Global Journal of Science Frontier Research: C
Biological Science
Volume 20 Issue 1 Version 1.0 Year 2020
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Antioxidant Activity and Total Phenolic Content of *Eucheuma Cottonii* and *Sargassum sp.* from South Sulawesi Indonesia

By Syaharuddin, Natalia Rombe, Yosefa M, Marianti Manggau, Rosany Tayeb & Sudir Sumarheni

Hasanuddin University

Abstract- Phenolic compounds have been extracted from red algae *(Eucheuma cottonii)* and brown algae (Sargassum sp.) and the antioxidant activity assay using DPPH method has been done. The extractions were carried out on dried and fresh samples by digestion method using ethanol: water (50:50) as solvents and followed by liquid-liquid extraction using hexane and ethyl acetate as solvents. The extraction results were qualitatively analyzed using Folin-ciocalteu and TLC methods, while quantitative analysis was performed by determination of total polyphenol levels and antioxidant activity using UV-Vis spectrophotometer. Identification with Folinciocalteu reagent showed the presence of polyphenol compounds in the ethanol fraction and ethyl acetate fraction. The TLC results showed a spot with a relatively equal Rf value between ethyl acetate fraction and gallic acid standard in both samples. The highest polyphenol content was obtained from the ethyl acetate fraction of fresh samples, 0.59% for red algae and 0.34% for brown algae while the ethanol fraction of dried samples had the lowest polyphenol content of 0.08% for red algae and 0.01% for brown algae.

GJSFR-C Classification: *FOR Code: 069999*

Strictly as per the compliance and regulations of:

Antioxidant Activity and Total Phenolic Content of *Eucheuma Cottonii* and *Sargassum sp.* from South Sulawesi Indonesia

Syaharuddin [α], Natalia Rombe [σ], Yosefa M [ρ], Marianti Manggau [Ω], Rosany Tayeb [¥] & Sudir Sumarheni [§]

Abstract- Phenolic compounds have been extracted from red algae *(Eucheuma cottonii)* and brown algae (Sargassum sp.) and the antioxidant activity assay using DPPH method has been done. The extractions were carried out on dried and fresh samples by digestion method using ethanol: water (50:50) as solvents and followed by liquid-liquid extraction using hexane and ethyl acetate as solvents. The extraction results were qualitatively analyzed using Folin-ciocalteu and TLC methods, while quantitative analysis was performed by determination of total polyphenol levels and antioxidant activity using UV-Vis spectrophotometer. Identification with Folinciocalteu reagent showed the presence of polyphenol compounds in the ethanol fraction and ethyl acetate fraction. The TLC results showed a spot with a relatively equal Rf value between ethyl acetate fraction and gallic acid standard in both samples. The highest polyphenol content was obtained from the ethyl acetate fraction of fresh samples, 0.59% for red algae and 0.34% for brown algae while the ethanol fraction of dried samples had the lowest polyphenol content of 0.08% for red algae and 0.01% for brown algae. In the antioxidant activity assay, the best results were obtained from the ethanol fraction of dried red algae sample with IC50 value of 52,36 ppm (strong antioxidant activity) and the ethyl acetate fraction of fresh brown algae sample with IC50 value of 7095 ppm. The lowest antioxidant activity were observed on the ethyl acetate fraction of dried red algae sample with IC50 value of 400,867 (very weak antioxidant activity) and the ethanol fraction of dried brown algae sample with IC50 value of 1918 ppm.

I. Introduction

Excessive free radicals in the body may cause the natural antioxidants produced by the body to be unable to reduce the danger of these free radicals, resulting in cell damages. Therefore, the intake of antioxidants from outside the body that can prevent, overcome and even repair the damage is required. Nowadays, there has been increasing interest in the use of marine natural compounds replacing chemicals either for treatment of specific diseases (Anis M., et al., 2018; Boopathy, N. S., & Kathiresan, K., 2013; Syaharuddin et al., 2018; Sun, Z., et al., 2018) or daily antioxidant intake. There are various resources of natural antioxidants, including the phenolic compounds of the red algae (*Eucheuma cottonii*) and brown algae (*Sargassum* sp.).

South Sulawesi is one of province in Indonesia which has a great potential as a *Eucheuma cottonii* seaweed producer. The total production of *Eucheuma Cottoni* in Indonesia reaches 3,082,113 tons which is about 50% of the world's seaweed product. The red algae (*Eucheuma cottonii*) and brown algae (Sargassum sp.) are also known to contain phenolic compounds that can act as antioxidants (Denny, 2013; Ermina P., et al., 2018). According to Irianti, et al. (2007), polyphenol compounds can be considered as the most important antioxidant component in plants. The antioxidant mechanism of polyphenol compounds is due to the presence of hydroxyl groups which are capable of binding to free radicals, thus forming more stable compounds.

Based on the polarity consideration of the hydroxyl groups, extraction of polyphenol compounds using polar solvents will be more effective than non-polar solvents (Nontji A, 2007). In addition, extraction methods can also affect the value of antioxidant activity. The study by Ermina P. et al., (2018) demonstrated that the n-hexane extract of *Eucheuma cottonii* which was extracted by maceration equipped by sonication method had antioxidant activity with IC50 of 119.208 µg/mL while extract using conventional maceration method had higher value of antioxidant activity with IC50 of 77,62 µg/mL. The extraction of polyphenol compounds from brown algae was also carried out by Indriawati (2015) using ethanol: water (50:50) with the temperature of 40oC by liquid-liquid extraction method, showing the antioxidant activity with IC50 value of 1770 mg/L (Indriawati, 2015). Based on these studies, the extraction of phenolic compounds from red algae (*Eucheuma cottonii*) and brown algae (*Sargassum* sp.) using the method of maceration by digestion using ethanol: water (50:50) as solvents at 40oC. The antioxidant activity assay was carried out by DPPH method. The purpose of this study was to obtain the extract of polyphenolic compounds from red algae (*Eucheuma cottonii*) and brown algae (*Sargassum* sp.) which has antioxidant activity.

II. Experimental

a) Collection and identification of samples

Samples of red algae *Euchema cottoni* and brown algae *Sargassum* sp. were collected from a cultivation area in Takalar Regency, South Sulawesi,

Author α σ ρ Ω ¥ §: Faculty of Pharmacy, Hasanuddin University, Makassar 90245, Indonesia. e-mail: saha_kasim@yahoo.co.id

Indonesia thus identified the taxonomic positions in the Biology Department, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, Indonesia. Fresh samples were washed using distilled water and cleaned form salt residue by soaking them in warm water. Washing and soaking were done 3 times. Fresh samples were immediately extracted after the sampling preparation process. While dried algae were obtained after vacuum drying at 70°C in the Laboratory of Biofarmaka, Faculty of Pharmacy, Hasanuddin University.

b) Extraction Process

The fresh and dried samples of *Eucheuma cottonii* and *Sargassum* sp. were blended into a fine powder with a grinder (Sico®) then were weighed (Sartorius®) to 60 g each, and soaked in mixture of ethanol: water (50:50) as a solvent. The maceration method at a temperature of 40°C equipped with constant stirring speed of 800 rpm. After 8 hr., the resulting liquid extract was then centrifuged at 4000 rpm for 5 minutes. The supernatant was evaporated using a rotary evaporator. The liquid extract obtained was then evaporated at dark shade in room temperature. During the evaporation process, ethanol is added to the extract until a thickened extract is obtained.

c) Separation of Compounds Based on Solubility

The obtained extract of dried *Euchema cottonii* was dissolved with 20 ml ethanol and then inserted in separation funnel. 20 ml of n-hexane was added and the mixture was homogenized and allowed to stand for 5 minutes. The n-hexane fraction was taken and then the ethanol fraction is added with 20 ml of ethyl acetate, homogenized and allowed to stand for 5 minutes and then the ethyl acetate fraction was separated from the ethanol fraction. Hexane fraction, ethyl acetate fraction and ethanol fraction were obtained as the final results.

Sargassum sp. extract was dissolved in 50 ml of ethanol and 50 ml of hexane was added and the mixture was shaken for 10 minutes and allowed to stand for 5 minutes. The upper layer was taken and evaporated. 50 ml of ethyl acetate was added to the lower layer and the mixture was shaken for 10 minutes and allowed to stand for 5 minutes. The upper layer was taken and evaporated. 50 ml of water was added to the bottom layer and the mixture was shaken for 10 minutes and allowed to stand for 5 minutes. The top and bottom layers were separated and evaporated at room temperature.

d) Identification of Polyphenol Compounds with the Folin-Ciocalteu Method

The dried fractions were analyzed for their polyphenol content using Folin-Ciocalteu reagents. Each of the fractions was taken sufficiently and dissolved in suitable solvent and then inserted in the test tube. The fractions were added with 7.5% Folin-Ciocalteu reagent and 1% NaOH sufficiently and then homogenized. The change of color to blue indicated the presence of polyphenol compounds in the extract.

e) Identification Using Thin Layer Chromatography

The fractions obtained from liquid-liquid extraction were added into vials separately and dissolved with adequate ethanol and then spotted on TLC plates that had been activated by heating at 110oC for 1 hour. TLC plates were developed using a mixture of methanol: Chloroform (3:1) as eluent. The spots were observed using UV lamps 254 and 366 nm.

f) Determination of Total Phenolic Content

The gallic acid solution was made by dissolving 10 mg of gallic acid in water in a 10 ml-volumetric flask (stock solution of 1000 ppm). For *Eucheuma cottonii,* the standard solution of gallic acid was prepared in the concentration of 0.5; 1.5; 3; 5; and 7 ppm by pipetting: 2.5 µl; 7.5 µl; 15 µl; 25 µl; 35 µl of stock solution respectively and for Sargassum sp. the standard solution of gallic acid was prepared in the concentration of 0.15, 0.5, 1.5, 3, 5, and 7 ppm by pipetting 7.5 µl, 25µl, 75 µl, 150 µl, 250 µl and 350 µl of stock solution respectively. Each of the standard solution was added into 5 ml-volumetric flask followed by 2.5 ml of folin reagent and the the mixture was homogenized. 2 ml of 1% NaOH was added to the mixture and diluted to the mark with water. The absorbances were measured with UV-Vis spectrophotometer.

The sample measurement procedure was performed by preparing a stock solution of 10,000 ppm, each of which 50 mg of ethanol fraction and ethyl acetate fraction were dissolved in 5 ml of ethanol. 3 dilution series were prepared by pipetting 500 µl of stock solution followed by 2.5 ml of 7.5% Folin-Ciocalteau reagent and 2 ml of 1% NaOH. The mixture was allowed to stand for 30 minutes and the absorbances were measured at maximum wavelength using UV-Vis spectrophotometer.

g) Antioxidant Activity Assay Using DPPH Method

8 mg of DPPH was dissolved in 50 ml of ethanol p.a in a volumetric flask (160 ppm). 1 ml of stock solution was pipetted and inserted into a 5 ml-volumetric flask and diluted to mark with ethanol p.a (32 ppm), then homogenized and allowed to stand for a few minutes. The absorbance was then measured with spectrophotometer at the wavelength of 516 nm.

Each 30 mg of the *Eucheuma cottonii* fractions was dissolved with ethanol in a 10 ml-volumetric flask (concentration of 3000 ppm), then a stock solution was prepared by pipetting 160 µl of the solution into a 5 ml-volumetric flask and diluted to mark with ethanol p.a. (concentration of 100 ppm). 1; 1.5; 2; 2.5; and 3 ml of stock solutions were pipetted respectively, followed by 1 ml of DPPH stock solution (160 ppm) and diluted to mark with ethanol p.a. The mixtures were allowed to

stand for 15 minutes at room temperature, then the absorbances were measured using UV-Vis spectrophotometer at the wavelength of 516 nm.

For *Sargassum* sp., 20 mg of the ethyl acetate fraction and ethanol fraction containing polyphenol compound were dissolved with ethanol in a 10 ml-volumetric flask, stock solution was prepared by pipetting 0.05 ml of the solution into a 5 ml-volumetric flask and diluted to mark with ethanol to obtain the concentrations of 20, 40, 60, 80, and 100 ppm and 1 ml of DPPH was added to each concentration and the mixtures were allowed to stand protected from light for 30 minutes. The absorbances were measured at 517 nm.

h) Experimental Analysis

One-way analysis of variance (ANOVA) was used to compare the antioxidant activity of *E. cottonii* and Sargassum sp. extracts, as well as the absorbance value of spectrophotometry results. The SPSS program (version 20.0; SPSS Inc., Chicago, IL, USA) was performed to further analyze the data. All p-values less than 0.05 were considered statistically significant.

III. Results and Discussion

In this study, the extraction of polyphenolic compounds from red algae *Eucheuma cottonii* and brown algae *Sargassum sp.* was carried out using the method of maceration by digestion with low temperature heating (40°C) at 800 rpm for 8 hours. This method was chosen because it had several advantages such as faster extraction time and stirring and slight warming could prevent the thickening of the solvent and precipitation of the extracted compound. The solvents used in the extraction wereethanol: Water (50:50). Previous study by Indriawati (2015) showed that the use of ethanol: Water (50:50) as solvents could provide the highest phenolic content compared to other concentrations. The solvent mixture was used because it is a polar compound which is capable of extracting polar polyphenol compounds.

The results of the extraction of *Eucheuma cottonii* and *Sargassum sp.*are shown in table 1.The results showed that the yield for dried samples was higher compared to fresh samples. The difference was probably caused by lesser water content in the dried samples than the fresh samples thus affecting the extraction process.

Table 1: Percentage yield extracted from dried and fresh of *Eucheuma cottonii* and *Sargassum sp.*

Sample	Sample Weight (g)	Extract weight (g)	Yield (% w/w)
Eucheuma cottonii dried sample	60	1,9	3,17
Eucheuma cottonii fresh sample	60	0,453	0,76
Sargassum sp. dried sample	60	9,1319	15,21
*Sargassumsp.*fresh sample	60	0,2616	0,44

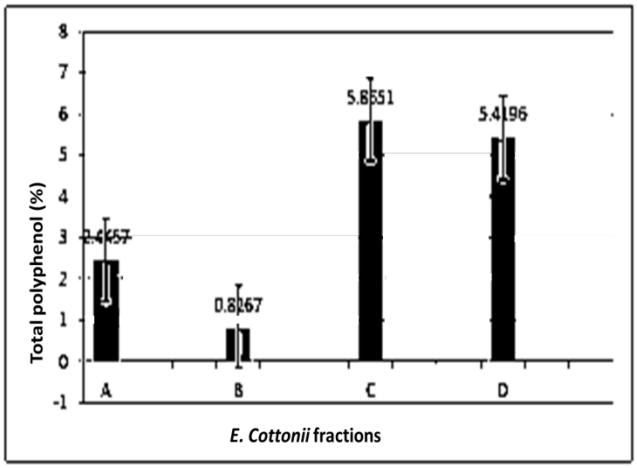
The qualitative identification of ethyl acetate fraction and ethanol fraction using Folin-ciocalteu method obtained a blue solution. The change of color was caused by the reduction of the phosphopolythdate phosphotungstate by the polyphenolic compounds present in Folin Cioucalteu and forminga blue-colored molybdenum. These results indicated that the ethyl acetate fraction and ethanol fraction of *Eucheuma cottonii* contain polyphenol compounds while for the hexane fraction there was no change of color which indicated the absence of polyphenol compounds. Positive results were obtained on ethyl acetate fraction and ethanol fraction of the *Sargassum sp.* samples. While negative results were observed on the hexane fraction. This difference was due to the hexane solvent being nonpolar, hence polyphenol compounds might not be present in the hexane fraction.

Table 2: Identification of and *Sargassum sp* fractions using TLC method

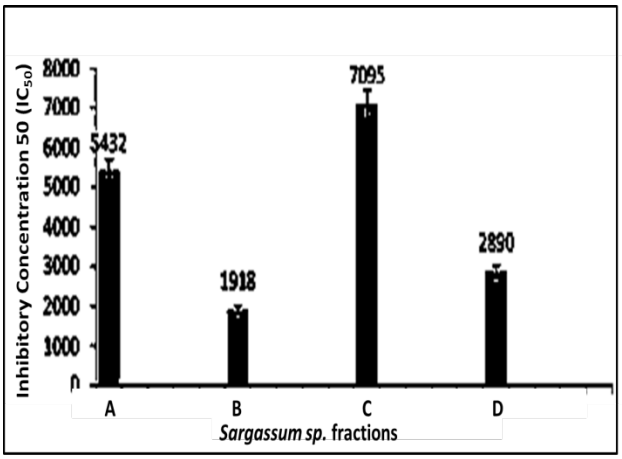
Sample	Fraction	Rf value
Dried *Eucheuma cottonii*	Ethyl acetate	0,61
	Ethanol	0,55
Fresh *Eucheuma cottonii*	Ethyl acetate	0,71
	Ethanol	0,55
Dried *Sargassum sp.*	Ethyl acetate	0,73
	Ethanol	0,68
Fresh *Sargassum sp.*	Ethyl acetate	0,71
	Ethanol	0,65
Gallic acid	-	0,68

The qualitative test by TLC method used silica gel GF 254 as the stationary phase, the mobile phase was a mixture of methanol: Chloroform (1:1) with gallic acid as the standard solution. It could be concluded from the Rf value that the ethyl acetate fraction of both samples might have the same compound with the standard solution.

Total polyphenol content of ethyl acetate fraction and ethanol fraction of *Eucheuma cottonii* were 0.24% and 0.08% respectively, while for the fresh sample the total polyphenol content of ethyl acetate fraction and ethanol fraction were 0.59% and 0.54% respectively. The measurements showed that the highest polyphenol content was found in the ethyl acetate fraction of the fresh sample of *Eucheuma cottonii* and the lowest content was observed in ethanol fraction of dried sample. For *Sargassum sp.*, the highest polyphenol content was obtained on ethyl acetate fraction of fresh sample with the value of 0.34% and the lowest content was obtained in ethanol fraction of dried sample with the value of 0.01%.



(1)



(2)

Figure 1: Comparison of polyphenol content of *E. cottonii* fractions (1) and *Sargassum sp.* fractions (2).

A = ethyl acetate fraction of dried sample; B = ethanol fraction of dried sample; C = ethyl acetate fraction of fresh sample; D = ethanol fraction of fresh sample

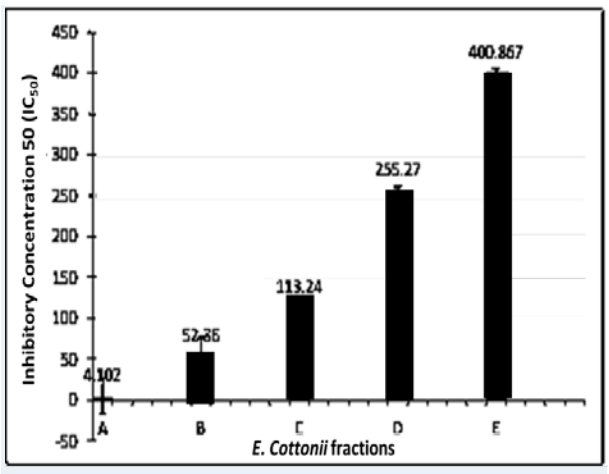
The antioxidant activity assay was performed using DPPH method. This method was chosen because it is a simple, fast and does not require many reagents. Moreover, the results of DPPH measurements show the general antioxidant activity of the sample and does not based on the type of radical inhibited (Juniarti, 2009). Antioxidant activity was measured using UV-Vis spectrophotometer and the percentage of DPPH radical binding will be used to calculate IC50 value. Determination of IC50 value aimed to determine the antioxidant activity of the fraction. IC50 shows the concentration of extracts that can provide DPPH damping by 50%. The smaller the value of IC50, the greater the antioxidant activity.

Table 3: The results of antioxidant activity assay of *Eucheuma cottonii*

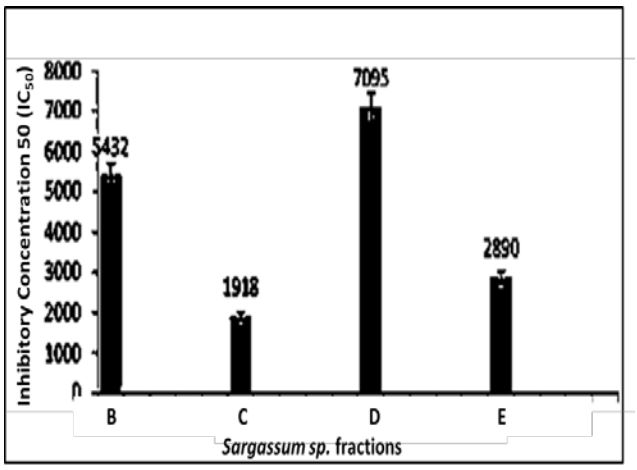
Sample	IC_{50}	AAI	Classification
Ethanol fraction Dried sample	52.36 ppm	0.611	IC_{50} (high antioxidant activity) AAI (moderate antioxidant activity)
Ethyl acetate fraction Dried sample	400.867 ppm	0.079	IC_{50} (very low antioxidant activity) AAI (very low antioxidant activity)
Ethanol fraction Fresh sample	113.24 ppm	0.283	IC_{50} (low antioxidant activity) AAI (low antioxidant activity)
Ethyl acetate sample Fresh sample	255.27 ppm	0.125	IC_{50} (moderate antioxidant activity) AAI (low antioxidant activity)
Vitamin C	4.102 ppm	7.801	IC_{50} (very high antioxidant activity) AAI (very high antioxidant activity)

Table 4: The results of antioxidant activity assay of *Sargassum* sp.

Sample	IC_{50}	AAI	Classification
Ethanol fraction Dried sample	1.918 ppm	0.016	IC_{50} (very low antioxidant activity) AAI (very low antioxidant activity)
Ethyl acetate fraction Dried sample	5.432 ppm	0.005	IC_{50} (very low antioxidant activity) AAI (very low antioxidant activity)
Ethanol fraction Fresh sample	2.890 ppm	0.011	IC_{50} (very low antioxidant activity) AAI (very low antioxidant activity)
Ethyl acetate sample Fresh sample	7.095 ppm	0.004	IC_{50} (very low antioxidant activity) AAI (very low antioxidant activity)
Vitamin C	4.102 ppm	7.801	IC_{50} (very high antioxidant activity) AAI (very high antioxidant activity)



(1)



(2)

Figure 2: Comparison of IC_{50}value between vitamin C and samples. 1 = red algae (*Eucheuma cottonii)*; 2 = brown algae(*Sargassum sp*.); A= Vitamin C; B = ethanol fraction of dried sample; C = ethanol fraction of fresh sample; D = ethyl acetate fraction of fresh sample; E = ethyl acetate fraction of dried sample.

The results of the antioxidant activity assay showed the highest IC50 value for ethanol fraction of dried *Eucheuma cottonii* and ethyl acetate fraction of fresh *Sargassum sp.* Meanwhile, the lowest IC50 values was obtained fromethyl acetate fraction of dried *Eucheuma cottonii* and *Sargasum sp*. The low antioxidant activity might result from improper separation processes, as well as other factors that may affect.

IV. Conclusion

Based on this study it can be concluded that the dried sample of red algae (*Eucheuma cottoni*) and brown algae (*Sargassum* sp.) had a higher yield percentage than fresh samples. The highest total polyphenol content was obtained from ethyl acetate fraction of fresh *Eucheuma cottonii* with the value of 0,59%, while the ethanol fraction of dried sample had the lowest polyphenol content of 0,08%. For Sargassum sp., the highest polyphenol content was obtained from ethyl acetate fraction of fresh sample with the value of 0,34% and the lowest was obtained from the ethanol fraction of dried sample with the value of 0,01%. The ethanol fraction of dried *Eucheuma cottonii* had better antioxidant activity than other fraction with IC50 value of 52,36 ppm (classified as high antioxidant activity) and AAI value of 0,611. While the ethyl acetate fraction of the dried sample had the lowest antioxidant activity compared to other fractions with IC50 value of 400.867 (very weak antioxidant activity) and AAI value of 0.079. For *Sargassum* sp., the antioxidant activity of all fractions was very weak.

Acknowledgement

We thank all staff of Faculty of Pharmacy, Hasanuddin University and the Centre for Research and Laboratory Biofarmaka for providing all the facilities.

References Références Referencias

1. Anis, M., Ahmed, S., & Hasan, M. 2018 Algae as nutrition, medicine and cosmetic: the forgotten history, present status and future trends. *World Journal of Pharmacy and Pharmaceutical Sciences*, *6*, 1934-1959.
2. Baihakki., Feliatra. and T. Wikanta. 2014 Journal of Extraction of Polyphenol from Sargassum Sp. and its Entrapment in the Nanochitosan. Faculty of Fisheries an Marine Science, Riau University.
3. Boopathy, N. S., & Kathiresan, K. 2013 Anticancer agents derived from marine algae. In *Functional Ingredients from Algae for Foods and Nutraceuticals*. pp. 307-337.
4. Denny. 2013 Kajian Isolasi Senyawa Fenolik Alga merah Euceuma Cottonii Berbantu Gelombang Micro Dengan Variasi Suhu Dan Waktu. Jurnal Teknik Kimia Vol. 3 Universitas Sultan Ageng Tirtayasa. Banten.
5. Ermina, P., Mufidah, Wijoyo, N., Sumarheni. 2018 Study of Sunscreen and Antioxidant Activity of Combination Extracts from the Red Algae *Eucheuma cottoni* and *E. spinosum.* Drug Invention Today, Vol (issue).10(9) pp. 1827-1830.
6. Harborne, J. 1987 Metode Fitokimia.Penerbit ITB. Bandung.
7. Indriyawati, N., 2015 Senyawa Fenolik dan Alginat Dari Ganggang Coklat Sargassaceae Indo-Pasifik: Ekstraksi, Pemurnian, Kuantifikasi dan Aktivitas Senyawanya. Program Studi Managemen Sumber-daya Pantai (MSDP), Fakultas Ilmu Kelautan UNDIP Desa Buddagan Kabupaten Pamekasan Madura.
8. Indriani, H., Sumarsih, E. 1999 Budidaya Pengolahan dan Pemasaran Rumput Laut. PT. Penebar Swadaya. Jakarta.

9. Irianti, T., Puspitasari., Andayana. dan Macchwiyah. 2007 Aktivitas Penangkapan Radikal 2-2' Difenil-1-Pikril Hidrazil (DPPH) Ekstrak Etanolik Daun Mengkudu (*Morinda citrifolia* L.). Yogyakarta: Fakultas Farmasi, Universitas Gadjah Mada.
10. Izzati, M. 2007 Skreening Potensi Antibakteri pada Beberapa Spesies Rumput Laut terhadap Bakteri Patogen pada Udang Windu. BIOMA. 9.
11. Keyrouz, R., Abasq, L, M., et al. 2011 Total Phenolic Contents, Radical Scavenging and Cyclic Voltammetry of Seaweeds from Brittany. The journal of Universitế de Bretagne Occidentale.
12. Koivikko, R. 2015 Brown Alga Phlorotannins Improving and Applying Chemical Methods. Departement of Chemistry, University of Turku, Finlandia.
13. Luning. 1990 Seaweeds, Their Environment, Biogeography and Ecophysiology. John Wiley and Sons. New York.
14. Nontji A. 2007 Laut Nusantara. Edisi Revisi. Penerbit Gedia, Jakarta.
15. Peng, J., Yuan, J. P., Wu, C. F., and Wang, J. H. 2011 Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health.
16. Rohmatussolihat. 2009 Antioksidan, Penyelamat Sel-Sel Tubuh Manusia. Bio Trends Volume 4 No 1.
17. Sayuti, K., Yenrina, R. 2015 Antioksidan Alami dan Sintetik. Padang : Andalas University Press.
18. Sun, Z., Dai, Z., Zhang, W., Fan, S., Liu, H., Liu, R., and Zhao, T. 2018 Antiobesity, Antidiabetic, Antioxidative, and Antihyperlipidemic Activities of Bioactive Seaweed Substances. *Bioactive Seaweeds for Food Applications* pp. 239-253.
19. Syaharuddin, Marzuki, A., Sumarheni, S., Evary, Y. M. 2018 Isolation and identification of *Bacillus alcalophilus* from K*appaphycusalvarezii* and their antibacterial activity against human pathogens, Asian Journal of Microbiology, Biotechnology and Environmental Sciences, vol. 20, no. 1, pp. 94–99.
20. Tapan, E. 2005 Kanker, Antioksidan dan Terapi Komplementer. Jakarta: PT. Elex Media Komputindo.
21. Tanduk, R., Ahmad, A. dan Dali, S. 2015 Isolasi dan Identifikasi Pigmen Karotenoid Alga merah *Eucheuma Cottonii* serta Potensinya sebagai Antioksidan dan Antikanker. Makassar: Fakultas MIPA Universitas Hasanuddin.
22. Vermerris, W., Nicholson, R. 2006 Phenolic Compound Biochemistry, Springer, The Netherlands.
23. Wijoyo, N., 2014 Uji Aktivitas Antioksidan dan Efek Tabir Surya dari Kombinasi Ekstrak *Eucheuma cottonii* dan *Eucheuma spinosum*. Fakultas Farmasi Universitas Hasanuddin.