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Isolation and Identification of Antioxidant Compound of Isolate Algae *Kappaphycus Alvarezii* from Madura Waters

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ABSTRACT

The objective of the study is to identify antioxidant compounds from the isolates of the ethyl acetate extract of *K. alvarezii* from Madura Ocean. Isolation and purification of *K. alvarezii* algae were carried out in four stages, by using column chromatography and thin layer chromatography. The passive phase of column chromatography is the silica gel, while the active phase is a solvent mixture of hexane-ethyl acetate-methanol, with 10% gradient (isolation Phase 1). The isolation phase two is a mixture of chloroform-methanol with 5% gradient, the active phase for isolation of stage three is a mixture of methanol-water solvent with 6% gradient and a solvent mixture of chloroform-methanol 2.5% is the isolation of the fourth stage for active phase. The results of the isolation of the fourth stage were detected by thin layer chromatography and $FeCl_3$ and H_2SO_4 spray reagents with heating for stain detection that appeared on the TLC plate. The fourth phase of the isolate was identified by UV-Vis spectrophotometer and FTIR spectrophotometer. UV spectrum data shows the presence of aromatic compounds while the infrared spectrum data shows the peak at wave number 1700 cm^{-1}, which is the carbonyl function cluster C = O, at wave number 3500 cm^{-1} shows an OH function cluster, and at wave number 3000 cm^{-1} or 1050 cm^{-1} shows an aromatic function cluster. The function cluster of C = O is a function cluster of carboxylic and amide, and the function cluster of OH and aromatic.

Keywords:* *UV spectrum, IR spectrum,* K. alvarezii, *Madura Waters

I. INTRODUCTION

K. alvarezii algae grow attached to the substrate at the bottom of the water in the form of dead coral on live corals with disc-shaped adhesive. In general, *K. alvarezii* algae found in intertidal areas or in areas which are always submerged by water (subtidal). The algae grows well in coastal areas that consist reefs, because in these areas some requirements for algae growth are fulfilled, such as water depth factor, light, substrate and water movement. The light intensity temperature and nutrients are important factors which influence the growth of *K.alvarezii* algae (Glenn dan Doty, 1990). Ohno, *et al.* (1994) report that *K. alvarezii* algae grows merely during summer at 20° – 30° C in the subtropical waters of Tosa Bay, Japan.

According to Karsten and Wiencke (1999), algae which grow in tropical climates have relatively high antioxidant activity which indicated by the connection between ultraviolet light absorption and the metabolic level of an antioxidant compound. The absorption level of algae to ultraviolet light influenced by the depth and turbidity of the waters, geographic location (Karsten dan Wiencke, 1999), time waktu (Aquilera *et al.* 2002), and ocean topography (Yuan dan Walsh, 2005). *K. alvarezii* has been examined and proven to contain antioxidant compounds, such as primary metabolites

compound that comprises polysaccharides that have low molecular weight, peptides, pigments, ascorbic acid, vitamin A and phenolics (Kumar *et al*. 2008 dan Sriwardhana *et al*. 2003).

Natural antioxidant compounds that contained in plant tissues are soluble in water or fat and in the form of being bound to walls of the plant cell. Antioxidant compounds need to be detached from plant tissue using the appropriate extraction method in accordance with the solubility of the compound. Antioxidant compounds that had been extracted by solvents are still mixed with interfering compounds so that they need to be isolated and purified by a proper isolation method. Antioxidant compounds purification is required in order to determine the chemical structure, which is the identity of the compound based on its physicochemical characteristic. Algae antioxidant compounds are various, depending on species, harvest time and growth conditions, light, climate, and post-harvest conditions (Kuda *et al*. 2005). Information regarding the antioxidant compound of *K. alvarezii* that grows in Indonesia, especially from the results of secondary metabolites, is still inadequate and has not been widely studied. Thus, The study aims to identify antioxidant compounds of an isolate of *K. alvarezii* algae from Madura waters.

II. MATERIALS AND METHOD

MATERIALS

The materials used in this research were ethyl acetate extract of *K. alvarezii* algae. Methanol, n-hexane, ethyl acetate, chloroform, aquades and acetone in pro analysis (PA) category, silica gel G60 plate size 20 cm x 20 cm, viewer reagent $FeCl_3$ and H_2SO_4 were used for insulation and identification of antioxidant compounds. Other materials are aluminum foil and plastic cling wrap sheets for sample preparation.

TOOLS

The used equipment consisted of chromatographic columns (height 40 cm diameter 3 cm), 10 ml vial bottles, drop pipettes, KLT plates size 20 cm x 20 cm, UV-Vis spectrophotometers (Shimadzu brand, 1601), FTIR spectrophotometers (multi spec), LCMS (Waters type LCT Premier XE brand), NMR (JEOL JNM ECA-500 brand).

RESEARCH METHOD

± 3 kg Algae powder from Madura was taken and put in a sealed container (plastic jerrycan). The methanol added to algae powder by a ratio of 1 part powder and 3 parts solvent (b/v). The mixture of algae powder and solvent was stirred for a while and left to be submerged in methanol for maceration which lasted for 3 days. The method by Faten *et al.* (2009) was used for the extraction and fractionation.

Isolation and Purification of the Best *K. alvarezii* Coarse Extract

Isolation and purification of *K. alvarezii* algae coarse extract were carried out on coarse extract of ethyl acetate *K. alvarezii* from Madura from the results of vacuum oven drying at 40° C. Ethyl acetate fraction was isolated and purified in four stages of isolation by column chromatography, followed by thin layer chromatography. The size of the chromatographic column that used is 40 cm lenght and 3 cm in diameter. Whereas the type of thin layer chromatography that used is G**60,** size 20 cm x 20 cm. The passive phase for column chromatography is silica gel and the active phase for isolation of stage I is a solvent mixture of hexane-ethyl acetate-methanol, with a 10% gradient. While the active phase for isolation of stage two is the solvent mixture of chloroform-methanol with a 5% gradient, and for the active phase for the isolation of stage three is a mixture of methanol-water solvent with a 6% gradient, and the active phase for isolation of stage four is a mixture of chloroform-methanol 2,5%.The isolation fraction was detected by thin layer chromatography, and the $FeCl_3$ and H_2SO_4 spray reagents were used accompanied by heating for stain detection that appeared on the KLT plate, while quercetin was used as the standard.

III. RESULTS AND DISCUSSION

1. UV-Vs Spectrum of Antioxidant Compound Isolate *K. alvarezii* of Madura

Results of UV-Vis spectra, *Kappaphycus alvarezii* isolate of Madura are presented in Figure 1.

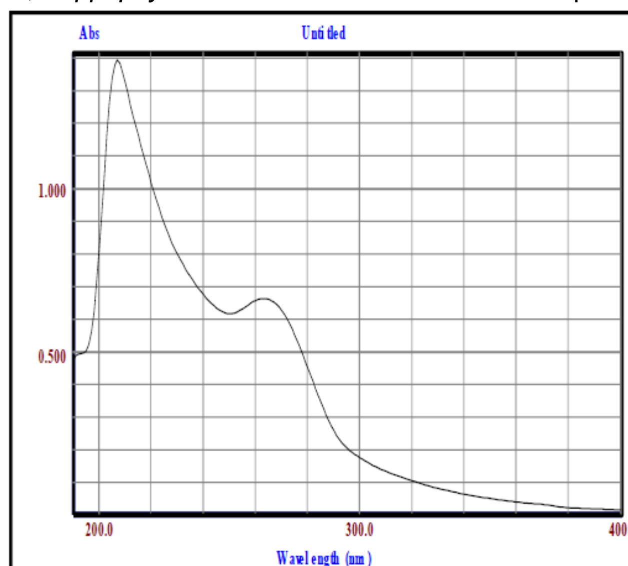

Figure 1. UV-Vis spectrum, antioxidant compound isolate of *K.alvarezii* from Madura

Figure 1 shows 2 maximum absorption peaks, in which the first absorption peak at a wavelength of 207 nm and the second absorption peak at a wavelength of 263 nm. The maximum absorption of an identified compound is evidence that the compound has a conjugated double bond. A conjugated double bond is an unsaturated cluster which go through electronic transition from orbitals n - n* and – ∗. The conjugated double bond is possessed by compounds with chromophore or auxochrome structures. The compound with a chromophore structure has function clusters such as -C=C-, -C⁼C-, -NO_2 or -C-, while the auxochrome structure has function clusters, namely -OH, -OR, -NH_2, -NHR, or -NR_2. The absorption of organic compounds at wavelengths above 200 nm is the area of electron excitation absorption in orbital π and orbital of bond systems π conjugated, including a conjugation expansion of the double bond system (silent electrons from oxygen, nitrogen and sulfur molecules). Based on absorption data for the chromophore structure, then the absorption at wavelength 207 shows absorption the amide function cluster ($RCONH_2$) which absorbs maximum at wavelength < 208 nm with transition n -- n*. The shifting towards a larger wavelength (batochromic shifting) is suspected because of the conjugation extension of the compound structure due to the effect of solvents, shifting reagents or the presence of auxochrome structures, so that the transition energy will be smaller and the wavelength will be larger (Supratman, 2010).

2. The Infrared Spectrum of the *K. alvarezii* Antioxidant Compound Isolate from Madura

The infrared spectrum of a molecule is the result of a transition between different energy levels (vibration) of an electron, therefore the infrared spectrum can be used to identify the function cluster of a molecule. The absorption of the function cluster of *K. alvarezii* isolate compound from Madura is presented in Figure 2.

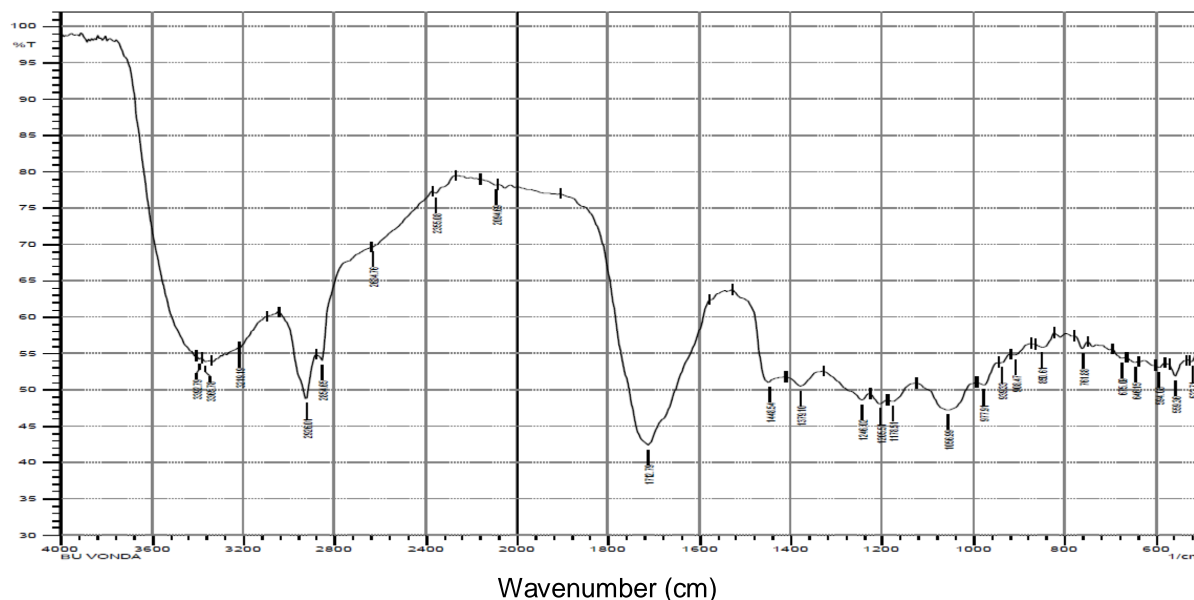

Figure 2. Infrared spectrum of *K. alvarezii* isolate from Madura

Based on the correlation map for the determination of function cluster, the absorption of function cluster of *K. alvarezii* isolate compound in Figure 2 can be categorized, according to the function cluster, as follows:

- Spectrum in the wave area of 800 - 900 cm^{-1} (850.61 cm^{-1}; 908.47 cm^{-1}; 939, 33 cm^{-1} and 977.91 cm^{-1}) shows the function cluster of C-H, aromatic.
- Spectrum in the wave area of 900 - 1300 cm^{-1} (977,91 cm^{-1} ; 1038, 99 cm^{-1}; 1178, 61 cm^{-1} ; 1205, 51 cm^{-1} dan 1246, 02 cm^{-1}) shows the function cluster of C-C, C-N and C-O, stretching.
- Spectrum in the wave area of 1250-1400 cm^{-1} (1205,51 cm^{-1}; 1246,02 cm^{-1}; 1379,1 cm^{-1} and 1448,54 cm^{-1}) shows the function cluster of O-H.
- Spectrum in the wave area of 1200 -1500 (1448 cm^{-1},1379,10 cm^{-1};1246,02 cm^{-1} dan 1205, 51 cm^{-1}) shows the function cluster of OH and CH, bending.
- Spectrum in the wave area of 1600 – 1800 cm^{-1} (1729, 79 cm^{-1})) shows the function cluster of C=O, stretching (cluster of aryl / ketone / carboxylic and amide).
- Spectrum in the wave area of 2500 – 3000 cm^{-1} (2926 cm^{-1} dan 2861,135 cm^{-1}) shows the function cluster of C-H, stretching.
- Spectrum in the wave area of 3000 - 3500 cm^{-1} (382, 79 cm^{-1} dan 3385,78 cm^{-1}) shows the absorption of function cluster O-H and N-H, stretching.

The figure 2 shows that absorption in the area between 3000 - 3500 cm^{-1} is the absorption of bound O-H cluster (hydrogen bond), because the bound O-H cluster provides absorption with lower and wider frequencies. Figure 2. also shows that in the wave area of 1448 - 1205 cm^{-1}, presumably, there is a shifting frequency due to the effects of induction, mesomeric and field effects that cause changing on bond strength and frequency. The shifting of frequency that occurs on absorption in the wave area of 1448 - 1205 cm^{-1}, allegedly due to additional alkyl cluster on carbonyl compounds so that the extension of the conjugation has occurred (hyperconjugation effect) and causes the C=O bond weakened and consequently, the strength of bond and frequency decrease. Presence of the

conjugation (unsaturated carbonyl group or aromatic ring) also causes the decrease of absorption frequency of vibration stretching C=O and C=C.

IV. CONCLUSIONS

Spectrum data of isolate *K. alvarezii* shows the existence of aromatic cpmpounds and the infrared spectrum data which shows the peak at wavenumber 1700 cm^{-1} is a carbonyl of function cluster C=O, wavenumber 3500 cm^{-1} shows function cluster of OH, and wavenumber 3000 cm^{-1} or 1050 cm^{-1} shows the aromatic function cluster. The function cluster of C=O is a carboxylic and amide, as well as the function cluster of OH and aromatic.

REFERENCES

[1.] Aguilera, J., Bischof, K., Karsten, U., and Hanelt, D. 2002. "Seasonal Variation in Ecophysiological Paterns in Macroalgae from an Artic fjord II. Pigmentation Accumulation and Biochemical Defense System Against High Light Stress" in *Marine Biol* 140: 1087 – 1095.

[2.] Faten, I.M., Abou-Elala,M., and Emad, A.S. 2009. "Antioxidant Activity of Extract Sei adn Purified Fraction of Marine Red Macro Algae, *Gracillaria verrucossa*", in *Australian Journal of Basic and Applied Sciences* (4): 3179 – 3185.

[3.] Glenn, E.P.and Doty,M.S., 1990. "Growth of the Seaweeds *Kappaphycus alvarezii, K. Striatum* and *Euchema Denticulatum* as Affected by Enviroment in Hawaii", in *Aquaculture* 84:245 – 255

[4.] Karsten, U. and Wiencke, C. 1999. "Factors Controlling the Formation of UV Absorbing Mycosporine Like Amino Acids in the Marine Red Algae *Palmaria Palmata* from Spitsbergen (Norway)", in *Journal of Plant Physiology* 155:407 – 415.

[5.] Kumar, K., Suresh, K., Ganesan, P.V. and Rao, S. 2008. "Antioxidant Potential of Solvents Extract of *Kappahycus alvarezii* (Doty) Doty an Edible Seaweed", in *Food Chemistry* 107:289 – 295.

[6.] Ohno, M., Largo, D.B., and Ikumoto, T. 1994. "Growth Rate, Carrageenan Yield and Gel Properties of Cultured *Kappacarrageenan* Producing Red Algae *Kappaphycus alvarezii* (Doty) Doty in the Subtropical Waters of Shikoku,Japan", in *J. Appl. Phycol* 6:1 – 5.

[7.] Sriwardhana, N., Lee, K.W., Kim, S.H., Ha, J.W., and Jeon, J.Y. 2003. "Antioxidant Activity of *Hizikia fusiformis* on Reactive Oxygen Species Scavenging and Lipid Peroxidation Inhibition", in *J. Food Science and Technology International* 9: 339 – 347.

[8.] Supratman, U. 2010. *Elusidasi Struktur Senyawa Organik Metode Spektroskopi untuk Penentuan Struktur Senyawa Organik*. Penerbit Widya Padjajaran.