Egyptian Journal of Aquatic Research (2016) xxx, xxx–xxx

HOSTED BY

ELSEVIER

National Institute of Oceanography and Fisheries

Egyptian Journal of Aquatic Research

http://ees.elsevier.com/ejar
www.sciencedirect.com

FULL LENGTH ARTICLE

Antibacterial effect of *Gracilaria verrucosa* bioactive on fish pathogenic bacteria

Maftuch *, **Isma Kurniawati, Awaludin Adam, I'ah Zamzami**

Dept. Aquaculture, Fisheries and Marine Science Faculty, Brawijaya University, Malang, East Java, Indonesia

Received 19 August 2016; revised 15 October 2016; accepted 31 October 2016

KEYWORDS

Antibacterial;
Gracilaria verrucosa;
Flavonoid;
Pathogenic bacteria

Abstract *Gracilaria verrucosa* seaweed is a type of seaweed commonly found in water. This study was conducted to investigate the effect of *G. verrucosa* on fish pathogenic bacteria to support fish farming. The method used in this research was the separation of *G. verrucosa* fractions using column chromatography. The active antibacterial fraction of *G. verrucosa* which is obtained from column chromatography indicated fractions containing antibacterial compounds. It was fraction number 3 by using an eluent 16 (ethanol): 4 (ethyl acetate). Furthermore, based on phytochemical screening, ultraviolet spectrophotometer and LC–MS analysis, antibacterial compounds contained in those fraction number 3 are Alkaloid, Flavonoid, Tannin, Phenolic compound. Based on LC–MS and UV–Vis analysis, flavonoid group, Quercetin-7-methyl-ether is a dominant group of the antibacterial compound on fraction no. 3. This fraction had moderate antibacterial activity against *Aeromonas hydrophila*, *Pseudomonas aeruginosa, Pseudomonas putida* and had weak antibacterial activity against *Vibrio harveyi* and *Vibrio algynoliticus* bacteria.
© 2016 National Institute of Oceanography and Fisheries. Hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Some secondary metabolites derived from marine algae plants have the potency to be the new material for pharmacy (Ely et al., 2004). Chemical compounds contained are the groups of polysaccharides, lipids, proteins, alkaloids and phenolic components (Almeida et al., 2011). The antibacterial characteristic which is obtained from plant products has bioactivity and can be widely studied for potential applications in cultivation systems (Reverter et al., 2014).

Castro et al. (2008) found that 31 species of methanol extracts derived from plants in Brazil had antibacterial activity against *Streptococcus agalactiae*, *Flavobacterium columnare*, and *Aeromonas hydrophila* fish pathogenic bacteria. Several recent studies have been revealed that seaweed and algae are potential sources that can be used as antimicrobial products (Al-Saif et al., 2014; Rabia et al., 2013).

Dubber and Harder (2008) showed that the methanol extract *Ceramium rubrum* (10 mg dry weight/mL) and hexane extract *Laminaria digitata* (31 mg dry weight/mL) gave a strong antibacterial activity against 16 bacterial pathogens. Other study conducted by Genovese et al. (2012) on the ethanol extract *Asparagopsis taxiformis* 100 mg/ml towards 9 types of pathogenic bacteria on fish had the best inhibition towards *V. alginolyticus* (17.0 ± 1.4 mm), *Vibrio vulnificus* (16.8

* Corresponding author.
E-mail address: maftuch@ub.ac.id (Maftuch).
Peer review under responsibility of National Institute of Oceanography and Fisheries.

http://dx.doi.org/10.1016/j.ejar.2016.10.005
1687-4285 © 2016 National Institute of Oceanography and Fisheries. Hosting by Elsevier B.V.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article in press as: Maftuch, et al., Antibacterial effect of *Gracilaria verrucosa* bioactive on fish pathogenic bacteriaGracilaria verrucosa as fish antibacterial –>. Egyptian Journal of Aquatic Research (2016), http://dx.doi.org/10.1016/j.ejar.2016.10.005

± 1.0 mm) and *Aeromonas salmonicida* bacteria (15.0 ± 0.9 mm).

In red algae, *Gracilaria verrucosa* is the third largest genus of class Rhodophyta. It is widely known that *G. verrucosa* contains many bioactive compounds with several bioactivities. Maftuch et al. (2012) and Saraswaty et al. (2015) reported that *G. verrucosa* enhances innate immune of shrimp and inhibit α glucosidase. There were not many types of research that examined antibacterial activity using in vitro on red algae called *G. verrucosa*. So in this study, the researchers would provide information about the compound of antibacterial activity contained in red algae *G. verrucosa* on various kinds of pathogenic bacteria in the water. *Gracilaria* is one of the genera with the largest number of species in the family *Gracilariaceae* (Rhodophyta) in tropical area habitat (Freile-Pelegrin and Murano, 2005). In this study, we investigate the antibacterial activity of *G. verrucosa* along with their antibacterial compounds which were extracted using specific eluent.

The bacterial species tested challenge is a type of pathogenic bacteria that easily invade the freshwater and brackish water fish. So by testing in vitro results of an extract of *Gracilaria verrucosa*, we get pertinent information about power extracts of *G. verrucosa* against freshwater and brackish water bacteria.

Materials and research method

G. verrucosa seaweed was obtained from cultivation area in Kraton Pasuruan, East Java, Indonesia. Cultivation of seaweed was conducted at fishpond with an area of about 225 Ha. The cultivation methods used are the spread and longline methods on fishpond, while technology uses a traditional farming system with polyculture shrimps, milkfish, and seaweed. The site is located at a latitude of 7° 40′ 42.64″ S and longitude 112° 51′ 13.85″ E.

The quality of the seaweed must satisfy the standard. Some of the things that affect the quality itself are the age of the plant, how to harvest and the state of the weather at the time of harvest. Seaweed is harvested when the age of plants has reached 1–1.5 months after being planted. Seaweed harvesting is done in the morning to minimize the moisture content. Harvesting is done in November when the water temperature is relatively cold. High content of agar in seaweed is in summer. Hence, the best time for harvesting seaweed is in summer (Bird and Ryther, 1990).

However, the condition of the growth of *G. verrucosa* is different in each region. In areas with relatively cool conditions, *G. verrucosa* grow at a temperature of 8–21 °C, optimum at a temperature of 12–20 °C at the beginning of May until mid-June (Ren-Zhi et al., 1984). Extraction was done using Velmurugan et al. (2012) method which had been modified. Extraction was done using maceration method with ethanol solvent during 2 × 24 h, then it was filtered using Whatman paper and evaporated using rotary evaporator.

G. verrucosa fraction separation using column chromatography

Separation fraction was performed using column chromatography (Velmurugan et al., 2012) which has been modified with a stationary phase of silica gel 70–230 mesh and mobile phase of ethanol: ethyl acetate at the ratio of 20:0, 18:2, 16:4, 14:6, 12:8, 10:10, 8:12, 6:14, 4:16, 2:18 and 0:20. Antibacterial activity against *A. hydrophila* bacteria was tested based on numbers of obtained fractions. The fraction with the best inhibition zone would be characterized and isolated for antibacterial activity against *A. hydrophila*, *Vibrio harveyi*, *Vibrio algynoliticus*, *P. aeruginosa*, *Pseudomonas putida* bacteria. Characterization of antibacterial compounds was done using phytochemical screening, ultraviolet spectrophotometer and LC–MS (Liquid Chromatography-Mass Spectrophotometer).

Phytochemical analysis

The phytochemical analysis used Nurdiani et al. (2012). It was aimed to observe the type of active compound contained in the fraction. Alkaloids, flavonoids, tannins, phenolic, steroids and saponins were analyzed. The detail of analysis was described elsewhere.

Spectrophotometer ultraviolet

One use of UV–Vis spectrophotometry i.e. can determine the chemical content of a material by measuring transmit or absorbent of a sample as a function of wavelength. UV rays wavelength ranges between 200 and 400 nm. The Rays seem (UV–Vis) to have a wavelength of 400–750 nm. The magnitude of the radiation absorption is proportional to the number of analyte molecules absorption, thus can be used for quantitative analysis.

Wavelength accuracy is done usually using samples containing a series of very sharp peaks such as aqueous perchloric and holmium oxide. Alternatively, it could use the measurement of the emission of the lamp. In addition, it can be done by measuring the emission from the lamp (Upstone and Seer Green, 2012).

UV–Vis spectrum measurement was performed at a wavelength of 200–800 nm. A total of 1 mg from the best fraction was dissolved in 100 ml of ethanol then it was measured its wavelength. Spectrophotometer used was Shimadzu UV-1601 PC with a medium scan speed and the sampling interval of 0.5 s (Chatterjee et al., 2011).

LC–MS (liquid chromatography–mass spectrophotometer)

LC–MS analysis was done to the best fraction which had the antibacterial characteristic. Isolates and eluent from LC went into the *capillary*. Then, isolate and eluent were sprayed through *Taylor cone* and analyzed using mass spectrophotometer (Pitt, 2009).

Antibacterial test

Antibacterial activity was observed using agar diffusion method following the method of Prihanto et al. (2012) and Genovese et al. (2012) with slight modification. Pure cultures of *A. hydrophila*, *V. harveyi*, *V. algynoliticus*, *P. aeruginosa*, *P. putida* bacteria were taken from the Laboratory of Microbiology Laboratory in Faculty of Medicine, University of Brawijaya, Malang. Furthermore, the cultures were grown in liquid media *Nutrient broth* (NB) and incubated at 35 °C for 3 h so that it could form the same turbidity with *Mc Farland* standard

Please cite this article in press as: Maftuch, et al., Antibacterial effect of *Gracilaria verrucosa* bioactive on fish pathogenic bacteriaGracilaria verrucosa as fish antibacterial –>. Egyptian Journal of Aquatic Research (2016), http://dx.doi.org/10.1016/j.ejar.2016.10.005

Download English Version:

https://daneshyari.com/en/article/8875269

Download Persian Version:

https://daneshyari.com/article/8875269

Daneshyari.com