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Antimicrobial Activity of Excoecaria Agallocha Mangrove Extract in Inhibiting the Growth of Aeromonas Hydrophila by in-Vitro

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ABSTRACT

One of the bacterial diseases that often attack freshwater fish is the red-sore disease, also known as aeromoniasis or Motile Aeromonas Septicemia (MAS). It is caused by bacteria from Aeromonas genus, such as Aeromonas hydrophila. A safe alternative treatment for pathogenic bacteria is the use of antibacterial compounds made from natural ingredients. The Excoecaria agallocha mangrove extract is one of potential alternatives that is useful as natural antimicrobials due to its various contents of antibacterial bioactive compounds. The objective of this study was to determine the antimicrobial activity of E. agallocha mangrove extract in inhibiting the growth of A. hydrophila. The research method was experimental with complete randomized design and factorial pattern of 3 factors (type of plant organ, bacterial strain, and concentration of extract), in 48 treatments and 3 replications. The solvents used consisted of methanol (polar) and n-hexane (non-polar). The plant organs used included leaves and stems. The bacterial strains consisted of GPl-04, GB-01, GL-01, GL-02, GJ-01, and GK-01 with concentrations of leaves and stems extracts of 0, 10, 20, and 30%. The parameter measured was the diameter of inhibition zone, which was done by using disc paper (Kirby Bauer method). Non-parametric Kruskall-Wallis Test and qualitative-descriptive data analysis were applied. The methanol extract of E. agallocha leaves in GJ-01 strain at 10 and 20% concentrations had antimicrobial activity with inhibition zone in 2.55 mm and 2.46 mm, respectively. The n-hexane extract of E. agallocha stem at 20 and 30% concentrations had antimicrobial activity with inhibition zones of 1.88 mm in GPl-04 strain and 1.58 mm in GB-01 strain, respectively. Extract of E. agallocha mangrove exhibited potential as a natural antibacterial to prevent aeromoniasis in fish.

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1. INTRODUCTION

One of bacterial diseases generally attack freshwater fish is the red-sore disease, also known as aeromoniasis or Motile Aeromonas Septicemia (MAS). It is caused by species in genus Aeromonas, such as Aeromonas hydrophila (Rasmussen-Ivey et al., 2016). These bacteria are motile, gram negative, rod-shaped, facultative anaerobic bacteria, oxidase-positive, and opportunistic (Stratev & Odeyemi, 2016; Pessoa et al., 2019). A. hydrophila infection in fish causes clinical signs such as depigmentation and erosion of skin, hemorrhagic, hyperaemic patches on fins, ulcers, abdominal cavity and congestion of liver, kidneys, and spleen (Anyanwu et al., 2015; Emeish et al., 2018; Mulia & Vauziyah, 2021).

Bacterial control applied in most cases uses antibiotics. In general, misuse and overuse of antibiotics can lead to bacterial resistance in addition to environmental pollution (Serwecińska, 2020). Moreover, it can accelerate evolution of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARG) in the environment and consequently increase the risk of transmitting environmental resistance to humans (Manaia, 2017). The use of antibiotics also leads to A. hydrophila resistance. Results of previous studies showed that A. hydrophila was resistant to ampicillin and erythromycin (Mulia et al., 2021).

A safe alternative treatment of pathogenic bacteria is the use of antibacterial compounds from mangrove plants, such as Excoecaria agallocha. Some parts of this plant have been studied for their antibacterial ability. Results of previous studies showed that E. agallocha contained alkaloids, flavonoids, tannins, and terpenoids (Bandaranayake, 2002). The root extract of E. agallocha macerated with N-hexane solvent contained alkaloids and steroids, while the methanol solvent contained flavonoids, alkaloids, and terpenoids (Dwisari et al., 2016). Extract of E. agallocha mangrove sap using chloroform solvent can inhibit the growth of Escherichia coli and Staphylococcus aureus bacteria (Puspitasari, 2017). Other studies also reported that the leaves of E. agallocha contain alkaloid compounds, tannins, and terpenoid in addition to the ability to inhibit S. aureus dan E. coli. Meanwhile, the stems can also inhibit S. aureus (Prihanto et al., 2011).

There has not been wide range use of E. agallcha extracts as antibacterial against A. hydrophila. Therefore, this study tried to test the inhibitory power of E. agallocha extract against A. hydrophila in GPl-04, GB-01, GL-01, GL-02, GJ-01, and GK-01 strains tested. Many strains were used here because each strain of A. hydrophila had different characteristics and pathogenicity.

The objective of this study was to determine the antimicrobial activity of E. agallocha mangrove extract in inhibiting the growth of A. hydrophila.

2. METHODS

This study applied experimental method of complete randomized design and factorial pattern with three factors of mangrove plant organs (leaves and stems), the A. hydrophila bacterial strains (GPl-04, GB-01, GL-01, GL-02, GJ-01, and GK-01), and mangrove extract concentrations (0, 10, 20, and 30%) with 48 treatments and 3 replications. Solvents used included methanol (polar) and n-hexane (non-polar).

2.1 Preparing E. Agallocha mangrove extract

The leaves and stems of E. agallocha were washed and cut into 4 cm pieces. Then, each leaf and stem were dried in oven at 60°C before crushed to become fine powder. As much as 100 g of mangrove leaf and stem powder was soaked in 500 mL of n-hexane (non-polar) for 2 x 24 hours at room temperature. The n-hexane extract was then separated from the pulp. The resulted extract was evaporated using n-hexane in a vacuum evaporator to reach thick extract for antibacterial testing purpose, while the dregs were soaked again in 500 mL of methanol (polar) for 2 x 24 hours at room temperature before the methanol extract and dregs were separated. The extract obtained was then evaporated with methanol by using a vacuum evaporator to obtain a thick extract used for antibacterial testing.

2.2. Phytochemical test

Phytochemical tests were carried out using a color test on a Thin Layer Chromatography (TLC) plate. The purpose of this test was to determine the presence of antibacterial bioactive compounds contained in the methanol and n-hexane extracts of the leaves and stems of E. agallocha.

2.3. Antimicrobial activity test of E. agallocha mangrove extract

Each strain of A. hydrophila was grown on Tryptone Soya Broth (TSB) (Merck, Merck Corporate, Kenilworth, USA) medium and incubated at 37°C for 24 hours. A total of 1 mL of each bacterial culture was grown on petri dish using the pour plate method and then Tryptone Soya Agar (TSA) (Merck, Merck Corporate, Kenilworth, USA) medium was poured into the petri dish. The thick extract of E. agallocha was diluted using distilled water to 5 mL of extract from methanol and also 5 mL for 10% Dimetil Sulfoksida (DMSO) from n-hexane. The sterilized disc paper was used to test the inhibition zone of each (GPl-04, GB-01, GL-01, GL-02, GJ-01, and GK-01) and at each concentrations (10, 20, and 30%), it was given with 2 drops of extract. The disc paper for methanol extract (0%) control and n-hexane extract (0%) control were dripped with distilled water and 10% DMSO, respectively. Then, the paper discs containing mangrove extract and solvent were placed on the surface

of the TSA medium and incubated at 37°C for 24 hours. After 24 hours, it was observed to identify the presence of clear zone around the paper disc.

2.4. Data analysis

The data obtained based on the calculation of the diameter of the inhibition zone were analyzed using the non-parametric analysis of the Kruskall-Wallis Test. Influencing factors included plant organs (leaves and stems), type of solvent (methanol and n-hexane), concentrations (0%, 10%, 20%, and 30%), and the type of A. hydrophila strains (GPl-04, GB-01, GL-01, GL-02, GJ-01, and GK-01).

3. RESULTS

3.1. Phytochemical test results extract of E. agallocha mangroves

Phytochemical test results on E. agallocha mangrove leaf and stem extract using methanol and n-hexane as solvents resulted in content of bioactive compounds (Tables 1 and 2). The methanol extract of E. agallocha leaves contained alkaloids, flavonoids, terpenoids, and tannins, while the stem extracts contained alkaloids and terpenoids (Table 1). This indicates that methanol solvent can dissolve polar to semi polar compounds, such as alkaloids, flavonoids, terpenoids, and tannins. Similar finding was reported by Bandaranayake (2002) indicating E. agallocha leaves were positive for alkaloids, flavonoids, terpenoids, and tannins. Likewise, the research results of Prihanto et al. (2011) showed that methanol leaf E. agallocha leaf extract contained alkaloids, terpenoids, and tannins, but no flavonoids were detected, while the stem extract of E. agallocha contained terpenoids. Leaf extract has antimicrobial potential unlike other parts (Prihanto et al., 2011). In addition, the differences of detected compounds were assumed to be due to varied locations of environment where E. agallocha grew. In this study, E. agallocha mangrove was originated from Cilacap mangrove forest, while in Prihanto et al. (2011) it came from the mouth of Porong River.

Table 1. Phytochemical test results of methanol extract of E. agallocha leaves and stems

Compound Group	Methanol Extract	
	Leaf	Stem
Alkaloids	+	+
Flavonoids	+	-
Terpenoids	+	+
Tannins	+	-

The n-hexane extract of the E. agallocha leaves and stems contained flavonoid and terpenoid compounds (Table 2). Results of this study were different from the compounds detected in methanol extract of E. agallocha leaves and stems (Table 1). Such difference was assumed to be due to the different solvents used. Sarker et al. (2006) stated that the efficiency and effectiveness of the extraction is strongly influenced by ability of solvent to diffuse into the cell.

Table 2. Phytochemical test results of n-hexane extract of E. agallocha leaves and stems

Compound Group	n-hexane extract	
	Leaf	Stem
Alkaloids	-	-
Flavonoids	+	+
Terpenoids	+	+
Tannins	-	-

3.2 Antimicrobial activity test in E. agallocha mangrove extract

The methanol extract of E. agallocha leaves and stems indicated antimicrobial activity on the growth of A. hydrophila (Table 3). The results showed that of all strains tested for inhibition zones, the widest inhibition was exhibited by leaf methanol extract at 10 and 20% concentrations by 2.55 and 2.46 mm on strain GJ-01, respectively (Table 3).

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Table 3. The results of the antimicrobial activity test of methanol extract of E. agallocha in inhibiting the growth of A. Hydrophila

Types of Plant Organs	Concen-tration (%)	Average Inhibitory Zone Diameter (mm)					
		GPl-04	GB-01	GL-01	GL-02	GJ-01	GK-01
Leaf	0	0	0	0	0	0	0
	10	0	0	0	0	2.55	0
	20	0	0	0	0	2.46	0.06
	30	0.08	0.5	1.59	0	1.09	0
Stem	0	0	0	0	0	0	0
	10	0.31	0	0.84	0	0.33	0.12
	20	0.14	0	0.25	0	0.59	0
	30	0	0	0.83	0	0	0

These results indicated that at 10 and 20% concentrations of methanol extract, E. agallocha mangrove was sufficient in inhibiting A. hydrophila growth. The formation of inhibition zone around the paper disc was due to the E. agallocha extract containing antibacterial compounds, such as alkaloids, flavonoids, terpenoids, and tannins. Bioactive compounds such as alkaloids, tannins, and terpenoids are antimicrobial agents are found in plants (Cowan, 1999; Negi et al., 2005). Bioactive molecules can inhibit the growth of pathogens (Mandal & Shi, 2020). Alkaloids can inhibit microbial growth due to their ability to intercalate cell walls and DNA, tannins complex with cell walls as well as to damage membranes, in addition to terpenoids that can damage the cell membranes.

However, the GL-02 strain did not form any inhibitory zone in either leaf or stem extract, possibly due to the high resistance of GL-02 strain to antibacterial compounds of E. agallocha leaf and stem extract. Thus, inhibition zone was not formed around the paper disc. Prihanto (2011) stated that extracts of leaves, stems, bark, and flowers of E. agallocha mangrove plant were able to inhibit the growth of S. aureus bacteria. While the leaf extracts were only able to inhibit the growth of E. coli. These are possible because E. coli and A. hydrophila are gram-negative bacteria containing peptidoglycan that enable the compounds contained in the extract of E. agallocha to be less effective in inhibiting the growth of A. hydrophila.

The n-hexane extract of E. agallocha leaves and stems exhibited different inhibitory effects on the growth of A. hydrophila (Table 4). The results showed strain GPl-04 having the widest inhibition zone diameter in leaves at 10% concentration by 1.12 mm. Meanwhile, the widest diameter in stems at 20% concentration was 1.88 mm. In strain GB-01, the leaf extract did not produce any inhibition zone, while the stem produced the widest inhibition zone at 30% concentration by 1.58 mm. In strain GL-01, the leaf extract did not produce any inhibition zone either, while the stem produced widest inhibition zone at 20% concentration by 0.18 mm. The diameter of the inhibition zone in GL-02 strain of the leaves and stems at 30% concentration were 0.2 mm and 1.23 mm, respectively. The widest diameter of inhibition zone in GJ-01 strain of the leaves and stems at 10% concentration were 0.38 mm and zero. In GK-01 strain, the leaf extract also did not produce any inhibition zone, while the stem produced widest inhibition zone at 10% concentration by 0.61 mm.

Table 4. The results of the antimicrobial activity test of the n-hexane extract of E. agallocha in inhibiting the growth of A. Hydrophila

Types of Plant Organs	Concen-tration (%)	Average Inhibitory Zone Diameter (mm)					
		GPl-04	GB-01	GL-01	GL-02	GJ-01	GK-01
Leaf	0	0	0	0	0	0	0
	10	1.12	0	0	0	0.38	0
	20	0.34	0	0	0	0.2	0
	30	0	0	0	0.2	0	0
Stem	0	0	0	0	0	0	0
	10	0.32	0.61	0	0	0	0.61
	20	1.88	0	0.18	0.13	0	0.3
	30	0	1.58	0	1.23	0	0.45

Of all the strains tested for their inhibition zones, widest inhibition zone was produced by stem organ, at 20% concentration in GPl-04 strain and 30% concentration in GB-01 strain. These indicated that at 20 and 30% concentrations, the n-hexane extract of E. agallocha mangrove was sufficient to inhibit the growth of A.

hydrophila. The formation of inhibition zone around the paper disc was due to the E. agallocha extract containing antibacterial compounds such as flavonoids and terpenoids.

The low average diameter of the inhibition zone on strains GB-01, GL-01, GJ-01, and GK-01 were due to the high level of strain resistance. In addition, the antibacterial compounds contained in the n-hexane extract of leaves and stems was also low, so that many do not form clear zone around the paper disc. By identifying the effectiveness of E. agallocha mangrove extract as a natural antibacterial against A. hydrophila, it is hoped that it will be useful as an alternative natural antibacterial for fish disease treatment and prevention.

4. CONCLUSION

Based on the results of phytochemical tests, the extract of the E. agallocha mangrove plant contains alkaloids, flavonoids, terpenoids, and tannins.

Extract E. agallocha mangrove had the antimicrobial activity to inhibit the growth of A. hydrophila.

5. RECOMMENDATION

It is required to take further research on the extracts of E. agallocha against other pathogenic bacteria in addition to research on extracts of other parts of the mangrove plant to observe its potential as antibacterial.

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