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Antibacterial Activity of Red Galangal (*Alpina purpurata*) Extract on the Growth of *E. tarda* Bacteria

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Abstract

This study aimed to analyze the antibacterial activity of red galangal extract (*A. purpurata*) against the growth of *E. tarda*. This study aimed to determine the antibacterial compounds and the antibacterial effectiveness of red galangal extract (*A. purpurata*) against the growth of *E. tarda* bacteria. Antibacterial activity test was carried out by MIC test and disc test. The MIC test used doses of 1 ppm, 10 ppm, 100 ppm, 500 ppm, and 1000 ppm with incubation for 24 hours. The disc test used doses of 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, and a positive control of 5 ppm chloramphenicol and a negative control without treatment. Photochemical test of red galangal extract (*A. purpurata*) contains flavonoid compounds, alkaloids, tannins, triterpenoids, and saponins. The results of the disc test showed that the highest inhibitory diameter was 7.55 mm at a dose of 500 ppm and was bacteriostatic because it decreased its inhibitory zone after 48 hours of incubation. The highest inhibitory effect was 56.56% at a dose of 500 ppm after 24 hours of incubation.

INTRODUCTION

Bacterial disease is a disease that often infects cultured organisms, one of which is *Edwardsiella tarda*. *E. tarda* is classified as Gram negative bacteria which have a short rod shape. These bacteria have a size of about 2-3 µm with a diameter ranging from 1 µm. *E. tarda* bacteria are facultative anaerobes (Park *et al*., 2012). Antibiotics are often given to treat bacterial disease but antibiotics will have side effects on the environment because the use of antibiotics will leave residues that will harm humans and the environment. The use of antibiotics can cause bacterial resistance to chemicals that will cause other pathogenic diseases. The use of antibiotics will cause chromosomal mutations (Yulvizar *et al*., 2014).

Natural ingredients can use as a substitute antibiotic by using materials that have antibacterial compounds. Natural ingredients are recommended because they are environmentally friendly, inexpensive, and not carcinogenic (Azhar *et al*., 2020). The antibacterial compound contained in the red galangal rhizome (*A. purpurata*) include alkaloids, flavonoids, tannin, saponin, and triterpenoid (Mardhiyyah *et al*., 2021). Red galangal rhizome can inhibit the growth of *Escherichia coli*, *Salmonella thyphimurium*,

Vibrio choleare, *Listeria monocytogenes*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Rialita *et al*., 2015). This study aims to determine the antibacterial activity of red galangal rhizome (*A. purpurata*) against the inhibition zone of *E. tarda* bacteria in vitro.

METHODOLOGY

Place and Time

This research was conducted in January - February 2022. This research was carried out at the Aquaculture Laboratory, Fish and Disease, Faculty of Fisheries and Marine Sciences, Brawijaya University. The phytochemical test was carried out at UPT Materia Media, Batu.

Research Materials

The material used in this research were red galangal powder from UPT Materia Medica Batu, DMSO, pro-analyst ethanol, aquades, TSA and TSB (Merck), Whatman no. 42, paper disc, aluminium foil, plastic warp, and *E. tarda* obtained from Faculty of Medicine, Brawijaya University. Tools used are a spectrophotometer (KJ-2097, Germany), incubator (Red Line), autoclave (GEA LS-B100), rotary evaporator, Erlenmeyer, test tube, and petri dish.

Research Design

The research design used an experimental method. The research design with Completely Randomized Design (CRD) with five treatments namely 1, 10, 100, 500, and 1000 ppm of red galangal extract concentrations, positive control which was given 5 ppm Chloramphenicol antibiotic and negative control without extract administration, with three replications.

Work Procedure

The manufacture of red galangal extract was carried out by the maceration method. Red galangal powder weighed as much as 150 g. Then, 750 ml of 96% ethanol was added to the sample and stored at room temperature for 5 days (shaking every day). The extract was filtered with filter paper Whatman no. 42 to produce filtrate one and the remainder was macerated again with 450 ml of 96% ethanol for 2 days (shaking every day) then filtered using filter paper to produce filtrate two. The collected filtrate is evaporated using a rotary evaporator (Lasut *et al*., 2019).

Inhibition test of red galangal extract (*A. purpurata*) against *E. tarda* using MIC and disc test. Minimum Inhibitory Concentration (MIC) is a test to determine the lowest dose that can kill the highest number of pathogens (Soelama *et al*., 2015). Red galangal extract (*A. purpurata*) was divided into 5 concentrations, namely 1, 10, 100, 500, and 1000 ppm with 2 controls, namely positive and negative controls, where positive control was given 5 ppm Chloramphenicol antibiotic and negative control without extract administration. After incubation for 24 hours, the turbidity level of the test media was observed and absorbance value measurements were carried out using a spectrophotometer using a wavelength of 600 nm. The disc test was carried out to determine the inhibitory power of red galangal extract (*A. purpurata*) by observing the clear zone formed around the disc paper. The concentration treatment was given based on the results of the MIC test. The diameter of the clear zone formed around the paper disc was measured using a caliper (Maftuch *et al*., 2018). The disc test dose used was divided into 5 concentrations, namely 100, 200, 300, 400, and 500 ppm with 2 controls, namely positive control with chloramphenicol antibiotics as much as 5 ppm and negative control without extract.

Scanning Electron Microscope (SEM) is a test to determine the morphological damage to bacterial cells. Observations of bacterial cell morphology were carried out after the administration of extracts containing active ingredients (Hariati *et al*., 2018). The SEM test used two treatments, *E. tarda* and *E. tarda*

bacteria given 500 ppm red galangal (*A. purpurata*) extract. Analysis of the damage was carried out by comparing photos between normal conditions and bacteria given red galangal extract (*A. purpurata*).

Data Analysis

The results are analyzed using one-way ANOVA to determine the effect of red galangal extract on the inhibition of *E. tarda* bacteria. The calculation of extract inhibition effectiveness is calculated based on the equation (Riyadi *et al*., 2021).

$$E = \frac{D}{Da} \times 100\%$$

Where:
E = effectiveness of inhibition (%)
D = diameter of plant material extract inhibition zone (mm)
Da = diameter of antibiotic inhibition zone (mm)

RESULTS AND DISCUSSION

Yield of Red Galangal Extract

The yield of red galangal extract was obtained by comparing the weight of the extract with the weight of the red galangal powder (Devi *et al*., 2020). The yield of red galangal extract with 96% ethanol solvent obtained the results presented in Table 1.

Table 1. The yield of red galangal extract.

Solvent Type	Total Solvent (ml)	Sample Weight (gr)	Extract Weight (gr)	Yield (%)
Etanol	1200	150	18,71	12,47

The yield of red galangal extract was 12.47%. The yield calculation aims to determine the amount of simplicia needed to produce a certain amount of thick extract (Yandri and Setyani, 2021).

Phytochemical Screening

Red galangal (*A. purpurata*) extract using 96% ethanol as solvent contains several compounds including alkaloids, flavonoids, tannins, and saponins as presented in Table 2.

Table 2. Phytochemical test of red galangal.

Compound Identification	Results
Flavonoid	(+) Positive
Alkaloid	
Meyer	(+) Positive
Dragendrof	(+) Positive
Bouchardat	(-) Negative
Tanin / Fenol	(+) Positive
Terpenoid	
Steroid	(-) Negative
Triterpenoid	(+) Positive
Saponin	(+) Positive

Note : (+) = there is chemical content (-) = there is no chemical content.

The results of the phytochemical test showed that the active compounds contained in red galangal were flavonoids, alkaloids, tannins, steroids/triterpenoids, and saponins. This is following the research of Fatimawali *et al*. (2020), the phytochemical results of red galangal extract with 96% ethanol solvent were found to contain flavonoid compounds, alkaloids, saponins, triterpenoids, and tannins.

Minimum Inhibitory Concentration (MIC)

The MIC test results were obtained from the absorbance value where the absorbance value indicated the extract's ability to inhibit bacterial growth. The

results of the MIC test using red galangal extract on *E. tarda* bacteria are presented in Table 3.

Table 3. The results of MIC test.

Concentration (mg/L)	Absorbance
Control (-)	0,893
1	0,612
10	0,583
100	0,436
500	0,471
1000	0,462
Control (+)	0,021

MIC test using red galangal extract obtained the lowest absorbance value at a dose of 100 ppm with a value of 0.436; close to positive control. The absorbance value decreased with increasing concentration of the given extract dose. This shows that red galangal extract can inhibit the growth of *E. tarda* due to the presence of antibacterial compounds in the extract. The lower absorbance value obtained from the red galangal extract (*A. purpurata*) indicates the inhibition of bacterial growth.

Based on the table above, red galangal extract (*A. purpurata*) with a dose of 100 ppm is the minimum dose to determine the dose of the disc test (Maftuch *et al*., 2018). The Optical Density (OD) value obtained is a combination of the color turbidity of the extract with bacterial turbidity. This is because the spectrophotometer is not able to distinguish between the color turbidity of the extract and bacterial turbidity (Warokka *et al*., 2016). The absorbance value at a concentration is not entirely due to bacterial growth. The absorption value is also influenced by the concentration of the extract, so it can affect the absorption of light by dead bacterial cells in the solution.

Antibacterial Test

The disc test was carried out to determine the clear zone formed around the dry disc paper. The clear zone formed indicates the ability of red galangal extract to inhibit the growth of *E. tarda* bacteria. The disc test was carried out for 24 hours of incubation and 48 hours of incubation with doses of 100, 200, 300, 400, and 500 ppm. The results of the clear zone measurements are presented in Table 4.

Table 4. Results of red galangal extract disc test.

Concentration (ppm)	Average of Inhibition (mm)		Classification Inhibition Zone Response
	24 hours	48 hours	
K (+)	0,00±0,00^{a}	0,00±0,00^{a}	Weak
100	4,36±0,09^{b}	4,07±0,08^{b}	Moderate
200	5,34±0,06^{c}	5,05±0,06^{c}	Strong
300	6,35±0,07^{d}	6,06±0,06^{d}	Strong
400	6,76±0,06^{e}	6,47±0,05^{e}	Strong
500	7,75±0,1^{f}	7,46±0,09^{f}	Very strong
K (-)	13,70±0,35^{g}	13,60±0,35^{g}	Very strong

Note: Classification of inhibition zone weak 1-3 mm, moderate 3-5 mm, strong 5-7 mm and very strong >7 mm (Nurhikmayani *et al*., 2019).

The diameter of the inhibition zone formed in all treatments with a time of 24 hours showed an increase with increasing concentration. The concentration of 100 was categorized as moderate with an average of 4.36 mm. Concentrations of

200, 300, and 400 ppm were categorized as strong with a mean of 5.34 mm, 6.35 mm and 6.76 mm. The concentration of 500 ppm was categorized as very strong with an average inhibition zone of 7.75 mm. The size of the inhibition zone is influenced by several factors. The main factor that affects the size of the inhibition zone is the difference in the size of the extract concentration. A high concentration of extract will be more powerful in inhibiting bacterial growth. Other factors that can affect the zone of inhibition include incubation temperature, time of insertion of discs, and the distance between discs (Alfiah *et al*., 2015).

The diameter of the inhibition zone formed in all treatments with a time of 48 hours showed a decrease in the inhibition zone. This is because red galangal extract (*A. purpurata*) is bacteriostatic. It happened because the bacteria continued to grow on the test medium but the density was reduced when compared to the positive control. Saidin *et al.* (2021) stated that the antibacterial mechanism is divided into two, bactericidal and bacteriostatic. Antibacterial agents that can kill bacteria are called bacteriocidal while antibacterial agents that can inhibit the growth of bacteria are called bacteriostatic. The inhibition effect of the extract is presented in Table 5.

Table 5. The effectiveness of inhibition.

Concentration (ppm)	Effectiveness of Inhibition	
	24 hours	48 hours
100	31,82%	29,92%
200	38,97%	37,13%
300	46,35%	44,55%
400	49,34%	47,57%
500	56,56%	54,85%

Based on Table 5, red galangal extract was classified as effective in inhibiting bacterial growth. The effectiveness of red galangal within 24 hours shows the higher the concentration is given, the greater the effectiveness. The highest effectiveness at treatment E with 500 mg/L red galangal extract was 56.56%. The effectiveness decreases with increasing time, after 48 hours the effectiveness decreases. This is because the nature of the red galangal extract is bacteriostatic. According to Kandou *et al.* (2016), the antibacterial action mechanism of red galangal extract in inhibiting bacteria is to damage the structure and change the permeability mechanism of the bacterial cell wall. The higher the dose is given, the greater the antibacterial activity, so the faster the microorganisms are killed and their growth is inhibited.

Scanning Electron Microscope (SEM)

The SEM test showed that there was an effect of giving red galangal extract is presented in Figure 1.

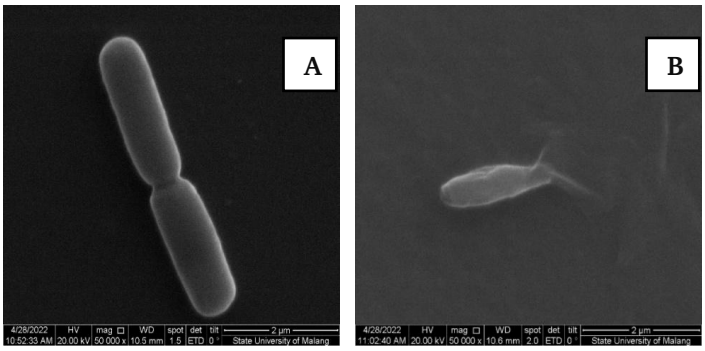

Figure 1. Morphology of *E. tarda*.

The morphology of *E. tarda* in Figure A shows an intact cell wall while Figure B shows the bacteria undergoing lysis due to the instability of the cell wall so that the metabolism of the bacteria is disrupted. Changes in the bacterial structure were caused by the influence of red galangal extract (*A. purpurata*) which has antibacterial compounds including alkaloids, flavonoids, and tannins.

Alkaloids in red galangal work by inhibiting enzymes that play a role in DNA replication, causing bacteria to be unable to divide, thereby inhibiting bacterial growth. Alkaloids are antimicrobial as DNA interchelators and inhibit bacterial cell enzymes (Isramilda *et al*., 2020). Alkaloids as antibacterials play a role in interfering with the formation of cross-bridges that make up the peptidoglycan component of bacterial cells, causing the cell wall layer to be incompletely lysed (Ariani and Riski, 2018). Flavonoids work to inhibit the nucleic acid synthesis and disrupt membrane function. Flavonoids form complex compounds with extracellular and dissolved proteins so that they can inhibit and damage the cytoplasmic membrane of microbial cells and inhibit microbial metabolism (Nirwana *et al*., 2018). Tannins work to deactivate adhesins, enzymes, and cell envelope proteins. Tannins are able to inhibit bacterial growth through hydrogen bonding with proteins in bacterial cells so that bacteria undergo protein denaturation and disrupted bacterial metabolism (Mogana *et al*., 2020).

CONCLUSION

The antibacterial activity of red galangal extract was bacteriostatic, indicating a decrease in inhibition after 48 hours of incubation. Red galangal (*A. purpurata*) extract showed the highest inhibition zone and effectiveness at treatment E with 500 mg/L reg galangal extract. Based on the SEM test, *E. tarda* bacteria with red galangal extract were lysed.

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