Identification and determination of growth hormones of *Kappaphycus alvarezi* cultivated in Ekas Bay East Lombok

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Abstract. The extract of red marine algae contained growth-enhancing properties which is associated to age and their morphotypes. *Kappaphycus alvarezi* cultured in Ekas Bay, Lombok, Indonesia was studied and aimed to determine the content of growth-promoting substances in the young and old thallus of brown and green morphotypes of *K. alvarezi*. The content of *K. alvarezi* thallus growth substances was determined by High Performance Liquid Chromatography (HPLC). This study showed that the old thallus in the brown *K. alvarezi* strain contained auxin 8.43 ppm and the young thallus 2.89 ppm, while the old thallus in the green strain *K. alvarezi* contained auxin 10.92 ppm and the young thallus contained 16.28 ppm. This study concluded that auxin was the only growth promoter detected in *K. alvarezi* seeds in both young and old thallus in green and brown morphotypes.

1. Introduction

Marine algae or seaweeds is one among the biodiversity richness inhabits marine life in Indonesia [1]. The most popular is red seaweed producing carrageenan, *Kappaphycus alvarezi*. This seaweed has been cultured by Indonesian seaweed farmers and spreads widely along the islands of the archipelago, including Madura, Bali, Lombok, Sulawesi, Sumbawa, Molucca and West Papua. The most cultivated of the species are from green and brown morphotypes, which has been characterized genetically and morphologically [2]. *K. alvarezi* cultivators generally use seeds obtained from the propagation of young thallus from previous harvests.

This species has phycollid which has been widely used in industries and other biochemical contents which have many biomedical advantages [3], and hormonal substances which play an important role in agriculture related to plant growth. *K. alvarezi* has been used as a fertilizer in horticulture and vegetables to increase soybeans, rice and tomatoes. *K. alvarezi* liquid fertilizer contains growth hormones, including gibberellins from the types of gibberellic acid GA3 and GA7 as
much as 128 and 110 ppm, cytokins from the zeatin and kinetin types at 117 and 73 ppm, and auxin in the form of Indole Acetic Acid (IAA) at 160 ppm [4].

Phytohormones or growth hormones produced by plants, including seaweed, play a role in controlling tissue growth. Cytokinin is hormone that plays a role in protein synthesis, cell cycle and division and stimulate growth; meanwhile, Auxin is a hormone that plays a role in cell elongation in roots and stems [5]. Cytokinins and auxins play a role in growth in Gracillaria perplexa and G. tenuistipitata [6].

K. alvarezi is known to contain growth hormone, but its content in thallus cultivated in Ekas Bay has never been known. Therefore, the purpose of this study was to identify and determine the levels of growth hormone contained in young and old thallus in brown and green morphotypes of K. alvarezi used in its cultivation in Ekas bay, East Lombok district.

2. Material and methods

The materials used were young and old thallus of K. alvarezi brown and green morphotypes, methanol (p.a), auxin, cytokinin, and filter paper. The tools used were a measuring cup with a volume of 1000 ml, a micropipette with a volume of 2 ml, a micropipette with a volume of 1 ml, and an HPLC Shimadzu LC-10 ATVP.

2.1. Extraction

Fresh K. alvarezi was washed, weighed 30 g and added 30 mL of methanol, then crushed with a blender. The extract was obtained by holding the solution. This liquid was then filtered at 0.45 µm millipores, and the filtrate was collected. The extract was diluted to 1000 ppm and then ready to be injected into the HPLC column.

2.2. Determination of growth hormone

Analysis of the cytokinin and auxin content of K. alvarezi was performed using HPLC Shimadzu LC-10 AT VP. It was equipped with a pump model LC-10AT, Rheodyne injector equipped with 20 L loop, SIL-10AT automatic injector, BDS type C-18 hypersil column (4.6 x 250 mm, 5µ m size) and a UV-Vis SPD detector. -10AT. The sample injection volume was 20μL, and elution was carried out in an isocratic system with a flow rate of 1 mL per minute at a temperature of 25-28 °C, where the mobile phase consisted of 0.1% methanol (solvent A) and water (solvent B) in a proportion of 0,64: 0.36 (v:v). Before use, the solvent was filtered with 0.45 µm millipore and then sonicated. The elution time was 15 min, and the wavelength of the UV-Vis detector used was 254 nm.

3. Results and Discussions

Growth hormone content in young and old thallus K. alvarezi green and brown morphotypes obtained the only auxin and showed variations in levels based on the age of the thallus and strain. The analysis of growth hormone content in the young and old thallus of K. alvarezi green and brown morphotypes can be seen in the table 1. Figure 1 shows the auxin chromatograms detected in young and old thallus of K. alvarezi green and brown morphotypes.

| Table 1. Growth Hormone of The Young and Old Thallus of K. alvarezi Green and Brown Morphotypes |
|-----------------------------------------------|
| Growth hormones | Content (µm/mL)  |
|                 | Green          | Brown          |
|                 | Young thallus | Old thallus   | Young thallus | Old thallus |
| Cytokinin       | -             | -             | -             | -           |
| Auxin           | 16,28         | 10,92         | 2,89          | 8,43        |

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The results of the analysis found that the tested thallus of *K. alvarezii* did not contain cytokinins. The auxin content in the green morphotype of *K. alvarezii* was more dominant than the brown, while the young thallus of *K. alvarezii* contained more auxin than the old thallus.

The analysis results showed that the tested thallus of *K. alvarezii* used for cultivation in Ekas Bay only contained growth hormone, namely auxin. Similar results were reported by [7] that *K. alvarezii* only contained auxin of 23.4 ppm and [8] reported the presence of auxin in *K. alvarezii* of 3.87 ppm, while [9] reported that *Gracillaria* sp in Brazil also contained cytokinins with up to 6 times ratio to auxin. These results explain that the external and internal factors of the Ekas Bay seawater are very suitable for the growth of *K. alvarezii* only to extend its thallus.

The auxin content in the green morphotype of *K. alvarezii* was more dominant than the brown, and the young thallus in the green of *K. alvarezii* contained more auxin than the old thallus contrary in the brown of *K. alvarezii* [10]. Reported that the auxin content of *K. alvarezii* in the brown was only about 0.4 ppm. [11] reported that the growth of the green was faster than the brown, although statistically not different. These results indicate that the auxin content in the green of *K. alvarezii* will decrease with the cultivation period, but in the brown of *K. alvarezii* the levels will increase.

4. Conclusions
Young and old thallus *K. alvarezii*, both green and brown morphotypes used for cultivation in Ekas Bay, East Lombok Regency, only contained auxin.

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