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Utilisation of Macroalgae from West Nusa Tenggara Towards Improved Human Health and Prosperity

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West Nusa Tenggara (WNT) is a province of central Indonesia which comprises two major islands and hundreds of small islands with rich marine flora. Exploration and characterisation of macroalgae or seaweeds collected from the marine waters of Lombok Island, Sumbawa Island, and small islands in WNT has been undertaken since 2015. There were at least 82 morphologically distinct macroalgal species in the WNT marine waters, including 32 species of red macroalgae, 18 species of brown macroalgae and 32 species of green macroalgae. Of those, only few species were cultivated, including *Kappaphycus alvarezii, Kappaphycus striatus, Eucheuma denticulatum* and *Gracilaria* sp., whilst most of them were wild species. Seaweed cultivation is important as a source of livelihood for many smallholder fisheries in WNT. Further characterisation of the cultivated and wild macroalgae identified 9, 10 and 11 species as the potential source of carrageenan, agar and alginate widely used in various industries. Domestication, propagation, cultivation and processing of these species may serve as an important source of income for WNT in the future. In addition, macroalgae from WNT showed the potential to be utilised as bio-fertiliser, as well as the source of UV protectant and anti-cancer agent. As of the date of writing, 10 macroalgal species from WNT were shown to contain plant growth promoting substances. Extracts of 10 macroalgal species were found to be able to absorb UV radiation and protect cells against the harmful effect of UV radiation based on the *in vitro* and *in vivo* analyses. In addition, liquid extract from *Acanthophora muscoides* was able to decrease mortality of *Artemia salina* and fucoidan from *Turbinaria murrayana* inhibited HeLa cells proliferation. These findings suggested that macroalgae from WNT may be utilised to improve human health and prosperity.

Keywords: seaweed diversity; carrageenan; anti-cancer; UV protection; plant growth promoting substances

III. INTRODUCTION

Indonesia is well-known for its rich assemblage of marine flora and fauna. More than 555 macroalgal species had been reported from the Indonesian marine waters. West Nusa Tenggara (WNT) is a province of central Indonesia which comprises two major islands, Lombok and Sumbawa, and more than 100 small islands locally known as *gili*. This province is bordered by Wallace's and Weber's lines, and surrounded by the Indonesian Throughflow branch currents. These unique geographical features are believed to be linked to the diversity of marine flora and fauna, including macroalgae, in WNT marine waters.

A macroalga or seaweed is a marine flora composed of thallus, a tissue that has no clear differentiation among root, shoot and leaves (Romimohtarto & Juwana, 2001). Based on the pigment content of the thallus, a macroalga can be classified as green, brown or red. These macroalgal

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groups have high economic potential as they are rich in minerals and organic compounds such as polysaccharides, hormones, vitamins and other bioactive compounds yet to be classified (Bold & Wynne, 1985). Macroalgae are an important commodity for WNT, and the development of a macroalgae-based industry is one of the priorities for improving the livelihood of people in WNT. Several species of macroalgae, particularly *Kappaphycus* and *Eucheuma*, are sources of raw material for hydrocolloids which are used as stabilisers, gelling agents, thickeners, binders and additives in various food and pharmaceutical industries (Mabeau & Fleurence, 1993). Today, *Eucheuma* and *Kappaphycus* are the major cultivated carrageenan-producing seaweed species, and the production and processing of these species has become a high value and profitable means of livelihood in many marginal sea farming communities in Indonesia. These species have been cultivated in more than 20 provinces, including WNT. However, the cultivated *Kappaphycus alvarezii* and *Kappaphycus striatus*, locally known as *kotoni* and *striatum*, are not of Indonesian origin. The two species were introduced to Indonesia from the Philippines and within the farms, *Kappaphycus* seed stocks are maintained solely by vegetative propagation, resulting in the seaweed aquaculture in Indonesia to be dominated by the same strains. *Eucheuma* and *Kappaphycus* have different habitat requirements; *Eucheuma* has greater invasive potential as it can tolerate a wide range of environmental conditions while *Kappaphycus* has more specific habitat. *Kappaphycus alvarezii* has been transplanted to more than 20 countries with two reported cases where *Kappaphycus* plants have spread from the introduction sites and adversely impacted the native habitats (Ask *et al*., 2003). Therefore, the use of native species will be more favourable to the environment. In spite of the importance of *Eucheuma* and *Kappaphycus* for the economy in Indonesia, no local or national strain has been released by the Indonesian authority for large-scale seaweed farming. In addition, information on the level of genetic diversity within natural and cultivated seaweed species, which will aid in species improvement, marine biodiversity conservation, and better understanding of the species for more sustainable management, is scarce. In view of this, exploration of the indigenous species is very important for sustainable development of a macroalgae-based industry in Indonesia, especially in WNT.

Macroalgae are an important natural source of metabolites with various biological and pharmacological activities. The metabolites isolated from macroalgae include plant growth promoting substances, polysaccharides (agar, carrageenan, alginate), halogenated furanones, kahalalides, lectins, fucoidans, kainoids and aplysiatoxins. In many countries, macroalgal extracts have been developed and utilised as natural fertilisers that are commercially available. These include Seasol (Tay *et al*., 1987), Kelpak (Beckett & van Staden, 1989), SM3, SM6, Maxicrop (Hankins & Hockey, 1990), ALGAENZIMS (Villareal-Sanchez *et al*., 2003), Algifert, Goemar GA14, Sea Spray, Cytex and Seacrop (Sivasankari *et al*., 2006). Foliar sprays of these macroalgae-based liquid fertilisers reportedly increase the uptake of nutrients and thus promote plant growth and development, as well as decrease the need for inorganic fertilisers (Thangaraju, 2008). It has been suggested that these features are due to the presence of plant growth substances including cytokinins and auxins as well as other nutrients in fertilisers made from brown macroalgae (Tay *et. al*., 1986; Thangaraju, 2008). In line with the well documented application of seaweed extracts as fertilisers, we also investigated the plant growth stimulating activity of macroalgae from WNT which may pave way for the development of local seaweed-based natural fertiliser in support of a sustainable agricultural system in the region.

Other potential uses of macroalgae we investigated are for UV-protection and anti-cancer properties. Macroalgae have been reported as a source of UV absorbent compounds such as mycosporine-like amino acids (MAAs) including shinorine (Cockell & Knowland, 1999). MAAs were originally found in red algae, and later found also produced in brown and green algae in low concentrations based on subsequent studies. Brown algae also produced UV-absorbent compounds other than MAAs, one of which is phlorotannins (Dunlap & Shick, 2002). In addition, many publications have reported that compounds extracted from red, brown and green macroalgae have anti-cancer properties. Many seaweed species, particularly the red and brown seaweeds, were known to be excellent

sources of polysaccharides such as galactans, agar, and carrageenan (Bouhlal *et al.*, 2011). The polysaccharides from marine algae are suggested to have great potential to be developed into anti-cancer medicine (Kwon & Nam, 2007; Paul, 2014; Xue *et al.*, 2012).

This paper summarised the findings of our on-going investigations on the diversity of macroalgae from WNT and their potential as the sources of hydrocolloids, plant growth promoting substances (fertiliser), UV protection agents and anti-cancer compounds. The investigations were undertaken with the aim to utilise macroalgae for the improvement of human health and prosperity.

IV. MATERIALS AND METHODS

A. Collection of Seaweed Samples

Seaweed samples were collected from Lombok, Sumbawa and small islands in WNT by scuba-diving. Upon collection in the field, specimens were identified morphologically, photographed, and the coordinates where the samples are obtained were recorded. The fresh seaweeds were washed with seawater and taken to the laboratory immediately. They were then rinsed with fresh water, air-dried, and used for characterisation of the hydrocolloid content, plant growth stimulating capacity, UV protection and anti-cancer properties.

B. Characterisation of Hydrocolloid Content in Macroalgae

The content of carrageenan and agar in red macroalgae, as well as alginate in brown macroalgae from WNT were examined. Hydrocolloids were extracted from 3 g of dried samples of various macroalgal species, and the hydrocolloid content of each seaweed species was expressed as the dry weight of hydrocolloid per dry weight of seaweed biomass.

Carrageenan was extracted according to the method described by Syamsuar (2005) with some modifications. Dried macroalgal sample was soaked in 180 mL of alkaline water (pH 9.0; 1:60 w/v) overnight, and the mixture was heated for 3–4 h at *ca.* 85°C with continuous stirring to form a seaweed paste. The filtrate separated from macroalgal residues was left to cool down for 10 min before 3 volumes of absolute ethanol was added to precipitate carrageenan. The mixture was stirred for 15 min, covered with a plastic wrap and incubated for 24 h at room temperature. Carrageenan was filtered on a piece of filter paper, transferred to a Petri dish and then dried in an oven at 60°C for 8 h.

Agar was extracted according to the method described by Winarno (1996). Dried macroalgal sample was soaked in 100 mL of 0.25% $Ca(OH)_2$ solution for 1 h, and rinsed with tap water to remove the lime. The macroalgal sample was then soaked in 100 mL of 5% H_2SO_4 for 15 min, filtered and boiled in water (pH 6.0–7.0; 1:40 w/v) for 2 to 4 h to form a paste. The agar was separated by filtration with a fine cloth, placed in a Petri dish and allowed to set at room temperature for 7 h before drying at 80°C for 8 h.

Alginate was extracted according to the method described by Winarno (1996) with some modifications. Prior to extraction, the seaweed sample was soaked in 5 mL of 1% $CaCl_2$ solution for 2 h with continuous stirring to remove laminarin, mannitol and other salts, and rinsed to remove excess calcium and dissolved salts. The macroalgal sample was then rinsed with 100 mL of 0.33% HCl and tap water, and processed into a slurry in a blender at low speed for 10 min. Two volumes of 4% Na_2CO_3 were added to the slurry and the mixture was incubated for 2 h at 40°C with continuous stirring to form a homogenous paste. The seaweed paste was then diluted with 3 volumes of distilled water, stirred to homogeneity and then filtered using a fine cloth. The filtrate was added with 5 mL of 0.33% HCl and incubated for 6 h at room temperature for alginate to precipitate. The gel was separated on a piece of filter paper, and dried at 60°C for 8 h.

C. Potential of Using Macroalgal Extracts as Fertiliser

Macroalgal extracts prepared in various solvents were applied to seeds for germination analysis and several plants for growth enhancement analysis. Water extracts of seaweed was typically prepared by processing the finely chopped air-dried seaweed biomass into a slurry for 30 min with an equal volume of water (1:1 w/v) in a blender. The homogenate obtained was then filtered using filter paper, followed by centrifugation at 5,000 rpm at room temperature for 10 min. Extracts of methanol, ethanol and high boiling solvent (HBS) were prepared by maceration.

The finely-chopped seaweed biomass was macerated with appropriate solvent (1:2 w/v) at room temperature for 48 h, and filtered using filter paper. The filtered macerate was centrifuged at 5000 rpm at room temperature for 10 min. The solvent was evaporated and the pellet was resuspended in distilled water (½ of the initial solvent volume) to form what is known as the stock extract at 100% concentration. The extracts were frozen at -20˚C for a maximum of 1-month storage, and diluted as appropriate when used for experiment.

For the germination experiments, the seeds of selected plants were immersed in 100 mL of extracts diluted at appropriate concentrations for 24 h. After immersion, the seeds were filtered to remove the excess water, air-dried for 10 min, and 100 seeds were planted on 3 layers of moistened paper towels placed in a germination dish (5 cm × 5 cm × 2.5 cm), with 5 replicates per treatment. The dishes were placed at room temperature and examined daily up to 15 d. For the field experiments, the seeds were planted in experimental pots containing 5 L of growing medium composed of the soil and chicken manure (1:1 v/v). Treatments with macroalgal extracts were done by spraying the foliage of the plants with the seaweed liquid extracts at regular intervals. The growth and yield parameters of the plants were monitored and compared with untreated plants (five replicates for each treatment). The bio-stimulating components were identified by high performance liquid chromatography (HPLC) against standards of commercial plant growth regulators prepared for indole acetic acid (IAA), naphthalene acetic acid (NAA), zeatin (ZA), gibberellic acid (GA_3), 2,4-dichlorophenoxyacetic acid (2,4-D) and abscisic acid (AbA).

D. Evaluating Ultraviolet (UV) Protection Property of Macroalgal Extracts

Samples of air-dried seaweed biomass were powdered, and macerated with various solvents such as ethanol, hexane and HBS at ambient temperature for 48 h. The suspensions were filtered using Whatman no. 1 filter paper every 24 h. Filtrates were evaporated with rotary evaporators until concentrated extracts were obtained for assessment of their UV protection property.

The UV protection property of seaweed extracts was first assessed by screening for the seaweed extracts at various concentrations that are able to absorb the UV light (wavelengths of 200–400 nm) based on the absorbance spectra generated using spectrophotometer. Selected seaweed extracts were then tested on the human cervical cancer (HeLa) cell line for their UV protection property. The HeLa cells (American Type Culture Collection) were cultured in Dulbecco's modified Eagle medium (DMEM; Wako) containing 10% foetal bovine serum and penicillin/streptomycin in a humidified growth chamber at 37°C and 5% CO_2 for 18–20 h. Cell proliferation was measured by trypan blue dye exclusion assay. The cells were washed with PBS/Hank buffer, harvested using 0.5 mg/mL trypsin solution, and cultured in 96-well culture plate containing DMEM for 2 h. The medium was discarded and replaced with new medium containing 20 μg/mL of macroalgal extracts or diluted commercial UV protective cream. The cells were subsequently irradiated with UV light at wavelength 302 nm for 15 and 30 min on a UV-transilluminator (ChemiDoc XRS+, Bio-Rad Laboratories), and incubated at 37°C for an additional 24 h after treatment. The cells were then stained with 1 μg/mL DAPI and the fluorescent dead cells were viewed under a BZ-9000 fluorescence microscope.

E. Evaluating Anti-Cancer Property of Macroalgal Extracts

The macroalgal extracts were prepared by maceration of seaweed biomass in methanol and dichloromethane (1:5 w/v) for 48 h. The extract was filtered using filter paper, and the filtrate was evaporated using vacuum evaporator for 48 h. The resulted crude extracts were stored in refrigerator until further use. The seaweed extracts were first assessed for toxicity using *in vivo* analysis by brine shrimp lethality test, and the anti-cancer property of selected seaweed extracts was then evaluated using *in vitro* analysis on the human cervical cancer HeLa cell line.

Toxicity analysis was carried out according to the method described by Sleet *et al.* (1983) using *Artemia salina* larvae, with minor modifications. The brine shrimp larvae were hatched for 48 h in a conical flask containing 500 mL of aerated artificial seawater at 29–30°C with illumination. Mortality assay was conducted by exposing the larvae to different concentrations of each macroalgal extract (5, 10 and 100 ppm for dichloromethane extract,

and 100, 200 and 300 ppm for methanol extract) for 48 h, and the mortality rate of brine shrimp larvae was assessed.

The anti-cancer property of polysaccharides and crude extracts of selected macroalgal species were analysed using the HeLa cell line. The cells were cultured overnight in DMEM supplemented with 10% foetal bovine serum at 37°C in a humidified atmosphere containing 5% CO_2, and then cultured in DMEM supplemented with polysaccharides and crude extracts of selected macroalgae from WNT in the concentration range of 0–200 µg/mL for 3 d. Early apoptotic activity was detected following calcein-AM/PI viability staining. The cells were stained with 2 µL of calcein-AM and incubated for 15 min in incubator enriched with CO_2 at 37°C, and then counterstained with the same volume of PI. The viability of cells was analysed using fluorescence microscopy and ImageJ software.

III. RESULTS AND DISCUSSION

A. Diversity of Macroalgae in WNT

Morphological analysis revealed at least 82 species of macroalgae in WNT marine water, which included 32 species of wild and cultivated red macroalgae (Figure 1), 32 species of green macroalgae (Figure 2), and 18 species of brown macroalgae (Figure 3). These macroalgae were obtained from different sample collections in Lombok, Sumbawa and small islands of WNT. Of those, 4 species were collected from cultivation sites, including *Kappaphycus alvarezii* (dominated by the brown Tambalang strain), *Kappaphycus striatus* (green and brown strains), *Eucheuma denticulatum* and *Gracilaria changii.* The cultivation of *K. alvarezii, K. striatus* and *E. denticulatum* were well established on the southern coast of Lombok Island, including West Lombok (Pengantap and Mawun Bay), Central Lombok (Gerupuk Bay), and East Lombok (Ekas and Seriwi Bay). In Sumbawa Island, the cultivated species were *K. alvarezii* and *K. striatus,* and the cultivation sites were at the western and northern coasts of Sumbawa including Kertasari, Jelenge, Kaung Bay, Santong Bay, Hu'u and Waworoda Bay. Cultivated *G. changii* was obtained only in one location at West Sekotong, West Lombok. Other species were collected from natural stands by scuba-diving, and thus were considered as wild species of macroalgae in WNT.

Figure 1. The 32 species of red macroalgae obtained from cultivation and natural sites of WNT marine waters

GREEN ALGAE (CHLOROPHYCEAE)

Valonia macrophysa | *Boergesenia forbesii* | *Boergesenia* sp. | *Valonia aegagropila* | *Caulerpa* sp. 1 | *Caulerpa okamurae*

Caulerpa racemosa | *Caulerpa sertularoides* | *Caulerpa serrulata* | *Caulerpa serrulata* | *Caulerpa* sp. 4 | *Caulerpa racemosa*

Boergesenia sp. | *Halimeda opuntia* | *Halimeda macroloba* | *Halimeda discoidea* | *Halimeda cylindracea* | *Halimeda* sp. 5

Ulva pertusa | *Ulva intestinalis* | *Ulva fasciata* | *Ulva* sp. 3 | *Chaetomorpha crassa* | *Chaetomorpha lingustica*

Codium lucasii | *Codium geppiorum* | *Codium fragile* | *Codium tomentosum* | *Codium geppiorum* | *Ulva reticulata*

Dictyosphaeria cavernosa | *Avrainvillea erecta* | *Udotea orientalis* | *Udotea indica*

Figure 2. The 32 species of green macroalgae obtained from natural sites of WNT marine waters

Figure 3. The 18 species of wild brown macroalgae obtained from natural sites of WNT marine waters

B. Characterisation of Hydrocolloid Content in Macroalgae from WNT

Many investigations suggested that macroalgae are a good source of hydrocolloids, including carrageenan and agar from the red seaweeds, and alginate from the brown seaweeds. Red seaweeds in the family Solieriaceae of order Gigartinales were reported to be a good source of carrageenan, including genera *Kappaphycus, Eucheuma, Hypnea* and *Gigartina* which could be found naturally in many parts of Indonesia. Several hydrocolloid-producing seaweed species were found in WNT during the current investigation. Carrageenan, agar and alginate were extracted from dried samples of representative macroalgal species, and the yield was presented as percentage per dry weight of seaweed biomass. The preliminary study revealed some potential species that produce carrageenan, agar and alginate, including 9 carrageenophytes (Figure 4), 10 agarophytes (Figure 5) and 11 alginophytes (Figure 6).

The commercially recognised "*Eucheuma*" species in the Pacific were found to contain different types of carrageenan. It is now recommended that the kappa-carrageenan producing species shall be renamed as *Kappaphycus*. At the molecular level, there was a clear distinction between the two groups of carrageenan-producing seaweeds, *Kappaphycus* and *Eucheuma*, based on the DNA sequences (Lim *et. al.*, 2013; Tan *et. al.*, 2013; Sunarpi *et al.*, 2013). In this study, 4 species of *Kappaphycus* and *Eucheuma* collected from the cultivation and natural sites produced high percentage of carrageenan (28–49% of dry weight). These macroalgae included 3 cultivated species of *K. alvarezii* (previously known as *E. cottonii*), *K. striatus* (previously known as *E. striatum*) and *E. denticulatum* (previously known as *E. spinosum*), and 2 wild species of *E. denticulatum* and *E. serra*. In addition, *Gracilaria* spp., *Sarcodia* sp. and *Hypnea* sp. obtained from WNT marine waters were also potential sources of carrageenan (Figure 4).

Agar is another important hydrocolloid produced by red seaweeds, which is widely used in the food, pharmaceutical and other industries. Ten potential agarophytes from WNT were *Gracilaria edulis, Gracilaria* sp. 1*, Gracilaria* sp. 2, *Gracilaria* sp. 3, *Gracilaria* sp. 4, *K. alvarezii*, *E. denticulatum*, *K. striatus*, *E. serra* and *Hypnea* sp., which produced high agar yield between 30 to 50% of the dry seaweed biomass (Figure 5).

Alginate is an important hydrocolloid from brown macroalgae which is used in many industries. The macroalgal species in WNT with the alginate yield between 8 and 43% dry weight were *Sargassum* (6 species), *Turbinaria* (2 species), *Dictyota* (2 species), and *Padina* (1 species) (Figure 6).

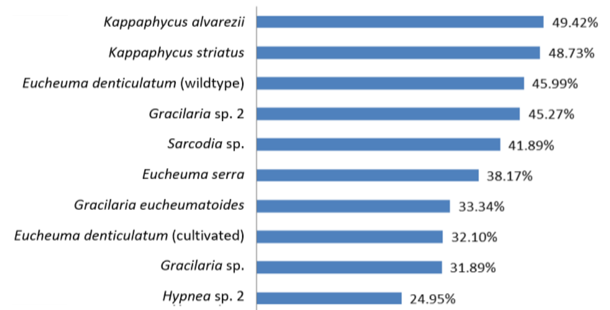

Figure 4. The carrageenophytic species of macroalgae from WNT and percentage of carrageenan obtained per dry weight of biomass for each species.

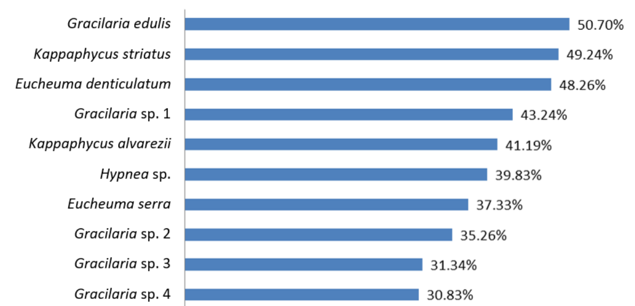

Figure 5. The agarophytic species of macroalgae from WNT and percentage of agar obtained per dry weight of biomass for each species.

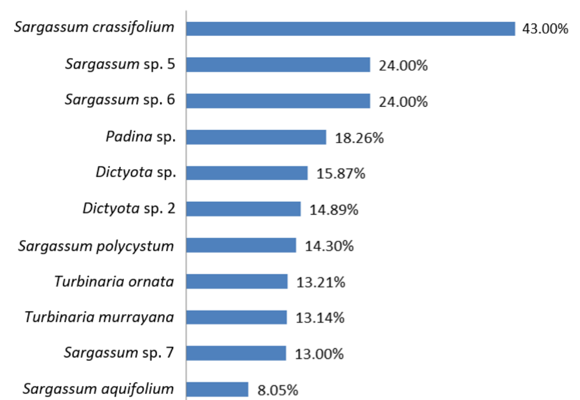

Figure 6. The alginophytic brown macroalgae from WNT and percentage of alginate obtained per dry weight of biomass for each species.

C. Potential Use of Macroalgae from WNT as A Source of Fertiliser

Macroalgae are suggested to contain macro- and micronutrients, as well as growth promoting substances for plant growth and development. Accordingly, liquid fertilisers have been produced in many countries using native macroalgae. In view of the prospect of developing seaweed-based fertiliser using the natural resources in WNT, several experiments had been conducted to investigate the application of extracts prepared from selected macroalgal species on the growth and yield of various agricultural crops. Table 1 listed 10 macroalgal species from WNT of which their liquid extracts showed promising results in promoting germination and growth, as well as yield enhancement of many plant species, including sesame, bean, rice paddy, glutinous rice paddy, tomato, mung bean, spinach and lettuce in our previous study (Sunarpi *et al.*, 2011). Water and HBS extracts of *Turbinaria murrayana* enhanced the germination and growth of sesame seeds (Figure 7); water extracts of *T. murrayana*, *Sargassum crassifolium*, *Sargassum aquifolium* and *Sargassum cristaefolium* promoted the growth of tomato, spinach and rice paddy plants; only water extract of *Hydroclathrus clathratus* was able to enhance the yield of rice paddy plants (Sunarpi *et al.*, 2010a; 2011). As with applied singly, water extracts of the above-mentioned brown macroalgal species were also able to increase the rice paddy growth as shown by the increased number of shoots and spikelets when applied in various combinations (Figure 8).

Table 1. Potential macroalgal species from WNT to be used as fertiliser

Group	Species
Chlorophyta	*Ulva fasciculata*
	Ulva fasciata
Phaeophyta	*Sargassum aquifolium*
	Sargassum crassifolium
	Sargassum cristaefolium
	Turbinaria murrayana
	Turbinaria ornata
	Padina sp.
	Hydroclathrus clathratus
	Hormophysa sp.

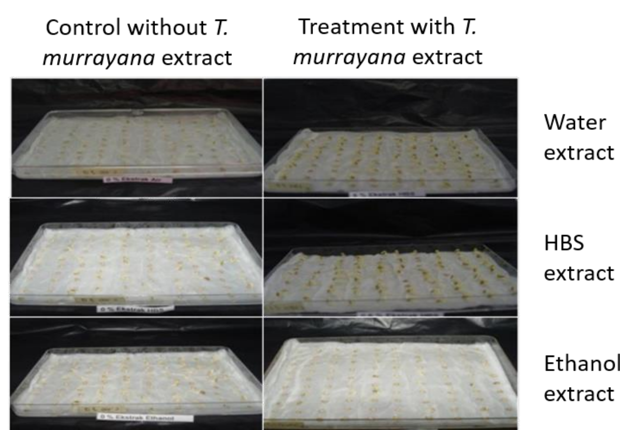

Figure 7. Increased germination of sesame seeds treated with the water fraction and HBS fraction of extract of *Turbinaria murrayana* collected in WNT marine waters.

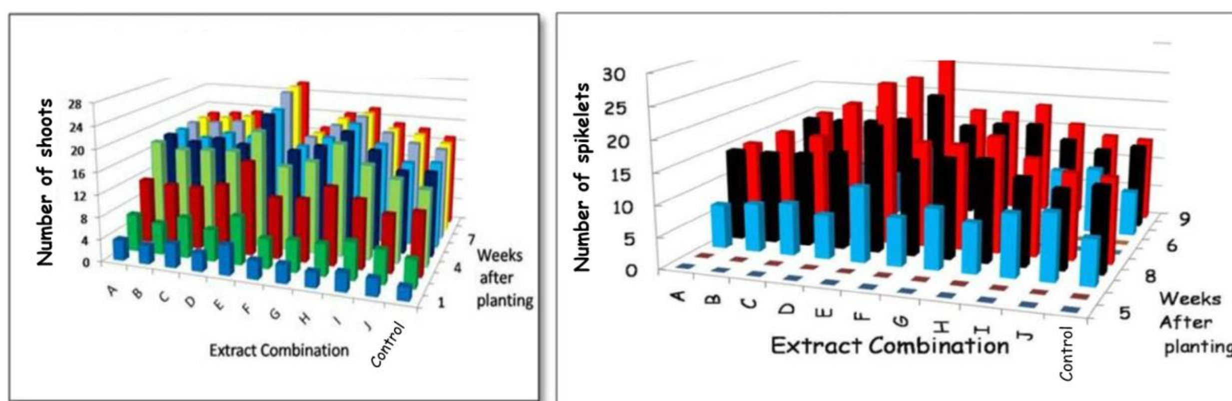

Figure 8. Various combinations of water extracts of macroalgae from WNT increased the number of shoots and spikelets of rice paddy plants: *Sargassum crassifolium* and *S. cristaefolium* (A), *S crassifolium* and *S. aquifolium* (B), *S. crassifolium* and *Turbinaria murrayana* (C), *S crassifolium* and *Hydroclathrus clathratus* (D), *S. cristaefolium* and *S. aquifolium* (E), *S. cristaefolium* and *T. murrayana* (F), *S. cristaefolium* and *H. clathratus* (G), *S. aquifolium* and *T. murrayana* (H), *S. aquifolium* and *H. clathratus* (I), and *T. murrayana* and *H. clathratus* (J).

Foliar sprays of the macroalgae-based liquid fertilisers have been reported to improve the uptake of nutrients, and thus promote plant growth and development, as well as decrease the need for inorganic fertilisers (Thangaraju, 2008). These features were attributed to the presence of plant growth promoting substances as well as other nutrients including cytokinin and auxin, as well as other nutrients, in the brown macroalgae used to develop those fertilisers (Prasad *et. al.*, 2010; Tay *et. al.*, 1986; Thangaraju, 2008). We conducted HPLC analysis on the liquid extracts of several seaweeds from WNT and confirmed that the promotive effects of the macroalgal extracts on plant growth and yield were partly due to the presence of plant growth promoting substances in the extracts (Nikmatullah *et al.*, 2014). HPLC was used to identify the plant growth regulators present in the water and methanol extracts from five species of macroalgae in WNT by comparing the chromatograms of the seaweed extracts and plant growth regulator standards prepared in corresponding solvents. Each standard was separated in the HPLC column with a different retention time. An example of using HPLC analysis to identify the presence of plant growth promoting substances in the water extract of *T. murrayana* was presented in Figure 9. The plant growth promoting substances identified in liquid extracts of 5 macroalgal species from WNT were presented in Table 2.

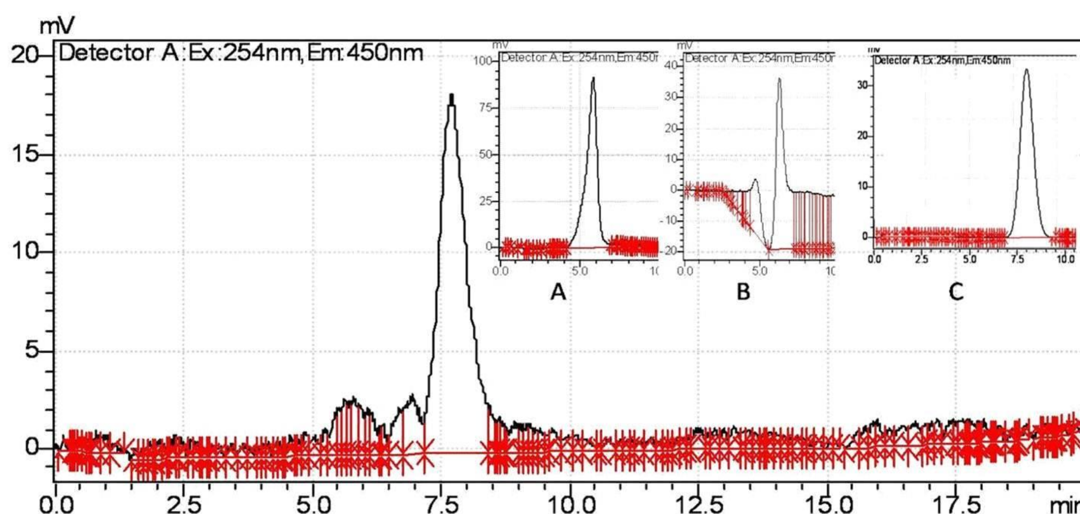

Figure 9. Comparison of the HPLC chromatograms for water extract of *Turbinaria murrayana* and standard plant growth hormones (inset) including IAA (A), NAA (B) and zeatin (C) indicated the presence of zeatin in the macroalgae extract, based on the presence of a single peak at the retention time of about 7.5 min.

Table 2. Summary of plant growth promoting substances present in water and methanol extracts of macroalgae from WNT as assessed by HPLC

Extract solvent	**Macroalgal species**	**Plant growth promoting substance**
Water	*Turbinaria murrayana*	NAA, IAA, zeatin*
	Hydroclathrus clathratus	Zeatin*
	Sargassum cristaefolium	Zeatin*
	Sargassum crassifolium	Zeatin*
	Sargassum aquifolium	Zeatin*
Methanol	*Turbinaria murrayana*	GA_3, AbA*
	Hydroclathrus clathratus	-
	Sargassum cristaefolium	Zeatin*
	Sargassum crassifolium	2,4-D, zeatin*
	Sargassum aquifolium	GA_3, zeatin, AbA*

* Main plant growth promoting substance detected in each macroalgal liquid extract.

D. Potential of Macroalgae from WNT as Skin Protector Against UV Radiation

Characterisation of the potential of using macroalgal extracts from WNT to protect skin from UV radiation has been undertaken in collaboration with Fukushima Medical University, Japan since 2009. Initial screening was undertaken to analyse the capability of seaweed extracts to absorb UVA (wavelengths between 320 and 400 nm), UVB (wavelengths between 280 and 320 nm) and UVC (wavelengths at 200 and 280 nm) using liquid extracts prepared from seaweed species such as *Actinotrichia fragilis*, *Gelidium latifolium* and *Eucheuma denticulatum* collected in WNT (Figure 10).

After identifying seaweed species that exhibited the UV-absorbing capacity, the ability of the macroalgal extracts to protect cell damage from UV radiation was examined *in vitro* using HeLa cells. The extent of cell damage was assessed from the degree of fluorescence staining using DAPI that only binds to the nucleus of cells with damaged plasma membrane. This was first tested by staining the nuclei of amphotericin B-treated cells with DAPI. Amphotericin B was used to induce cell death, and the dead cells can be stained with DAPI that fluoresced under fluorescent microscopy. Figure 11 showed that the system for analysing cell death induced by amphotericin B using fluorescence staining was successful based on the observation of green fluorescence of dead cells. Following this, the same method of staining the nuclei of dead cells was utilised as the basis to evaluate the potential of macroalgal extracts in protecting cell damage from UV radiation. The results confirmed that extracts of several macroalgal species from WNT were able to lower cell death after exposure to UV radiation for 30 min with an efficiency similar to that of commercial anti-UV product (Figures 12 & 13).

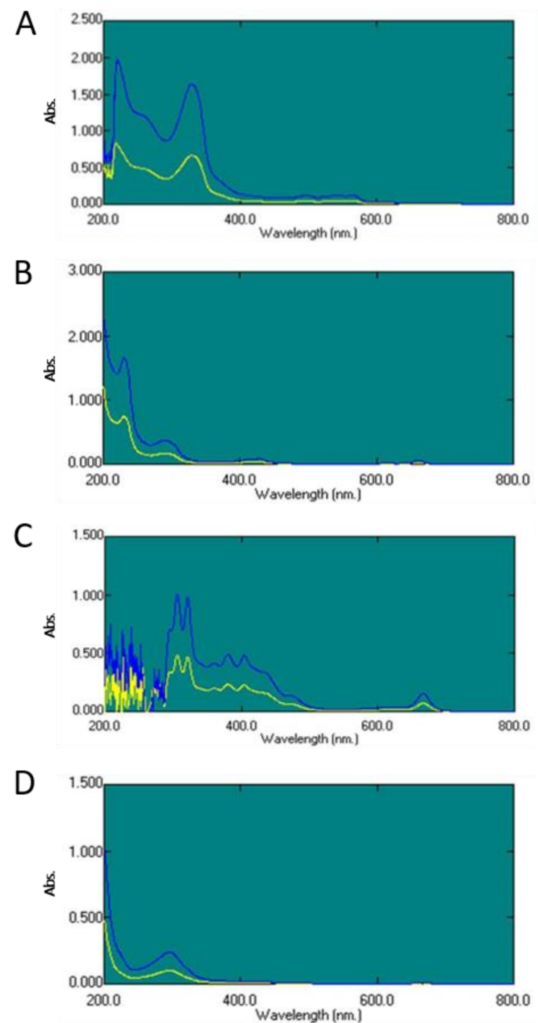

Figure 10. Absorbance spectra showing capability of (A) HBS extract of *Actinotrichia fragilis* to absorb UVA and UVC, (B) hexane extract of *Gelidium latifolium* to absorb UVA and UVB, (C) ethanol extract of *Actinotrichia fragilis* to absorb UVA and UVB, and (D) ethanol extract of *Eucheuma denticulatum* to absorb UVB and UVC. Yellow and blue lines represent absorbance spectra of liquid extracts at the concentrations of 10 and 20%, respectively.

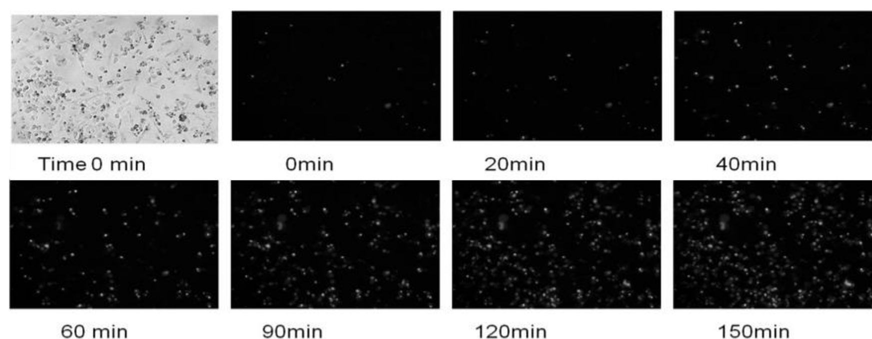

Figure 11. Bright field and fluorescence images of dead HeLa cells stained with DAPI following treatment with amphotericin B to disrupt the nuclear membrane. Time indicates duration of treatment.

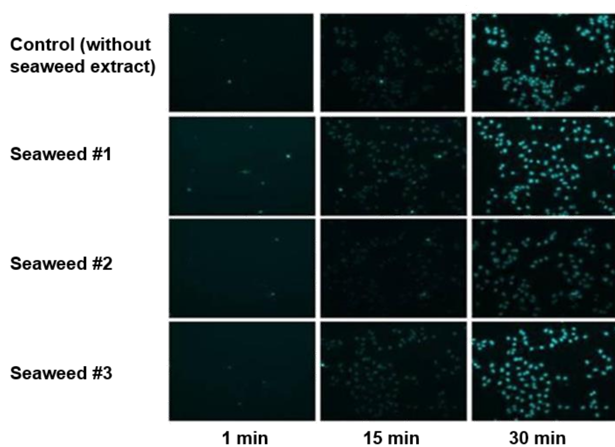

Figure 12. Assessing cytoprotective property of the ethanol extracts of selected seaweed species following the exposure of HeLa cells to UV radiation for 30 min. The treatment with extract of *Turbinaria murrayana* (seaweed no. 2) resulted in a relatively lower cell death with less fluorescence from DAPI staining compared to the control and treatments with extracts of *Acanthophora spicifera* (seaweed no. 1) and *Turbinaria ornata* (seaweed no. 3), indicating the potential of *T. murrayana* extract as a UV-protectant for cells.

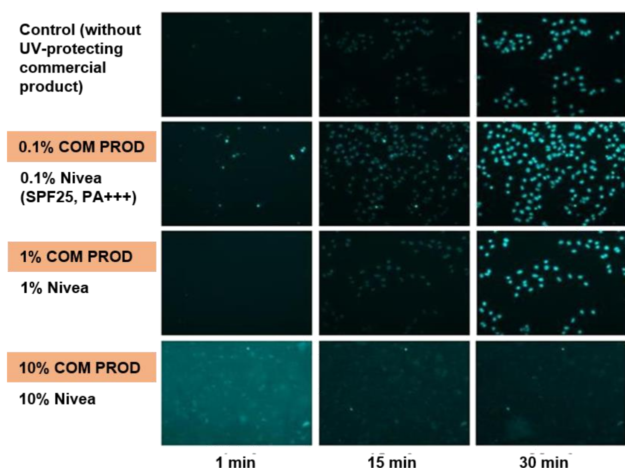

Figure 13. Assessing cytoprotective property of commercial anti-UV product at different concentrations following the exposure of HeLa cells to UV radiation for 30 min. Cell death was lower in treatment with 10% commercial anti-UV product.

The UV protection potential was assessed for all macroalgal species from WNT. The results revealed that the extracts of 10 macroalgal species from WNT were highly capable of absorbing UV radiation and providing protection from cell death as shown by *in vitro* analysis, including *Acanthophora* sp., *Acanthophora spicifera*, *Gracilaria* sp., *Kappaphycus striatus*, *Hypnea* sp., *Sarcodia* sp., *Halimeda* sp. 1, *Halimeda* sp. 2, *Turbinaria murrayana* and *Turbinaria ornata* (Sunarpi *et al*., 2010b). Further characterisation is now underway to identify the bioactive compounds in these species and to develop UV protective products from seaweed extracts.

E. Potential Use of Macroalgae from WNT as an Anti-Cancer Agent

Many seaweeds species, particularly the red and brown seaweeds, are excellent sources of polysaccharides such as galactans, agar, and carrageenan with anti-cancer properties (Bouhlal *et al*., 2011), and the polysaccharide-producing macroalgae had been reported to hold the potential as the sources of anti-cancer agent.

Two approaches were used for analysing the anti-cancer property of macroalgae from WNT: *in vivo* analysis using brine shrimp (*Artemia salina*) larvae for initial mass screening, and *in vitro* analysis using HeLa cancer cells to further analyse the cytotoxicity of seaweed extracts. Preliminary investigation showed that extracts of many macroalgae species such as *Acanthophora muscoides* from WNT (at concentrations ranged between 10 to 1000 ppm) were able to decrease mortality of brine shrimp larvae (Figure 14). The brine shrimp cytotoxicity assay was used for initial mass screening in this study, as it is considered as an excellent method for preliminary investigations of toxicity, detection of fungal toxins, heavy metals, pesticides, cytotoxicity testing of dental materials, and fractionation of active cytotoxic and anti-tumour agents. This assay has been widely used due to the ease of handling and low cost, it also utilises only a small amount of test materials (Meyer *et al*., 1982).

Following the initial screening, the potential of polysaccharides and crude extracts of macroalgae to prevent cancer cell proliferation was assessed by *in vitro* analysis using the HeLa cell line. The investigations were carried out in Fukushima Medical University, Japan. Several polysaccharides and crude extracts (organic, acidic and basic extracts) were used for the analysis. Cell proliferation was assessed with fluorescence microscopy to analyse the HeLa cells before and after treatment with polysaccharides and crude extracts of macroalgae. The cytotoxicity of seaweed extracts was assessed with double-staining using calcein-AM and PI that simultaneously stained the live cells green and dead cells red.

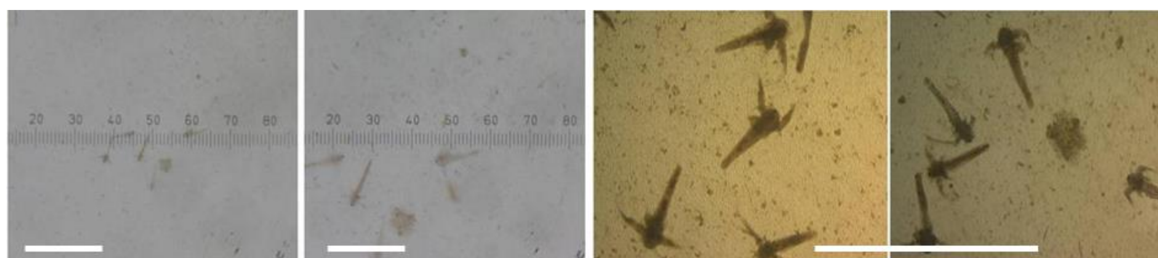

Figure 14. Growth of brine shrimp larvae after 48 h of treatment with methanol extract of *Acanthophora muscoides* at concentrations 0, 100, 200 and 300 ppm. Scale bars represent 2 cm.

Figure 15 showed an example of cytotoxicity assay on cells treated with 50 μg/mL fucoidan extracted from *Turbinaria murrayana*. After 3 d of incubation, the control showed few dead cells, while more dead cells were observed in the treatment group, indicating the anti-cancer property of fucoidan. In addition, untreated HeLa cells showed normal elongated cell morphology while the treated HeLa cells showed irregular cell size and cell shrinkage.

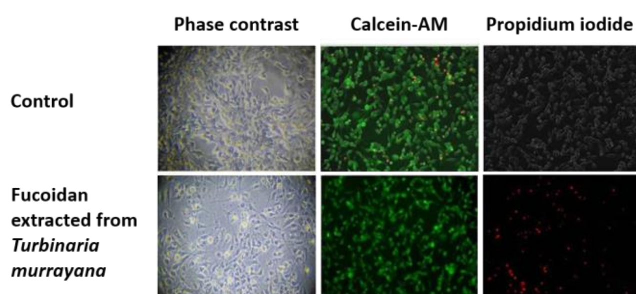

Figure 15. Fluorescence microscopy observation of HeLa cells untreated and treated with 50 μg/mL fucoidan from *Turbinaria murrayana* for 3 d. Green fluorescence indicated cells stained with calcein-AM, while red fluorescence indicated dead cells stained with PI. The number of dead cells was higher when the cancer cell culture was treated with fucoidan.

Various red and brown macroalgal extracts have been suggested to have antioxidant and anti-tumour activities, as characterised on cell growth and DNA synthesis (Kwon & Nam, 2007), inhibition of angiogenesis/anti-angiogenesis (Xue *et al.*, 2012) and tumour cell proliferation (Paul, 2014). The capability of seaweed extracts to prevent cancer cell proliferation has been suggested to be a result of the biochemical properties of the compounds such as sulphated or non-sulphated polysaccharides (including galactans, glucan, agar, carrageenan, fucoidan) in the extracts (Bouhlal *et. al.*, 2011; Moghadamtousi *et al.*, 2014). Properties of polysaccharides from macroalgae vary and different sources of polysaccharides, including fucoidan, will prevent tumour proliferation in different ways (Moghadamtousi *et al.*, 2014). The polysaccharides from marine algae are suggested to have great potential to be developed into anti-cancer medicine (Kwon & Nam, 2007; Paul, 2014; Xue *et al.*, 2012). Our preliminary studies also indicated that the highly diverse group of marine macroalgae identified in WNT holds great potential as a source of anti-cancer agents.

IV. CONCLUSION

WNT marine waters have highly diverse group of wild macroalgae, some of which were potential sources of hydrocolloids like carrageenan, agar and alginate. A number of the seaweeds also showed the potential to be used as fertiliser with the plant growth promoting substances identified within the seaweed extracts. The extracts of several red macroalgae were found to possess cytoprotective property against UV radiation, while the polysaccharides and crude extracts of other seaweeds showed the potential to inhibit proliferation of HeLa cancer cells. The results of these ongoing studies suggested the great potential of macroalgae in WNT that awaits to be further explored and exploited in the effort of developing a macroalgae-based industry towards the prosperity of Indonesia.

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