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ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF THE RED ALGA *GRACILARIA VERRUCOSA* FROM THE NORTH COAST OF JAVA, SEMARANG, INDONESIA

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Abstract: Marine algae and bacteria are inexhaustible sources of chemical compounds that produce a wide variety of biologically active secondary metabolites, an important target for pharmaceutical industries. They contain bioactive compounds that play an important role against various diseases and against the aging process because they protect cells from oxidation. The purpose of this research is to analyze the potential antibacterial properties of the red alga Gracilaria verrucosa and its associated surface bacteria against six bacterial strains Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Vibrio harveyi, Vibrio parahaemolyticus and Vibrio alginolyticus. And also the antioxidant capacity of the algae by inhibiting the 2,2-diphenyl-1-picrylhydrazyl (DPPH). The extracts obtained were analyzed qualitatively by phytochemical screening. The total phenolic was determined by Folin-Ciocalteu reagent. Result showed that the methanol extract was the most active. It was active in 4 out of 6 strains objects antibacterial tests. The ethyl acetate extract, however, showed antioxidant activity (EC50 = 173 ppm) supported by the phytochemical study of the compounds of the extract. Content and the presence of phenolic compounds (14.58 mg Gallic acid equivalent / g of dry sample) and flavonoids (presence) known for their antioxidant effects were in accordance with the data obtained. The phytochemical study showed that G. verrucosa contained many bioactive compounds with diverse biological properties. Symbiotic bacteria isolated from the alga showed no antibacterial activity.

Résumé: Les algues marines et les bactéries sont des sources inépuisables de composés chimiques qui produisent une grande variété de métabolites secondaires biologiquement actifs, une cible importante pour les industries pharmaceutiques. Ils contiennent des composés bioactifs qui jouent un rôle important contre diverses maladies et contre le processus de vieillissement, car ils protègent les cellules contre l'oxydation. Le but de cette recherche est d'analyser les propriétés antibactériennes potentielles de l'algue rouge Gracilaria verrucosa et ses bactéries de surface associées contre six souches bactériennes Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Vibrio harveyi, Vibrio parahaemolyticuset Vibrio alginolyticus. Aussi, le pouvoir antioxydant de l'algue par l'inhibition de la 2,2-diphényl-1-picrylhydrazyle (DPPH). Les extraits obtenus ont été analysés qualitativement par criblage phytochimique. Le phénolique total a été déterminé par le réactif de Folin-Ciocalteu. Les résultats ont montré que l'extrait de méthanol a été la plus active. Il a été actif dans 4 des 6 souches objets de tests antibactériens. L'extrait d'acétate d'éthyle, cependant, a montré une activité anti-oxydante (EC50 = 173 ppm), soutenu par l'étude phytochimique des composés de l'extrait. Le contenu et la présence de composés phénoliques (14,58 mg d'acide gallique équivalent / g d'échantillon sec) et les flavonoïdes (présence) connus pour leurs effets antioxydants étaient en conformité avec les données obtenues. L'étude phytochimique a montré que G. verrucosa contient de nombreux composés bioactifs ayant des propriétés biologiques différentes. Les bactéries symbiotiques isolées de l'algue n'ont montré aucune activité antibactérienne.

Key Word: *●Antibacterial ● Antioxidant ● Red Algae ● Gracilaria verrucosa*

INTRODUCTION

Numerous studies have revealed the anti-bacterial properties in different macro-algae (Bansemir *et al.*, 2005; Farid *et al.*, 2009; Villarreal-Gomez *et al.*, 2010). On the other hand, algae living near the sea surface are constantly exposed to ultraviolet rays and oxidation air, which usually leads to the formation of free radicals and other oxidants. However, no damage due to oxidation was observed in the structure of seaweed which suggests that they possess defense mechanisms against oxidation (Mallick & Mohn, 2000; Matanjun *et al.*, 2007)

In addition, these algae welcome to the surface of many different strains of bacteria that also produce potentially active molecules. It would be a mutually beneficial relationship between algae and bacteria. These relationships based on the ability of algae to produce organic compounds and oxygen used by bacteria called then "symbiotic bacteria." In part against the bacteria play an important role in maintaining the health of the host organism by the production of bioactive secondary metabolites (Bolinches *et al.*, 1988).

Seaweeds, considered as source of bioactive compounds, produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae (Newman *et al.,* 2003). Seaweeds have been screened extensively to isolate life saving drugs or biologically active substances all over the world (Shanmugam *et al.*, 2001; Khotimchenko *et al.*, 2002).

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It is necessary to find a natural resource grown in mass and thus be exploited by the pharmaceutical and bio-medical. Thus, the purpose of this study is an alga grown in a traditional aquaculture sea of Indonesia: *Gracilaria verrucosa,* mainly grown for food industry.

The objectives are to evaluate (1) the antibacterial activities of the macro-algae and its associated bacteria on its surface towards six bacterial species: *E. coli, S. aureus, P. mirabilis, V. harveyi, V. parahaemolyticus, V. alginolyticus*; (2) the antioxidant activities of *G. verrucosa*. *E. coli, S. aureus*, and *P. mirabilis* are human pathogens species showing resistance to antibiotics. While *V. harveyi, V. parahaemolyticus* and *V. alginolyticus* are marine bacteria responsible for infections in marine organism or humans through ingestion or contact with marine animals infected.

MATERIALS AND METHODES

Algal collection

The study was conducted in May 2012 - June 2012. Sampling of the red alga *G. verrucosa* was performed in a traditional aquaculture / "tradional pond" Mangkang district, Semarang, Central Java, Indonesia. The samples were washed with seawater after rewashed with fresh water to remove salt remaining algae. Then, they were cut into small pieces (0.5 cm) and were dried in the open air. Isolation of symbiotic bacteria, stress tests and sensitivity tests were performed at the Laboratory of Microbiology of the University FPIK-Diponegoro, Semarang. The phytochemical screening and analysis of total phenolic were performed in the laboratory of Chemistry of the University of Diponegoro MIPA.

Isolation and purification of bacteria symbiotic algae

1 gr of fresh *G. verrucosa* was introduced into a test tube containing 9 ml of sterile water. Different dilutions were then made up to 10-7 dilution using sterile pipettes. For repeatability, two dilution ranges were made. A volume of 100 µL of each dilution was then inoculated in Petri dishes containing the universal medium Zobell 2216E seawater-based volume which was spread with a sterile glass rake. The Petri dishes were incubated for 48 h at 26 ° C.

The bacterial purification was achieved by sub-culturing single colonies on solid medium to another to obtain pure cultures. Bacterial colonies are selected based on morphology and purified by the method of streaking to obtain pure cultures of bacteria (Madigan *et al*., 2000). According to Waluyo (2007), the morphology of bacterial colonies is determined by the shape, surface, edge and color of the colony. Pure cultures are then stored by subculturing in nutrient Milieus NA and grown in nutrient broth (NB).

Antibacterial activity of symbiotic bacteria

Microbiological assay of antibacterial activity of the symbiotic bacteria was through the technique of double layer Dopazo *et al.* (1988). Zobell on a medium based solid seawater paper discs impregnated with symbiotic bacteria isolates were deposited. After 24 h, the medium Zobell based soft freshwater previously inoculated with the bacteria to be tested was poured into the first medium. The Petri dishes were incubated for 5 days at room temperature atmosphere. Every 24 hours, the diameters of the zones of inhibition of microbial growth are measured (Volk & Wheleer 1984).

Preparation of algal extracts

One part of the dried algae was suspended by stirring in distilled water (50 $g.L^{-1}$ of dried weight) with an Ultra-turrax (2 hours) at 4°C. After centrifugation (30 min, 3000 g, 4°C) and filtration (Whatman cat n° 1822 047), the supernatant was lyophilised and we obtained then the aqueous extract (Extract A). For organic extracts, the dried algae were suspended by stirring in ethanol 95° (200 g in 300 mL) with an Ultra-turrax (2 hours) at 4°C. After centrifugation (30 min, 3000 g, 4°C), the resultant pellet was re-extracted five times in the same way. The alcoholic extracts were combined and evaporated under vacuum at low temperature (<40°C). Distilled water (100 mL) was then added and was partitioned with methylene chloride (4 x 100 mL). The aqueous phases were collected, lyophilised, re-suspended in absolute ethanol (100mL), filtered and concentrated under vacuum at low temperature (Extract B). The organic phases were collected, then they were dried during 24 hours under Na_2SO_4, filtered and concentrated under vacuum at low temperature (Extract C). These three phases were stored at -40°C before use (Hellio *et al*., 2000).

Antibacterial activity

The antibacterial activity of extract and symbiotic bacteria were evaluated on *E.coli, S. aureus, P. mirabilis, V. harveyi, V. alginolyticus* and *V. parahaemolyticus.* The bacterial strains were cultured in solid Zobell freshwater-based. The media were incubated for 48 hours at room temperature (27°C). The extract of *G. verrucosa* then seeded by spreading the cultures of pathogenic bacteria.

After incubation for 48h, the potential antibacterial activities were revealed by the appearance of a zone of inhibition. The microbiological assay of antibacterial activity of symbiotic bacteria was by diffusion method according to the Kirby-Bauer technique. Each pathogen was inoculated by spreading on a solid medium Zobell. The extracts of *G. verrucosa* were inoculated onto sterile paper discs. Different concentrations of each extract were used, i.e. 5, 20, 35, 50, 65 µgr per disk. The installation of disks on the middle Zobell was aseptically using sterile forceps. The negative control corresponds to a disk soaked in nutrient broth; the positive control corresponds to a disk soaked in the antibiotic Amoxicillin. The Petri dishes were incubated for 4 days at room temperature. Every 24 hours, the diameters of zones of inhibition of microbial growth were measured (Volk & Wheleer, 1984).

Determination of antioxidant activities

The determination of the activity of DPPH was performed according to the protocol described by Molyneux (2003). Control or blank was prepared by reacting 0.2 mL of methanol with 3.8 mL of DPPH. Then, the solution was allowed to stand for 30 minutes in the dark. Finally, the absorbance of this solution was measured at a wavelength of 516 nm using a spectrophotometer Shimadzu UV-VIS 1601. It corresponded to the maximum absorbance A_0.

The determination of the antioxidant activity of the extract *Graciliara* in methanol and extracted with ethyl acetate was carried out by preparing solutions of the two different samples at concentrations 20, 40, 60, 80 and 100 ppm. Then, 0.2 mL of each solution was mixed with 3.8 mL of DPPH to 50 microns. After 30 minutes, the absorbance at 515 nm of solutions is measured. For comparison, the same solution of quercetin at concentrations of 20, 40, 60, 80 and 100 ppm was made. The percentage reduction in the percentage of DPPH or anti-radical activity was calculated using the following equation:
DPPH (%) = {(A0-AP)/A0} x 100 %

A_0 is the absorbance of the blank
A_P is the absorbance of the sample

The extract concentration providing 50% of the activity of free radical scavenging (EC50) was calculated from the graph of percentage reductions depending on the concentration.

Determination of Total Phenols

The amount of total phenols in the extract of *G. verrucosa* methanol and ethyl acetate was determined using the colorimetric method of Folin-Ciocalteu (Singleton *et al.*, 1999).

A total of 0.2 ml of each extract is reacted with 15.8 ml of distilled water and 1 ml of Folin-Ciocalteu reagent, and the solution is homogenized and allowed to stand for 8 minutes. Then, 3 ml of 20% Na_2CO_3 was added to the solution. The solution was stirred until the solution became homogeneous and left to stand for 30 minutes at room temperature. Finally, the absorbance of the extract solution was measured at a wavelength of 765 nm using a UV-VIS spectrophotometer. The measurements are performed in three replicate. The total phenolic content was expressed as mg gallic acid / g extract.

Phytochemical screening of algal extract

The method used in this experiment was the addition of certain reagents giving a positive reaction if the extract belongs to the chemical class Wanted (Harborne, 1973). This analysis determined the presence or absence of different compounds: alkaloids, saponins, flavonoids, tannins, steroids and terpenoids.

Statistical Analysis

The parameters of antibacterial activity were evaluated in triplicate, and results were expressed as the average and standard deviation. The mean values and standard deviation were calculated using the program Microsoft Office Excel 2010.

RESULTS

Antibacterial activities of symbiotic bacteria

From *G. verrucosa*, we obtained 19 isolations of bacteria. Isolated colonies were not chosen at random. Strains that differed morphologically by the color, shape, terrain, surface and size were selected. In addition, the proportions of the boxes with respect to the size, shape and color were generally met. Strains were mainly isolated from dilutions 10-3 and 10-4.

Symbiotic bacteria isolated from *G. verrucosa* presented no antibacterial activity against strains of pathogenic bacteria tested. Qualitative tests antibacterial symbiotic bacteria against pathogenic bacteria were all negative. Therefore, it was not necessary to conduct quantitative tests. It was however noticed the growth of most symbiotic strains. This suggested that the bacteria associated with *G. verrucosa* coexisted with bacteria.

Table 1. Antibacterial Activity of *G. verrucosa* against *E. coli.*

Concentrations (µg)	Inhibition Zone (mm)											
	GM* 24**	**GEA* 24**	**GH* 24**	**GM 48****	**GEA 48**	**GH 48**	**GM 72****	**GEA 72**	**GH 72**	**GM 96****	**GEA 96**	**GH 96**
10	16,00	0,00	0,00	14,00	9,34	0,00	13,90	10,12	0,00	13,20	11,66	0,00
30	12,20	0,00	0,00	13,12	11,27	0,00	11,50	10,75	0,00	10,60	10,10	0,00
50	14,00	0,00	0,00	13,40	12,18	0,00	12,63	11,70	0,00	12,28	10,27	0,00
70	13,30	0,00	0,00	12,67	13,32	0,00	12,73	12,37	0,00	12,25	11,23	0,00
90	11,80	0,00	0,00	12,32	14,15	0,00	13,49	13,17	0,00	11,19	12,33	0,00

** GM = G. verrucosa in Methanol; GEA = G. verrucosa in Ethyl Acetate; GH = G. verrucosa in N-hexane*
*** 24, 48, 72, 96 = time of incubation in hours*

Table 2. Antibacterial Activity of *G. verrucosa* against *P. mirabilis.*

Concentrations (µg)	Inhibition Zone (mm)											
	GM* 24**	**GEA* 24**	**GH* 24**	**GM 48****	**GEA 48**	**GH 48**	**GM 72****	**GEA 72**	**GH 72**	**GM 96****	**GEA 96**	**GH 96**
10	0,00	0,00	0,00	0,00	0,00	0,00	10,40	0,00	0,00	0,00	0,00	0,00
30	0,00	0,00	0,00	0,00	0,00	0,00	11,21	0,00	0,00	9,32	0,00	0,00
50	0,00	0,00	0,00	0,00	0,00	0,00	13,00	0,00	0,00	0,00	0,00	0,00
70	0,00	0,00	0,00	0,00	0,00	0,00	10,85	0,00	0,00	0,00	0,00	0,00
90	0,00	0,00	0,00	0,00	0,00	0,00	11,24	0,00	0,00	0,00	0,00	0,00

** GM = G. verrucosa in Methanol; GEA = G. verrucosa in Ethyl Acetate; GH = G. verrucosa in N-hexane*
*** 24, 48, 72, 96 = time of incubation in hours*

Table 3. Antibacterial Activity of *G. verrucosa* against *V. harveyi.*

Concentrations (µg)	Inhibition Zone (mm)											
	GM* 24**	GEA* 24	GH* 24	GM 48**	GEA 48	GH 48	GM 72**	GEA 72	GH 72	GM 96**	GEA 96	GH 96
10	0,00	0,00	0,00	11,17	0,00	0,00	10,82	0,00	0,00	10,80	0,00	0,00
30	0,00	0,00	0,00	9,65	0,00	0,00	9,65	0,00	0,00	10,53	0,00	0,00
50	0,00	0,00	0,00	10,19	0,00	0,00	10,60	0,00	0,00	10,99	0,00	0,00
70	0,00	0,00	0,00	10,10	0,00	0,00	10,23	0,00	0,00	10,63	0,00	0,00
90	0,00	0,00	0,00	10,50	0,00	0,00	10,57	0,00	0,00	10,70	0,00	0,00

** GM = G. verrucosa in Methanol; GEA = G. verrucosa in Ethyl Acetate; GH = G. verrucosa in N-hexane*
*** 24, 48, 72, 96 = time of incubation in hours*

Table 4. Antibacterial Activity of *G. verrucosa* against *V. parahaemolyticus.*

Concentrations (µg)	Inhibition Zone (mm)											
	GM* 24**	GEA* 24	GH* 24	GM 48**	GEA 48	GH 48	GM 72**	GEA 72	GH 72	GM 96**	GEA 96	GH 96
10	0,00	0,00	0,00	0,00	0,00	0,00	10,00	0,00	0,00	10,00	0,00	0,00
30	0,00	0,00	0,00	0,00	0,00	0,00	10,23	0,00	0,00	10,50	0,00	0,00
50	0,00	0,00	0,00	0,00	0,00	0,00	9,33	0,00	0,00	10,90	0,00	0,00
70	0,00	0,00	0,00	0,00	0,00	0,00	9,11	0,00	0,00	9,85	0,00	0,00
90	0,00	0,00	0,00	0,00	0,00	0,00	10,00	0,00	0,00	9,18	0,00	0,00

** GM = G. verrucosa in Methanol; GEA = G. verrucosa in Ethyl Acetate; GH = G. verrucosa in N-hexane*
*** 24, 48, 72, 96 = time of incubation in hours*

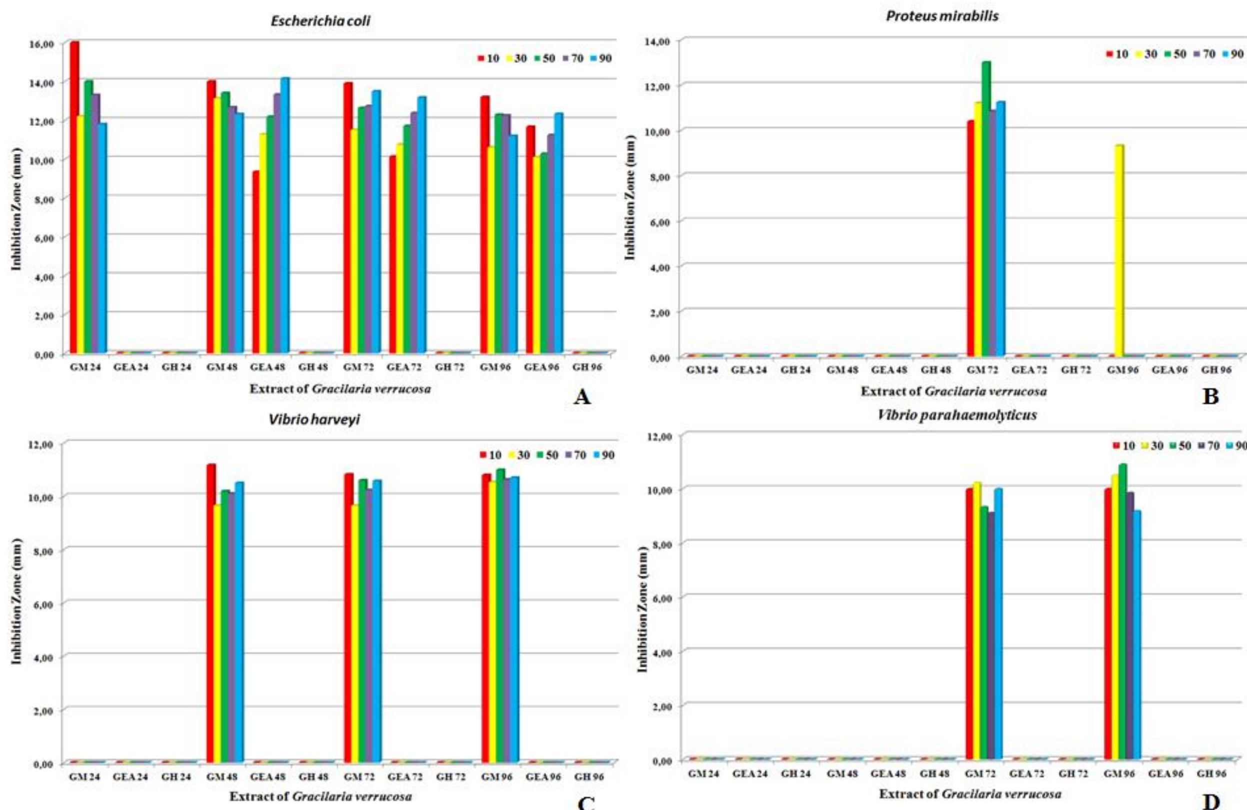

Figure 1. Antibacterial Acitivity of *G. verrucosa* against; **(A)** *E. coli,* **(B)** *P. mirabilis,* **(C)** *V. harveyi*, **(D)** *V. parahaemolyticus.*

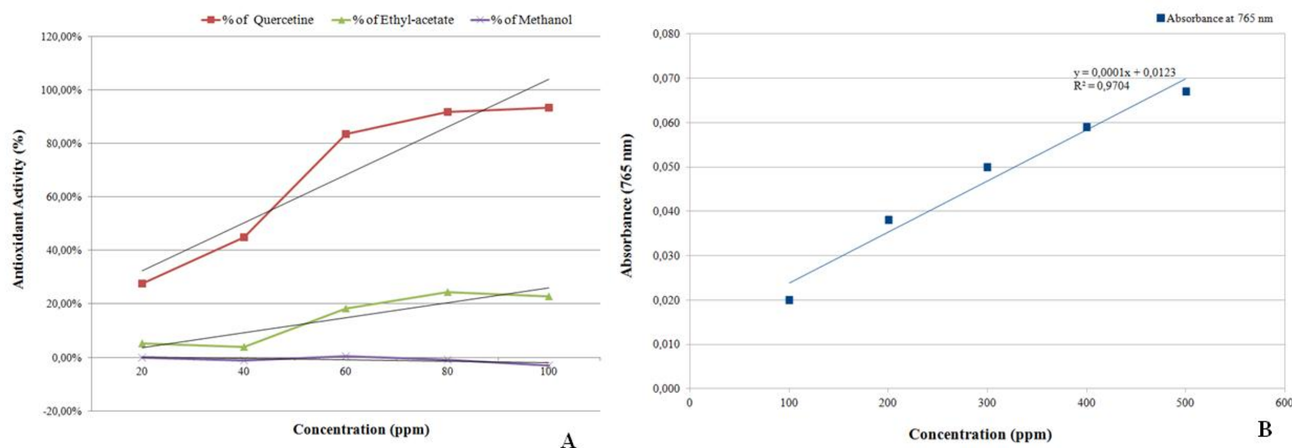

Figure 2. (A) Antioxidant activity of G. verrucosa represented in percentage of discoloration (B) Total Phenolic Content of G. verrucosa

Antibacterial of G. verrucosa

Based on the qualitative tests methanol extract was the most active. It was active against strains *of E. coli, P. mirabilis, V. harveyi* and *V. parahaemolyticus*. The extract in ethyl acetate was only active against *E. coli.* It was observed that the methanol extract of *G. verrucosa* had the best antibacterial activity, especially visible to the strain of *E. coli*. Indeed, a 16 mm diameter growth inhibition of *E. coli* appeared at a concentration of 10 µg after 24 hours. Extract of *G. verrucosa* in all three solvents, i.e; methanol, ethyl acetate and n-hexane, showed no antibacterial activity against *S. aureus* and *V. alginolyticus* indicated by the absence of inhibition zone. Even though in the 10 µg methanols extract of *G. verrucosa* in 24 h incubation did show an inhibition zone of 8.30 ± 0.6 mm (data not shown).

Table 1, 2, 3 and 4 showed the results of antibacterial activity of *G. verrucosa* against *E. coli, P. mirabilis, V. harveyi* and *V. parahaemolyticus*.

Phytochemical of the methanol and ethyl acetate extract

Depending on the nature of the solvent, the composition of the extract changed. In the methanol extract, the analysis revealed the presence of compounds such as saponins, steroids, flavonoids, polyphenols and alkaloids. While in ethyl-acetate extract, the tests revealed the presence of steroids, quinones, polyphenols and flavonoids. The results were recorded as – (negative) or + (positive).

Antioxidant activities of the extract with ethyl acetate and methanol

Measuring the effectiveness of the antioxidant nature of a substance was given by the parameter EC50; EC50 is the effective concentration of substrate to produce a 50% reduction of DPPH. The stronger antioxidant activity of the substrates was performed by the lowest concentration. The test was initiated with the measurement of DPPH absorbing activity in methanol as absorbance control. Additional controls were provided by the 515 nm absorbance of a standard solution of quercetin at different concentrations. Test of antioxidant activity was carried out on the methanol extract and ethyl acetate *G.verrucosa.*

From the equations of linear regressions, the effective concentrations of solutions producing 50% reduction of DPPH were calculated. The EC50 of quercetin was 39.33 ppm, while the ethyl acetate was 172.67 ppm. The standard solution of quercetin was clearly a high antioxidant activity. The extract obtained by the solvent ethyl acetate showed antioxidant activity that was lower than quercetin, however its activity was potentially high. Furthermore, the correlation coefficient of methanol was overly low to allow data mining on EC solution.

Totals phenolic G. verrucosa

The analysis of the calibration curve (Figure 2) of Gallic acid gave the regression equation Y = 0.0001 x 0.0123. The coefficient of determination (R^2) was 0.97. If R-squared was close to 1, it indicated that the regression equation was linear. A calibration curve was to determine the unknown concentration of phenolic compounds of *G. verrucosa*. The result of the analysis of the total phenolic compounds was expressed in equivalents mg gallic acid / g of dry sample.

DISCUSSION

The extracts and active constituents of various algae have been shown to possess the antibacterial activity in vitro against Gram-positive and Gram-negative bacteria. The production of antimicrobial activities was considered as an indicator of the capacity of the seaweeds to synthesize bioactive secondary metabolites (del Val *et al.*, 2001). There are numerous reports of compounds derived from macro algae with a broad range of biological activities, such as antibacterial (Nair *et al.*, 2007), antiviral (Richards *et al.*, 1978), antitumoral (Espeche *et al.*, 1984), anticoagulant (Athukorala *et al.*, 2006) and antifouling (Marechal *et al.*, 2004). Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae (Newman *et al.*, 2003). Also, considering their great taxonomic diversity, investigations

related to the search of new biologically active compounds from algae can be seen as an almost unlimited field.

The growth of pathogenic bacteria was inhibited by the release of secondary metabolites from the extract of *G. verrucosa*. This was indicated by the formation of inhibition zones around the extract. The methanol extract of *G. verrucosa* was most active against pathogenic bacteria inhibiting the growth of 4 out of 6 pathogenic bacteria. The presence of secondary metabolites in algae is a form of adaptation to the environment. According to Murniasih (2005), secondary metabolites serve as defense against infection, but they also allow interactions with other organisms, and finally they help the process of reproduction.

There was no inhibition on the growth of pathogenic bacteria in symbiotic bacteria. Pathogenic bacteria are capable of neutralizing the secondary metabolites of symbiotic bacteria. When two competing bacterial species grown in the same medium, a species will produce a compound that can be toxic to other species, so that the growth of the species will be disturbed. In this case, pathogenic bacteria may have developed defense mechanisms to deal with the elements that threaten their survival. It is also possible that symbiotic bacteria have no effect on pathogenic bacteria.

The antibacterial activity of the methanol extracts and extracted with ethyl acetate was decreasing in function of time of incubation. This revealed that the secondary metabolites of the extract have a bacteriostatic effect and not bactericidal. They therefore inhibited growth and reproduction of pathogenic bacteria but do not kill.

According to Jawetz *et al.* (1996), the mechanism of antimicrobial action can be achieved by four different ways: first by the inhibition of the synthesis or activation of enzymes which damage the bacterial cell wall, thus eliminating the ability to grow and causes lysis of the cell. Second is by direct action on the cell membrane by affecting cell permeability leading to leakage of intracellular compounds. Third is the disruption of the functioning of the bacterial ribosome, thereby inhibiting protein synthesis. Fourth is by interfering with the metabolism of nucleic acids leading to a loss of function of the synthesis of the cell.

However, the best antibacterial activity was performed by methanol. This may be due to the fact that methanol is a highly polar solvent. Inhibition zones appeared on four of six strains of pathogenic bacteria. However, these four strains are all Gram-negative. Gram-negative have their outer membrane partially hydrophilic substances such as lipopolysaccharide and "porins" that are barriers to hydrophobic compounds. It is possible that the polar compounds present in the methanol extract through porins in the outer membrane, resulting in transport and easier entry antibacterial compounds into the bacterial cell.

The evaluation of ant*ioxidant activity of the red alga* G. verrucosa was determined by measuring the free radical scavenging using DPPH. The free radical scavenging by antioxidant depends on two types of mechanisms (Popovici *et al.*, 2009), i.e. the release of the hydrogen atom of the hydroxyl group and the release of an electron.

An EC50 of 172.67 ppm was found for solvent extracted ethyl acetate. An EC50 of 39.33 was found for quercetin. However, no anti-radical activity was found in the methanol solvent. Several factors affect the evaluation of the antioxidant potential. The absorbance at 515-520 nm of DPPH may decrease under the action of light and may vary depending on the type of sample solvent.

Quercetin as one of the member of flavonoids, is known to be a powerful antioxidant. The EC50 value for the solvent extract ethyl acetate is higher than that of quercetin. However, this value remains high. Thus, in extract ethyl acetate, the compounds may have an antioxidant role. *G. verrucosa* is a potential source of natural antioxidants.

The content of phenolic compounds *G. verrucosa* is determined using the Folin-Ciocalteu reagent and is expressed in equivalent Gallic acid (GAE). The antioxidant activity of phenolic compounds is often due to phenolic acids and flavonoids (Faujan-Huda, 2009).

Phenolic compounds have a wide range of biological activities such as antibacterial activity, anti-inflammatory, antiviral, and anti-carcinogenic. Many of these biological actions have been attributed to the antioxidant properties of phenolic compounds. A number of studies have pointed out that alga polyphenols are associated with antioxidant activity and play an important role in stabilizing lipid peroxidation (Hoez, 2010). Thus, the search for antioxidant activity related to the research of phenolic compounds and total flavonoids. The study showed the presence of 14.52 mg gallic acid equivalent / g dry sample extracted with ethyl acetate and 7.86 mg gallic acid equivalent / g sample Dry in the methanol extract.

According to Abu Elalla and Shalaby (2009), macro-algae *G. verrucosa* contains phenolics. The solvent extract of Ethyl Acetate would own 31-mg Gallic acid equivalent / g of dried seaweed. In addition, the results of this study show that phenolic compounds play an important role in the antioxidant activities of seaweed *G. verrucosa*. The extract of *G. verrucosa* obtained with methanol, contains 21.63 mg of phenol per gram of sample. The different result obtained may be due to the fact that the environmental parameters of the sampling of the species *G. verrucosa* are different according to the study. Indeed, pH, salinity, temperature are parameters that can influence the production of secondary metabolites of organisms (Heo, 2006).

The phytochemical study showed that the methanol extract contained saponins, steroids, phenolic composite including flavonoids and alkaloids. While the extracted ethyl-acetate contained steroids, quinones, phenolic compounds and flavonoids. It also showed that the methanol extract had antibacterial activity in contrast to ethyl acetate. Many biological properties have been found in these compounds in *G. verrucosa* including antibacterial and antioxidant activities.

CONCLUSION

The study highlighted the antioxidant and antimicrobial of *G. verrucosa*. The ethyl acetate extract was found to posses a strong antioxidant activity. The methanol extract was effective antibacterial activity against both human pathogenic

bacteria against pathogenic bacteria and marine. The phytochemical study showed that *G. verrucosa* contained many bioactive compounds with diverse biological properties. It is possible that these compounds play an important role in antioxidant and antibacterial activities. Thus, the alga *G. verrucosa* is a natural product that could be a new source for applications in the pharmaceutical, food and aquaculture.

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REFERENCES

[1] Abou Elalla, F.M. & E.A. Shalaby. Antioxidant Activity of Extract and Semi- Purified Fractions of Marine Red Macroalga, *Gracilaria verrucosa*. *J. Bas. Appl. Sciec.* 3 (4): 3179-3185. 2009.

[2] Athukorala, Y, K.W. Lee, S.K. Kim & Y.J. Jeon. Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. *Biores. Technol*. 98: 1711-1716. 2006.

[3] Bansemir, A., M. Blume, S. Schröder & U. Lindequist. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquacul.* 252: 79-84. 2006.

[4] Del Val, G.A., G. Platas, A. Basilio, A. Cabello, J. Gorrochategui, I. Suay, F. Vicente, E. Portillo, J.M. del Rio & G.G. Reina. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Inter. Microbiol.* 4:35-40. 2001.

[5] Espeche, M.E, E.R. Fraile & A.M.S. Mayer. Screening of Argentine marine algae for antimicrobial activity. *Hydrobiol*. 116/117: 525-528. 1984.

[6] Farid, Y., S. Etahiri & O. Assobhei. Activité antimicrobienne des algues marines de la lagune d'Oualidia (Maroc): Criblage et optimisation de la période de la récolte. *J. Appl. Biosci*. 24: 1543 – 1552. 2009.

[7] Harborne, J.B. *Phytochemical methods*. London. Chapman and Hall, Ltd. pp. 49-188. 1973.

[8] Heo, S.J. Antioxidant Activities of Red Algae from Jeju Island. *Algae.* 2: 149-156. 2006.

[9] Hellio, C., G. Bremer, A.M. Pons, Y. Le Gal & N. Bourgougnon. Inhibition of the development of microorganisms (bacteria and fungi) by extracts of marine algae from Brittany, France. *Appl. Microbiol. Biotechnol*. 54: 543-549. 2000.

[10] Jawetz, E., J.L. Melnick & E.A. Adelberg. *Medical microbiology.* Appleton & Lange, East Norwalk, CT. 1989.

[11] Mallick, N. & F.H. Mohn. Reactive oxygen species: Response of algal cells. *J. Plant Physiol.* 157:183-193. 2000.

[12] Matanjun, P., S. Mohamed, N.M. Mustapha, K. Muhammad & C.H. Ming. Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. *J. Appl. Phycol*., 20: 367–373. 2008.

[13] Marechal, J.P., G. Culioli, C. Hellio, H. Thomas-Guyon, M.E. Callow, A.S. Clare & A. Ortalo-Magné. Seasonal variation in antifouling activity of crude extracts of the brown alga *Bifurcaria bifurcata* (Cystoseiraceae) against cyprids of *Balanus amphitrite* and the marine bacteria *Cobetia marina* and *Pseudoalteromonas haloplanktis*. *J. Experimen. Mar. Biol. Ecol.* 313:47-62. 2004.

[14] Murniasih T. Substansi Kimia Untuk Pertahanan Diri dari Hewan Laut Tak Bertulang. *Oseana.* XXX, 2 : 19-27. 2005.

[15] Nair, R., R. Chabhadiya & S. Chanda. Marine algae: Screening for a potent antibacterial agent. *J. Herb. Pharmaco*. 7:73-86. 2007.

[16] Newman, D.J, G.M. Cragg & K.M. Snader. Natural products as sources of new drugs over the period 1981-2002. *J. Nat. Prod.* 66:1022-1037. 2003.

[17] Popovici, C., I. Saykova & B. Tylkowski. Evaluation de l'activité antioxydant des composés phénoliques par la réactivité avec le radical libre DPPH. *Revue de Génie Industriel.* 4 :25-39. 2009.

[18] Richards, J.T., E.R. Kern, L.A. Glasgow, J.C. Jr. Overall, E.F. Deign & M.T. Hatch. Antiviral activity of extracts from marine algae. *Antimicrobial Agents and Chemotherapy*. 14:24-30. 1978.

[19] Villarreal-Gómez, L.J, I.E. Soria-Mercado, G. Guerra-Rivas & N.E. Ayala-Sánchez. Antibacterial and anticancer activity of seaweeds and bacteria associated with their surface. *Revista de Biología Marina y Oceanografía*. 45 (2): 267-275. 2010.

[20] Wheeler, M.F. & W.A. Volk. *Basic Microbiology*. Harper & Row: New York. 686 pp.1984.