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Diversity of Seaweed in West Nusa Tenggara and its Potency

by

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More than 60 marine macro algae species have been characterised from West Nusa Tenggara including 36 Rhodophyta, 18 Phaeophyta and 34 Chlorophyta. Many of these species are of economic importance as source of foods and industrial products which some are commercially cultivated using different cultivation system. Many others, mostly naturally inhabitant non-cultivating spesies, retain bioactive compounds which are of potential UV protectants, skin mousturizers and growth promoting substances. Exploration and morphologycal characterisation of West Nusa Tenggara marine macro algae will be discussed. Potential species as sources of foods, hydrocolloid, alginate, UV protectant and skin moisturizer as well as growth promoting substances have been screened and characterised. In addition, improved cultivation and post-harvest methods to increase production and quality of commercially cultivated species have also been developed and introduced in several commercial seaweed production centres in West Nusa Tenggara. Prospect and chalangges in macro algae cultivations to support the West Nusa Tenggara 'PIJAR" programme will be discussed.

INTRODUCTION

West Nusa Tenggara (WNT), a province in the central Indonesia, composes of two main islands, Lombok and Sumbawa, and many small islands (locally called Gili). The province has an area of *ca.* 20,153.13 km^2 and is situated between 115°46' to 119°5' of East Latitude and 8°10' to 9°5' of South Latitude. It is bordered to the south by the Indian Ocean, to the north by the Flores Sea, to the west by the Lombok Straits, and to the east by the Sape Straits.

The province has specific geographic features, which mark discontinuities in genetic variation of flora and fauna the area. The WNT is located between the Wallace's and Weber's Lines which has been suggested to influence biodiversity of flora and fauna in the province. Indonesia has been put in the first rank for the endemic species, and 65 % of the endemic species in Indonesia is located in Wallacea regions. In addition, the province is also bordered by an Indonesian "throughflow current" which influence the WNT marine fauna and flora diversity, including seaweed.

The NTB with coastal length of 3.333 km has about 23,628 km^2 potential areas for seaweed cultivation but only about 6,900 ha (less than 20%) has been utilized by about 6,000 seaweed farmers (Anonim, 2010). In WNT Sea and coast, there are at least 88 species of seaweed identified (Sunarpi et al., 2005, 2006, 2010a), Of those, only few species has been cultivated in WNT as sources of foods, carrageenan and agar while function of most of them remain uncharacterized. Many published reports indicate the important of seaweed as raw materials in many industries including foods and cosmetics (De-Roeck-Holtzhauer, 1991) as well as to be potential source of plant hormones and organic fertilizers (Hong *et al.*, 2007, Thangaraju, 2008), biofuel (Horn *et al.*, 2000) and paper. In this paper, our investigation to identify and then characterize seaweed from West Nusa Tenggara is reported. The 88 species

identified, morphologically fall onto three classes: *Rhodophyceae* (red algae: 36 species), *Phaeophyceae* (brown algae: 18 species) and *Chlorophyceae* (green algae: 36 species). Characterizations of the species as potential source of hydrocolloids, UV protecting, biofertilizer and plant growth promoting substances will be elucidated.

METHOD

1. Seaweed species exploration, identification and collections.

Seaweed samples used were collected from coastal areas of West Nusa Tenggara and morphologically identified, picture taken for collection and aiding the identification, freshly or frozenly stored for functional characterisation.

2. Spectra determination of UV wavelength.

Wavelength of UV A, UV B and UV C radiation was determined using spectrophotometer.

3. Seaweed extraction for UV protection and biofertilizer characterisation.

Samples were extracted by adding extraction buffer (1:1, w/v), as appropriate, and grinding onto find slurry, filtered and transferred into chilled tubes, then centrifuged at the top speed for 10 mins. Supernatant (designated as a 100% extract) was transferred into falcon tubes, diluted as required then freshly used for the indicated experiments.

4. Determination of seaweed potency to protect cell death.

Potency of seaweed extract to prevent cell death was determined *in vitro* using HeLa cells. Investigation was undertaken in the Fukushima Medical University, Japan and Bioscience and Biotechnology Center, Nagoya University, Japan.

5. Characterization of seaweed extracts as fertilizer and growth hormones.

a) **Preparation of growth medium and planting.**

Each experiment was undertaken in plastic houses. Plants were grown in 10-L capacity pot, supplemented with 8 L medium (soil, sand, manure; 1:1:1 (v/v)), inorganic fertilizers added, hand watered daily and maintained for two weeks before being treated with seaweed extracts.

b) **Germination experiment.**

Germination experiments were undertaken for 10 days in the Laboratory. Seeds were incubated in 10% of representative seaweed extracts for 12 hours with shaking then 100 seeds placed in a germination plate (10 x 12 cm^2) overlaid with several layers of aquadest-wetted cotton and placed in the dark. Measurement of germination rate and primary root length were undertaken daily.

c) **Plastic-house experiment.**

Several experiments, designed by completely-randomized design (CRD), were undertaken in the plastic houses in Jatisela, Mataram.

d) **Treatment with seaweed extracts.**

Freshly prepared extract was diluted with solvent as appropriate; tween-20 (0.01%, v/v) added and mixed before being sprayed throughout the plants in the morning. Unless otherwise stated, treatment was started from two weeks after replanting to pot, in weekly interval, and treatment stop one week before termination of experiment. In regard to different growth stages application, treatment was started according to growing stages of the plant assessed. For vegetative stage, plant was sprayed from two-weeks old until plant started to flower while for

generative-stage treatment, application of seaweed extract was started from the time of flowering and continued until growth ceased.

Graph and Statistical analysis. All graphs presented were constructed using Microsoft Excel software and data analyzed for variance differences as appropriate.

RESULTS AND DISCUSSION

1. Biodiversity of seaweed in West Nusa Tenggara

The exploration was started in 2004 to identify and characterised seaweed collected from West Nusa Tenggara Coast and sea. The species are claasified onto three classes: *Rhodophyceae* (red algae: 36 species), *Phaeophyceae* (brown algae: 18 species) and *Chlorophyceae* (green algae: 36 species) (Table 1).

Table 1. Seaweed species collected and identified from West Nusa Tenggara region.

No	Class	Species
1	Red Algae (*Rhodophycea*)	*Achantophora muscoides, A. specifer. Achantophora sp.1. Achantophora sp.2, Achantophora sp.3, Achantophora sp.4, Actinotricia fragilis, Actinotricia sp.1, Eucheuma cottonii (moumere), Eucheuma cottonii (brown), Eucheuma cottonii (green), Eucheuma striatum, Eucheuma spinosum (cultivated), Eucheuma spinosum (natural), Gracilaria eucheumeuides, Gracilaria salicornia , Gracilaria sp1, Gracilaria sp.2, Gracilaria sp3, Gracilaria sp4, Gracilaria sp5, Gracilaria sp6, Gracilaria sp7, Gelidium sp.1, Gelidium sp.2, Hypnea sp.1, Hypnea sp.2, Hypnea sp.3, Hypnea sp.4, Rhodymenia sp.1, Rhodymenia sp.2, Ptilophora sp, Sarcodia sp.1, Sarcodia sp.2.*
2	Brown algae (*Phaeophyceae*)	*Dyctiota sp.1, Dyctiota sp.2, Dyctiota sp.3, Hydroclathrus sp., Hormopisa sp, Padina aquifolium, Padina sp., Sargassum sp.1, Sargassum sp.2, Sargassum sp.3, Sargassum sp.4, Sargassum sp.5, Sargassum sp.6, Turbinaria murayana, Turbinaria ornata, Turbinaria conoides, Turbinaria sp., Colpomenia peregrina*
3.	Green Algae (*Cjlorophycea*)	*Boergesenia forbesii , Boergesenia sp.1, Boergesenia sp.2, Boergesenia sp.3, Caulerpa okamura, Caulerpa serrulata, Caulerpa sp.1, Caulerpa sp.2, Caulerpa sp.3, Caulerpa sp.4, Caulerpa sp.5, Caulerpa sp.6, Boergesenia sp, Halimeda sp.1, Halimeda sp.2, Halimeda sp.3, Halimeda sp.4, Halimeda sp.5, Ulva fasciulata, Ulva fasciata, Ulva sp1, Ulva sp2, Ulva sp.3, Ulva sp.4, Codium sp.1, Codium sp.2, Codium sp.3, Codium sp.4, Codium sp.5, Enteromorpha sp., Dictiosphaeria covernosa, Penicilus sp., Udotea sp.1, Udotea sp.2*

Out of the 88 species, five commercially cultivated red algae species including *Eucheuma cottonii (moumere),* Eucheuma *spinosum* and *Eucheuma cottonii (green)* (source of carrageenan) as well as *Gracilaria eucheumeuide* and *Gracilaria salicornia* (source of agar). The remaining, 83 species, are functionally uncharacterized and naturally inhabitant species. Those are subjected to our investigation.

2. Screening for potential seaweed as source of hydrocolloids

As only five species (three was introduced from other regions) have been cultivated, characterisation of natural WNT species as potential source of hydrocolloids is crucial to obtain adaptive candidate for breeding and improvement. Initial investigation was undertaken to mass characterise the species by extracting each species for carrageenan, agar and alginate. Results of the characterisation identified 13 red algae species as source of carrageenan and agar, and 10 brown algae species as source of alginate (Table 2).

Table 2. Potential seaweed from West Nusa Tenggara as source of hydrocolloids

Class	Species	Hydrocolloid
Chlorophyceae	-	-
Rhodophyceae	*Gracilaria gigas, Gracilaria verucosa, Gracilaria salicornia, Gracilaria eucheumioides, Gracilari edulis, Glacilaria sp, Gelidium sp, Hypnea sp*	Agar
	Hypnea asperi	Agar, karaginan
	E.cottonii, E. Spinosum, E. Serra	Karaginan
Phaeophyceae	*Sargassum polycistum, Sargassum crassifolium, Sargassum latifolium. Sargassum sp, Turbinaria murayana, Turbinaria ornate, Turbinaria sp., Harmopisa sp., Dyctiota sp., Padina sp*	Alginate

3. Screening for potential seaweed as UV protecting agents

Many reseraches reported that many marine organisms such as *Tridentata marginat* (House, 1999), *Glomerella cingulata* (Young dan Patterson, 1982), extract corals (Shick et al., 199 and zoozooplanktons (Schmid et al, 2003) are potential source of Mycosporin, a strong U protecting agent. Therefore, West Nusa Tenggara seaweed may also attain a potency as UV-protectant. Initial screening was undertaken by investigating capability of the species to absor UV and identied red, brown and green seaweeds capable to absorb UV radiation as determine spectrophotometrically (Tabel 3) (Sunarpi et al., 2008).

Class	Species
Chlorophyceae	*Caulerpa serulata, Boergesenia sp, Dictyosphaeria covernosa, Caulerpa racemos Codium sp4, Ulva sp1., Halimeda sp1, Caulerpa sp, Chaetomorpha sp Enteromorpha sp2,*
Rhodophyceae	*Acanthophora sp, A. specifera, Gracilaria sp, Eucheuma striatum, Hypnea s Sarcodia sp. , Gelidium latifolium, Actinotrichia fragilis, Actinotrichia s Phyllophora sp, Gracilaria salicornia, Eucheuma spinosum, E. serra, E. coton Chondrococcus hornemonii*
Phaeophyceae	*Hydroclathrus clathrantus, Turbinaria ornata, Padina sp, Zonaria sp, Sargassu aquifolium, Sargassum cristaefolium, Sargassum polycystum (Folioid), Dictyo Turbinaria gracilis sp and Turbinaria sp*

Following this, the potency of the extract obtained from those species to protect cell deat were then determined *in vitro* using HeLa cells. Most of the species was capable to protect HeLa cell damage and some even have stronger protecting capacity than some commercial UV protecting products. Examples of the assay are presented as Figure 1.

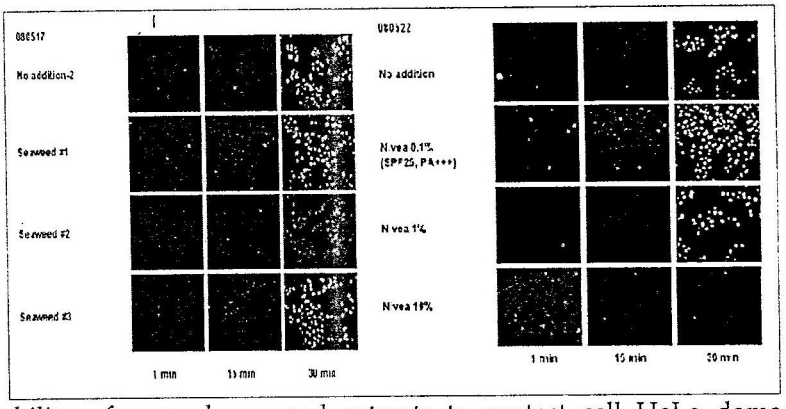

Figure 1. Capability of several seaweed extracts to protect cell HeLa damage against UV radiation (left panels) compared with some commercial UV protection products (right panels)

Overall, the results indicate capability of several seaweed extracts (as indicated in Table 3) to absorb UV and protect cell death when exposed to UV radiation. Investigation is now underway to identify biochemical properties and bioactive compounds of the potential species.

4. Screening potential seaweed species for biofertilizer

Following screening for hydrocolloids, 56 species, mostly green and brown seaweeds, was screened for theirs potency as fertilizer. Supernatant of water extract from each species was used in imbibitions media for germination or sprayed frequently onto several plant species. Of those, ten species capable to promote seed germination, plant growth and/or increase yield were selected for further characterisation (Table 4)

Table 4. Potential seaweed species for fertilizer

No	Class	Species
1.	Green algae (Chlorophyta)	*Ulva ferticulata Ulva fasciata*
2.	Brown algae (Phaeophyta)	*Turbinaria murayana, Turbinaria ornate, Sargassum aquifolium, Sargassum sp1, Sargassum sp2, Padina sp, Hydroclthrus clantartus Hormopisa sp.*

Initially, water soluble extract was used for the screening. However, many substances including plant hormones are insoluble in water and will not be available in the water extract. Therefore, several extraction methods were tested, then capability of diluted fractions as stimulator of seed germination, plant growth and yield was tested on many plant species. In General, additional of water and HBS extracts promoted seed germination and/or stimulated growth and yield of many plant species. Examples of those are shown in Figures 2 and Figure 3.

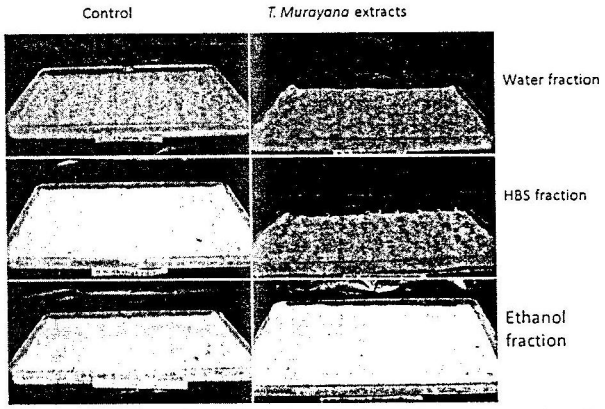

Figure 2. Germination of sesame seeds following imbibitions in water (left panels) and different fractions of *Turbinaria muriana* extracts (right panels).

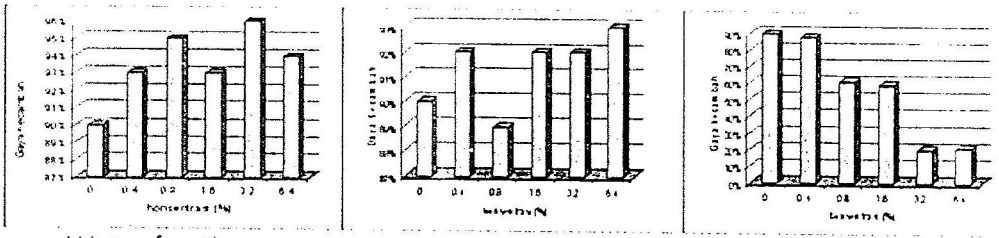

Figure 3. Germination rate of bean seeds in different concentration of water fraction, HBS fraction and Ethanol fraction of *Sargassum aquifolium*.

These results indicated that water and HBS extracts may contain growth promoting substances while ethanol may contain growth inhibition substances. As there were similar patterns of stimulation indicated by both water and HBS extracts, further characterization attempts were undertaken using water as the solvent. Water is easily available thus will be more convenient for direct application, in particular, by farmers. Further characterization indicated that growth promoting effect of water fraction from the ten seaweed extracts were not only concentration but also species dependant (example in Figure 4). Most of the species tested increased growth and yields when sprayed with seaweed extract with concentration at or below 6%.

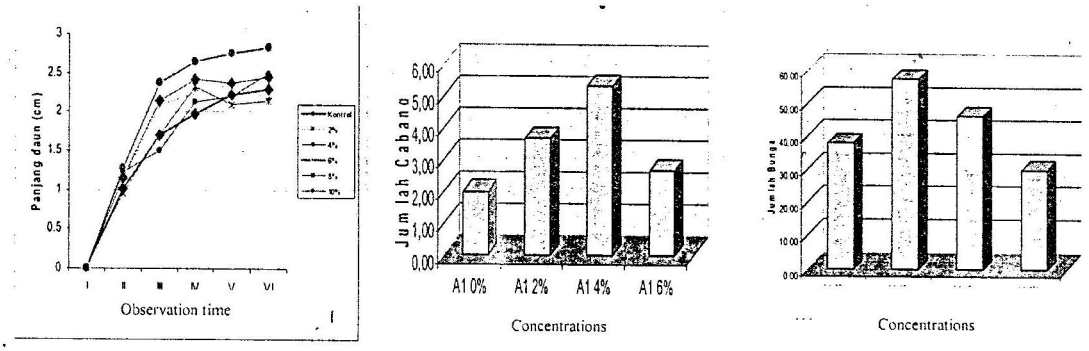

Figure 4. Growth and yield of spinach and tomato sprayed with different concentration water extract of seaweed. Growth of spinach spread with *Hydrochlarus sp.* (left), tomato spread with *Turbinaria murayana* (middle) and yield of tomato spread with *Ulva ferticulata*.

Previous results indicated selected promoting capability of each species which was concentration and developmental stages dependency. To confirm this, other sets of experiment were undertaken. For this experiment, one concentration of extract was used (4 %) and selected as previous experiment show this was the best concentration for promoting growth and yield. In the experiment, extracts were sprayed at three different stages of plant development vegetative stage only, both vegetative and generative stages or at generative stage only (data not shown).

Overall, the results indicated promoting effect of the ten seaweed extracts on seed germination, plant growth, development and yields. This promoting capacity is probably due to growth promoting substance contained in the extracts. Selective capacity of each species to promote certain stages of plant development in selective species indicates differential properties of the species. Many reports suggest that many species of red and brown seaweeds particularly utilize many plant growth regulatora such as auxin, sitokinin and giberellin (Tay et al., 1985; Thangaraju, 2008; Prasad et al., 2010). In addition, several red and brown seaweeds also contained micro nutrients, oligoshaccarides or enzyme D-glycananes which also promoted growth of many plant species (Petit et. al., 2005; Laporte et al., 2007; Prasad et al., 2010). In these report Brown seaweeds studied were included *Kappaphycus alvarezii, Sargassum wightii Grev. Ascophyllum nodosum, Ascophyllum nodosum*. In our system, not only Brown seaweeds but also two green seaweeds observed indicating the growth promoting properties. However, it is still

unclear why and how extracts from those species could selectively promote germination, plant growth and development. Biochemical analysis is underway to analyse bioactive compounds contained by each species. Various formulas are being investigated in different cultivating system including hydroponics. This will allow for formulation of seaweed extracts specifically design for different crop species.

CONCLUSION

The 88 seaweed from West Nusa Tenggara attain a great potential not only as potential source of hydrocolloids but as UV protecting and biofertilizer. The potential seaweed species for sources of hydrocolloids are red algae while the species for UV protecting and fertilizers mostly fall in the brown and green seaweed. Selective properties of the species offer great opportunity but also challenges for further development of the native species as potential marine-based economic activities for the local communities.

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