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ANTIBACTERIAL ACTIVITY FROM ETHANOL AND ETHYL ACETATE EXTRACTS OF *Padina pavonica* HAUCK FROM KABUNG ISLAND AGAINST *Escherichia coli*

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ABSTRACT

Macroalgae is marine biological resources that play a crucial role and have an important economic value. They synthesize bioactive compounds with different kind of biological activities, such as antioxidant, antitumor, including antibacterial. In the health sector, infectious diseases from bacteria is one of the problems that still increasing. In addition, E. coli besides having the capacity to be pathogenic agent, it also showed mutlidrug resistence (MDR). Antibiotic resistance is a serious problem worldwide. The increase of this phenomenon leads to the exploration of new antibiotics using natural resources as an effort to overcome the problem. Padina pavonica Hauck belongs to Phaeophyceae spreeding along Kabung Island waters, but its existence has not been widely used. The objective of this study was to evaluate antibacterial activity from ethanol and ethyl acetate extract of P. pavonica Hauck from Kabung Island, West Kalimantan against E. coli. The extraction was made using ethanol and ethyl acetate solvents, and the antibacterial activity test was realized with concentration series of 5 ppm, 10 ppm, 15 ppm, and 20 ppm. The quantitative assessment of the antibacterial test showed that both ethanol and ethyl acetate extracts of P. pavonica Hauck had potential antibacterial activity against E. coli. The inhibition zone of ethanol extract was 11.6 mm, while ethyl acetate extract was 12.6 mm, respectively. These two solvents were included to the bacteriostatic category.

Keywords: antibacterial; ethanol; ethyl acetate; *Escherichia coli*; Kabung Island ;*Padina pavonica*.

INTRODUCTION

Macroalgae is one of the potential of marine biological resources and responsible for primary productivity (Sudhakar *et al*., 2018). Generally, macroalgae can be found in intertidal and shallow waters (Anggadiredja *et al*., 2006). In marine ecosystem, macroalgae play a crucial role (John and Al-Thani, 2014) and have as well an important economic value (Irwandi *et al*., 2017). In addition, macroalgae as a renewable source of valuable compounds, they are widely utilized in various applications (Leandro *et al*., 2020), such as food supplement (Rupérez, 2002), medicine (Melka, 2009; Haryani *et al*., 2014), pharmaceutical, and cosmetic industry (Bueno *et al*., 2017; Garcia-Vaquero *et al*., 2020).

Padina is classified to brown macroalgae (Phaeophyceae), due to the presence of fucoxantin pigment (Barsanti and Gualtieri, 2014; Vidotti *et al*., 2014). This genus has the ability to produce valuable compounds (Biris-Dorhoi *et al*., 2020) which are potential to be developed. The main content of brown macroalgae is alginate (Basmal *et al*., 2013; Stiger-Pouvreau *et al*., 2016), but also it contains a significant amount of protein, phenol, tannin and vitamin C (Fayaz *et al*., 2005; MacArtain *et al*., 2007). Several studies have reported the bioactive compounds with different kind of biological activities of the genus *Padina*, such as antioxidant (Corsetto *et al*., 2020; Sofiana *et al*., 2021), antitumor (Shannon Shannon and Abu-Ghannam., 2016), and antibacterial (Kiroquero, 2015; Klomjit *et al*., 2021; Wijayanti *et al*., 2021).

In the health sector, infectious diseases of various types of microorganisms, including bacteria is one of the problems that still increasing. One of the bacteria causing infection is *Escherichia coli*. *E. coli* is a Gram-negative bacteria belonging to the family Enterobacteriaceae, has a rod-shaped with a length of 2 µm and 0.5 µm in diameter (Escherich, 1885), motile and non-motile, grows well at a temperature of 37 ºC (Croxen *et al*., 2013). This bacteria is actually harmless in the human body, but it has the capability to be pathogenic agent causing infection and inflammation (Szmolka and Nagy, 2013; Ahlstrom *et al*., 2018). Moreover, *E. coli* showed as well mutlidrug resistence (MDR) (Suárez-Pérez *et al*., 2021). Antibiotic resistance is a serious problem worldwide. The increase of this phenomenon leads to the exploration of new antibiotics using natural resources as an effort to overcome the problem.

According to previous studies, *Padina* sp. contains alkaloids, saponins, terpenoids and flavonoids (Sofiana *et al*., 2021) which allows them to be developed as a natural antibacterial (Salosso *et al*., 2020). These compounds can inhibit the growth of several types of bacteria, such as *Pseudomonas* sp. (Izzati, 2007; Salem *et al*., 2011), *Staphylococcus aureus* (Wijayanti *et al*., 2021), *Bacillus subtilis* (Klomjit *et al*., 2021), *Vibrio cholera*, *Salmonella typhi* (Haryani *et al*., 2015), and *E. coli* (Haryani *et al*., 2014; Sameeh *et al*., 2016). The extraction method and the type of solvent used greatly affect the content of the dominant compounds and their biological activity. Hidayah *et al*. (2016) reported that the extraction of *Sargassum* using ethanol solvent had the greatest antibacterial activity against *S. aureus* compared to ethyl acetate and n-hexane. Furthermore, the use of ethyl acetate resulted in a total phenol content greater than extraction with isopropyl alcohol, methanol, ethanol and acetone solvents (Savitri, 2017).

P. pavonica Hauck spreeding along Indonesian coasts, including Kabung Island waters. However, its existence has not been widely used by the community and considered as a weed or nuisance plant. For this reason, this study aims to evaluate the antibacterial activity of ethanol and ethyl acetate extract of *P. pavonica* Hauck from Kabung Island, West Kalimantan against *E. coli* bacteria.

RESEARCH METHODS

Samples Collection and Identification

This research was conducted in June - October 2020. Samples of *P. pavonica* Hauck were collected from Kabung Island waters, West Kalimantan, Indonesia. Identification and extraction of the samples, as well as the test of antibacterial activity were carried out in the Laboratory of Biotechnology, Faculty of Mathematics and Natural Sciences, Tanjungpura University.

Samples Preparation

Samples of *P. pavoniva* Hauck were cleaned, then dried at room temperature for 3x24 hours, and drying was continued with an oven at 50°C for 2x24 hours until a constant dry weight was obtained. Furthermore, samples were blended into a fine powder, then stored in a clean and tightly closed container.Extracts were made by maceration method using ethanol and ethyl acetate solvents at room temperature. Each 500 g of sample powder was immersed in a dark glass using 70% ethanol and ethyl acetate for 3x24 hours. The ratio of the sample and solvent was 1:10. The immersion was filtered, then the extract was evaporated using a rotary evaporator at a temperature of 50°C until there was no condensation of solvent in the condenser. The results were then thickened by heating using oven for 3 hours at 50°C (Warsidah *et al*., 2020).

Antibacterial Activity Test

The antibacterial activity test of the ethanol and ethyl acetate extracts of *P. pavonica* samples was carried out using the well diffusion method (Warsidah *et al*., 2020). This method was based on the process of diffusion of antibacterial compounds from the extract into solid media that has been inoculated with the test bacteria. The antibacterial ability of the extract can be indicated by the presence or absence of a clear zone around the well where the sample diffuses (Balaouri *et al*., 2016). The extract solutions of ethanol and ethyl acetate were made with concentration series of 5 ppm, 10 ppm, 15 ppm, and 20 ppm using Dimethyl Sulfoxide solvent. Meanwhile, 25 mL of Nutrient Agar (NA) media in a petri dish was poured *E. coli* bacteria with a volume of 500 µL, then a well was made with a diameter of 6 mm.

In each well, sample extracts of 5 ppm, 10 ppm, 15 ppm and 20 ppm were poured, with a volume size of 30 µL per well, respectively. Tetracycline 10 g as a positive control was used and 20 µL of 1% DMSO solvent as a negative control. The observations were made for 1-2 x 24 hours and the determination of antibacterial activity was measured based on the inhibition zone. The inhibition zones formed at various concentrations of ethanol and ethyl acetate extracts of *P. pavonica* were continued with bacteriostatic and bactericidal activity tests after 1-2 x 24 hours. Bacteriostatic activity was indicated by the growth of bacteria, while bactericidal was marked by the absence of test bacteria in the previously inhibition zone.

RESULT AND DISCUSSION

The increasing level of bacterial resistance in the medical sector has stimulated the development of the search for novel antibacterial active compounds. Macroalgae is one of the important marine organisms that intensively used as a source of bioactive compounds with various types of biological activity. These bioactive compounds can be obtained by extraction. Many researches have reported that macroalgae contain a wide range of bioactive compounds, such as fucoxanthin (Peng *et al*., 2011; Rajauria and Abu-Ghannam, 2013), phlorotannins (Choi *et al*., 2010), sterols (Kavita *et al*., 2014), and terpenes (Rodrigues *et al*., 2015) which has antibacterial activity.

The bioactive compounds in macroalgae have different characteristics and polarities, therefore it was necessary to adjust the organic solvent used to find the target compound. According to previous studies, ethanol and ethyl acetate extract of *P. pavonica* from Kabung Island indicated the presence of alkaloids, steroids, flavonoids, phenols, and saponins compounds (Sofiana *et al*., 2021). Specifically, the extraction of phenolic compounds affected by the polarity of the solvent and the degree of extraction components polarity (Klomjit *et al*., 2021). Regarding to its nature, phenol was classified as a polar compound, therefore it was required a polar solvents to extract, such as ethanol, water, methanol, and acetone. Furthermore, semi-polar solvent such as ethyl acetate can also be used to extract phenolic compounds in a natural materials. Phenolic compounds in plants have polar properties due to the presence of a hydroxyl group in their chemical structure. The extraction method could be as well realized using 70% etanol (Robinson, 1995). In addition, ethyl acetate as a semipolar solvent, has also the ability to extract semi-polar chemical content, such as saponins.

In this study, the quantitative assesment of the antibacterial activity of each extract against pathogenic bacteria was done by measuring the inhibition zone. *E. coli* was selected as a target bacteria due to its capacity to affect human health and even caused death. This bacteria was commonly found in the gastrointestinal tract of the human body, will cause diarrheal and extraintestinal diseases if present in excessive amounts (Parija, 2009; Ahlstrom *et al*., 2018). Moreover, pathogenic strain of *E. coli* O157:H7 was responsible to significant mortality worldwide (Croxen *et al*., 2013). In addition, *E. coli* has become resistant to several types of antibiotics, causing new problems in the sector of medicine (Brooks *et al*., 2007).

The extraction of antibacterial activity from macroalgae were solvent dependent (Cox *et al*., 2010). In our study, phenolic compounds were detected in two solvents. Ismail *et al*. (2019) has reported that ethanol extract from *P. pavonica* exhibited some natural compounds, including phenols. Another study pointed that ethyl acetate extracts also contained polyphenols molecule (Fayad *et al*., 2017). The antibacterial activities of the macroalgae were associated with the presence of phenolic content (Smith, 2004; Kim *et al*., 2008).

Table 1. Inhibition Zone of the Growth of *E. coli* Bacteria from Ethanol and Ethyl Acetate Extracts of *P. pavonica*

Diameter of the inhibition zone (mm)									
Ethanol extract				Ethyl acetate extract				Positive control	Negative control
5 ppm	10 ppm	15 ppm	20 ppm	5 ppm	10 ppm	15 ppm	20 ppm		
5.2	6.6	8.2	11.6	6.0	8.0	10.4	12.6	18.0	0

According to the results of antibacterial activity toward *E. coli* bacteria showed that both ethanol and ethyl acetate extracts had the highest inhibition zone at the concentration of 20 ppm (Table 1). Ethyl acetate extract presented the inhibition zone of 12.6 mm. This fraction was suspected to contain more antibacterial compounds than ethanolic extract thus showing better growth inhibition against *E. coli*. Semipolar solvents will dissolve semipolar materials that have a greater affinity for their interaction with the bacterial cell wall (Fitrial *et al*., 2008). Most of marine brown macroalage possess the antibacterial activity belonging to intermediate or susceptible category (Silva *et al*., 2020) and drived inhibition zone greater than 15 mm (Al-Khazan *et al*., 2016; Akremi *et al*., 2017). The antibacterial activity of *P. pavonica* has been reported. Methanolic extracts inhibited the growth of *E. coli* strain O157:H7 with diameter of inhibition zone >15 mm (Zeid *et al*., 2014). The extract *P. pavonica* has been as well invstigated and indicated microbial activity (Ertürk and Tas, 2011; Omar *et al*., 2012; Kosanic *et al*., 2019).

Antibacterial active compounds from macroalgae have different mechanisms of action. The general mechanism of antibacterial compounds in inhibiting bacterial growth is destroying the cell (Wei *et al*., 2016), altering the cell membrane (Bogolitsyn *et al*., 2019), altering the cell wall composition and inhibiting the formation of the germ (Lopes *et al*., 2013), interfering the protective proteins of the cell membrane, and inhibiting the function of DNA or RNA (Eom *et al*., 2014). Phenolic compounds can interact with bacterial proteins and form phenol-protein complexes. Furthermore, protein-phenol as a weak bond will undergo rapid decomposition. Free phenol will eventually enter the bacterial cell, resulting in precipitation and protein denaturation, and due to protein coagulation, the cell membrane will be lysis (Juliantina *et al*., 2008). In addition, free phenols will interfere working system of the cell, inhibit cell growth, damage the membrane of the cytoplasm, agglomerates proteins, inhibit the synthesis of nucleic acids and protein (Cushnie and Andrew, 2005).

Other compounds contained in the *P. pavonica* extract, such as terpenoids have also the potential capability to inhibit bacteria growth. The mechanism of action was by interfering membrane or cell wall synthesis so they become imperfect cells (Ajizah, 2004). The antibacterial activity of *P. pavonica* extract can also be strengthened by the presence of saponins, as a chemical component that is generally contained by marine organims. Saponins work as antibiotics through the mechanism of decreasing the surface tension of the bacterial cell wall. This condition causes the bacterial membrane permeability to be damaged and other active substances in the extract can penetrate into cells to damage proteins and enzymes (Madduluri *et al*., 2013).

The test of the ethanol and the ethyl acetate extract of *P. pavonica* showed that two solvents were included in the bacteriostatic category. This was because after 3x24 hours of observation, the inhibition zone that previously formed was was re-grown with *E. coli* tested bacteria.

CONCLUSION

The quantitative assessment of the antibacterial test showed that both ethanol and ethyl acetate extracts of *P. pavonica* Hauck had potential antibacterial activity against *E. coli*. The inhibition zone of ethanol extract was 11.6 mm, while ethyl acetate extract was 12.6 mm, respectively. These two solvents were included in the bacteriostatic category.

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