Diversity of brown macroalgae in Kupang Bay waters and alginate content potential and its phytochemistry

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Abstract. This study was aimed at knowing the diversity, alginate content, and phytochemical content of brown macro algae found in Kupang Bay waters. Macroalgal sampling was carried out at the lowest tide in Paradiso beach, Kelapa Lima, Pasir Panjang, Bolok, and Tabulolong, through survey along the coast. Each species found was photographed for identification. After species identification, each macroalga was cleaned and dried, then sublimated for alginate content analysis and secondary metabolite test covering alkaloid, saponin, flavonoid, tannin, terpenoid, and steroid compounds. Results found 11 species of brown macroalgae, namely *Padina australis*, *Padina* sp., *Turbinaria ornata*, *Hydroclathrus clathratus*, *Hormophysa cuneiformis*, *Sargassum* spp., *S. cristaefolium*, *S. polycystum*, *S. crassifolium*, *Dictyopteris acrostichoides*, and *Dictyota ciliolata.* The alginate content of two species, *H. clathratus* and *D. ciliolata,* was not examined because of insufficient amount. All brown macroalgae contained different alginate with species and the highest occurred in *Sargassum* spp. and *Padina* spp. reaching 33.33%, while the lowest was in *Turbinaria ornate* and *S. cristaefolium,* only 13.33%. Besides, they held alkaloid, saponin, steroid, terpenoid, tannin and flavonoid compounds that could be developed as medicinal materials.

Key Words: species identification, occurrence, content amount, metabolite compounds.

Introduction. Macroalgae usually known as seaweed are one of the coastal and marine resources that have good economic or ecological benefits (Pakidi & Suwoyo 2016). Economically, macroalgae can be developed as food materials, livestock feed, medicines, fertilizer and raw materials for industry (Kemer et al 2015). Ecologically, they function as primary producers and provide habitats for several marine animals (Prathep et al 2011).

Macroalgae have long been utilized by people and industries for various needs as well. The use of macroalgae for industry is particularly caused by its hydrocolloid content, such as carrageenan, agar and algin. Agar is produced by red alga species, such as *Gracilaria* spp.*, Gelidium* spp., and *Hypnea* spp. (Basman et al 2013), carrageenan by *Eucheuma* spp., and alginate by brown macroalgae, such as *Sargassum* spp., *Turbinaria* spp.*, Hormophysa* spp. and *Padina* spp. (Rasyid 2007; Laksanawati et al 2017).

Nevertheless, alginate produced from brown macroalgae is still limited because its culture has not been developed yet. Alginate is one of hydrocolloids, a colloid system in which water is the dispersion medium. The use of alginate is extensive enough, either in food or non-food industries and drug industries (Lone et al 2016). In the food industry, alginate is largely utilized as emulsion stabilizing agent in ice cream, suspension in brown milk, viscosity control in yoghurt, and etc. In the non-food industry, alginate is used as thickening agent in textile printing pasta, uniformity and smoothness regulator of the paper surface, penetration and stability control of glue made from starch or latex, and etc. (Basman et al 2013).

Beside holding alginate, brown macroalgae also contain marine natural products (Manilal et al 2016). Bhakuni & Rawat (2005) found antibacterial compounds in algae, such as amino acids, terpenoids, phlorotannins, acrylic acids, phenols, steroids, halogenated ketones and alkanes, cyclic polysulphides and fatty acids. Phloroglucinol, alginate, mannitol and phenolic compounds are also bioactive compounds that occur in brown algae and show antibacterial, antifungal and antimicrobial activities (Zubia et al 2008).

In Indonesia, there are actually many alginate-producing brown macroalgae and containing active compounds (secondary metabolites), but their utilization has not been optimal yet compared with the use of red macroalgae. Kupang Bay waters represent one of Indonesian waters possessing the potential of brown macroalgae that have not been optimally utilized yet. Umar & Salosso (2009) found 6 genera of brown macroalgae in Kupang Bay waters, *Dictyota* sp., *Hormophysa* sp., *Hydroclathrus* sp., *Padina* sp., *Sargassum* sp., and *Turbinaria* sp. Compared with the genera found in Indonesian waters, *Cystoseira* sp., *Dictyopteris* sp., *Dictyota* sp., *Hormophysa* sp., *Hydroclathrus* sp., *Padina* sp., *Sargassum* sp., and *Turbinaria* sp. (Romimohtarto & Juwana 2001), nearly all the genera, but *Cystoseira* and *Dictyoteris* occur in Kupang Bay waters.

To optimize the use of brown macroalgae found in Kupang Bay waters, it is necessary to study the species diversity and hydrocolloid content, such as alginate and their active compounds for the development of its potential utilization. This study aims to know the species diversity, alginate content, and secondary metabolites of brown macroalgae in Kupang bay waters that their utilization potentials can be informed.

Material and Method

Sample collection and identification. Macroalgae sampling was conducted twice, in February and May, 2016, respectively, at the lowest tide in each study site, Paradiso, Kelapa Lima, Pasir Panjang, Bolok and Tabulolong coastal waters. The samples were haphazardously collected along the coast, and each of them was photographed. The samples of high numbers were then taken to the Laboratory of Fisheries and Marine Science, Universitas Nusa Cendana, Nusa Tenggara Timur, for further analyses, while those collected in very low numbers were photographed and identified. They were kept in plastic bags, cleaned, sorted with genus, weighed in fresh condition, wind-dried, and then ready to examine the alginate content and their nutrition.

Alginate content analysis. Alginate content was done in brown macroalgae. Three g of dry brown macroalgae was immersed in 15 mL of 1% $CaCl_2$ solution for 2 hours while stirred in order to remove laminaran, mannitol, and other salts. Then, the samples were washed in water to remove calcium and dissolved salt, followed by washing in 0.33% HCl. Before chopped, the macroalgae were washed in water and immersed in 4% Na_2CO_3 solution at 1:2 ratio at 40^{o}C for 2 hours while stirred using a magnetic stirrer up to yielding homogenous pasta. Moreover, the pasta was diluted in distilled water 3 times the initial volume while stirred, sieved through fine cloth, and the filtrate was added with 5 mL of 0.33% HCl, left for 6 hours up to deposition formed. Finally, the deposit, the alginate rendement, was sieved through filter paper and dried in the oven at 60^oC (Widyastuti 2009).

Phytochemical analysis. Phytochemical tests of brown macroalgae included alkaloid, saponin, flavonoid, tannin, terpenoid and steroid compounds. These analyses were only done on 6 samples representing each genus of the brown macroalgae found. These applied Culvenor-Fiztgerald method for alkaloid, foam test for saponin, FeCl addition for tannin compound, and Lieberman-Burchard method for terpenoid and steroid (Harborne 1987).

Results and Discussion

Diversity of brown algae. Species and occurrence of brown macroalgae in Kupang Bay waters are presented in Table 1, which demonstrates that there are 11 species of brown macroalgae found in Kupang Bay waters. In general, the species of brown macroalgae found at the first sampling (February) were also found at the second sampling (May). The analysis of the occurrence frequency of the brown algae in Kupang Bay waters (Table 1) indicated that *Padina australis* was distributed in all locations but Tabulolong coast, where was found an unidentified species of *Padina*. The species was found only in one location and only appeared at the first sampling was *Hydroclathrus clathratus*.

Table 1

Species and occurrence of brown macroalgae found in Kupang bay water in February (I) and May (II)

No	*Species*	*Locations*										*Occurrence (%)*	
		Paradiso		*Kelapa Lima*		*Pasir Panjang*		*Bolok*		*Tabulolong*			
		I	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*
1	*Padina australis*	++	+++	++	+++	++	+++	++	+	-	-	80	80
2	Padina sp.	-	-	-	-	-	-	-	-	++	++	20	20
3	*Turbinaria ornata*	-	++	++	+++	-	-	-	-	++	+++	40	60
4	*Hydroclathrus clathratus*	-	-	-	-	-	-	-	-	++	-	20	-
5	*Hormophysa cuneiformis*	-	-	-	-	++	++	-	-	++	+++	40	40
6	*Sargassum cristaefolium*	+++	+++	+++	+++	-	-	-	-	++	+++	60	40
7	*Sargassum* sp.	-	-	-	-	++	++	++	++	+++	+++	60	60
8	*Sargassum polycystum*		-			-	++			-	++	40	40
9	*Sargassum crassifolium*		-							++	++	20	20
10	*Dictyopteris acrostichoides*	-	-	-	-	+	++	+	+	++	+	60	60
11	*Dictyota ciliolata*	-	-	-	-	+	+	-	-	+	+	40	40
	Number of species	2	3	3	3	5	6	3	3	9	9		

Note: +++ = many; ++ = moderate; + = few (no enough samples collected); - = not present.

The highest number of brown macroalga species was found in Tabulolong coastal waters, followed by Pasir Panjang and the lowest in Paradiso coast. It is supported with number of substrate types in both coasts (Table 2). Although number of substrate types in Tabulolong coast was the same as in Pasir Panjang, number of brown macroalga species found was different as a result of human activities around. Sampling location in Tabulolong coast was far from residential area and human activities, but Pasir Panjang coast was very close to the residential area.

Table 2

Habitat types and number of habitat types with observation location

No	*Locations*	*Habitat*				*No. habitat types*
		Mud	*Sand*	*Dead corals*	*Seagrass*	
1	Paradiso			v		1
2	Kelapa Lima		v	v		2
3	Pasir Panjang	v	v	v	v	4
4	Bolok	v			v	2
5	Tabulolong	v	v	v	v	4

Alginate content analysis of brown macroalgae. Two species of the brown macroalgae, *H. clathratus* and *D. ciliolata,* were not examined their alginate content because of insufficient amount of samples found, and their amount was only used for phytochemical analysis. The other nine species found in Kupang Bay waters contained alginate in different percents (Figure 1). In general, percent of alginate content of brown macroalgae was higher at the second sampling than the first sampling.

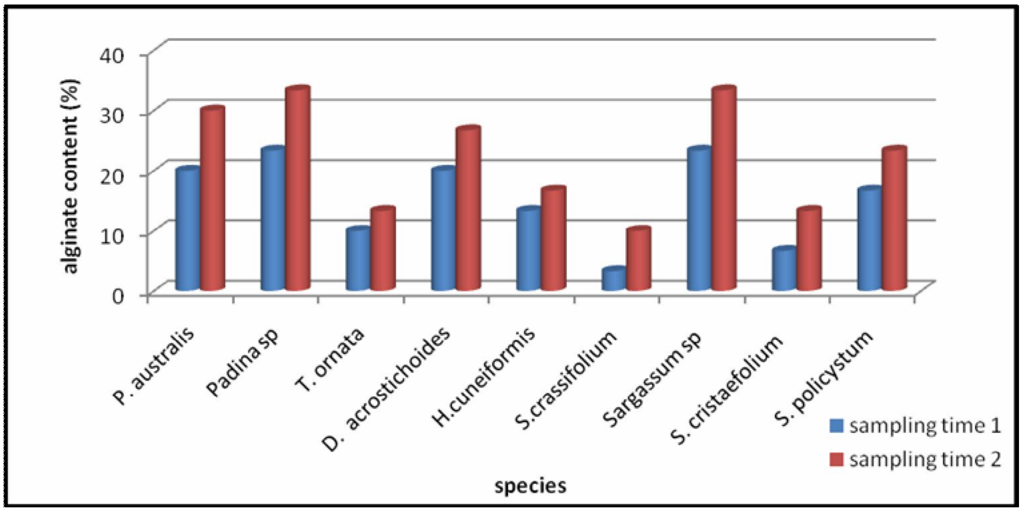

Figure 1. Alginate content of brown macroalgae in Kupang Bay waters.

Secondary metabolite content analysis. The results of qualitative analysis of macroalgal secondary metabolites found in Kupang Bay waters can be seen in Table 3.

Table 3

Secondary metabolite content of the macroalgae found in Kupang Bay waters

No	*Species*	*Secondary metabolite*					
		Alkaloid	*Saponin*	*Flavonoid*	*Tannin*	*Terpenoid*	*Steroid*
1	*P. australis*	+	+	+	+	+	+
2	*T. ornata*	+	+	+	+	+	+
3	*H. clathratus*	+	+	+	+	+	+
4	*H. cuneiformis*	+	+	+	+	+	+
5	*Sargassum* sp.	+	-	+	+	+	+
6	*D. acrostichoides*	+	+	+	+	+	+

Note: + = present; - = absent.

Discussion. The number of brown macroalgae species found in Kupang Bay waters was higher than in several other places in Indonesia, but lower than those reported by Sukiman et al (2014) in Sekotong waters. They found 15 genera in these waters. Thirteen species were also reported occurring in Raigad Coast of Konkan, Maharashtra (Ambhore & Whankatte 2016) and 19 species were recorded from Blue Lagoon, Malaysia (Asmida et al 2017).

In Anambas group of islands only 6 species were found: *Dictyota dichotoma, Hormophysa triquetra, P. australis, Sargassum echinocarpum, S. polycystum, T. ornata* (Kadi 2009). In Untung island waters, Java, were also found 6 species of brown macroalgae: *Padina minor*, *Dictyota dichotoma*, *Sargassum binderi, S. asperifolium, S. ilicifolium,* and *S. polycystum* (Marianingsih et al 2013). In Pasige island lagoon, Tagulandang district, Sitaro regency, only 2 species of macroalgae, *Padina minor* and *T. ornata* were found (Tampubolon et al 2013), and in the coastal waters of Dofamuel island, south Jailolo, west Halmahera regency only one genus of Phaeophyta was found (Sinyo & Somadayo 2013). Number of brown macroalgae species recorded in Kupang bay waters is lower than that found in some waters outside Indonesia, such as Raigad coast of Konkan, Maharashtra, 13 species (Ambhore & Whankatte 2016) and Blue Lagoon, Malacca strait, Malaysia, 19 species (Asmida et al 2017).

Based on the number of samples collected from 5 sampling sites, the most abundant species, distributed in all sites, and found in each sampling time, was *P. autralis* with occurrence frequency up to 80% (Table 2), while the lowest number of species occurrence was found only in Tabulolong waters at the first sampling, *H. clathratus*. Difference in number and distribution of these macroalgae could result from their adaptive capability to environmental conditions and the distributional ability of the spores. According to Atmadja (1999), the distribution of macroalgae species is affected by environmental characteristics and the characteristics of the macroalgae. The suitability between both factors will determine the macroalgal growth including the ability to settle at the early development stage.

Variations in macroalgal occurrence are also dependent upon habitat conditions and seasons. According to Soegiarto et al (2011), algae live as phytobenthos by plugging or embedding their body into the mud, sand, corals, dead coral fragments, rocks, or wood. Hence, habitat difference will highly determine the seaweed growth. It is in agreement with Kadi (2000) that substrate structures highly influence the variations in the occurrence of macroalgae species. Thus, similar habitat substrate structure will have species diversity approaching to similarity.

Comparison among sampling sites showed that Tabulolong waters held the highest diversity of macroalgae due to higher habitat variations. This is in line with Papalia (2015) that level of occurrence and diversity usually highly depend on habitat and substrate complexity. The occurrence of macroalgae is seasonal as well (Litaay 2014). This finding revealed that several species of brown macroalgae were found in high numbers at the first sampling (February), but they occurred in low numbers at the second sampling time (May), and even *H. clathratus* was not found. Similarly, species found in low numbers at the first sampling time occurred in high numbers at the second sampling time.

Human activities are also affecting factors. This situation was seen in Pasir Panjang, where variations in number of habitats are similar to Tabulolong, but Pasir Panjang had less number of species. Pasir Panjang waters are very close to the residential area, so that environmental pressure of anthropogenic factors on the macroalga diversity was very high. This finding is supported by Langoy et al (2011) that human activities tend to influence the macroalgal occurrence.

Furthermore, the macroalgae found in Kupang Bay waters contained alginate (Figure 1), but its concentration is different with species. Also, the same species had different concentration of alginate at different sapling time. In this study, the alginate content of the macroalgae collected in Kupang Bay waters was found in general lower at the first sampling time than the second one. It could result from age difference, in which the macroalgae at the first sampling was younger than those collected at the second sampling. This finding reconfirms the statement of Maharani & Widyayanti (2010) that alginate content in brown algae is dependent upon season, habitat, age, and species.

The present study found the highest alginate concentration in *Sargassum* sp. and *Padina* sp. reaching 33.33% at the second sampling and 23.33% at the first sampling and the lowest in *S. crassifolium,* only 3.33%, at the first sampling and 10% at the second sampling. The range of alginate concentration in brown macroalgae collected from Kupang Bay waters was higher than that in Lombok Sea, only from 4% to 18% (Widyastuti 2009). Nevertheless, our finding was not so different from that of alginate content in brown algae from Gunung Kidul, Yogyakarta, from 16.93 to 30.5% (Mushollaeni & Rusdiana 2011). Besides, alginate content in brown macroalgae is also affected by culture environmental factors and extraction process (Kasim et al 2017).

Based on qualitative test on phytochemical content in macroalgae as presented in Table 3, it is found that brown algae contain alkaloid, saponin, steroid, terpenoid, tannin, and flavonoid compounds. All these compounds enable the brown macroalgae to be developed in the pharmacy because the bioactive compounds of these macroalgae could inhibit the pathogenic microorganisms. According to Vijayakumar et al (2012), seeweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activies. This finding is also supported by Bajpai (2016) that marine algae held antimicrobials as terpenoid, phenol, polysacharride, alkaloid, and fatty acid.

Conclusions. Eleven species of brown macroalgae were recorded in Kupang bay waters, *Padina* sp.*, P. australis, T. ornata, H. clathratus, H. cuneiformis, S. cristaefolium, Sargassum* sp., *S. polycystum, S. crassifolium, D. acrostichoides,* and *D. ciliolata.* These species contained different amount of alginate with species. The highest was recorded in *Sargassum* sp. and *Padina* sp., reaching 33.33% and the lowest in *T. ornata* and *S. cristaefolium,* only 13.33%. They also contained alkaloid, saponin, steroid, terpenoid, tannin and flavonoid compounds, for possible development of the brown algae in pharmacy.

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