INTRODUCTION

Macroalgae is one of the potential of marine biological resources 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 multildrug resistance (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 spreading 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 qualitative 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.

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. Generally, macroalgae can be found in intertidal and shallow waters (Anggadirejda 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-Pouveau 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 °C (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 multildrug resistance (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 antibiotic (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 (Klonjit 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 spreading 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. pavonica 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 fucosanthen (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% ethanol (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 assessment 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).
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 macroalgae possess the antibacterial activity belonging to intermediate or susceptible category (Silva et al., 2020) and drove 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 investigated 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 organisms. 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 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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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                                | 6.6            | 8.2                  | 10.4             | 12.6            |
| 10 ppm                               | 7.2            | 9.8                  | 11.4             | 12.6            |
| 15 ppm                               | 8.0            | 10.4                 | 11.6             | 12.6            |
| 20 ppm                               | 8.8            | 10.4                 | 12.6             | 12.6            |

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