**In vitro** antioxidant activity of macroalgae *Sargassum duplicatum* and *Palmaria palmata* extracts collected from Sepanjang Beach, Gunungkidul, Yogyakarta

C Darsih*, A W Indrianingsih, C D Poeloengasih, D J Prasetyo and N Indirayati

Research Unit for Natural Product Technology, Indonesian Institute of Sciences, Gunungkidul, Yogyakarta, Indonesia

*Email: cici.darsih@gmail.com

**Abstract.** Marine macroalgae are considered as important sources for bioactive phytochemicals. In this research, two potential marine macroalgae, i.e. *Sargassum duplicatum* and *Palmaria palmata* taken from Sepanjang beach, Gunungkidul, Yogyakarta, Indonesia were explored as sources of phenolic compound, and their antioxidant activities were evaluated. Three different solvents, i.e. chloroform, ethyl acetate, and methanol were applied as extraction medium. For each species, its total phenolic content (TPC) and antioxidant activity were determined using Folin-Ciocalteu reagent and DPPH scavenging assay, respectively. The result showed that methanolic extracts exhibited the highest yield in both species. The highest TPC (726.54 mg GAE/g) was obtained from *S. duplicatum* extracted using ethyl acetate. The antioxidant activity from *S. duplicatum* and *P. palmata* have similar value with IC$_{50}$ values of 790.34 μg/mL and 789.29 μg/mL. The scavenging activities of all extracts related to the total polyphenol content, whereas the polarity of solvent affected the resulted yield and TPC of the extracts. However, our results demonstrated that all extracts were inactive against DPPH radicals (IC$_{50}$ > 250 μg/mL).

1. **Introduction**

Marine macroalgae are considered as important sources for bioactive phytochemical with promising application in drugs, cosmetics, and food. Macroalgae were classified into four major groups, i.e. green algae (Chlorophyceae), red algae (Rhodophyceae), blue-green (Cyanophyceae), and brown algae (Phaeophyceae) [1]. Metabolites on macroalgae such as polyphenols, polysaccharides, carotenoids, flavonoids, pigments, and fatty acids have been proven exhibit biological activities [2,3]. Moreover, macroalgae also contain vitamins, protein, inorganic elements, and carbohydrates [3,4]. These metabolites possess biological activities such as antifungal, antibacterial, anti-inflammatory, antidiabetic, antioxidant, antiviral, and antimalarial [5-9]. The various metabolites of macroalgae and its concentration were affected by environmental, season, age of macroalgae, and geographical location [10].

The metabolites from brown alga *S. duplicatum* have been reported showing various pharmacological activities such as antioxidant, antibacterial, anti-inflammatory, anticancer, and tyrosinase inhibition [11-15]. Meanwhile, some studies reported that metabolites from red alga *P. palmata* or known as dulse exhibit biological activities such as antioxidant, angiotensin-converting enzyme (ACE) inhibition, anti-
inflammatory, and inhibit primary human neutrophil activation [16-18]. Both of these macroalgae can be found in Sepanjang Beach Gunungkidul, Yogyakarta. In Gunungkidul, \textit{P. palmata} traditionally consumed as a snack food, whereas \textit{S. duplicatum} has not been widely used yet by the Gunungkidul community. The aim of this study was to determine the total phenolic content (TPC) and the antioxidant activity of \textit{S. duplicatum} and \textit{P. palmata} extracts because study about both of macroalgae and its biological activity were rarely conducted. As a valuable compound for preservation and protection against oxidation, the study about antioxidant is to be concerned.

2. Materials and Method

2.1. Materials
The fresh macroalgae \textit{S. duplicatum} and \textit{P. palmata} (Figure 1) were collected from Sepanjang Beach, Gunungkidul, Yogyakarta, Indonesia on November 2018. The samples were then identified in Plant Systematic Laboratory, Biology Faculty, Gadjah Mada University. The fresh macroalgae were rinsed using seawater followed by distilled water to remove debris and sands. Samples were dried in room temperature for seven days, ground, packed in plastic bags, and stored at room temperature until further analysis.

![Macroalgae S. duplicatum (a) and P. palmata (b).](image)

2.2. Extraction of samples
In this work, three different solvents were used, \textit{i.e.} chloroform, ethyl acetate, and methanol with the ratio of 1:10 (w/v). The powder samples were sonicated at room temperature for 15 minutes, followed by maceration in room temperature for 24 hours. The filtrate and the solid residues were filtered using Whatman 1. The crude extracts of \textit{S. duplicatum} and \textit{P. palmata} were obtained by evaporating the filtrate.

2.3. Total Phenolic Content (TPC)
The TPC of crude extracts from \textit{S. duplicatum} and \textit{P. palmata} were determined using Folin-Ciocalteu reagent. The extract samples (500 μL) were added with 500 μL of the Folin-Ciocalteu reagent and mixed thoroughly. The samples were added with 1.5 mL of 20% sodium carbonate. The final volume was added up to 10 mL with distilled water. The mixture was incubated for two hours in room temperature. The absorbance of the samples was measured using ELISA reader at 765 nm. Gallic acid was used as a standard [19].

2.4. Assay for antioxidant activity
The antioxidant activity of \textit{S. duplicatum} and \textit{P. palmata} extracts was determined using DPPH method. The sample solutions consisted 20μL of crude extracts and 180 μL DPPH solution (40 mM in methanol) were
incubated in the dark at room temperature for 30 minutes. A microplate reader was used to measure the absorbance of the solutions at 517 nm. Ascorbic acid was used as a positive control and absolute methanol as a blank solution. The percentage activity of crude extracts to scavenge the DPPH radical can be seen in the equation (1).

\[
\text{Radical scavenging activity (\%) } = \frac{A_0 - A_1}{A_0} \times 100 \quad \text{(Eq. 1)}
\]

where \(A_0\) is the absorbance of the control and \(A_1\) is the absorbance in the presence of the sample [20].

3. Results and Discussion

Table 1 summarizes the effect of solvent polarity on the yield of extracts from \textit{S. duplicatum} and \textit{P. palmata}. The extract yield ranged between 0.58 to 17.22% (w/w). It was found that the methanolic extracts resulted higher yield than two other solvents. This is probably due to the polarity of methanol as a solvent. Since solvent polarity affected the extraction efficiency, methanol was effective enough to extract the compounds from the samples. As a solvent with high polarity, methanol would solubilize both polar and nonpolar compounds, so that resulted higher extraction yield. These results were in line with the previous study by Sharma and Cannoo [21], which is working with methanol, chloroform and hexane in the extraction of \textit{Nepeta leucophylla}. These results also go along with a study by Do [22], which is using methanol, pure ethanol and acetone in the extraction of \textit{Limnophila aromatica}.

Figure 2 presents the TPC of all extracts. The results showed that the TPC of ethyl acetate extracts from both algae were higher than that of the other extracts. The TPC of ethyl acetate extract from \textit{S. duplicatum} was the highest and reached up to 726.54 mg GAE/g. This result goes along with the previous research that the phenolic acid and quercetin were confirmed in ethyl acetate extract of \textit{Sargassum plagiophyllum} [23]. Meanwhile, the TPC of methanol extracts from \textit{S. duplicatum} was the lowest and reached up to 118.99 mg GAE/g. This indicates that phenolic constituents in the samples of \textit{S. duplicatum} and \textit{P. palmata} prefer to be extracted with semi-polar degree solvents such as ethyl acetate. This result was also reported in several literatures [24,25]. A study also revealed that complex formation among phenolic constituents possibly occurred and facilitated the extraction of phenolic constituents in ethyl acetate solvents [26].

| Macroalgae | Extracts   | Yield (% w/w) |
|------------|------------|---------------|
| \textit{S. duplicatum} | Chloroform | 1.22          |
|            | Ethyl acetate | 1.22          |
|            | Methanol    | 13.25         |
| \textit{P. palmata}    | Chloroform | 0.62          |
|            | Ethyl acetate | 0.58          |
|            | Methanol    | 17.22         |

The antioxidant activity of \textit{S. duplicatum} and \textit{P. palmata} can be seen at Table 2. The results reported that the antioxidant activity of ethyl acetate extracts from \textit{S. duplicatum} and \textit{P. Palmata} were similar with IC\textsubscript{50} values of 790.34 and 789.29 µg/mL, respectively. These results were higher than those of chloroform and methanol extracts, although the antioxidant activity of chloroform extract from \textit{S. duplicatum} is higher than of \textit{P. palmata}. The antioxidant activity of the ethyl acetate extracts higher than others because TPC on this extract was higher than others.
This study showed that there was a correlation between TPC and the antioxidant activity of the ethyl acetate extracts for both algae types. TPC affected antioxidant activities. TPC have ability to donate hydrogen, quench singlet oxygen and act as metal chelators, which can act as antioxidant agent [19]. The results were in line the previous research that TPC and antioxidant activity on brown algae higher than green and red algae [27-29]. In this study, all extracts were inactive against DPPH radicals (IC$_{50}$>250 µg/mL) [30].

### Table 2. Radical scavenging activity of *S. duplicatum* and *P. palmata* extracts.

| Samples     | Extracts      | IC$_{50}$ (µg/mL) |
|-------------|---------------|-------------------|
| *S. duplicatum* | Chloroform    | 821.301           |
|             | Ethyl acetate | 790.34            |
|             | Methanol      | >1000             |
| *P. palmata*  | Chloroform    | >1000             |
|             | Ethyl acetate | 789.29            |
|             | Methanol      | >1000             |
| Ascorbic acid |               | 8.28              |

### 4. Conclusion

The yield and TPC on *S. duplicatum* and *P. palmata* extracts were affected by the polarity of solvents. Chloroform, ethyl acetate, and methanol extracts of *S. duplicatum* and *P. palmata* showed inactive antioxidant properties toward DPPH radical. It suggested that other biological activities could be conducted on these extracts.

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References

[1] Thomas N V and Kim S K 2013 Beneficial effects of marine algal compounds in cosmeceuticals. *Mar. Drugs* **11**(1) 146-64

[2] Cikoš A M and Jokić S 2018 Overview on the Application of Modern Methods for the Extraction of Bioactive Compounds from Marine Macroalgae *Mar. Drugs* **16**(10) 348

[3] Wulanjati M P, Indrianiingsih A W, Darsi C, Apriyana W and Batrisya 2020 Antioxidant and antibacterial activity of ethanolic extract from *Ulva* sp. IOP Conf. Series: Earth and Environmental Science **462** 012028

[4] Wells M L, Potin P, Craigie J S, Raven J A, Cartwright P, Craigie J S, Smith A G, Camire M E and Brawley S H 2016 Algae as nutritional and functional food sources: revisiting our understanding *J. Appl. Phycol.* **29**(2) 949-82

[5] Abdel-Raouf N, Ibraheem I B M, Abdel-Hameed M S and Elhayany K M N E 2008 Evaluation of antibacterial, antifungal and antiviral activities of ten marine macroalgae from Red Sea, Egypt. *Egypt. J. Biotechnol.* **29** 157-72

[6] Barbalace M C, Malaguti M, Giusti L, Lucachinni A, Hrelia S and Angeloni C 2019 Anti-inflammatory activities of marine algae in neurodegenerative diseases. *Int. J. Mol. Sci.* **20**(12) 3061

[7] Pirian K, Moesn S, Sohrabipour J, Rabiei R and Blomster J 2017 Antidiabetic and antioxidant activities of brown and red macroalgae from the Persian Gulf *J. Appl. Phycol.* **29**(6) 3151-9

[8] Ponce N M A, Pujol C A, Damonte E B, Flores M L and Stortz C A 2003 Fucoidans from the brown seaweed *Adenocystis utricularis*: extraction methods, antimicrobial activity and structural studies *Carbohydr. Res.* **338**(2) 153-65

[9] Spavieri J, Allmendinger A, Kaiser M, Itoe M A, Blunden G, Mota M M and Tasdemir D 2013 Assessment of dual life stage antiplasmodial activity of british seaweeds *Mar. Drugs* **11**(10) 4019-34

[10] Generalić Mekinić I, Skroza D, Šimat V, Hamed I, Čagalj M and Perković Z P 2019 Phenolic content of brown algae (Pheophyceae) species: extraction, identification, and quantification *Biomolecules* **9**(6) 244

[11] Firmansyah S B, Firmansyah R A and Hayati N 2016 Antioxidant activity and antibacterial seaweed methanol extract (*Sargassum duplicatum* J. Agardh) and its potential as a natural preservative alternative to salted eggs *J Nat Sci Mathematics Res* **1**(1) 133

[12] Johnson M, Aasha Kanimozhi S, Renisheya Joy Jeba Malar T, Freitas S P R, Tintino S R, Menezes I R A, da Costa J G M and Coutinho H D M 2019 The antioxidative effects of bioactive products from *Sargassum polycystum* C. Agardh and *Sargassum duplicatum* J. Agardh against inflammation and other pathological issues *Complement. Ther. Med.* **46** 19-23

[13] Usoltseva R V, Anastyk S D, Shevchenko N M, Surits V V, Silchenko A S, Isakov V V, Zvyagintseva T N, Thin H P D and Ermakova S P 2017 Polysaccharides from brown algae *Sargassum duplicatum*: the structure and anticancer activity in vitro *Carbohydr. Polym.* **175** 547-56

[14] Chan Y Y, Kim K H and Cheah S H 2011 Inhibitory effects of *Sargassum polycystum* on tyrosinase activity and melanin formation in B16F10 murine melanoma cells. *J. Ethnopharmacol.* **137** 1183-8.

[15] Yende S R, Harle U N and Chaugal B B 2014 Therapeutic potential and health benefits of Sargassum species *Pharmacog. Rev.* **8**(15) 1-7

[16] Beaulieu L, Sirois M and Tamigneaux É 2016 Evaluation of the in vitro biological activity of protein hydrolysates of the edible red alga, *Palmaria palmata* (dulse) harvested from the Gaspe coast and cultivated in tanks *J. Appl. Phycol.* **28**(5) 3101-15

[17] Harnedy P A, O’Keeffe M B and FitzGerald R J 2017 Fractionation and identification of antioxidant
peptides from an enzymatically hydrolysed Palmaria palmata protein isolate Food Res. Int. 100 416-22

[18] Harnedy P A, Soler-Vila A, Edwards M D and Fitzgerald R J 2014 The effect of time and origin of harvest on the in vitro biological activity of Palmaria palmata protein hydrolysates Food Res. Int. 62 746-52

[19] Nisa, K., et al. 2017 Investigation of total phenolic and flavonoid contents, and evaluation of antimicrobial and antioxidant activities from Baeckea frutescens extracts IOP Conf. Series: Earth and Environmental Science 101 012002

[20] Indrianingsih A W, Tachibana S and Itoh K 2015 In vitro evaluation of antioxidant and α-glucosidase inhibitory assay of several tropical and subtropical plants Proced. Environ. Sci. 28 639-48

[21] Sharma A and Canno D S 2017 A comparative study of effects of extraction solvents/techniques on percentage yield, polyhenolic composition, and antioxidant potential of various extracts obtained from stems of Nepeta leucophylla: RP-HPLC-DAD assessment of its polyhenolic constituents. J. Food Biochem. 41(2) e12337

[22] Do Q D, Angkawijaya A E, Tran-Nguyen P L, Huynh L H, Soetaredjo F E, Ismadji S and Ju Y H 2014 Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica J. Food Drug Anal. 22(3) 296-302

[23] Chakraborty K, Maneesh A and Makkar F 2017 Antioxidant Activity of Brown Seaweeds. J. Aquat. Food Prod. Technol. 26(4) 406-19

[24] Wijaya Y A, Widydinata D, Irawaty W and Ayucitra A 2017 Fractinarion of phenolic compounds from Kaffir Lime (Citrus hystrix) peel extract and evaluation of antioxidant activity 2017 7

[25] Anagnostopoulou M A, Kefalas P, Papageorgiou V P, Assimopoulou A N and Boskou D 2006 Radical scavenging activity of various extracts and fractions of sweet orange peel (Citrus sinensis) Food Chem. 94(1) 19-25

[26] Zhu K X, Lian C X, Guo X N, Peng W and Zhou H M 2011 Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ Food Chem. 126(3) 1122-6

[27] Gupta S and Abu-Ghanam N 2011 Bioactive potential and possible health effects of edible brown seaweeds. Trends Food Sci. Technol. 22(6) 315-26

[28] Balboa E M, Conde E, Moure A, Falque E and Dominguez H 2013 In vitro antioxidant properties of crude extracts and compounds from brown algae Food Chem. 138(2-3) 1764-85

[29] Li Y, Fu X, Duan D, Liu X, Xu J and Gao X 2017 Extraction and identification of phlorotannins from the Brown Alga, Sargassum fusiforme (Harvey) Setchell Mar. Drugs 15(2) 49

[30] Phongpaichit S, Nikom J, Rungrijdahm N, Sakayaroj J, Hutadilok-Towotana N, Rukachaisirikul V and Kirtikara K 2007 Biological activities of extracts from endophytic fungi isolated from Garcinia plants FEMS Immunol. Med. Microbiol. 51(3) 517-25