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Abstract. This research aims to determine the composition of phenolic and antioxidant activity of five seaweeds (*Gracilaria salicornia, Halymenia durvylae, Halimeda macroloba, Turbinaria decurens* and *Sargassum olygocystum*) that were collected from North Sulawesi, Indonesia. Seaweeds were dried and extracts were prepared using TP maceration method, with methanol solvent. The analyses perfor 16 d consist of extraction yield, total phenolic content (TPC), scavenging radical activity of DPPH (1,1-diphenyl-2-picrylhydracyl), ferric reducing antioxidant power (FRAP) and ferrous ion chelating (FIC) activity. The results of analy 6 s present the highest content of TPC in *H. macroloba* (186.80±15.54 µg GAE (gallic acid equivalent) g⁻¹ extract). *T. decurens* had the highest radical DPPH scavenging activity (IC₅₀ 10.01±0.54 mg mL⁻¹). The highest value of FRAP was for *H. macroloba*, 28.52±1.46 µg GAE g⁻¹ extract. *S. olygocystum* and *T. decurens* exhibit higher values of FIC (IC₅₀ 5.18±0.21 and 7.02±0.43 mg mL⁻¹, respectively). The conclusion is that *G. salicornia, H. durvilae, H. macroloba, T. decurens* and *S. olygocystum* extracts are a possible source of phenolic compounds and al natural antioxidants. **Key Words**: algae, antioxidant activity, extracts, phenol.

Abstrak. Peneltian ini bertujuan untuk menghitung kandungan fenol dan aktifitas antioksidan lima jenis rumput laut ((*Gracilaria salicornia, Halymenia durvylae, Halimeda macroloba, Turbinaria decurens* and *sargassum olygocystum*), yang diambil dari perairan Sulawesi Utara Indonesia. Rumput laut dikeringkan kemudian diekstraksi menggunakan metanol dengan metoda maserasi. Jenis analisis penelitian ini terdiri dari: hasil ekstraksi, Kandungan total fenol, aktifitas peredam radikal DPPH (1,1-diphenyl-2-picrylhydracyl), daya reduksi dan aktifitas pengkelat ion. Hasil analisis menunjukkan bahwa kandungan total fenol tertinggi terdapat pada *H.macroloba* ((186.80±15.54 µg EAG (equivalent asam galat) g⁻¹ ekstrak). *T. decurens* mempuntai aktifitas antioksidan peredam radikal DPPH tertinggi, sebesar IC₅₀ 10.01±0.54 mg mL⁻¹). Nilai Daya reduksi tertinggi pada *H.macroloba* (28.52±1.46 µg EAG g⁻¹ ekstrak). Sedangkan *S. olygocystum dan T. decurens mempunyai nilai pengkelat in lebih tinggi, dengan nilai masing-masing* (IC₅₀ 5.18±0.21 and 7.02±0.43 mg mL⁻¹). Kesimpulan penelitian ini menunjukkan bahwa *G. salicornia, H. durvilae, H. macroloba*, *T. decurens and S. olygocystum* mempunyai potensi sebagai sumber senyawa fenolik dan antioksidan alami.

Kata Kunci: rumput laut, fenol, aktifitas antioksidan, ekstrak.

Introduction. Seaweeds are beneficial to humans, environment and animal nutrition. They are used as fertilizers and soil acclimatizing substances in many countries (Anantharaman et al 2010; Robledo & Freil 39 eligrin 1997). Seaweeds contain large amounts of nutrients and natural bioactive compounds, such as carotenoids, dietary fibers, proteins, essential fatty acids, vitamins and minerals (Devi et al 2000; Chandini et al 2008; Mantanjun et al 2008). These compounds are interesting for the pharmaceutical, medical, cosmetic, nutraceutical, food and agricultural industries (Boonchum et al 2011; Murugesan et al 2015; Kelman et al 2012).

Phenolic substances are generally obtained from edible red, green and brown seaweeds. The antioxidant characteristics of these seaweeds have been associated to their phenolic composition. Phenolic compounds or polyphenols have attained substantial attention due to their physiological purposes, counting antioxidant, antimutagenic, antitumor and anticancer activities (Souza et al 2011). Seaweeds could eliminate free

radicals by acting as free radical scavengers (Molyneux 2004) or by donating a hydrogen atom to the free radical (Re et al 1999).

All organisms have multifaceted regularities of antioxidant enzymes, for example thioredoxin enzyme. Some of these enzymes are preserved throughout growth and are needed for a normal development. Antioxidants in biological systems have various purposes, could be a counter for oxidative destruction and have a contribution in cell pathways. The most important action of antioxidants in cells is to inhibit the destruction caused by reactive oxygen groups (Haliwell et al 1992; Borek 1993). Reactive oxygen groups consist of hydrogen peroxide, superoxide anion and free radicals, such as hydroxyl. These molecules are changeable and extremely reactive, and can injure cells by chain reactions, like lipid peroxidation or creation of DNA adducts that could initiate cancer, endorsing mutations or cell mortality. Thus, to decrease or avoid these injuries, all cells constantly and consistently use antioxidants (Halliwell 1991; Aruoma 1993; Reaven & Witzum 1996).

Bioactive compounds from seaweeds possess a wide assortment of pharmacological properties, like anticancer, antibacterial, antifusial, anti-viral, antiinflammatory, anticoagulant, antioxidant, bone regenzating, hepatoprotective and neuroprotective properties (Liu et al 2012; Chakraborty et al 2013).

Seaweed is one of the potential natural bioactive resources. In Indonesia, some types of macroalgae, such as *Gracilaria salicornia*, *Halymenia durvilae*, *Halimeda macroloba*, *Turbinaria decurens* and *Sargassum olygocystum* grow abundantly and have a high economic value for various industries. Any studies evaluating the antioxidant potential of th 37: Indonesian seaweeds would increase their usefulness rate. Hence, the present study aims to examine the total phenolic content (TPC) and antioxidant ability of these five seaweeds.

Material and Method

Sample preparation and extraction. *G. salicornia*, *H. durvilae*, *H. macroloba*, *T.* **29** *curens* and *S. olygocystum* were collected from Nain Village, Manado, North Sulawesi. The samples were rinsed with fresh water to remove their holdfast, epiphytes, salt, debris and shellfish present. Afterwards, the samples were transported to the laboratory of Fis[20] y and Marine Science Faculty, Unsrat-Manado. Samples were shade-dried for two days and dried in an oven at 50°C for 3 days. After the drying process was complete, the samples were grounded to fine powder.

The samples were extracted using methanol (1:10 w/v) and were incubated overnight at room temperature. Extraction was repeated threastimes until the sample was colorless. The procedure was carried out in triplicates. The extracts were filtered and concentrated using a vacuum rotary evaporator (Buchi, Inggris) at 40°C. The extracts were stored in colored vial for future analysis.

Chemicals and reagents. Follin-Ciocalteu's phenol, gallic acid, sodium carbonate, methanol, ferric chloride hexahydrate, trichloroacetic acid (TCA), potassium ferricyanide, ferrous sulfate, v50 min C and butylated hydroxytoluena (BHT) were purchased from Merck (Spain). 51-diphenyl-2-picrylhydrazyl (DPPH), ferrozine iron reagent and phosphate buffer were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other solvents and chemicals were of analytical grade.

Total phenolic content (TPC). The TPC of the extracts was determined using the Follin Ciocalteau reagent according to the method of Dev36t al (2008), with a modification. Briefly, 75% of Follin Ciocalteu's phenol reagent (1 mL) was added to 57 mL of extract (0.1 g dry sample (4 10 mL methanol) and vortexed. Next, 7% Na₂CO₃ (1 mL) was added and the reaction mixture was incubated at room temperature for 30 minutes. The absorba13 was read at 750 nm using a spectrophotometer (Shimadzu type 1240, Japan). TPC was expressed as µg gallic acid equivalent (GAE) g⁻¹ dried extract.

DPPH radical scavenging assay. The DPPH-scavenging capacity of the extracts was measured based on the scavenging potential of 27able 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals by the seaweeds antioxidants. The capacity of the extracts to scatter the DPPH radical was determined by the method of Chew et al (2008) with a modification. Briefly, 2 mL of 0.93 µM DPPH in methanol w26 added to 0.5 mL extract of varying dilutions. The mixture was then vortexed and left in the dark for 20 minutes at room temperature. The absorbance was measured at 517 nm using a spectrophotometer thimadzu type 1240, Japan). Vitamin C and BHT were used as control positive. Antioxidant activity was expressed as a percentage of the DPPH scavenging activity relative to the control, using that following equation:

% radical scavenging activity = $\left(\frac{A \text{ control} - A \text{ sample}}{A \text{ control}}\right) x 100$

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Ferric reducing antioxidant power (FRAP). The seaweeds 46 xtract antioxidant activities at various dilutions were detern 12 ed by using FRAP assay following the method of Kumar et al 2008, with modifications. 1 mL of 0.2 M phosphate buffer (p 35.6) and 1 mL of K₃[Fe(CN)₆] (1%) were mixed with 1 mL of 20 racts. The reaction mixture was incubated at 50°C for 20 minutes, after which 1 mL of 10% TCA was adde 45 and centrifuged at 3000 rpm for 10 minutes. 1 mL of solution from the upper 22 ver was mixed with 1 mL of sterile water and 0.5 mL of 0.1 % FeCl₃6H₂O. The alter the assured at 700 nm. BHT was used as a positive control. The FRAP value was expressed as μ g GAE g⁻¹.

Ferrous ion chelating (FIC) assay. The chelating of ferrous ions by extracts was investigated following the method described by Chew et al (2008). Bri 34, 0.5 mL of seaweed extract at various concentrations 55.5 mL of 0.1 mM FeSO₄ M and 0.5 mL of 0.25 mM fero 1 ion reagent were mixed. The reaction mixtures were incubated for 20 minutes. The absorbance of the solution was measured at 562 nm. The percentage of inhibition of ferrozin-Fe²⁺ complex formation was determined using the following formula:

% inhibition =
$$\left(\frac{A \text{ control} - A \text{ sample}}{A \text{ control}}\right) \times 100$$

Statistical analysis. All experiments were conducted in triplicate. The means of parameters, extraction yields, TPC and antioxidant activity are presented as mean±standard deviation, using Microsoft Excel 2007.

Results and Discussion

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Extraction yield. Table 1 shows the extraction yield of the five seaweed extracts on a dry basis weight. Among the methanol extract of the five seaweeds, *H. macroloba* had the highest yield extraction ($18.45\%\pm0.43$), followed by *H. durvilae* ($6.34\%\pm0.43$), *T. decurens* ($5.72\%\pm0.23$), *S. olygocystum* ($4.95\%\pm0.03$) and *G. salicornia* ($3.56\%\pm0.12$), respectively.

Extraction yield of seaweed extracts on a dry weight basis

Table 1

Seaweeds	Division	Yields (%) w/w
Gracilaria salicornia	Rhodophyta	3.56±0.12
Halymenia durvilae	Rhodophyta	6.34±0.43
Halimeda macroloba	Chlorophyta	18.45±0.43
Turbimaria decurens	Phaeoephyta	5.72±0.23
Sargassum olygocystum	Phaeoephyta	4.95±0.03

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Total phenolic content. TPC of methanol extracts for the five seaweeds are presented in Table 2. The rest show that *H. macroloba* exhibited the highest amount of TPC (186.80±15.84 μ g GAE g⁻¹ extract), whereas the lowest TPC was for *G. salicornia* (14.73±1.04 μ g GAE g⁻¹ extract).

Total phenolic content (TPC) of seaweed extracts

Table 2



Note: GAE - gallic acid equivalent.

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DPPH radical scavenging activity. DPPH is a stable nitrogen free radical, which can be successfully scavenged by antioxidants. DPPH have been used comprehensively as a free radical to calculate the activity of antioxidants from plant extracts. A newly ready DPPH solution displays an intense purple color. This purple color usually weakens/vanishes while an antioxidant is present in the solution. Hence, antioxidant compounds can scavenge DPPH free radicals and transform them in a colorless product. Therefore, the faster is the decline of the absorbance, the more strong the anti-xidant activity is (Seenivasan et al 2013). The capacity of the extract to scavenge DPPH radicals was established by the 43 cline of absorbance at 517 nm (Molyneux 2004). Figure 1 shows the DPPH scavenging activity of the five seaweed extracts. The most effective activity was presented by *T. decurens*, followed by *G. salicornia*, *H. durvilae*, *S. olygocystum* and *H. macroloba*, with the folf9ving IC₅₀ values: 10.01 ± 0.53 ; 12.81 ± 0.93 ; 14.17 ± 1.06 ; 15.38 ± 1.17 ; 18.54 ± 1.25 mg mL⁻¹, respectively.

Ferric reducing antioxidant power. The reducing capability is deliberated as an important indicator of potential antioxidant activity of a constituent or a sample. The attendance of reductants (antioxidants) instigates the reduction of the Fe³⁺/ferricyanide multifarious to the ferrous type. Consequently, by evaluating the establishment of Perl's Prussian blue at 255 nm, the quantity of Fe²⁺ can be observed (Seenivasan et al 2013). Bable 3 shows the antioxidant activity of the extracts using FRAP assay. The highest reducing power was observed in the case of *H. macroloba*, followed by *S. olygocystum*, *T. decurens*, *G. salicornia* and *H. durvilae*, with the following values: 28.52±1.46; 19.95±1.72; 16.77±1.47; 7.24±0.61 and 5.39±0.341 µg GAE g⁻¹ extract, respectively. The positive control, BHT, showed higher antioxidant activity than all the seaweed samples.

Ferrous ion-chelating activity. *G. salicornia*, *H. durvilae*, *H. macroloba*, *T. decurens* and *S. olygocystum* extracts have ferrous ion-chelating abilities, as presented in Figure 2. The ability of all the five species of seaweeds increased with the concentrations, which increased from 2.5 to 10 mg mL⁻¹. The extracts with a higher ferrous ion-chelating ability were from brown algae *S. olygocystum* and *T. decurens*, followed by *G. salicornia*, *H. durvilae* and *H. macroloba*, the IC₅₀ values being 5.18±0.21, 7.02±0.43, 13.12±0.54, 14.39±1.34 and 34.25±2.78 mg mL⁻¹, respectively.



Figure 1. DPPH radical scavenging activity (%) of seaweeds extracts.

The metaanol yields of extraction for the seaweeds were different, the same as in other cases (Chew et al 2008; Chandini et al 2008; Ganesan et al 2008). Mantanjun et al (2008) detected a yield of total methanol extract for red algae, *Euchema spinosum*, of 1.88%, for green algae, *Caulepa lentillifera*, of 30.86% and brown algae, *Dictiota dichotoma*, of 40.33%. The difference in the yields of various extracts is ascribed to the polarities of different constituents in the plants.

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Ferric reducing antioxidant power (FRAP) of seaweeds extracts

Species	FRAP (μg GAE g ⁻¹ extract)
Gracilaria salicornia	7.24±0.61
Halymenia durvilae	5.39±0.34
Halimeda macroloba	28.52±1.46
Turbinaria decurens	16.77±1.47
Sargassum olgoycystum	19.95±1.72
BHT	41.32±3.87

Note: GAE - gallic acid equivalent.

Halimeda spp. have been examined over the past years as sources of high phenolic compounds with biological activity of diverse types (Table 4). The antioxidant properties of phenols are an effect of their capability to work as reducing agents, hydrogen donors and free radical quenchers. Phenols can also work as metal chelators, which avoid the catalytic purpose of metal in the process of instigating radicals (Devi et al 2008). Many hydrophilic polyphenolic compounds were observed in seaweeds, such as epigallocatechin gallate, epicatechin and phlorotannins (Boonchum et al 2011). *H. macroloba* contains high levels of polyphenolic compounds, up to 28000 µg of epigallocatechin and 1880 µg of catechol. It also presents caffeic acid and hesperidin 33 oshie et al 2002).

Some studies fervently sustain an involvement of polyphenols in the avoidance of cardiovascular diseases, cancer 32 inflammation and osteoporosis (Das et al 2012). Moreover, polyphenols seem to have a function in the hindrance of neurodegenerative diseases and diabetes mellitus (Scalbert et al 2005). Most likely, cells react to polyphenols primarily over straight contacts with receptors or enzymes implicated in motion transduction, which may produce consequences in the alteration of the redox position of the cell and may activate a sequence of redox-reliant reactions. Antioxidant and prooxidant impressions of polyphenols have been explained with dissimilarity effects

on cell physiologic developments. As antioxidants, polyphenols may recover cell endurance; as prooxidants, they may generate apoptosis and avoid tumor growth (Lambert et al 2005).





The five species of seaweeds, especially T. decurens, show high antioxidant activity as determined by the DPPH assay. Polyphenols are forceful antioxidant components (Boonchum et al 2011). However, the antioxidant activities were not the effect of just phenolic compounds. For example, T. decurens had low phenolic content, but presented high antioxidant activity. As it was explained by Kuda & Ikemori (2009), the activities may be initiated by other hydrophilic compounds, for instance peptides, fucoidan and Maillard reaction products and it may be correlated to polysaccharides and pigments (Chattopadhyay et al 2010). Sanger et al (2018) reported that the TPC of ethanol extracts of T. decurens was 60.79±4.57 ug GAE g-1 extract, the carotene content was 6.948 \pm 0.654 µg g⁻¹ dry weight and the scavenging radical activity of DPPH was IC₅₀ 0.3033 ± 0.023 mg mL⁻¹. According to Chattopadhyay et al (2010), the sulfated polysaccharides responsible for the antioxidant activity of T. conoides have been isolated and identified as fucoidan, laminaran, and alginate. The major antioxidants isolated from the Hawaiian specimen of T. ornata were carotenoid and fucoxanthin (Kelman et al 2012). Crude and purified sulfated polysaccharides from T. ornata and T. conoides have been shown to be excellent antioxidants and have been suggested as natural antioxidant agents (Chattopadhyay et al 2010) for using as food supplements to raise the shelf-life of food products and nutraceuticals against oxidative stress liseases (Chakrabortty et al 2013). Seaweed polysaccharides have an essential function as free radical scavengers (in vitro) and antioxidants in the deterrence 23 oxidative destruction in live organisms (Yuan et al 2005). The crude polysaccharide of *T. ornata* could be deliberated as a prospective antioxidant and anti-inflammatory substances (Ananthi et al 2010).

The reducing capacity properties show that the antioxidant constituents are electron donors and can decrease the oxidized intermediates of the lipid peroxidation process, so they can act as primary and secondary antioxidants. In terms of antioxidant activity, *H. macroloba*, *T. decurens* and *S. olygocystum* are more active than the red algae *G. salicornia* and *H. durvilae*. Boonchum et al (2011) reported **15** the reducing power of aqueous extracts from *H. macrolo***15** (1 mg mL⁻¹) was 0.092 mg gallic acid g⁻¹ dry weight and its TPC was 0.077±0.001 mg gallic acid g⁻¹ dry weight. *Halimeda* sp. possesses high phenolic content and low quantities of other antioxidants, like ascorbate, β -carotene, chlorophylls and selenium. Phenolic acids are the most important constituents of *Halimeda* spp., the substantial antioxidant activity being exhibited by *Halimeda* spp. extracts, especially by the phenolic acids (salicylic, cinamic, gallic

pyrogallic, ferulic and caffeic acids). The genus *Halimeda* contains phytopharmaceuticals and possess pharmacological properties, such as antimicrobial activity, stimulation of apoptosis, anti-trichomonal and anti-inflammatory activity; it is also valuable in biomedicine, in hepato, neuro and athero protection (Silva et al 2017).

Table 4

Total phenolic content (TPC) and biological activity in some species of the genus Halimeda

Species	Antioxidant activity	TPC	Reference
H. macroloba	DPPH IC ₅₀ 18.54±1.25 mg mL ⁻¹ FIC 34.25±2.78 mg mL ⁻¹	186.80±15.54 µg GAE g ⁻¹ extract	This study
H. macroloba	DPPH (10 mg mL ⁻¹) 57.37±3.13% FRAP 14.89±2.78 g GAE/100 g extract	18.42±0.65 g GAE/100 g extract (70% methanol extract)	Sanger et al (2013)
H. macroloba	DPPH 1.0212±0.044 mg mL ⁻¹ FRAP 0.248±0.014 µM Fe2+mg ⁻¹	62.78±5.38 μg GAE g ⁻¹ acetone extract	Sanger <mark>et al</mark> (2018)
H. macroloba	DPPH 1.9776±0.1402 FRAP 0.214±0.013 µM Fe2+mg ⁻¹	11.58±1.032 µg GAE g ⁻¹ ethanol extract	Sanger <mark>et al</mark> (2018)
H. macroloba	Anti-lipid IC ₅₀ 155.590 \pm 16.129 mg mL ⁻ peroxidation activity 0.018 \pm 0.002b mg trolox g ⁻¹ dry weight	0.085±0.003 mg gallic acid/g dry weight	Boonchum et al (2011)
H. opuntia	β-carotene-linoleic acid system (20 µg phenols) 73.5% DPPH (THF extract) IC ₅₀ 12.8-15.2 mg phenolic compounds	74.3 mg/g dry weight seaweed	Silva <mark>et al</mark> (2017)
H. monile	β-carotene-linoleic acid system 74.4% (20 μg phenols IC ₅₀ 7.7-13.2 mg phenolic compounds	66.7mg/g dry weight seaweed	Silva <mark>et al</mark> (2017)
H. incrassata	DPPH 19-53% Inhib. oxidation-Cu LDL 0.87±0.09mg/mL Inhib. oxidation-AAPH-LDL 0.16±0.01 mg/mL	10-40 µg poliphenolics	Costa- Mugica (2012)
H. incrassata	β-carotene-linoleic acid 95% (10 μg polyphenols)	255 µg/g fresh seaweed	Vidal et al (2011)

GAE - gallic acid equivalent; FRAP - ferric reducing antioxidant power; THF - tetrahydrofuran.

The binding of the antioxidant constituents to metal ions was estimated using the FIC assay. An extract with higher binding activity would avoid or restrain reactions, like Fenton's reaction, which produces reactive hydroxyl radicals. In this study, brown algae (*S. Olygocystum* and *T. decurens*) had higher binding activities than red algae (*G. salicornia* and *H. durvilae*) and green algae (*H. macroloba*). As it was shown by Chew et al (2008), brown algae *Padina antilarium* had the highest chelating ability compared with red algae *Kappaphycus alvarezzie* and green algae *Caulerpa racemosa*. Phlorotannins, commonly found in brown seaweeds, are powerful chelators of heavy metals (Toth & Pavia 2000; Sathya et al 2013). In contrast, the TPC values of *S. olygocystum* and *T. decurens* were lower than that of *H. macroloba*. According to Wang et al (2009) and Chin et al (2015), phlorotannins did not seem to be extremely effective metal chelators, hence indicating the existence of some compounds other than phenols. Polysaccharides, pigments, proteins or peptides in the extracts also have the capacity to

chelate metal ions (Chakraborty et al 2013). Polysaccharides (alginates, fucoidan) and phytochelatins are more valuable than phlorotannins regarding detoxification and copper accumulation in *Ascophylum nodosum* (Wang et al 2009; Chin et al 2012). Actually, compounds counting phenolic acids, flavonoid quercetin and phenolic glycosides are also noticed to chelate transition metal ions like Fe²⁺. These active compounds might have a synergistic effect, contributing with an essential function in antioxidant scavenging activities of seaweed extracts (Kuda & Ikemori 2009).

Conclusions. *G. salicornia, H. durvilae, H. macroloba, T. decurens* and *S. olygocystum* extracts can be used as a source antioxidants. The highest content of TPC was in *H. macroloba* (186.80±15.54 µg GAE g⁻¹ extract). *T. decurens* had the highest radical DPPH scavenging activity (IC₅₀ 10.01±0.54 mg mL⁻¹). The highest value of FRAP was *H. macroloba*, 28.52±1.459 µg GAE g⁻¹ extract. *S. olygocystum* and *T. decurens* exhibit a strong FIC ability, with IC₅₀ 5.18±0.21 and 7.02±0.43 mg mL⁻¹ values, respectively.

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