnel with serrated edges and air bubbles (bladder), which are located in the fluid. The edge of the leaf creates a lip, and the middle of the leaf curves inward, defining this kind of macroalgae. The leaves are arranged in a circle on the stem. The width of the leaves is about 1 cm, with a length of 0.8 cm. This brown macroalga lives on coral substrates.[9,10]

DPPH (1,1-diphenyl-2-picrylhydrazil) with spectrophotometry is a method for measuring antioxidant activity. This test method has long been used in the determination of antioxidant activity. The principle of this method is the DPPH free radicals' uptake of hydrogen from antioxidants. The antioxidants donate protons or hydrogen to DPPH, thereby breaking free radical chains to form non-radical compounds.[6]

Figure 1: *Padina australis* Hauck.

Figure 2: *Turbinaria ornatta*

© 2022 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The results of the antioxidant activity tests are expressed in terms of the percentage of antioxidant activity, as shown in Table 1. The percentage value (%) of inhibition indicates the number of hydrogen atoms provided by the active compound to the DPPH radical, which can stabilize the DPPH compound to become DPPH-H. The DPPH radical inhibitory activity of each test macroalgae sample was expressed in IC_{50} values. A low IC_{50} value indicates strong antioxidant activity.[6] The antioxidant test results (Table 1) on macroalgae in the coastal area of Kora-kora showed that *Padina australis* and *Turbinaria ornata* had antioxidant activity because the IC_{50} was less than 50 ppm. However, the antioxidant activity of *Padina australis* was much stronger with an IC_{50} value of 16.63 ppm compared to *Turbinaria ornata* with an IC_{50} value of 43.22 ppm. The differences observed in IC_{50} results from various studies are caused by extrinsic and intrinsic factors. Extrinsic factors include variations in the water's nutrient availability, climate, and weather where the samples are grown. According to the study by Lumbessy (2020), red algae have very different nutritional profiles and bioactive components, despite being members of the same species. Intrinsic factors that affect the IC_{50} value include differences in chemical constituents, sample age, harvest time, and sample size. Besides that, differences in extraction methods and solvents used and the length of the drying process at high temperatures, which can damage the content of polyphenolic compounds, are other influences.[12,13]

Brown macroalgae are organisms that live in shallow waters, such as the intertidal zone and beaches, where they are frequently exposed to long periods of ultraviolet light from the sun. Exposure to sunlight and air can cause the formation of free radicals or other reactive oxygen species (ROS). Because intracellular biomolecules are oxidized by ROS in cells, this can result in tissue damage and even cell death. However, ROS radiation does not cause oxidative damage to the structural components of macroalgae. This observation demonstrates that the cells of macroalgae have a defense mechanism against oxidative stress. The ability of these organisms to synthesize antioxidants and form a defense system against exposure to free radicals reflects the adaptability of seaweed to solar radiation. [13]

Antioxidants have been identified as a therapy to inhibit the development of Alzheimer's disease. The pathophysiology behind the development of Alzheimer's disease is due to increased levels of ROS in the brain. Brain neuron cells are very vulnerable to free radicals due to a low antioxidant defense system. Therefore, increased levels of ROS in brain cells can lead to lipid peroxidation, neurodegeneration, and finally cell death. [14] The term "phytochemicals" refers to a description of the variety of organic compounds that are produced, stored, and utilized by organisms. These include their chemical composition, biosynthesis, modifications, and metabolism, as well as their natural distribution and biological functions for the isolation and comparison of the composition of chemical compounds from various types of plants. The reason for performing a phytochemical analysis is to determine the characteristics of the bioactive components of a crude extract that have toxic effects or other pharmacological effects that are useful when tested with biological or bioassay systems.[15] Table 2 shows the results of the phytochemical analysis of the macroalgae, *Padina australis*, and *Turbinaria ornate*. The two macroalgae contain alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, and phenolics. These compounds have the potential to act as neuroprotectors. Neuroprotective potential can be obtained from phenolic acid compounds, flavonoids, and phlorotannins contained in both macroalgae. Several studies have shown that these compounds can inhibit acetylcholineesterase and butyrylcholineesterase. [3,16,17]

Cholinesterase inhibitory activities such as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are important enzymes involved in the regulation of acetylcholine (ACh) in the synaptic cleft of neurons to maintain cognitive function. However, the loss or rapid degradation of acetylcholine causes cholinergic dysfunction, which results in memory impairment. Therefore, cholinesterase has been developed to reduce cholinergic deficits by restoring ACh levels and improving cognitive function.[3]

Table 1: Antioxidant activity testing of macroalgae found in coastal area of Kora-kora beach.

	***Padina australis* Hauck**			***Turbinaria ornata* (Turner) J. Agardh**		
Concentration (ppm)	**Mean absorption**	**% Mean inhibition**	**Mean IC_{50}**	**Mean absorption**	**% Mean inhibition**	**Mean IC_{50}**
25	0.438	48.23		0.487	42.43	
50	0.303	64.18		0.384	54.6	
75	0.245	71.04	16.63582	0.323	61.82	43.22342
100	0.229	73.4		0.282	66.66	
125		81.44			80.37	
DPPH control	0.846	-		0.846	-	

Table 2: Phytochemical analyses of macroalgae found in coastal area of Kora-kora beach

Compound	***Padina australis Hauck***	***Turbinaria ornata (Turner) J. Agardh***
Alkaloid (Dragendorf, Wagner, Meyer)	+++	+++
Flavonoid	+	+
Tannin	+	+
Saponin	+	+
Steroid	+	+
Triterpenoid	+	+
Phenol	+	+

Besides that, the content of phenolic acid compounds, flavonoids, and phlorotannins contained in macroalgae *Padina australis* and *Turbinaria ornata* showed strong BACE-1 (*β-site amyloid precursor protein cleaving enzyme-1*) inhibitory activity. BACE-1 has been identified as a key therapeutic target for Alzheimer's disease. Increased BACE-1 activity and expression of tumor necrosis factor (TNFa) may contribute to the onset of mild cognitive impairment early in AD. In addition, increased BACE-1 activity also contributes to an increase in the number of plaques around neurons and reduces the cognitive ability of Alzheimer's disease patients.[3] These two brown macroalgae also have the potential to be neuroprotectors due to their antioxidant activity, which includes phenolic phlorotannins and terpenoids. This antioxidant can operate as a free radical antioxidant, collect radicals via DPPH, have iron-reducing abilities, and prevent lipid oxidation. Furthermore, this antioxidant activity can reduce neurotoxicity by inhibiting apoptosis, decreasing nitric oxide and malondialdehyde production, and increasing antioxidant status.[3,4,14]

© 2022 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Conclusion

The findings of this study reveal that *Padina australis* and *Turbinaria ornata* are two types of brown macroalgae that are abundant in the coastal area of Kora-kora, Indonesia. The DPPH technique showed that these two species have significant levels of antioxidant activity. Both macroalgae contain flavonoids, tannins, triterpenoids, and phenolics, according to qualitative phytochemical analysis. They had the potential to act as neuroprotectors.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors wish to thank the Medical Faculty of Samratulangi University for the facilities to execute the research.

References

1. Dorhoi E, Michiu D, Pop C, Rotar A, Tofana M, Pop O, Socaci A, Farcas A. Macroalgae : A sustainable source of chemical compounds with biological activities. Nutr. 2020; 12(10):3085-3095.
2. Ganesan A, Tiwari U, Rajauria G. Seaweed nutraceuticals and their therapeutic role in disease prevention. Food Sci Hum. 2019; 8(3):252–263.
3. Olasehinde T, Olaniran A, Okoh A. Macroalgae as a valuable source of naturally occurring bioactive compounds for the treatment of Alzheimer's disease. Marine Drugs. 2019; 17(11):1-18.
4. Hanan MA, Dash R, Haque N, Sohaq AM, Rahman A, Ferreres F, Andrade P. Neuroprotective Potentials of Marine Algae and Their Bioactive Metabolites: Pharmacological Insights and Therapeutic Advances. Marine Drugs. 2020; 18(7):2-50.
5. Syad A, Rajamohamed B, Shunmugaiah P, Kasi PD. Neuroprotective effect of the marine macroalga *Gelidiella Acerosa* : identification of active compounds through bioactivity-guided fractionation. Pharm Biol. 2016; 54(10):2073–2081.
6. Munteanu I and Apetrei C. Analytical Methods used in determining antioxidant activity: a review. Int J Mol Sci. 2021; 22(7):1-30.
7. Shaikh JR and Patil MK. Qualitative tests for preliminary phytochemical screening: an overview. Int J Chem Stud. 2020; 8(2):603-608.
8. Kepel R, Lumingas L, Watung P, Mantiri D. Community structure of seaweeds along the intertidal zone of mantehage islands, north Sulawesi, Indonesia. AACL Bioflux. 2019; 12(1):87-101.
9. Wijayanti D, Giftania W, Oki P. Phytochemical screening of secondary metabolite *Padina australis* from puteran island madura. J Pharm. 2020; 2(2):60-69.
10. Handayani T. Knowing macroalgae *Turbinaria* and their usages. OSEANA. 2018; 43(4):1-10.
11. Sianipar A, Satriawan N, Sumartono J, Kambira P. Assessment of antioxidant activity of sumbawa macroalgae in relation to their bioactive compounds and pharmacological effects. Jurnal Riset Kesehatan Nasional. 2022; 6(2):151–157.
12. Kustiyah E, Saing B, Afriyanti A, Joyosemito S. Protein Characterization of Brown and Red Algae from Pari Island waters as an antioxidant substance. J Rekayasa Hijau. 2018; 2(3):247-253.
13. Miyashita K, Beppu F, Hosokawa M, Liu X, Wang S. Nutraceutical charateristics of brown seaweed carotenoid fucoxanthin. Archieves of Biochemistry and Biophysics. 2020; 686(108364):1-10.
14. Alghazwi M, Kan YQ, Zhang W, Gai WP, Garson JM, Smid S. Neuroprotective activities of natural products from marine macroalgae during 1999–2015. J Appl Phycol. 2016; 28(6):3599–3616.
15. Shaikh JR and Patil MK. Qualitative tests for preliminary phytochemical screening: an overview. Int. J. Chem. Stud. 2020; 8(2):603-608.
16. Sahnouni F, Debib A, Saim S, Bouhadi D, Menadi S. Phytochemical Content, Antioxidant and Antibacterial Activities of Three Red Macroalgae from Algerian West Coast. Trop J Nat Prod Res. 2021; 5(2):336–341.
17. Abid KY and Tawffiq ZS. Comparative study, phytochemical screening and antioxidant activities of three types of apple seeds extracts. Trop J Nat Prod Res. 2022; 6(9):1401-1404.

© 2022 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License