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Study of *Padina australis* using UV-VIS, HPLC and Antibacterial

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ABSTRACT: This study aimed to explore *Padina australis* using UV-VIS, HPLC and Antibacterial. Brown algae (*Padina australis*) obtained from Padike, Talango, Madura. Sampling was done using GPS. Extraction method was done using CaCO3 as neutralizing plus premises of methanol and acetone (7/3 v / v in 300 ml). Analysis was done using UV-VIS and Shimadzu spectrophotometer with a wavelength of 300-800 nm. The disc test showed that the concentration of *Padina australis* extract inhibits the growth of salmonella at a concentration of 10.000 ppm or equal to 4.6 pm ethanol, and at a concentration of 10,000 ppm is equal to 6.23 nm *salmonella typhi* with methanol. Reversed-phase HPLC method with the brand Shimadzu LC-20A (ODS.C-18) used for identification of chlorophyll a fucozanthin 412.5 nm and 439 nm. The identification of brown alga *Padina australis* obtained wavelength value chlorophyll b 444.583 nm, fucoxanthin 450,455 nm, chlorophyll a 618,664 nm, feofitin 665 nm and β caroten 426,451 nm, while the results of the identification of crude brown alga *Padina australis* has a retention time chlorophyll c 6.432 sec, fucoxanthin 10.22 sec, chlorophyll a 38.5 sec, feofitin 56.82 sec and β caroten 62.144 sec.
Key words: Antibakterial, HPLC, *Padina australis*, UV-VIS.

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INTRODUCTION

The Brown algae is very popular in Japan, China and Korea as one of the components of the main intake diet daily [1]. Besides brown algae contain the pigment caroteneoid as a source antioxidants and anticancer [2].

Substances antimikroba is a compound biological or chemical that can inhibit the growth and activity of microbes. Substances antimikroba special for bacteria can be bactericidal (kill bacteria) and bacteriostatic (inhibits the growth of bacteria) [3]. Some algae from the Indonesian coastal areas found to have compound active a character as antimikroba against bacteria patogen [4]. Brown algae type of *Padina australis* have a secondary metabolit like Steroids, Carotene substance bioaktif anti bacterial, fungi, virus or cancer [5].

Padina australis is compounds steroids, terpenoid, polifenol, and saponin [6]. All this chemical allows *Padina australis* to be developed as antibakterial natural because the compounds bioaktif were conceived, is able to inhibit the growth of bacterial. Brown algae can also contain pigments other than as an antibacterial.

The aim of this study was to explore *Padina australis* using UV-VIS, HPLC and Antibacterial.

MATERIAL AND METHODS

Sampling Methods With GPS

Sampling methods with the GPS (Global Positioning System) with the purpose to determine the layout coordinates of the point were observed (113.94444°BT – 7.08795°LS, 113.94231°BT – 7.08913°LS, 113.94548°BT – 7.08911°LS and 113.94347°BT – 7.08999)

Extraction

Samples seaweed *Padina australis* washed and drained, cutted at ± 1.5 cm size with scissors, then the 100 grams sample was weighed and mashed with a mortar pestle. During the process of refining ± 0.5 gram sample was added as a neutralizing CaCO3 because the extraction process will run optimally at neutral pH. *Padina autralis* inserted into the glass beaker covered with aluminum foil. Added methanol (CH 3 OH) and acetone (CH3COCH3) ratio of 7/3 (v / v) in a 300 ml glass beaker is then covered using cling wrap to minimize evaporation of the solvent and covered with aluminum foil to minimize exposure to cahaa. After maceration is then performed filtering (filtration) using Whatman paper no. 42 to separate the results of the filtrate with the filtrate residue thus produced is mixed with clean without residue for 12 hours in order to maximize the results of maceration.

Analysis *Padina australis*

Spectrophotometer UV-Vis and HPLC analysis: UV-Vis spectrophotometry is used to identify pigments, subsequently identified based on wavelength and absorbance values. First dried using a gas powered 104, included in the Cuvet ± 3 ml. Further included in the instrument Shimadzu UV-Vis 1601 tested in the wavelength range 300-800 nm. *Padina australis* analysed using HPLC with a brand Shimadzu LC-20A (ODS C-18). The first step taken in the workplace HPLC system which extracts coarse pigments were dissolved in 5 ml of mobile phase (acetone: methanol: ammonium acetate, 80: 10: 10 v/v). In high performance liquid chromatography, the mobile phase in addition to functioning as a carrier of the components of a mixture to the detector, the mobile phase is one of the critical success factors analysis process [7]. The next phase of 20 mL solution was injected on HPLC pigment with the silence that was the ODS phase (C-18) 5 µm with gradient elution system of methanol, acetone and ammonium acetate (1 M) and a flow rate of 1.0 ml / min.

Activity Anti Bacterial

The content of secondary metabolites of seaweed potentially as diverse bioactive metabolites with very broad activity as an antibacterial and antiviral. Sea grass green, red or brown is a potential source of bioactive compounds that are beneficial for the development of the pharmaceutical industry such as anti-bacterial, anti-bacterial, anti-tumor, anti-cancer and agrochemical industries, especially for fungicides and herbicides [8].

The mechanisms that lead to inhibiting the growth of bacteria after being fed extracts of *P. Australis* is the content of bioactive compounds, one of which is the compound phenol and its derivatives. Phenol and derivatives compound binds to a protein on the bacteria through non-specific binding of proteins to form complexes of phenol. At low concentrations, the protein complex formed phenol with weak bonds and immediately undergo decomposition. Phenol then damage the cytoplasmic membrane and cause leakage of the contents of the cell, thereby inhibiting the growth of bacteria. Whereas at high concentrations the substance teesebut coagulated with cellular proteins and the cytoplasmic membrane through lysis. *Padina australis* which contains phenolic compounds, inhibit bacterial growth by interfering with the function of the cytoplasmic membrane. The presence of phenolic compounds is causing the destruction of the cytoplasmic membrane. Ion H of phenol and its derivatives (flavonoids) will attack the polar groups (a phosphate group) so that the phospholipid molecules in the cell walls of bacteria will break down into glycerol, carboxylic acid and phosphoric acid. In such circumstances, phospholipids are not able to maintain the form of the cytoplasmic membrane cytoplasmic membrane consequently will leak and the bacteria will experience growth retardation and even death [6].

RESULTS

The results of the sample pigment suspected as β-carotene, chlorophyll a, chlorophyll b and fukosantin do identification, which is used Spektrofotometer UV-Vis brand of Shimadzu 1601 with long waves are used which 300-800 nm. Analysis with spektrofotometri UV-Vis using the solvent acetone as blanko. The next step is the analysis by using HPLC a Shimadzu LC-20 with A phase of silence (stationer) used that ODS (C-18) and phase motion (mobile) acetone: metanol: ammonium asetat (80:10:10 v/v).

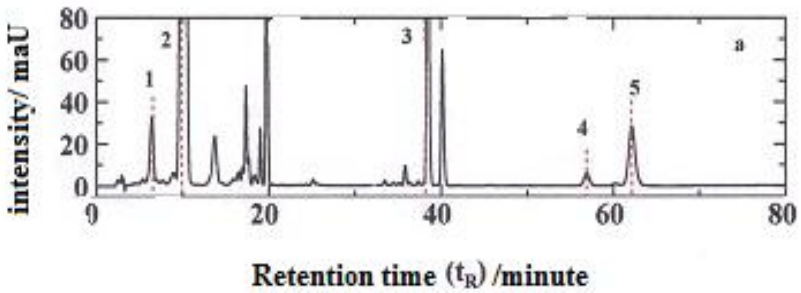

Figure 1. Crude extract chromatograms of *Padina australis*

At each peak or peak indicates a pigment contained in brown seaweed *Padina australis* species. The sequence of each peak or peak in the order of degree of polarity. Of pigment which has the highest degree of polarity to the polarity of the pigment which has the lowest level, because in this study applied a reversed-phase system or a "reversed phase". Reversed phase chromatography using a stationary phase that is less polar than the mobile phase her, because in general the HPLC method using hydrophobic stationary phase.

The next step in the work system that extracts HPLC coarse pigment dissolved in 5 ml of mobile phase (acetone: methanol: ammonium acetate, 80: 10: 10 v / v). Then about 20 mL solution was injected on HPLC pigment with the stationary phase ODS (C-18) 5 µm with gradient elution system of methanol, acetone and ammonium acetate (1 M) and a flow rate of 1.0 ml / min. According to the Son (2004), elution gradient which increase the strength of the mobile phase for chromatographic analysis takes place. The effect of the gradient elution was shortened the retention time of the compounds strong retained on the column. Phytochemical test was conducted on the Uju flavonoids, alkaloids, steroids, terpenoids, saponins and tannins. The test resultsof phytochemical content contained in extracts of *Padina australis* can be seen in Table 1.

Table 1. Testing phytochemical content in the extract *Padina australis*

Type of test	Result	Description
Alkaloid	-	Brown positive. white/yellow and red/orange
Tanin	-	Greenish black positive
Saponin	+	Positively characterized by the presence of foam
Flavonoid	+	Positive green
Terpenoid	+	Positive red-green
Steroid	+	Positive Blue or green

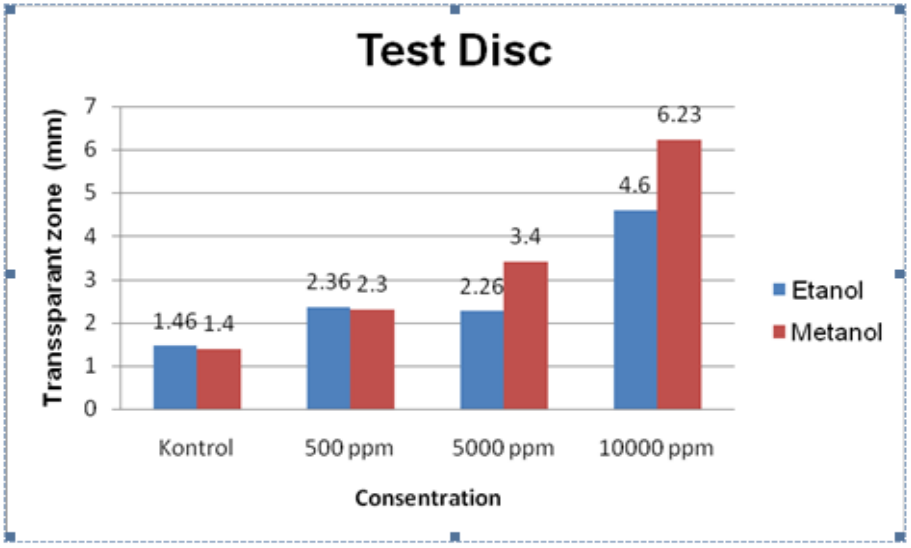

Figure 2. Tes Disc shows the relationship consentration and transparantzone

Test antibacterial activity against *Salmonella typhi* use *Padina australis* extract was used to determine the best solvent between methanol and ethanol in different concentrations in inhibiting the growth of bacteria. The graph above is antibacterial test results with the agar diffusion method or test discs graph using the average yield of 3 replications for each solvent and concentration.

DISCUSSION

To strengthen the results of the TLC, further identification is needed using UV-Vis spectrophotometry. Identification have been a simple process and does not need to make special preparation. Samples included in the cuvet spektro then placed in a special place on the tool then didetektor and spectral pattern will be read and wavelength produced. In addition the sample used for the identification process is very little ± 20 lm. Advantage of identification using UV-Vis spectrophotometer is a simple and very small concentration [9].

The results of the sample pigment suspected as isolate the from the β-carotene, chlorophyll a, chlorophyll b and fukosantin done to identify the next, which is identification with Spektrofotometer UV-Vis with long waves are used which 300-800 nm. Results identification of brown alga *Padina australis* with Spectrophotometer obtained wavelength value chlorophyll b 444,583 nm, fucoxanthin 450,455 nm, chlorophyll a 618,664 nm, feofitin 665 nm and β caroten 426,451 nm.

Using the quiet phase and the phases of the motion are virtually the same [10]. On every peak or peak suggests that the presence of pigment contained in kelp brown type of *Padina australis*. The sequence of each

peak or peak in the order of degree of polarity. Of pigment which has the highest degree of polarity to the polarity of the pigment which has the lowest level, because in this study applied a reversed-phase system or a "reversed phase". Reversed phase chromatography using a stationary phase that is less polar than the mobile phase her, because in general the HPLC method using hydrophobic stationary phase [11].

In Table 1 shows that the extract of *Padina australis* mengandung saponins, flavonoids, terpenoids. Its mechanism of flavonoid compounds thought to denature bacterial cell protein and cell membrane damage irreparably. Flavonoids also are lipophilic which would damage the membranes of microbes. In the flavonoid-containing phenol. Phenol is an acidic alcohol so-called karbonat. Fenol acid also has the ability to denature the protein and cell membrane damage. Acidic conditions by the presence of phenol can affect the growth of bacteria [12].

Terpenoids as an anti-bacterial mechanism that reacts with a transmembrane protein on the outer membrane of the bacterial cell wall, forming a strong bond polymers resulting in the destruction of transmembrane proteins [13]. Damage to the trans membrane protein is a compound entry and exit doors, will reduce the permeability of the bacterial cell wall resulting in bacterial cells will lack nutrients. So that inhibited bacterial growth or death. Saponins as antibacterial mechanism that lowers the surface tension, resulting in the increase in permeability or leakage of cell and intracellular compounds will come out [14].

On the pictures chart the test disc of showing that the concentration of extract *Padina australis* is effective inhibit the growth of *Salmonella typhi* using the solvent ethanol is at a concentration was 10,000 ppm as big as 4,6 ppm. As for the concentration of extract *Padina australis* is effective inhibit the growth of *salmonella typhi* use solvent metanol is to concentrate the 10,000 ppm as big as 6,23 mm. *Salmonella typhi* has the compound a handicap zone at extremely high concentrations. The higher the concentration of a material anti-bacterial activity then an anti-bacteria getting stronger anyway. And the results show that *Padina australis* is able to inhibit the growth of anti-bacteria gram positive and negative [15].

CONCLUSION

The identification of brown alga *Padina australis* with Spectrophotometer obtained wavelength value chlorophyll b 444,583 nm, fucoxanthin 450,455 nm, chlorophyll a 618,664 nm, feofitin 665 nm and β caroten 426,451 nm, while the results of the identification of crude brown alga *Padina australis* has a retention time chlorophyll c 6,432 sec, fucoxanthin 10,22 sec, chlorophyll a 38,5 sec, feofitin 56,82 sec and β caroten 62,144 sec.

Competing interests

The authors declare that they have no competing interests.

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