

GROWTH PROMOTING CAPABILITY OF AQUADEST-EXTRACTS FROM DIFFERENT MACRO ALGAE OBTAINED IN LOMBOK ISLAND, INDONESIA TO GROWTH OF RICE-PADDY PLANT

KEMAMPUAN EXTRACT AQUADEST BEBERAPA MAKRO ALGA PULAU LOMBOK, INDONESIA DALAM MERANGSANG PERTUMBUHAN TANAMAN PADI

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ABSTRACT

Concern on the impact of excessive and unwise usage of synthetic fertilizers and growth hormones to environment, food safety and food quality leads to an increase utilization of organic fertilizer and/or natural bioactive stimulants. One potential source for natural fertilizer and bioactive growth stimulant is macro algae. Out of 88 species of macro-algae obtained in Nusa Tenggara Barat Province, five of them namely *Turbinaria murayana*, *Sargassum cristaefolium*, *S. crassifolium*, *S. aquifolium*, and *Hydrochlarus sp* are potential to be developed as organic fertilizer and growth promoting substances. This paper examines the property of aquadest-extract of *T. murayana*, *S. cristaefolium*, *S. crassifolium*, *S. aquifolium*, and *Hydrochlarus sp* and their effect on growth and production of rice-paddy. Application of combined-extracts of *T. murayana*, *S. cristaefolium*, *S. crassifolium*, *S. aquifolium*, and *Hydrochlarus sp* can promotes growth and development of rice-paddy. This capability is partly due to the presence of Zeatin-like substance in the extracts as examined by High Performance Liquid Chromatography (HPLC).

ABSTRAK

Meningkatnya kesadaran masyarakat terhadap dampak penggunaan pupuk dan hormon pertumbuhan sintetis yang berlebihan terhadap kualitas dan keamanan pangan serta lingkungan mendorong meningkatnya penggunaan pupuk organik dan perangsang pertumbuhan yang berasal dari bahan alam. Makro alga (rumput laut) adalah satu sumber pupuk dan perangsangan pertumbuhan tanaman yang alami. Dari 88 spesies jenis rumput laut yang ditemukan di perairan laut Provinsi Nusa Tenggara Barat, lima spesies diantaranya memiliki potensi untuk dikembangkan menjadi pupuk dan perangsang pertumbuhan alami, jenis-jenis tersebut adalah *Turbinaria murayana*, *Sargassum cristaefolium*, *S. crassifolium*, *S. aquifolium* dan *Hydrochlarus sp*. Makalah ini membahas analisa kandungan hormon perangsang pertumbuhan tanaman pada ekstrak aquadest rumput laut *T. murayana*, *S. cristaefolium*, *S. crassifolium*, *S. aquifolium*, dan *Hydrochlarus sp*, serta pengaruh ekstrak tersebut terhadap pertumbuhan dan produksi padi. Aplikasi ekstrak kombinasi *T. murayana*, *S. cristaefolium*, *S. crassifolium*, *S. aquifolium*, dan *Hydrochlarus sp* merangsang pertumbuhan dan perkembangan tanaman padi. Kemampuan ini diduga disebabkan karena ekstrak-ekstrak tersebut mengandung sitokinin dengan berat molekul yang sama dengan Zeatin, seperti ditunjukkan oleh hasil analisisakromatografi cair menggunakan High Performance Liquid Chromatography (HPLC).

Key Words: Macro Algae, Synthetic Fertilizer, Cytokinin, Rice-paddy, High Performance Liquid Chromatography (HPLC)

Kata-kata Kunci: Makro Alga, Pupuk Sintetis, Sitokinin, Padi, High Performance Liquid Chromatography (HPLC)

INTRODUCTION

Excessive usage of synthetic fertilizer in crop cultivation has increased the demand for synthetic fertilizer as well as the production cost, which in turn decreased profitability of crop production. On the other hand, concern on the usage of excessive synthetic fertilizers and growth regulators have increased significantly, partly due to their possible adverse impact to the environment such as

continuous reduction in soil quality (fertility), soil and water pollutions, others environmental concerns as well concern on the safety of those foods to human beings. Therefore, strategic alternative is urgently needed to solve this problem, and this could involve the use of organic fertilizer and/or natural bioactive stimulants which are reported to be capable to increase capability of plant to absorb nutrient efficiently therefore decrease required-dosage of fertilizer (Thangaraju, 2008).

In many countries, liquid fertilizers from local macro-algae's grown in those countries have been developed. These include Seasol in Australia (Tay et al., 1986), Kelpak in Europe (Beckett and van Staden, 1989), SM3, SM6 and Maxicrop in the United States (Hankins and Hockey, 1990), Algaenzims in Mexico (Sanchez et al., 2003) and Algifert, Goemar GA14, Seaspray, Cytec and Seacorp in India (Sivasankari et al., 2006). Foliar sprays of those macro alga-based liquid fertilizers were reported to increase the absorption of nutrients, and thus promote plant growth and development as well as decreased the need for inorganic fertilizers (Thangaraju, 2008). These capability was suggested due to the presence of plant growth substances including cytokinin and auxin presented in brown macro algae's used to developed those fertilizers (Tay et al., 1986; Thangaraju, 2008; Prasad et al., 2010) as well as other nutrients. However, there is insufficient evidence to suggest that the locally inhabitant-brown algae's of Lombok Coast retains the same quality, as they grown in different ecology.

The experiment have previously reported that there are at least 88 macro alga species identified in Nusa Tenggara Barat, Indonesia, marine water (Sunarpi et al., 2005, 2006) and five species of brown-algae's are potential to be used as organic fertilizer and growth promoting substances (Sunarpi et al., 2010a). Aquadest- extracts of *Turbinaria murayana*, *Sargassum crassifolium*, *S. aquifolium*, and *S. cristaefolium* promoted growth of tomato, spinach and rice-paddy plant, and only water extract of *Hydrochlorus* sp was able to enhanced yield of rice-paddy plant (Sunarpi et al., 2006, Jufri et al., 2010, Sunarpi et al., 2010b). However, it is still unclear to why those extracts could promote different stages of different plant growth and development. In addition, it is unknown if combination of those extracts could enhance both growth and yield of rice-paddy plants. This paper reports the effect of combined-extracts of five brown algal obtained from coastal areas of Lombok Island – *T. murayana*, *S. cristaefolium*, *S. crassifolium*, *S. aquifolium*, and *Hydrochlorus* sp – to growth and production of rice-paddy plants. In this report want to extent our previous finding on the promoted effects of the five species by evidence that growth promoting substances are existed in the water extracts of those five species.

MATERIALS AND METHODS

Design, time and place of study

The study was designed using Completely Randomized Design (CRD) with 10 treatments of different combinations of aquadest extract of seaweed species and four replicates, as follow:

- A : Aquadest extracts of *Turbinaria murayana*
- B : Aquadest extracts of *Sargassum cristaefolium*
- C : Aquadest extracts of *Sargassum crassifolium*
- D : Aquadest extracts of *Sargassum aquifolium*
- E : Aquadest extracts of *Hydrochlorus* sp.
- F : Aquadest extracts of *T. murayana* + *S. cristaefolium*
- G : Aquadest extracts of *T. murayana* + *S. crassifolium*
- H : Aquadest extracts of *T. murayana* + *S. aquifolium*
- I : Aquadest extracts of *T. murayana* + *Hydrochlorus* sp
- J : without macro alga extract (control treatment)

Macro alga samples were taken from several sampling points in the coast and marine water of Lombok Island. Fresh macro alga was placed in the sample collection box, and transported to the Laboratorium Immuno-biology, Faculty of Mathematics and Sciences, University of Mataram. The samples were freshly extracted or preserved in refrigerator (-20°) until required. Rice planting and treatment were done in a plastic house at Jatisela Village, Gunung Sari Subdistrict, West Lombok District, Nusa Tenggara Barat, Indonesia.

Preparation of macro alga extract

One hundred grams of macro alga samples were trimmed onto fine pieces and blended with 100 mL of distilled water (ratio of 1:1 (w/v)) to form fine slurry. The slurry was filtered and the supernatant was transferred onto falcon tubes and centrifuged at 5,000 rpm for 5 mins at 4°C to separate the extracts with cell debris. Following this, the supernatant was transferred onto a Falcon tube and equally combined with other extracts, as indicated in the treatment. The stocks were preserved (20°C) for 2 to 4 weeks. These extracts were designated as extracts with 100% concentration and used as stocks to prepare the working concentration. The required concentration (15%) was prepared by mixing 15 mL of the stock with 85 mL of water. The dilution was prepared freshly, just before being sprayed onto the rice-paddy plants as appropriate. Macro alga extracts used for HPLC analysis was filtered using Millipore filter (diameter of 0.22 µM) and diluted as appropriate before being injected onto HPLC column.

Planting and plant treatment

The medium used to grow rice paddy in this study was potting mix composed of soil, sand and manure (1:1:1; w/w/w). The three components were homogeneously mixed, weighed *ca.* 8 kg, and placed in a plastic pot (size of 10 L). Rice seeds were

showed by spreading them in the nursery pots containing planting medium as described previously. After 21 days, rice seedlings (2 clumps) were planted in the medium that had been prepared in the pot. Synthetic NPK fertilizers; each with a dose of 2.4 g urea and 0.36 g TSP per pot; were applied to the potting mix before the transplanting and at 28 days after transplanting. For the second application of synthetic fertilizer, KCl of 1.2 g/pot was also added. During the course of experimentation, the plant was maintained according to the standard procedure for rice-paddy cultivation.

Treatment of macro alga extract was done by spraying the whole plant with aquadest extracts of macro alga, as treated. The treatments was applied weakly, started from a week after transplanting, with spray volume of 25 to 50 mL per pot, according to the plant age and size. Growth and yield parameters observed were plant height, number of tillage, the number of spikelet, number of grains (seeds) per spikelet and weight of 1000 seeds.

Analysis of plant growth substances by High Performance Liquid Chromatography (HPLC)

HPLC analysis was used to identify the plant growth promoting substance presented in each aquadest extract of macro alga. Six plant growth hormones, namely Indole Acetic Acid (IAA), 6-Naphtalene Acetic Acid (NAA), Gibberlic Acid (GA₃), Zeatin (ZA) and Absciscic Acid (AbA) were used as a standard in the analysis. Each plant growth hormone were weight and diluted in aquadest containing 0,1 % of ethanol or NaOH 1 M (as appropriate) to a final concentrations of 25 mM, 50 mM and 100 mM.

Each HPLC analysis was undertaken using a Shimpack CLC-ODS column (Shimadzu, Japan). Each sample were injected automatically, after diluted as appropriate, onto the HPLC column and separated at column temperature of 30°C, pressure of 50 kg/cm² with continuous retention speed of 0,5 mL per minutes, and using methanol and aquadest (7:1. v/v) as a mobile phase. Analysis was done by comparing chromatogram of each sample with the standards.

Data analysis

Data were analyzed by Analysis of Variance (Anova) followed by Least Significant Difference (LSD) (at 5 % level of confidence).

RESULTS AND DISCUSSION

Growth and Yield of Rice Plants

The effect of different aquadest-extract of macro algae was evaluated in rice-paddy plant by

comparing the growth and yield of control plant (without extract treatment) with plants prayed with different extracts or combination of extracts from macro algae. Most of the seaweed extracts tested was not significantly alter the plant height (Figure 1). On the other hand, application of aquadest extract of the three *Sargassum* and *Hydrochlathurus*, either applied solely or in combination with *Turbinaria murayana* extracts, enhanced the number of tillage, plant biomass, number of spikelet and number of seeds. Interestingly, application of *Turbinaria murayana* extracts did not alter rice-paddy growth and yield (Table 1).

Overall, the results indicate different promoting effects of different water soluble extracts from five species of brown alga collected from marine water of Lombok Island, at which all extracts or combination of . *S. crasifolium*, *S. cristaefolium*, *S. aquifolium*, and *Hydrochlathurus sp.* promoted rice paddy growth and development while water soluble extract of *Turbinaria murayana* did not promote growth and yield of rice paddy plants. to elucidate the property of aquadest extracts from these five macro alga species, the hplc analysis was undertaken and reported in this article.

HPLC Analysis of Macro Alga Extracts

Before being used to identify the presence of plant growth promoting substances in the aquadest extract of the macro alga, the HPLC protocol was optimized using five standards of plant growth regulator, i.e. IAA (Indole-3-acetic acid) , NAA (Naphtalene acetic acid), GA₃ (Gibberellic acid), Zeatin and AbA (Absciscic acid) at concentrations of 25mM to 100mM. The HPLC was able to detect the presence of those five plant growth regulator as they are separated and detected at different time (Figure 2). Similarly, chromatograms of each standard at concentration of 25mM to 100 mM indicate that peak height for each standard was correspondence to the concentration of plant growth regulator in the standard (data not shown).

Following the PGR standard detection, the present of plant growth promoting substances in the aquadest-extracts of *Hydrochlathrus Sp*, *Turbinaria murayana*, *Sargassum. cristaefolium*, *Sargassum crassifolium*, and *Sargassum aquifolium* were undertaken using same protocols as used for the PGR standard chromatography analysis. The chromatogram of each HPLC analysis revealed only one peak form aquadest extract of each sample (Figure 3 and 4). The peak of four samples, *Hydrochlathurus sp*, *Sargassum cristaefolium*, *S. crassifolium* and *S aquifolium*, appeared after 7.5 min of retention in the HPLC column, and this retention time was similar to those of Zeatin standard (Figure 3 and 4).

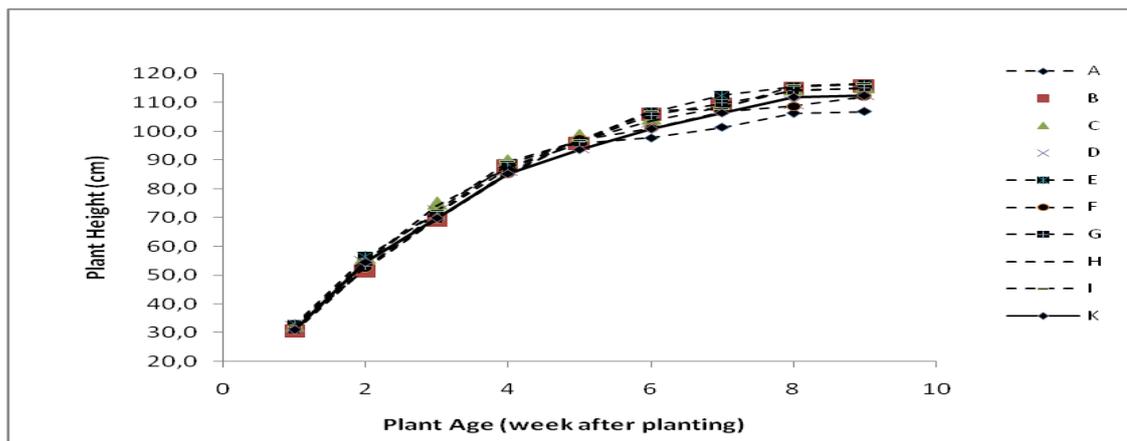


Figure 1. Changes in the height of rice-paddy plant after treatment with different aquadest extract of seaweed. A=Aquadest extracts of *Turbinaria murayana*, B= Aquadest extracts of *Sargassum cristaefolium*, C=Aquadest extracts of *Sargassum crassifolium* D =Aquadest extracts of *Sargassum aquifolium*, E=Aquadest extracts of *Hydrochlathurus sp.*, F= Aquadest extracts of *T. murayana* + *S. cristaefolium*, G=Aquadest extracts of *T. murayana* + *S. crassifolium*, H=Aquadest extracts of *T. murayana* + *S. aquifolium* , I= Aquadest extracts of *T. murayana* + *Hydrochlathrus sp*, K= control treatment (without macro alga extract).

Table 1. Number of tillage, plant biomass, number of spikelet and number of seeds from rice-paddy plants treated with different extracts of seaweed.

Treatment	No of tillage	Plant biomass (g)	No of spikelet	No of seeds
A*	18,3 a**	61,3 a	14,7 a	135,5 a
B	18,7 a	72,1 c	17,0 bc	147,5 b
C	19,3 ab	64,8 ab	16,7 bc	148,8 b
D	19,0 a	68,1 ab	18,3 c	153,2 b
E	24,0 c	89,1 d	23,3 d	182,2 c
F	17,0 a	64,7 ab	17,0 bc	173,9 c
G	20,3 b	67,7 ab	17,0 bc	147,2 b
H	21,7 b	65,4 ab	18,7 c	142,0 a
I	19,0 a	60,9 a	15,7 ab	148,8 b
K	17,0 a	71,2 bc	13,7 a	131,9 a
LSD 5%	1,43	7,21	3,2	14,7

* A = Aquadest extracts of *Turbinaria murayana*, B= Aquadest extracts of *Sargassum cristaefolium*, C=Aquadest extracts of *Sargassum crassifolium* D =Aquadest extracts of *Sargassum aquifolium*, E=Aquadest extracts of *Hydrochlathurus sp.*, F= Aquadest extracts of *T. murayana* + *S. cristaefolium*, G=Aquadest extracts of *T. murayana* + *S. crassifolium*, H=Aquadest extracts of *T. murayana* + *S. aquifolium* I= Aquadest extracts of *T. murayana* + *Hydrochlathrus sp*, K= control treatment (without macro alga extract).

***) the value at the same row followed by the same number is not different based on Least Significant Difference at 5%

HPLC analysis of the aquadest extracts from *Turbinaria murayana* resulted in one peak which was appeared after 2 mins retention in the HPLC column, and this was similar to the retention of Abscisic acid standard (Figure 5)

These results indicate that the four brown algae, *Hydrochlathrus sp.*, *S. cristaefolium*, *S. crassifolium* and *S. aquifolium* may contain Zeatin-like cytokinin. Interestingly, HPLC results indicate that extract of those four macro

alga species retains about the same concentration of Zeatin as they all produce the similar peaks height. However, the presence of Zeatin-like substance was absent in the aquadest extract of *Turbinaria murayana*, and interestingly the aquadest-extract of *Turbinaria murayana* may contain Abscisic Acid. .

Many published findings suggest the capability of different extracts of green, red and brown macro alga to promote plant growth and

development (Thangaraju, 2008; Kavipriya et al., 2011) as well as to induce morphogenesis *in vitro* (Vinoth et al., 2012). The experiment have previously reported the selective capability of green and brown seaweed extracts from *Ulva fasciata*, *Paddina sp.*, *U. ferticulata*, *Turbinaria murayana*, *T. ornate*, *Sargassum polycistum*, *S. aquifolium*, *S. crassifolium*, *Chaetorpha sp.* and *Hydrochlathurus sp.* to promote rice paddy growth and development (Sunarpi et al., 2010b). In this experiment, the aquadesh extract and

combined extracts of *Hydrochlathurus sp.* and three species of, *Sargassum sp.* were able to increase the number of tillage, number of spikelet and number of seeds obtained from rice-paddy plants, but, interestingly, growth of rice paddy plants were not significantly altered by aquadest extracts of *Turbinaria murayanan* or combination of *T. murayana* extracts with *Hydrochlathurus sp.* and *Sargassum sp.* peak appeared after 2 min similar to the AbA standard (right panels)

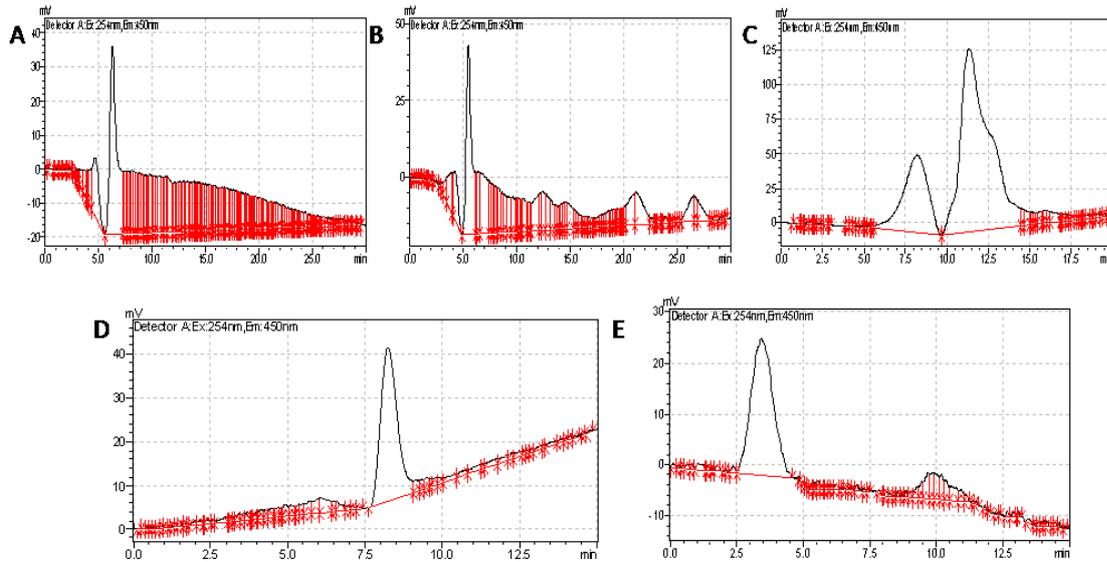


Figure 2. Chromatogram of IAA (A), NAA (B), GA_3 (C), Zeatin (D) and AbA (E) showing different retention time for each plant growth regulator.

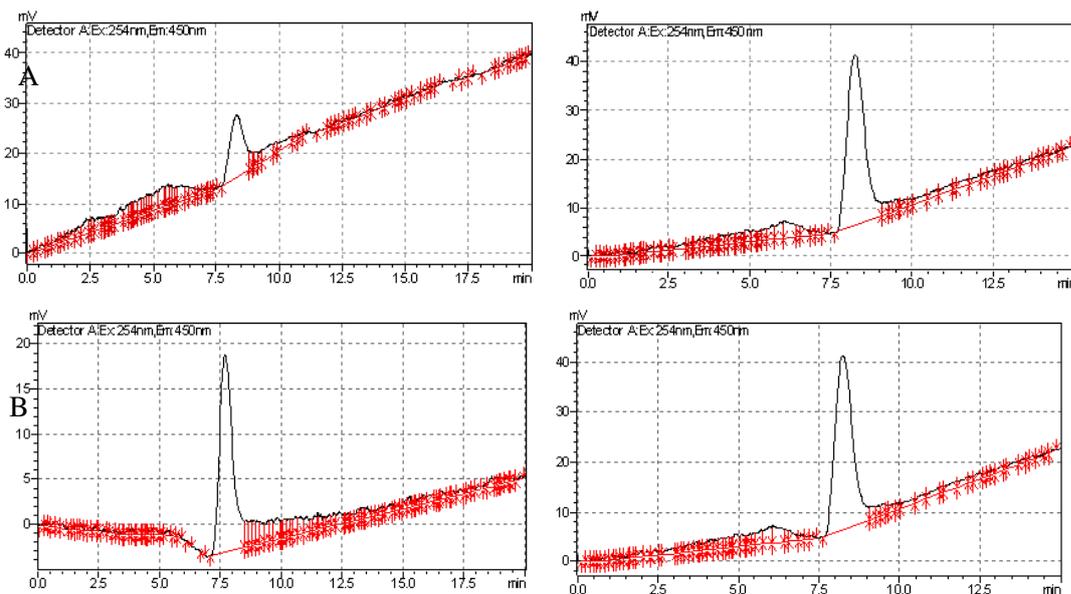


Figure 3. Chromatogram of aquadest extract of *Hydrochlathurus sp.* (A, left) and *S. cristaefolium* (B, left) showing only one peak appeared after 7.5 min similar to the Zeatin standard (right panels)

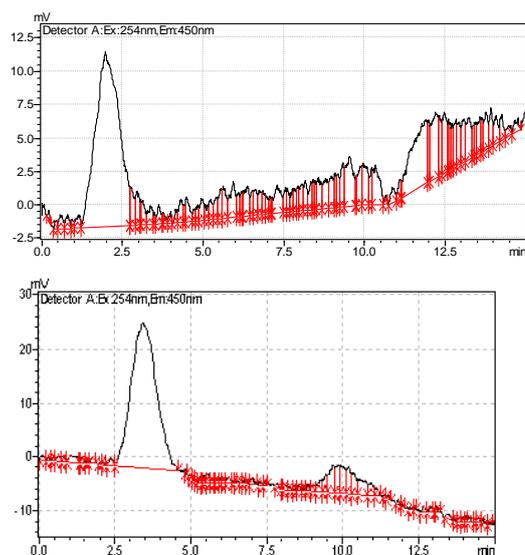


Figure 5. Chromatogram of aquadest extract of *T. murayana*(left) showing only one

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The capability of macro alga extract to promote plant growth and development are suggested due to the presence of plant growth regulator such as auxin and sitokinin in the macro alga extracts (Tay et al., 1986; Thangaraju 2008; Prasad et al., 2010). The presence of plant growth regulator in many species of macro algae have been detected using different methods, including conventional bioassay and colorimetric test (Gordon and Paleg, 1957; Bernart and Gerwik, 1990), GLC (Gas Liquid Chromatography), GC-MS (Gas Chromatography Mass Spectrophotometry), HPLC (High Performance Liquid Chromatography) and LC-MS (Liquid Chromatography Mass Spectrometry) methods (Stirk et al., 2003; Prasad et al., 2010). THE occurrence of such compound in macro alga grown in marine water of lombok island has not yet been reported previously. In the present study detection of plant growth promoting substance from polar (aquadest) extract of *Turbinaria murayana*, *Hydrochlathrus sp.*, *Sargassum aquifolium*, *S. crassifolium* and *S. cristaefolium* obtained in Lombok marine waters are undertaken using HPLC. Analysis of five different plant growth regulator standards including IAA, NAA, Zeatin, GA₃, ABA, indicated that the HPLC can detect between 25 to 100 mM of the standard, and more importantly the HPLC can distinguish these five plant growth regulator as each of them can be separated after different retention time in the HPLC column. Furthermore, the chromatograms indicated that aquadest extracts of *Sargassum crassifolium*, *S. cristaefolium*, *S. aquifolium* and *Hydrochlathrus sp.* may content Zeatin, as all aquadest extracts produce peak at similar retention time as Zeatin standard.

Zeatin is a naturally occurring cytokinin, and cytokinin is a group of plant hormone with an important role in various plant growth and development; such as cell division, shoot initiation and proliferation, chloroplast biogenesis and plant senescence (Mok and Mok, 2001). Therefore, application of seaweed extracts containing cytokinin may promote cell division, shoot initiation and proliferation. In this experiment, application of aquadest extracts of *Hydrochlathrus sp.*, *S. crassifolium*, *S. cristaefolium* and *S. aquifolium* increased number of tillage, number spikelet and number of seed in the rice paddy. This enhancement may indicate the increase rate of cell division, shoot initiation and proliferation (tillage and spikelet number) in the rice-paddy plant sprayed with those seaweed extract.

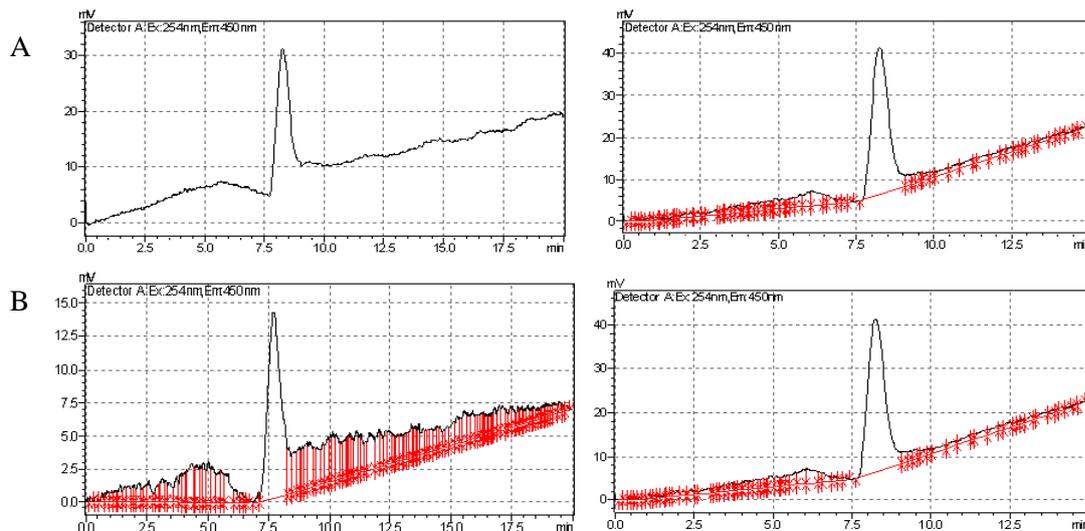


Figure 4. Chromatogram of aquadest extract of *S. crassifolium* (A, left) and *S. aquifolium* (B, left) showing only one peak appeared after 7.5 min similar to the Zeatin standard (right panels)

Therefore, the plant with higher number of tillage will produce higher number of spikelet and seeds. The increase cell division and proliferation in seaweed-treated rice paddy-plant is may be due to the effect of cytokinin to promote cell division, differentiation and to enhance nutrient absorption and mobilization in the rice-paddy plant. It has been reported that exogenous application of cytokinin could increase nutrient mobilization and improve nutrient use efficiency which therefore decreases the need for inorganic fertilizer (Thangaraju, 2008). However, it is still unclear whether application of seaweed extract from Lombok Island could also increase the nutrient use efficiency by rice-paddy plant, and if so, then application of seaweed extracts may decrease the required dosage for inorganic fertilization. Further study is now underway to investigate the capability of brown macro algae extracts from Lombok Island to increase nutrient absorption and mobilization.

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