International Journal of Marine Science, 2020, Vol.10, No.6, 1-7
http://www.aquapublisher.com/index.php/ijms

Research Report Open Access

The Potential of Bioactive Compounds of *Halimeda micronesica* and *Halimeda macroloba* Species of Seaweeds, Obtained from Maspari Island, South Sumatra to Express Antioxidant Activities, and The Phytochemical Screening of Their Active Extracts

Delini Oktaviana Lubis, Muhammad Hendri ✉, Rozirwan
Marine Science Department, Faculty of Mathematics and Natural, Sriwijaya University, Indralaya-Prabumulih Street KM 34 Inderalaya Ogan Ilir 30662, Indonesia
✉ Corresponding author email: muhammad.hendri@unsri.ac.id
International Journal of Marine Science, 2020, Vol.10, No.6 doi: 10.5376/ijms.2020.10.0006
Received: 04 Aug., 2020
Accepted: 11 Sep., 2020
Published: 16 Oct., 2020

Preferred citation for this article:
Lubis D.O., Hendri M., and Rozirwan, 2020, The Potential of bioactive compounds of *Halimeda micronesica* and *Halimeda macroloba* species of seaweeds, obtained from Maspari Island, South Sumatra to express antioxidant activities, and the phytochemical screening of their active extracts, International Journal of Marine Science, 10(6): 1-7 (doi: 10.5376/ijms.2020.10.0006)

Abstract *H. micronesica* and *H. macroloba* species are a group of green seaweed (Chlorophyta) found in Maspari Island waters, which have been proven to show some biochemical activities. Therefore, this study aims to analyze the antioxidant activity, phytochemical compounds and total phenolic content of their extracts. The procedures used herein includes sampling and handling, maceration of both samples using ethyl acetate (Hmi-EA and HmaEA) and methanol (Hmi-M and Hma-M) solvents, extraction, antioxidant test using DPPH method, phytochemical test and IC50 analysis. The results indicate that Hmi-EA, which is one of the 4 extracts tested has the ability to inhibit free radicals. Furthermore it has an IC value of 52.81 ppm, and contains alkaloid, steroid and flavonoid compounds. In conclusion, this extract has the potential to express antioxidant activities.
Keywords *H. micronesica*; *H. macroloba*; DPPH; Antioxidant; Maspari Island

Background

Halimeda sp lives in shallow waters. This biota can grow well in protected water types such as bays and sheltered island. Maspari Island is the only island located in South Sumatra Province. The island has a large natural resource potential, one of which is seaweed. There are at least 19 types of seaweed in Maspari Island. One of them comes from the genus Halimeda. Seaweed type *Halimeda micronesica* dominate the waters, while the seaweed type *Halimeda macroloba* does not dominate.

Antioxidants are compounds that can counteract free radicals and reduce the negative effects of oxidants in the body (Julyasih et al., 2009). Antioxidants have molecular structures that can give electrons to free radical molecules without disturbing their function and can break the chain reaction of free radicals (Ramadan, 2015).

This research is expected to provide information about the antioxidant activity of seaweed extract of *H. micronesica* and *H. macroloba* as input for further development and increase the added value of seaweed type *H. micronesica* and *H. macroloba* as the natural source of antioxidant.

1 Materials and Methods

1.1 Time and place

The study was conducted on March-October 2019. Seaweed samples were taken in the waters of Maspari Island, South Sumatra. The analysis of antioxidant test was conducted at Marine Biophysics Laboratory, Marine Oceanography and Instrumentation Laboratory, Marine Science and Basic Chemistry Laboratory, Chemistry Department, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Inderalaya. The sampling location is located at coordinates 106º13’0,743”BT and 3º12’57,151”LS. The location of the study is presented in Figure 1.

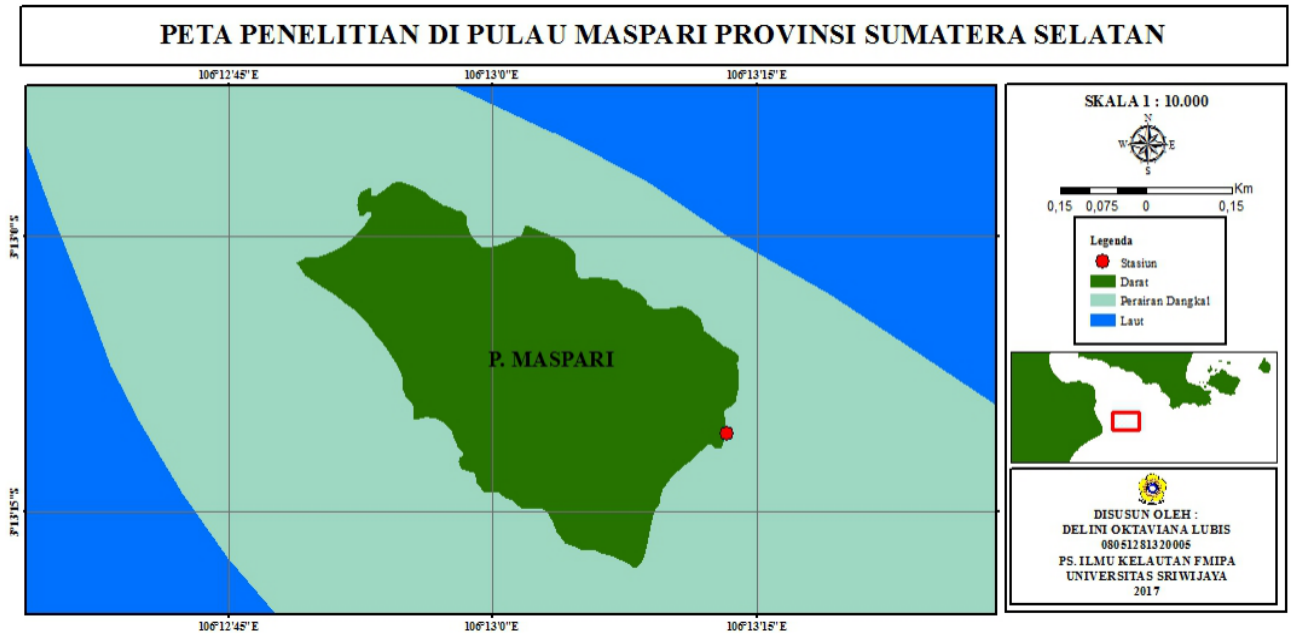

Figure 1 Research site

1.2 Sampling and handling samples

Seaweed is cleaned by using seawater to prevent dirt and epiphytes from sticking out. Then the sample is taken to Marine Biotech Laboratory, Marine Science Study Program, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Inderalaya for further extraction.

1.3 Seaweed extraction

Seaweed extraction process is very important to get the rough extract of seaweed to be tested as an antioxidant. Here's how to extract seaweed.

1.3.1 Mixed maceration

The sample uses fresh seaweed because according to Suryaningrum et al. (2006), fresh samples yield more extracts than dry samples. Samples are macerated by using 2 solvents with different polar properties namely ethyl acetate (semi polar) and methanol (polar). Samples of 100 grams are dissolved in 400 ml of ethyl acetate (1: 4 b/v) solvent for 2x24 hours and then filtered with Whatman 40 filter paper to obtain macerate and pulp. The dregs are again macerated with methanol solvent and then filtered by the same treatment (Septiana and Asnani, 2013). The process of maceration is done in 1 repetition.

1.3.2 Evaporation of macerate

The produced macerates are evaporated using a Rotary Evaporator. The temperature used in the evaporation process is 40°C so that the compound contained in it is not damaged (Pramesti, 2013).

1.4 Potential test of antioxidant activity

Seaweed extract is weighed as much as 0.03 gr and 30 mL of methanol is added which resulted in a stock solution with a concentration of 1 000 ppm. DPPH solution is prepared by dissolving 0.003 gr of DPPH crystals into 10 mL of methanol and obtaining a solution with a concentration of 300 ppm. The concentration series is made by dilution of the main liquor to 750, 500, 250 and 50 ppm (Suhendra and Arnata, 2009).

Sample extract solution, sample control, negative control, blank and positive control are homogenized and incubated in dark spaces at room temperature for 30 minutes. Each solution is introduced into quvet (v=4 mL) of 3.75 mL. Each concentration is done in 3 times repetition. The active extracts are re-assessed with concentrations of 40, 80, 120, 160 and 200 ppm to determine the antioxidantlamic activity. The active extracts are tested at concentrations of 40, 80, 120, 160 and 200 ppm.

According to Sari et al. (2015), Husni et al. (2014), Pramesti (2013), Nurjanah et al. (2011) and Salamah et al. (2008), DPPH uptake is measured using a UV-Vis spectrophotometer at λ_{maks} 517 nm. The antioxidant activity of an extract is based on the value of percentage calculation of DPPH uptake inhibition and the ability of an extract to reduce 50% of DPPH radical through IC_{50} value.

1.5 Phytochemical test

1.5.1 Steroid and triterpenoid test

Extract and chloroform are dropped on the drop plate to be set aside for a few minutes to dry. After it got dried, 10 drops of anhydrous acetic acid and stirred until homogeneous are added. After that, 3 drops of 96% sulfuric acid are added. Positive samples contain steroids if they are blue or green and positively contain triterpenoids when they are red or violet.

1.5.2 Flavonoid test

Positive samples contain flavonoids when yellow to orange are formed. Flavonoid test is done by adding 5 mL 70% of ethanol to the extract and heating it. After that, the extract is filtered and the filtrate is taken. 2 drops of 10% NaOH are added.

1.5.3 Saponin test

The extract is added with distilled water and it is boiled for 2 minutes. After it gets cold, 1 drop of HCl 2N is added and shake until homogeneous. The positive extract contains saponins if the foam is formed.

1.5.4 Alkaloid test

The extract is added with 10 mL of chloroform ammonia solution and homogenized for 1 min. The filtrate is taken by using a spotting pipette and then filtered. After that, the filtrate is added with H_2SO_4 96% amounted to 5 drops. Water and chloroform are separated and meyer, dragondorf, and wagner reagents are added. Observe the changes in the extract formed by the deposit.

1.5.5 Tanin test

2 mL extract solution was added to a test tube with 4 drops of 1% NaCl. Then, 3 drops of gelatin solution are also added to be homogenized. The positive extract contains tannin if there is deposit at the bottom of the test tube.

1.6 Data analysis

1.6.1 Percentage calculation of antioxidant inhibition

According to Fithriani et al. (2015), the inhibition percentage of DPPH absorbance by the extract to determine antioxidant activity is calculated using the following formula:

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

Where, A=Abs Negative control; B=Abs blank; C=Abs extract; D=Abs control extract

According to Mega and Swastini (2010), a compound has antioxidant activity when its percentage is more than or equal to 50%. If the percentage of resistance 0-<50% means no potential as an antioxidant and 100% indicates the need for dilution of the sample to know the limits of activity concentration.

1.6.2 IC_{50} Value calculation

According to Sugiat et al. (2010), IC_{50} is the value of extract concentration to inhibit free radicals by 50%. The equation of quadratic line formed from percentage data of inhibition and concentration is used to determine IC_{50} value. The equation of the squared line can be expressed as:

$$Y=a+bX$$

According to Jun et al. (2003) in Samin et al. (2013), the antioxidant power criterion is IC_{50}<50 ppm=strong, IC_{50} 50-100 ppm=active, IC_{50} 101-250 ppm=medium, IC_{50} 251-500=weak and IC_{50}>500 ppm=inactive.

2 Results

H. *macroloba* lives on a sandy substrate and has a long thallus of 76.65 mm and a width of 30.42 mm. *H. macroloba* has holdfast with a smooth fiber containing sand. Branching *H. macroloba* has a dichotomous type and has a flat oval thallus. Measurements of H. macroloba are presented in Figure 2.

Halimeda micronesica has a lush, small, curved and green thallus. Thallus will turn whitish when *H. micronesica* dies. The seaweed has a single, hard holdfast. The branching of thallus has a trichotomous type. The thallus segment of *H. micronesica* has a width of 66.15 mm and a segment length of 93.60 mm. The thallus segment forms a semicircle like a kidney shape. The results of *H. micronesica* measurements are presented in Figure 3.

Figure 2 *Halimeda macroloba*

Figure 3 *Halimeda micronesica*

The result of measurement of waters quality in Maspari Island obtained average temperature value of 29.33°C, DO (Dissolved oxygen) content of 4.25 mg/L, salinity 32.67‰, and pH value of 7 and density of 1 024.67 kg/m^3. The value of the measurement of the parameters of the waters is presented in Table 1.

The waters of Maspari Island can be categorized as the suitable place for the growth of seaweed. This is in accordance with the statement of Marianingsih et al. (2013) that the macroalgae can grow optimum at temperatures ranging from 25℃~31℃ and pH ranges from 7~8. Meanwhile, according to BSNI (2010), water quality requirements for seaweed production with temperatures ranging from 26℃~32℃, salinity ranges from 28~34 mg/L and the pH ranges from 7~8.5. Ladunta et al. (2015) state that good water quality for marine algae growth with temperatures ranging from 29.8℃~31℃, DO content 4.2~5.4 mg/L, salinity ranges from 31~35 ppt and pH ranges from 7.5 to 8.1.

The multilevel maceration performed in this study resulted in a crude extract of H. micronesica with an ethyl acetate solvent of 0.73 gr and a methanol of 1.05 gr. H. macroloba rough extract with ethyl acetate solvent of 0.98 gr and methanol 1.22 gr. The yield of *H. micronesica* extract was 1.78% while *H. macroloba* was 2.2%, as presented in Table 2.

H. macroloba sample extracts have more quantities than *H. micronesica* extracts. This is caused by the filtering process at the time of maceration leaving the extract on the filter. In addition, the maceration time and evaporation process also affect the amount of the produced cruide extract. The longer the maceration time the more dissolved extract compound will be. Too long evaporation process will cause the extract to be too dry. Thus, it will be difficult to move from the flask to the vial tube. If it is too fast then the extract will be difficult to dry.

Based on Table 3 the lowest IC_{50} values are found in *H. micronesica* samples with ethyl acetate solvent which is worth 52.81 ppm whereas the highest IC_{50} value is found in *H. micronesica* sample with methanol solvent amounted to 2 142 442.09 ppm. Samples that potentially have antioxidant activity are samples of *H. micronesica* with an ethyl acetate solvent. The other three samples are not potential antioxidants because they have too high IC50 values. This may occur due to a multilevel maceration process that causes compounds that have the ability as an antioxidant already dissolved in an ethyl acetate solvent. So when the *H. micronesica* sample is dissolved using methanol, the antioxidant compound is gone.

Phytochemical test results of seaweed extract of *H. micronesica* ethyl acetate containing alkaloid, steroid and flavonoid. *H. micronesica* methanol extract contains only alkaloid compounds. Phytochemical test results are presented in Table 4. Based on Table 3 ethyl acetate extract *H. micronesica* has active antioxidant activity. It can be caused by the content of the compounds contained in the extract of flavonoid compounds that function as antioxidants (Winarno, 1996 in Salamah et al., 2008; Redha, 2010; Firdiyani et al., 2015). *H. micronesica* methanol extract has weak antioxidant activity because based on phytochemical test results only contain alkaloid compounds that function as antibacterial as proposed by Robinson (1995) in Darsana et al. (2012).

Table 1 Average Measurement of Seawater Parameters on Maspari Island

Site	Temperature(°C)	DO (mg/L)	Salinity (‰)	pH	Density (kg/m^3)
1	29.33	4.25	32.67	7	1024.67

Note: Source: Research Results, 2019

Table 2 Extraction Results and Yield Extracts of *H. micronesica* and *H. macroloba* Using Ethyl Acetate and Methanol Solvents

Sample	Wet Weight (gr)	Ethyl Acetate (gr)	Methanol (gr)	Total (gr)
H. micronesica	100	0.73	1.05	1.78
H. macroloba	100	0.98	1.22	2.2

Note: Source: Research Data Result, 2019

Table 3 Results of linear regression calculations and IC_{50} extract of *H. micronesica* and *H. Macroloba*

Sample	Equation	R^2	IC_{50} (ppm)
Hmi EA	y = 1.071x+3.155	0.884	52.81
Hmi M	y = 0.275x+3.259	0.698	2 142 442.09
Hma EA	y = 0.275x+3.546	0.861	193 763.84
Hma M	y = 0.352x+3.155	0.982	174 372.21

Note: Source: (Data Research Result, 2017); Information: Hmi EA= *H. micronesica* Ethyl Acetate; Hma EA= *H. macroloba* Ethyl Acetate; Hmi M= *H. micronesica* Methanol; Hma M= *H. macroloba* Methanol

Table 4 Phytochemical Test Results of *Halimeda micronesica* Ethyl Acetate and Methanol Extracts

Parameter	Hmi EA	Hmi M
Alkaloid	+	+
Steroid	+	-
Terpenoid	-	-
Tanin	-	-
Saponin	-	-
Flavonoid	+	-

Note: Source: (Research Result Data, 2019)

3 Conclusion

Based on the result of the research, the conclusion is as follows:

I. *micronesica* extract in an ethyl acetate solvent (Hmi-EA) has a percentage of free radical inhibition ranging from 43%~89.2%. The percentage of free radical inhibition on *H. micronesica* extract in methanol solvent (Hmi-M) ranging from 11.14%~21.61%. *H. macroloba* extract in ethyl acetate (Hma-EA) solvent has inhibition percentage ranging between 16.57%~29.01% and *H. macroloba* extract in methanol solvent (Hma-M) ranging from 10.68%~21.79%.

Hmi-EA extract has potential antioxidant activity with IC_{50} value of 52.81 ppm. All three other extracts have no antioxidant activity potential.

The results of phytochemical test of the Hmi-EA extract contain alkaloid compound, steroid and flavonoid. Flavonoid can function as antioxidants.

Authors' contributions

Muhammad Hendri participated in the design of the study and performed the statistical analysis, conceived of the study, participated in its design and coordination and helped to draft the manuscript, writing and correspondence. Rozirwan participated in the sequence alignment. All authors read and approved the final manuscript.

Acknowledgements

The authors wish to thank Universitas Sriwijaya for support throughout the research, in particular the Faculty of Mathematics and Natural Sciences, especially the Department of Marine Sciences and the faculty staff. Special thanks go to Yoga Pebrian Firmansyah, S. Kel, Adi Murdianto, S. Kel, Tri Rizky Oktariansyah, Mas Ebiet and Mas Kacong (Pahawang) for their assistance during the research.

References

Badan Standarisasi Nasional, 2010, Produksi Rumput Laut Kotoni (*Eucheuma cottonii*) - Bagian 2: Metode *Long-Line*. Jakarta : SNI 7579.2

Darsana I.G.O., Besung I.N.K., and Mahatmi H., 2012, Potensi Daun Binahong (*Anredera Cordifolia* (*Tenore*) *Steenis*) dalam Menghambat Pertumbuhan Bakteri *Escherichia coli* secara *In Vitro*, Indonesia Medicus Veterinus 1(3): 337-351

Firdiyani F., Agustini T.W., and Ma'ruf W.F., 2015, Ekstraksi senyawa bioaktif sebagai antioksidan alami *Spirulina platensis* segar dengan pelarut yang berbeda, JPHPI, 18(1)
https://doi.org/10.17844/jphpi.2015.18.1.28

Fithriani D., Amini S., Melanie S., and Susilowati R., 2015, Uji Fitokimia, Kandungan Total Fenol Dan Aktivitas Antioksidan Mikroalga Spirulina Sp., Chlorella Sp., dan Nannochloropsis Sp. Jurnal Pascapanen Dan Bioteknologi Kelautan Dan Perikanan, 10(2): 101-109
https://doi.org/10.15578/jpbkp.v10i2.270

Husni A., Putra D.R., and dan Lelana I.Y.B., 2014, Aktivitas antioksidan *Padina* sp pada Berbagai Suhu dan Lama Pengeringan, JPB Perikanan, 9(2): 165-173
https://doi.org/10.15578/jpbkp.v9i2.109

Julyasih K.S.M., Wirawan I.G.P., Harijani W.S., and Widajati W., 2009, Aktivitas antioksidan beberapa jenis rumput laut (*seaweeds*) komersial di Bali, Seminar Nasional Akselerasi Pengembangan Teknologi Pertanian Dalam Mendukung Revitalisasi Pertanian' Surabaya, 2 Desember 2009, Fak. Pertanian & Lppm UPN Veteran Jawa Timur

Jun M., Fu H.Y., Hong J., Wan X., Yang C.S., and Ho C.T., 2003, Comparison of antioxidant activities of isoflavones from kudzu root (*Pueraria lobata* Ohwi), Journal of food science, 68(6): 2117-2122
https://doi.org/10.1111/j.1365-2621.2003.tb07029.x

Ladunta H., and Hasim dan Koniyo Y., 2015, Pertumbuhan alga laut *kappaphycus alvarezzi* dengan metode vertikultur pada kedalaman yang berbeda di kabupaten boalemo, Gorontalo: Universitas Negeri Gorontalo

Marianingsih P., Amelia E., and Suroto T., 2013, Inventarisasi dan identifikasi makroalga di perairan pulau untung jawa, Di dalam: *Prosiding Semirata*; Lampung, 2013, Lampung: Universitas Lampung, 1(1)

Mega I.M., and Swastini D.A., 2010, Phytochemical screening and free antiradical activity of Gaharu leaf methanol extract (*Gyrinops versteegii*), Chemistry Journal, J Kimia, 4(2): 187-192

Nurjanah N., Izzati L., and Abdullah A., 2011, Aktivitas antioksidan dan komponen bioaktif kerang pisau (Solen spp), ILMU KELAUTAN: Indonesian Journal of Marine Sciences, 16(3): 119-124

Pramesti R., 2013, Aktivitas antioksidan ekstrak rumput laut Caulerpa serrulata dengan metode DPPH (1,1 difenil 2 pikrilhidrazil), Buletin Oseanografi Marina, 2(2): 7-15
https://doi.org/10.20884/1.jm.2012.7.1.104

Redha A., 2013, Flavonoid: struktur, sifat antioksidatif dan peranannya dalam sistem biologis, Jurnal Belian, 9(2): 196-202.

Robinson T., 1995, Kandungan organik tumbuhan tinggi, Bandung: ITB, 14: 1-6

Salamah E., Ayuningrat E., and dan Purwaningsih S., 2008, Penapisan awal komponen bioaktif dari kijing taiwan (*Anodonta woodiana* lea) sebagai senyawa antioksidan, *Buletin Teknologi Hasil Perikanan*, 11(2): 119-132

Samin A.A., Bialangi N., and Salimi Y.K., 2013, Penentuan kandungan fenolik total dan aktivitas antioksidan dari rambut jagung (*Zea mays* L.) yang tumbuh di daerah gorontalo, Gorontalo: Jurusan Pendidikan Kimia FMIPA, Universitas Negeri Gorontalo

Sari B.L., Susanti N., and dan Sutanto, 2015, Skrining Fitokimia dan Aktivitas Antioksidan Fraksi Etanol Alga Merah *Euchema spinosum*, Pharm Sci Res, 2(2): 59-68
https://doi.org/10.7454/psr.v2i1.3331

Septiana A.T., and Asnani A., 2013, Aktivitas Antioksidan Ekstrak Rumput Laut *Sargassum duplicatum*, Jurnal Teknologi Pertanian, 14(2): 79-86

Sugiat D., Hanani E., and dan Mun'im A., 2010, Aktivitas Antioksidan dan Penetapan Kadar Fenol Total Ekstrak Metanol Dedak Beberapa Varietas Padi (Oryza sativa L), Majalah Ilmu Kefarmasian, 7(1): 24-33

Suhendra L., and Arnata I.W., 2009, Potensi Aktivitas Antioksidan Biji Adas (*Foeniculum vulgare* Mill) sebagai Penangkap Radikal Bebas, *Agrotekno* ISSN 0853-6414 15(2): 66-71

Suryaningrum T.D., and Kristiana H., 2006, Uji aktivitas senyawa antioksidan dari rumput laut Halymenia harveyana dan Eucheuma cottonii, Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan, 1(1): 51-64
https://doi.org/10.15578/jpbkp.v1i1.231

Winarno F.G., 1996, Teknologi Pengolahan Rumput Laut, Jakarta: Pustaka Sinar Harapan