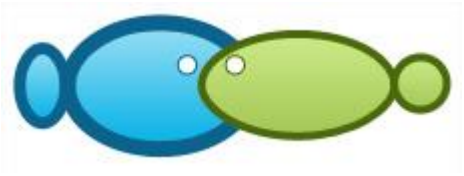

Phytochemical screening and antibacterial activity of brown algae (*Padina australis*) from Atep Oki Coast, East Lembean of Minahasa Regency

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Abstract. The present study aimed to identify the bioactive compounds contained in the brown algae *Padina australis* and to determine the antibacterial activity of the ethanol extract of *P. australis*. Extraction was carried out by maceration using ethanol as a solvent. The phytochemical screening of extracts, including alkaloids, flavonoids, tannins, phenols, terpenoids, steroids and saponins, was done using Harborne method. Antibacterial test was carried out using the agar diffusion method by means of a well: the extract contained in a petri dish was placed in the well filled with a solid agar medium previously inoculated with bacteria from species *Staphylococcus aureus, Streptococcus mutans* and *Escherichia coli*. The results of the phytochemical screening showed that the brown algae *P. australis* contained alkaloids, flavonoids, steroids, saponins and tannins while antibacterial activity test showed that the brown algae extract had the ability to inhibit the growth of *S. aureus, S. mutans* and *E. coli*.
Key Words: bioactive, environment, flavonoid, macroalgae.

Introduction. Infectious diseases are caused by microbes and mostly affect people in tropical regions, including Indonesia. Bacteria are one of the causes of infectious diseases. Currently, many pathogenic bacteria in humans show drug resistance due to inappropriate use of antibiotics (Sartoratto et al 2004). The development of science and technology allowed to identify changes in disease patterns and resistance of germs to antibiotics due to an nappropriate use of antibiotics, causing infections treatments to be no longer effective (Melliawati 2009). Therefore, the discovery of new antibiotics that did not cause resistance in pathogenic microbes is one of the alternative solutions in dealing with this problem.

The marine environment is a rich source of bioactive components, many of which having chemical structures that are not found in terrestrial environments (Jadulco 2002). According to Trainor (1978), algae are grouped into thallophyta plants (spiny plants) because morphologically, the algae body structure cannot be distinguished between roots, stems and leaves. Basically, alga consists of a stem called a thallus with various shapes (Landau 1992). Their texture is soft or hard, chalky and stringy (Nontji 2002).

Algae (seaweed), especially macroalgae, have the potential to be used as a promising source of bioactive compounds in the medical world (Leary et al 2009). Kardono (2004) states that there are about 2,500 types of bioactive compounds from the sea that have been isolated and identified, of which 93% are obtained from algae (seaweed). One of the results of secondary metabolites currently being studied are bioactive substances with antibacterial, antifungal or antiviral potential.

Several studies conducted previously had identified bioactive compounds in algae and tested their ability to inhibit pathogenic and resistant bacteria (Singkoh 2011; Panden et al 2019; Singkoh et al 2019a,b). The results of the research of Julyasih et al (2020) and Kartikaningsih et al (2020) showed that the algae *Eucheuma cottonii*, *E. spinosum, Caulerpa* spp., *Gracilaria* spp. and *Sargassum cristaefolium* have the ability to inhibit *E. coli* and *Salmonella typhosa* bacteria.

The use of algae (seaweed) in the health sector is still very limited, while their potential as raw material for medicine in Indonesia, especially in the North Sulawesi area, is very large. Consequently, it is necessary to conduct research studies in order to determine the bioactive compounds present in the brown algae *P. australis* as a source of natural antibiotics. *P. australis* (Figure 1) has a fan-like thallus, forming thin sheet segments (lobes). It is 5-9 cm high, yellowish brown, consisting of several flabellate lobes with a blade width of 3.2 cm. The alga has double concentric lines on the bottom surface with a distance from one another ranging between 2-3 mm. The liming that occurs on the surface of the leaf has rhizoid holdfast (Kepel et al 2012). The purpose of this study was to identify bioactive compounds contained in the brown algae *P. australis* and to test its antibacterial activity against *Staphylococcus aureus, Streptococcus mutans* and *Escherichia coli.*

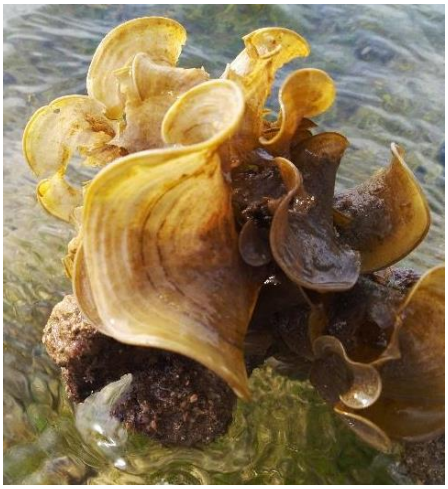

Figure 1. *Padina australis*.

Material and Method

Sampling. Sampling was carried out from March to May 2020, by the cruising method in the coastal waters of Atep Oki Village, Lembean Timur Minahasa. Samples of *P. australis* brown algae were taken from the marine waters, at low tide. Algae samples were removed from the substrate and immediately cleaned for impurities using sea water. Furthermore, the samples were put in a plastic bag and then placed in a cool box. Samples were transported to the Laboratory of Pharmacy Microbiology, Faculty of Mathematics and Natural Sciences, University of Sam Ratulangi, for screening of bioactive compounds and antibacterial activity tests.

Algal extraction. Fresh samples were first cleaned and washed thoroughly, drained, weighed and dried using an oven at 48^0C. The dried samples were then crushed using a blender into a fine powder and stored as a dry powder. The powder of *P. australis* algae was then macerated for 24 hours by immersing in ethanol p.a. (99.8%) with a 2:1 ratio of solvent to sample, then the macerate was filtered using Whatman paper no. 1 to obtain the first filtrate. The first filtrate was stored in the bottle while the sample residues were soaked again with the same solvent and ratio. The same procedure was applied for the third maceration for 24 hours. The filtered maceration product were then collected and evaporated using a rotary vacuum evaporator at a temperature of 45^0C to obtain a crude algae extract. To prevent the loss of compounds, the extract was stored at 18^0C before being used for the antibacterial tests against *S. aureus*, *S. mutans* and *E. coli*.

Phytochemical screening. Phytochemical screening of *P. australis* extract included examination of the alkaloid, flavonoid, tannin, terpenoid, steroid and saponin compounds. The analytical method used was based on the procedure by Harborne (1984).

Antibacterial test. S*. aureus*, *S. mutans* and *E. coli* bacteria were obtained from the Laboratory of Pharmacy Microbiology, Faculty of Mathematics and Natural Sciences, University of Sam Ratulangi Manado. Antibacterial test was carried out using the well method. The algae extract of *P. australis* has been placed in a petri dish, then in the well, within a solid agar medium that has been inoculated with the bacteria *S. aureus*, *S. mutans* and *E. coli*. The results of the extraction of algae were dripped using a micropipette of 100 µl in a well that had been made on the bacterial culture medium. In different wells, a ciprofloxacin antibiotic solution was included as a positive comparison and ethanol as a negative comparison. All treatments were repeated 3 times. After that it was incubated at 37^0C for 24 hours. For each treatment, the observed test parameters were the inhibition zone diameter (mm), and the distance of the inhibition zone. They were measured using a ruler or calipers (Bachtiar et al 2012). If the test solution inhibits bacterial growth, a clearing zone will appear around the well. This means that there is no bacterial growth in the clearing zone. Further, it was compared with the clearing zone formed by the control antibiotic. If the test sample would not inhibit bacterial growth, there was no visible bright zone around the well hole.

Results. Phytochemical screening was used to determine the content of secondary metabolites present in the brown algae *P. australis* samples. The extraction method used is the maceration method, by immersing the sample in the solvent. The solvent used is ethanol solvent, where this solvent can dissolve almost all organic compounds in the sample, both polar and non-polar. Ethanol is a volatile solvent, so it can be easily freed from the extract. The algae extract of *P. australis* used in phytochemical screening was an extract with a concentration of 100%. Phytochemical screening found that *P. australis* contained several bioactive compounds (Table 1).

Table 1

Bioactive compounds of *Padina australis* ethanol extract

Compounds	*Reactor*	*Information*
Alkaloids	Mayer	+
Alkaloids	Wagner	+
Alkaloids	Dragendorf	-
Flavonoids	Mg, HCl	+
Phenols	$FeCl_3$ 1%	+
Terpenoids	CH_3COOH, HCl	-
Steroids	CH_3COOH, HCl	+
Saponins	HCl	+
Tannins	$FeCl_3$ 1%	+

(+) positive result, there is a compound content; (-) negative result, no compound content.

The found bioactive compounds from marine algae *P. australis* are as shown in Table 1: alkaloids, flavonoids, phenols, steroids, saponins and tannins. According to Saloso et al (2011), the methanol extract of *P. australis* contains steroids, terpenoids, saponins and polyphenols. Maharany et al (2017) reported that the n-hexane extract of *P. australis* is positive for flavonoids, phenol hydroquinones, triterpenoids, saponins and tannins.

Antibacterial test. The results of the antibacterial activity test showed that the *P. australis* algae extract test solution had antibacterial activity against the three tested bacteria. This was indicated by the present of clearing zone around the well. The clear zone or the area where bacteria are not present is called the barrier area. The size of the inhibition area is influenced by the absorption of the antibacterial substance into the agar and by the sensibility of the bacteria to the antibacterial substance. The size of the zone of inhibition of the ethanol extract of *P. australis* is relatively smaller, compared to the size of the zone of inhibition formed by the control antibiotic compound. The results of measuring the inhibition zone diameter can be seen in Table 2.

Table 2

Antibacterial activity of *Padina australis* extract

Name of bacteria	*Average diameter (mm)*		
	Padina australis	Ciprofloxacin	Ethanol
Staphylococcus aureus	11.6	12	-
Staphylococcus mutans	11.8	11	-
Escherichia coli	11.3	14	-

Discussion. Davis & Stout (1971) stated that the bacterial inhibition is categorized as weak for an inhibition zone of less than 5 mm; moderate for 5 to 10 mm, strong for 10 to 20 mm and very strong a for more than 20 mm. According to these criteria, ethanol extract of *P. australis* to inhibit *S. aureus, S. mutans* and *E. coli* bacteria is categorized as strong, with an inhibition zone of 11.6 mm 11.8 and 11.3 mm. The results of this study were supported by several previous studies: Ismail et al (2016) used methanol extract from *Padina pavonica* as an antibacterial with positive results against *S. aureus*, *Staphylococcus typhimurium*, and *Micrococcus* sp.; Zen et al (2015) reported that the crude extract of *P. australis* brown algae was able to inhibit the growth of *Staphylococcus epidermidis* bacteria; Dulger & Dulger (2014) tested the antibacterial ability of the ethanol aquous extract of *P. pavonica* and the results were positive for *S. aureus*; Kayalvizhi et al (2012) reported that *Candida albicans* was resistant to chloroform extract of *P. boergessenii*, while *Aspergillus flavus*, *E. coli* and *S. aureus* were sensitive to methanol extract; El-Fatimy & Said (2011) showed that the methanol and chloroform extracts (2:1) of *Padina* sp. had a significant effect when tested against *E. coli* and *S. aureus*. The results of the test carried out by El-Fatimy & Said (2011) also showed that the diameter of the inhibition zone in Gram positive bacteria was greater than that in Gram negative. This indicated that the antibacterial compounds of *P. australis* were more sensitive to Gram positive bacteria than to Gram negative bacteria. This was supported by the research of Taherpour et al (2016), where the extract of *Padina* sp. was more effective against Gram positive bacteria than against Gram negative bacteria. Gazali & Safutra (2016) stated that the concentration of active ingredients contained in the *P. australis* extract, the sensitivity of the *Vibrio harveyi* bacteria to the extract, and the diffusion rate of the active ingredients contained in the extract will affect the width of the inhibition zone formed. Another category of factors that affect the sensitivity of the extract is formed by the environmental conditions of the bacterial test media, namely temperature, incubation time and age of the bacteria. The ability of *P. australis* brown algae extract to inhibit bacterial growth is influenced by the bioactive compounds content of *P. australis* algae. This was consistent with Cox et al (2010) who reported that the potential of algae inhibition as an antimicrobial was influenced by the content of phenolic compounds, terpenoids, alkaloids, steroids and flavonoids.

Suryati et al (2017) reported that the biological activity of alkaloid compounds is due to the presence of alkaline groups containing nitrogen. If there is a contact between the base group and the bacteria, there will be a reaction with the amino acid compounds from the bacterial cell wall and with the bacterial DNA, which is the main constituent of the cell nucleus, the center for regulating all cell activities. Changes in the composition of amino acids will change the genetic balance of DNA acids so that the bacterial DNA will be damaged. DNA damage in the bacterial cell nucleus will encourage lysis of the cell nucleus, so that cell damage will occur. Cell damage results in bacterial cells being unable to metabolize so that they will experience lysis (destruction). Another mechanism of alkaloids as antibacterial is related to the alkaloid component known as DNA intercalator, which inhibits the bacterial cell topoisomerase enzymes (Karou & Savadago 2005). Antibacterial algae activity, according to Akremi et al (2017) and Deyab et al (2016), is thought to be due to the presence of phenolic and flavonoid compounds. Flavonoids contain C_{15}, consisting of two phenolic nuclei connected to three carbon units. Flavonoids are the largest group of phenolic compounds found in nature. Based on the carbon structure, the flavonoid compounds are divided into 6 main sub groups, namely flavones, flavonols, flavanones, isoflavones, and anthocyanidins. Flavonoids function as

antibacterial by binding to proteins in bacteria so that they inhibit enzyme activity, disrupting the bacterial metabolic process. Also, the lipophysical properties of flavonoids can damage the bacterial cell membrane, due to the content of lipids which allow flavonoids to channel through the membrane. According to Chusnie & Andrew (2005), flavonoids can also disrupt the energy metabolism by inhibiting the use of oxygen by bacteria. Phenolic compounds in high concentrations can penetrate and damage the bacterial cell walls and precipitate protein in bacterial cells, while phenol in lower concentrations can also activate important enzyme systems in bacterial cells (Oliver et al 2001). The presence of phenol compounds can cause damage to the cytoplasm. H- ions from phenol compounds and their derivatives will attack polar groups (phosphate groups) so that the phospholipid molecules in the bacterial cell walls will break down into glycerol, carboxylic acids and phosphoric acids (Pakidi & Hidayat 2016). The steroids mechanism of inhibiting microbes consists of damaging the plasma membrane of microbial cells, causing the cytoplasm to leak out of the cell, causing cell death. Steroid molecules have nonpolar (hydrophobic) and polar (hydrophilic) groups so that they have a surfactant effect that can dissolve the phospholipid components of the plasma membrane. The mechanism of action of saponins as antibacterials is to reduce surface tension so that the intracellular compounds will come out (Nuria et al 2009). Cavalieri et al (2005) revealed that this compound diffuses through the targeted cell's outer membrane and walls, then binds to the cytoplasmic membrane and disrupts its stability. This causes the cytoplasm to leak out of the cell resulting in cell death. Tannins have antibacterial activity, forming complex compounds with proteins through hydrogen bonds, when these are formed between tannins and proteins, altering the protein structures and disrupting the bacterial metabolism. Tannins inhibit the reverse transcriptase enzyme, the DNA topoisomerase, and also the cell wall polypeptides, so that the cell walls imperfections cause bacterial cells' lysis and eventually their death, due to osmotic and physical pressure (Nuria et al 2009). Tannins are produced by algae as a form of defense against microbes. Thus it can be concluded that the algae content of bioactive compounds affects the ability of *P. australis* algae to inhibit the bacterial growth.

Conclusions. Brown algae *P. australis* taken from the coastal waters of Atep Oki Village, East Lembean, Minahasa Regency, contained bioactive compounds consisting of alkaloids, flavonoids, phenols, steroids, saponins and tannins. The ability of ethanol extract of *P. australis* to inhibit the growth of *S. aureus*, *S. mutans* and *E. coli* bacteria was categorized as a strong.

Acknowledgements. The authors would like to thank to the Sam Ratulangi University for the financial support through PNBP Fund, for the fiscal year 2020. We also thank the head of Advance Laboratory of Faculty of Math and Science for allowing us to use the laboratory facilities during the research.

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Received: 09 December 2020. Accepted: 22 February 2021. Published online: 28 February 2021.
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Singkoh M. F. O., Katili D. Y., Rumondor M. J., 2021 Phytochemical screening and antibacterial activity of brown algae (*Padina australis*) from Atep Oki Coast, East Lembean of Minahasa Regency. AACL Bioflux 14(1):455-461.