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Characterization of *Caulerpa Racemosa* Fraction as Aeromonas Hydrophila Anti-Bacterial

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ABSTRACT: *Caulerpa racemosa* is one type of seaweed that is commonly found in Talango, Sumenep, Madura, Indonesia. This type could be expected to be used as an Aeromonas hydrophila anti-bacterial, so it is necessary to characterize the compound. The method used in this research is the method of the separation of *Caulerpa racemosa* fractions using chromatography column. Characterization of *Caulerpa racemosa* anti-bacterial compounds against Aeromonas hydrophila bacteria examined by using chromatography column method, and it has been found that the fraction containing anti-bacterial is fraction 7, with a ratio of eluent 4 (ethyl acetate): 6 (ethanol). Furthermore, based on the characterization tests using ultraviolet spectrophotometer, infrared spectrophotometer and LC-MS, it is known that the antibacterial compounds contained in Caulerpa racemosa fraction is flavonoid luteolin group.

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INTRODUCTION

Freshwater fish farming activities face many problems which commonly faced by fish farmers. One problem that often occurs is a bacterial disease caused by bacteria Aeromonas hydrophila. Aeromonas hydrophila bacteria, generally, causes infection in the entire body followed by the bleeding in the internal organs of fish. These acteria can be spread quickly at high stocking density which can lead to death of the seeds up to 90% [1] which can result in losses in aquaculture.

Seaweed generally produce hydrocolloid compound as primer metabolic products (metabolites). This primer metabolite is commonly processed to be gelatin, carrageenan, alginate, and others. In addition to the benefits of the products of primary metabolites, seaweed also contains secondary metabolites products. Approximately 500 natural products (chemical compounds) derived from seaweed has been identified and the largest percentage of these products are derived from the active compound (bioactive substances) which is the result of secondary metabolites of various types of seaweed [2]. One type of seaweed that is found in the water of Sumenep Madura is *Caulerpa racemosa* that can be used as a natural treatment alternative in this bacterial disease.

MATERIAL AND METHODS

Extraction

Caulerpa racemosa which is used, derived from Talango Island, Sumenep, Madura. *Caulerpa racemosa* extraction [3] is *Caulerpa racemosa* that has been dried and pulverized. A total of 50 grams of *Caulerpa racemosa* soaked with 200 ml of ethanol for 2 x 24 hours, and then evaporated.

Fraction separation of Caulerpa racemosa with chromatography column

Fraction separation is done by using chromatography column [4] with the stationary phase silica gel 70-230 mesh and mobile phase ethyl acetate: ethanol at a ratio of 100: 0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100. Fractions are further characterized as an anti-bacterial using ultraviolet spectrophotometer, infrared spectrophotometer and LC-MS.

RESULTS AND DISCUSSION

Ultraviolet Spectrophotometer

Based on the measurement data obtained ultraviolet absorption spectrophotometer wavelength of 341.40 nm and 268 nm. Flavonoids have a wavelength of 250 nm - 270 nm and 330-350 nm [5]. The results of ultraviolet spectrophotometer can be seen in Figure 1.

Infrared Spectrophotometer

Infrared spectrum of the isolated anti-bacterial compounds can be seen in Figure 2, which provides the interpretation of the data that is important in some absorption wave number 3423.41 cm-1 which shows the strain -OH group. Strain C = C are shown in wave number 1542.95 cm-1. Strain C = O shown in wave number 1739.67 cm-1. CO is shown on local strain wave number 1236.29 and 1176.50 cm-1 cm-1 and strain CH shown in wave number 2921.96 and 2852.52 cm-1 cm-1. The presence of the carbonyl group (C = O) is the typical presence of flavonoid compounds [6]. It is also supported by the results of the study [7] that the presence of functional groups OH, aliphatic CH, C = C aromatic and positive CO contains a flavonoid compound. The results of infrared spectrophotometer can be seen in Figure 2.

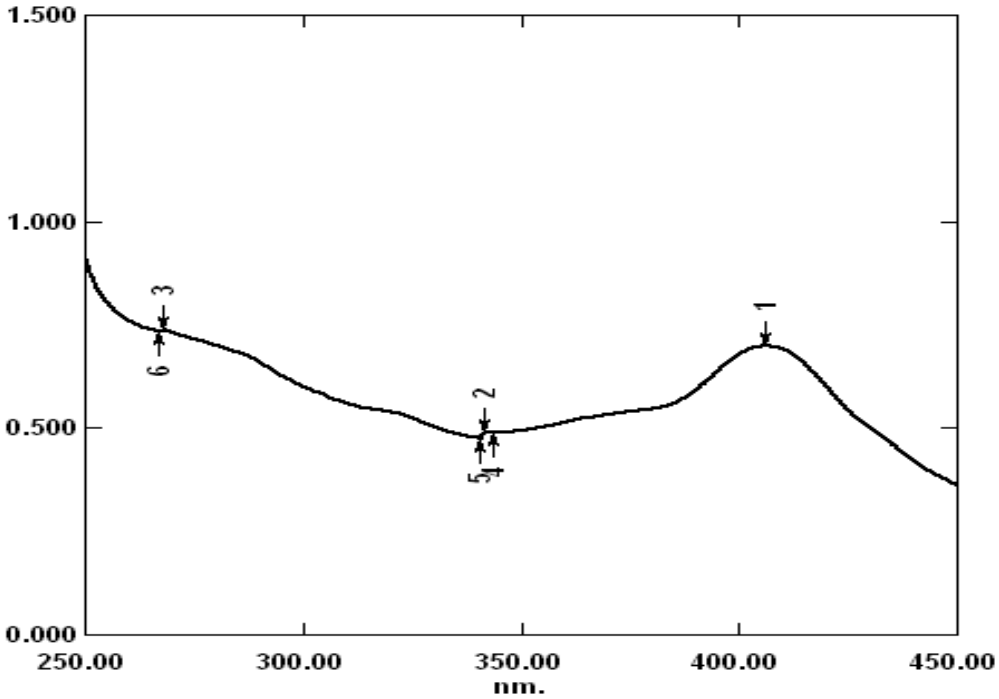

Figure 1. Spectra UV-Vis of *Caulerpa racemosa* fractions 4: 6

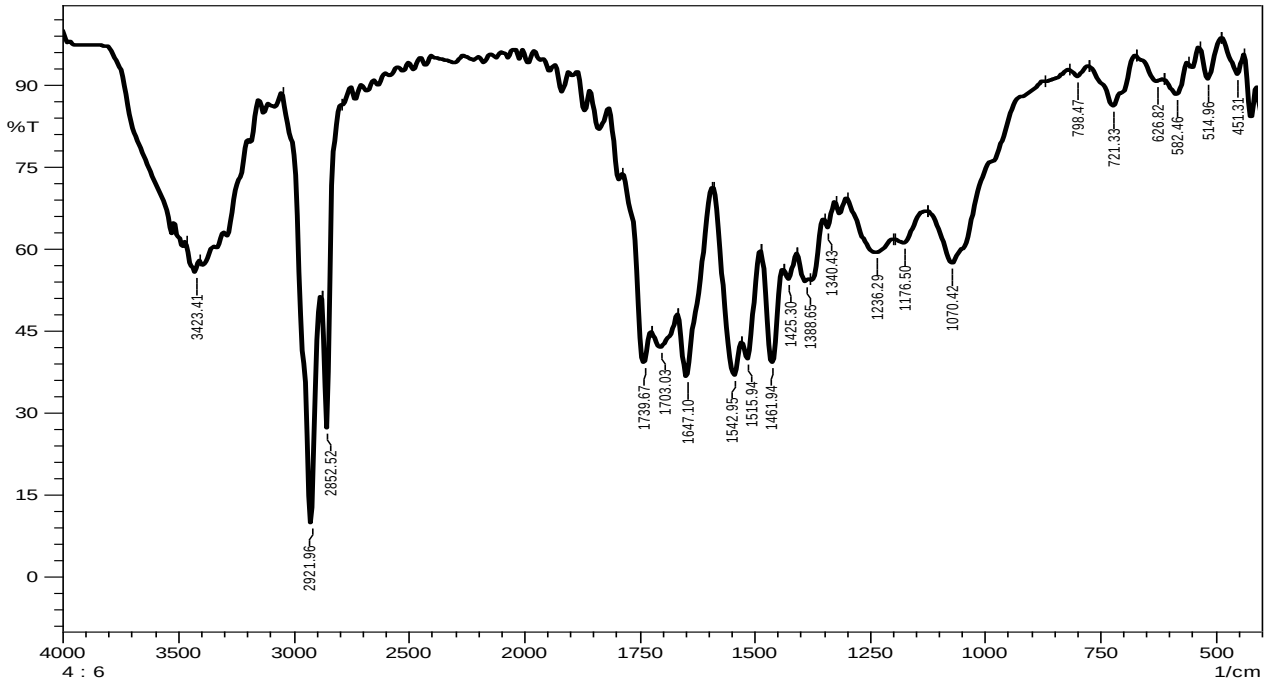

Figure 2. Spektra FTIR of Caulerpa racemosa fraction 4: 6

Liquid Chromatography - Mass Spectrophotometry

From antibacterial compounds as the result of isolation, obtains LC-MS with m / z values of 357,851 with RT 2,697. In accordance with the results of the study of Gardana et al. [8] on the analysis of polyphenolic fraction

from different sources using LC-MS, it is found that a compound which has a maximum wavelength of 268, 350 is included luteolin. It is also supported by the results of the study of Rabaneda et al. [9] on the test results LCMS phenolic compounds in cocoa which indicates that the value of m / z 447, 429, 357, 327 and 285 are Luteolin-6-C-glucoside and Luteolin-8-C-glucoside compounds. LC-MS results can be seen in Figure 3.

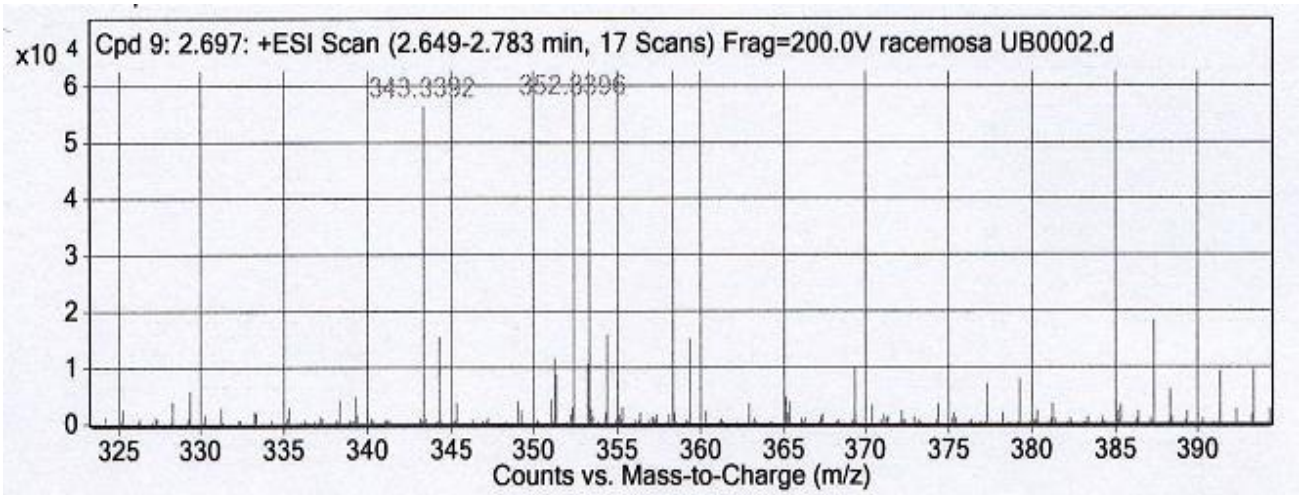

Figure 3. Results of Liquid Chromatography-Mass Spectrophotometer

DISCUSSION

Luteolin is a type of flavonoid found in many plants, including fruits, vegetables and medicinal plants [10]. Luteolin is a flavonoid derivative compounds derived from plants. This compound has many pharmacological activity and antioxidant [11]. Mechanism of flavonoids in disrupting the function of the cytoplasmic membrane is the presence of H^+ ions from phenol and its derivatives (flavonoids) will attack the polar groups (phosphate groups) so that the phospholipid molecules in the cell wall of the bacteria will break down into glycerol, carboxylic acid and phosphoric acid. In such circumstances, phospholipids are not able to maintain the shape of the cytoplasmic membrane as a result of the cytoplasmic membrane will leak and the bacteria will experience growth retardation and even death [12].

CONCLUSION

Based on the characterization tests using ultraviolet spectrophotometer, infrared spectrophotometer and LC-MS on C. *racemosa* fraction, it can be seen that the anti-bacterial compound contained is a flavonoid luteolin group.

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