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The Antibacterial Activity of Dayak Onion Ethanol Extract (Eleutherine Palmifolia (L.) Merr) and Red Ginger (Zingiber Officinale Rosc Var. Rubrum) on Growth Gi Tract Pathogen Bacteria

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ABSTRACT

Antibiotic resistance in gastrointestinal bacterial infections can be overcome by alternatives using medicinal plants such as Dayak bulbs (Eleutherine palmifolia (L.) Merr) and red ginger rhizome (Zingiber officinale Rosc. Var. Rubrum). This study aims to examine the antibacterial activity of a combination of ethanol extracts of Dayak bulbs with red ginger rhizome and the effectiveness of the extract combinations in inhibiting the growth of Pseudomonas sp. and Bacillus sp. This research is an experimental study with a completely randomized design (CRD). Data analysis in this study used the ANOVA test. The results showed ethanol extract of Dayak onion bulbs, red ginger rhizome, and a combination of the two extracts that have antibacterial activity, which can inhibit the growth of Pseudomonas sp. and Bacillus sp. The combination of ethanol extract from Dayak onion bulbs and has the greatest antibacterial activity at a 3:1 combination of 56.95% against the growth of Pseudomonas sp. and 109.72% against the growth of Bacillus sp. 95% against the growth of Pseudomonas sp. and 109.72% against the growth of Bacillus sp. 95% against the growth of Pseudomonas sp. and 109.72% against the growth of Bacillus sp.

INTRODUCTION

The gastrointestinal tract is considered the largest immunological organ in the body and the gastrointestinal mucosal immune system is often the first line of defense against microbial infections. Pseudomonas sp. and Bacillus sp. is a type of bacteria that causes many infections in the digestive tract, especially in cases of food and beverage poisoning such as milk poisoning and diarrhea (Suwito, 2010).

Management of patients with bacterial infections such as diarrhea can be done with treatment with antibiotics, but until now there are still mistakes in their inappropriate use which can lead to bacterial resistance to antibiotics.(Ministry of Health of the Republic of Indonesia, 2016).Efforts to overcome the problem of antibiotic resistance in gastrointestinal bacterial infections can be done through traditional methods, namely using medicinal plants.

Plants that are known to the public and have medicinal properties include Dayak bulbs (Eleutherine palmifolia (L.) Merr) and red ginger rhizome (Zingiber officinale Rosc. Var. Rubrum). Dayak onion is a traditional plant typical of the people of Kalimantan. This plant has been used for generations by the Dayak community as a cure for various types of

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diseases. Dayak onions can be used fresh, simplicia, candied or powdered form(Galingging, 2009). Dayak onions contain bioactive compounds, including flavonoids, terpenoids, tannins, steroids and alkaloids which function as antifungal and antibacterial.(Harlita et al., 2018). Red ginger also contains antibacterial compounds including essential oils, oleoresin, and flavonoids (Setyawan, 2015).

Previous research conducted on testing the activity of medicinal plants as an alternative treatment showed that the ethanol extract of Dayak onion (E. palmifolia (L.) Merr) bulbs was able to inhibit the growth of Methicillin-Resistant Staphylococcus aureus (MRSA), Bacillus cereus, Shigella sp., Pseudomonas aeruginosa (Harlita et al., 2018)*;*and E. Coli (Amanda, 2014). Ethanol extract of red ginger rhizome (Z. officinale Rosc. Var. Rubrum) has also been shown to inhibit the growth of Staphylococcus Aureus and E. coli (Handrianto, 2016).

Some of these studies have investigated the ethanol extract of Dayak onions (E. palmifolia (L.) Merr) and red ginger rhizome (Z. officinale Rosc. Var. Rubrum) in inhibiting Gram-positive and Gram-negative bacteria as well as combinations of various medicinal plants, but Until now, the combination of Dayak onion root extract (E. palmifolia (L.) Merr) and red ginger rhizome (Z. officinale Rosc. Var. Rubrum) as an antibacterial against the growth of Pseudomonas sp. and Bacillus sp.

Based on this background, the researchers were interested in conducting research on testing the antibacterial activity of a combination of ethanol extract of red ginger (Z. officinale Rosc. Var. Rubrum) and Dayak onion (E. palmifolia (L) Merr) bulbs on the growth of Pseudomonas sp. and Bacillus sp.

METHODS

This type of research was experimental which was conducted with a one-factor Completely Randomized Design (CRD), namely a combination of ethanol extract of Dayak onion (E. palmifolia (L.) Merr) bulbs and red ginger rhizome (Z. officinale Rosc. Var. Rubrum) with a concentration variation of 25, 50.75, and 100 mg/mL which were diluted in sterile distilled water and combination variations, namely with a ratio of 2:1 and 3:1. The positive control is chloramphenicol30g/mL,$\mu$and the negative controls were sterile aquadest and Polysorbate 80. Each treatment was repeated six times to obtain 48 treatment combinations.

Plant identification

Determination of Dayak onion bulbs (E. palmifolia (L.) Merr) and red ginger rhizome (Z. officinale Rosc. Var. Rubrum) was carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Mulawarman University.

Making Simplicia

Red ginger rhizomes and Dayak onion bulbs are washed first until they are clean so that there is no dirt that can contaminate the extract. Furthermore, the red ginger rhizome was peeled and sliced with a thickness of 7-8 mm then dried using an oven at 50oC for 3 x 24 hours until it lost about 60-70% weight (moisture content around 7-12%). Dayak onion bulbs were peeled and sliced with a thickness of 1-2 mm then dried in an oven at 50oC for 2 x 24 hours. The powder obtained was weighed and stored in a dry bottle(Harlita et al., 2022).

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Extraction of Antibacterial Compounds

Weigh 2000 grams of Dayak onion bulb powder and 1000 grams of dried red ginger rhizome, then put each powder in a glass bottle and macerate using 96% ethanol solvent with a ratio of 1:2 (w/v). Samples were macerated for 3x24 hours. The dregs are separated by filtering with filter paper. All the filtrates obtained were concentrated using a rotary evaporator at 50◦C with a rotating speed of 30 rpm until a thick extract was obtained. The viscous extract obtained from each sample was weighed, the yield value was calculated, put into a sterile bottle to prevent contamination and stored (Galingging, 2009).

The extract obtained was first tested for ethanol-free by taking a small amount of extract in a tube then adding sulfuric acid and acetic acid, then heating. If the solution has no ester odor, then the extract is ethanol free (Kurniawati, 2015).

Preparation of Test Solutions

The concentrated extracts of Dayak onion and red ginger obtained were each made into a stock concentration solution of 100 mg/mL. The stock solution was then diluted with sterile distilled water, each with a concentration of 25, 50, 75 and 100 mg/mL. The combination of ethanol extract of Dayak onion and red ginger is made by mixing Dayak onion extract and red ginger extract. The combination of extracts was made with a ratio of 2:1 and 3:1 which were taken from stock solutions of each Dayak onion bulb ethanol extract and red ginger ethanol extract with a concentration that had an inhibitory effect on Pseudomonas sp. and Bacillus sp.

Extract combination antibacterial activity test

Antibacterial activity test was carried out using the Kirby-Bauer diffusion disc method. The extract concentrations used were 25, 50, 75, 100 mg/dl, and the combination ratios used were 2:1 and 3:1. The test bacteria is Pseudomonas sp. ATCC 27853 and Bacillus sp. ATCC 11774. Antibacterial activity test was carried out at the East Kalimantan Provincial Health Laboratory, Microbiology section. as positive control is chloramphenicol 30 µg/mL and sterile aquadest negative control.

Prepare the standardized test bacterial suspension and MHA plate culture media. The test bacterial culture was taken using a sterile cotton swab, then inoculated by means of a spread on MHA plate medium. Sterile paper discs with a diameter of 6 mm, dripped with 15% of the combined extract solution, then placed on the surface of the MHA plate media and pressed using tweezers so that it sticks perfectly to the surface of the media. Then incubated at 37oC for 24 hours. The diameter of the inhibition zone formed was measured using a ruler at the longest limit and the shortest limit of the inhibition area formed in millimeters (mm)µl (Harlita et al., 2022).

Data analysis

The diameter of the inhibition zone obtained from the measurement results is converted into a percentage of inhibition activity using the following equation:(Ashshobirin et al., 2014)

$$I = \frac{(d2 - d1)}{d1} x100\%$$

Information :

I : inhibitory activity (%)

d1 : disc paper diameter (6 mm)

d2 : diameter of inhibition zone (mm)

The percentage of inhibitory activity was then analyzed using analysis of variance (ANOVA) at the 95% confidence level and the DMRT follow-up test. Data on the treatment that had the best antibacterial activity from the combination of Dayak onion bulb extract and red ginger rhizome was then calculated for its antibacterial effectiveness by comparing the diameter of the inhibition zone with the diameter of the inhibition zone produced by the positive control antibiotic, namely chloramphenicol 30 µg/mL. The effectiveness of the antibacterial extract against antibiotics was calculated using the following equation(Oroh et al., 2015):

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

Information :
E : antibacterial effectiveness (%)
D : the diameter of the inhibition zone of the combination of Dayak bulb extract andred ginger rhizome (mm)
Da : diameter of positive control antibiotic inhibition zone (mm)

RESULTS AND DISCUSSION

The results of plant identification with No. 135/UN.17.8.5.7.16/HA/XI/2019 states that the Dayak bulb plant is a species of Eleutherine palmifolia (L.) Merr and No. 134/UN.17.8.5.7.16/HA/XI/2019 states that the red ginger rhizome used is a species of Zingiber officinale Rosc. Var. Rubrum (Harlita et al., 2022).

Figure 1. Dayak onions (a) fresh; (b) after drying; (c) after being mashed (Harlita et al., 2022)

Figure 2. Red ginger (a) fresh; (b) after drying; (c) after being mashed (Harlita et al., 2022)

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The samples that have been identified are then made into simplicia by drying using an oven. The drying process aims to reduce the water content in the Dayak onion bulbs and red ginger rhizomes so that the simplicia is not easily damaged and can be stored for a longer time. The simplicia obtained was then extracted by maceration technique using 96% ethanol as the solvent. Ethanol is an organic solvent that can attract most of the bioactive compounds contained in simplicia and its polarity increases with increasing water content.

The results obtained from the maceration process were then concentrated with a rotary evaporator to obtain a thick red-brown extract of Dayak onion bulbs and a thick yellow-brown extract of red ginger rhizome. The yield values obtained from the ethanol extract of Dayak onion bulbs were 1.04% and 1.65% of red ginger rhizome as shown in Table 1.

Table 1. Yield Value of Dayak Onion Bulb Extract and Red Ginger Rhizome

Extract type	**Simplicia weight (gram)**	**Extract weight (grams)**	**Yield (%)**
Onion bulbs	2000	20,8	1.04
Red ginger rhizome	1000	16.5	1.65

Testing the antibacterial activity of the ethanol extract of Dayak onion bulbs, the ethanol extract of red ginger rhizome, and their combination against the test bacteria Pseudomonas sp. and Bacillus sp. carried out using the Kirby Bauer diffusion method which is characterized by the presence of a clear zone (*inhibition zone*) around the disc. Antibacterial testing was carried out on each extract and the combination of the two extracts with several concentration variations, namely 25, 50, 75, and 100 mg/mL. The diffusion method was chosen as the antibacterial test method because it is the most sensitive for assessing the antibacterial potential of crude extracts of natural ingredients (Valgas et al., 2007).

The results of the antibacterial activity test of the ethanol extract of Dayak onion bulbs and the ethanol extract of red ginger rhizome at different concentrations against the gastrointestinal pathogenic bacteria Pseudomonas sp. and Bacillus sp. showed the presence of a clear zone around the disc and the results obtained varied inhibition zone diameter measurements which can be seen in Figure 1 and Figure 2.

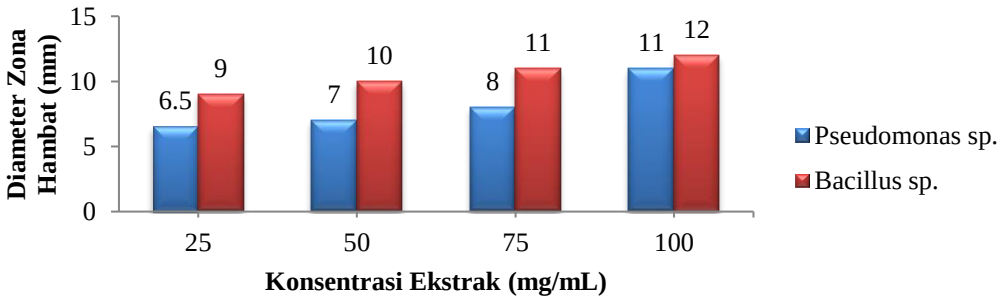

Figure 1. Diameter of inhibition zone of Dayak onion bulb ethanol extract on growth*Pseudomonas*sp. and Bacillus sp.

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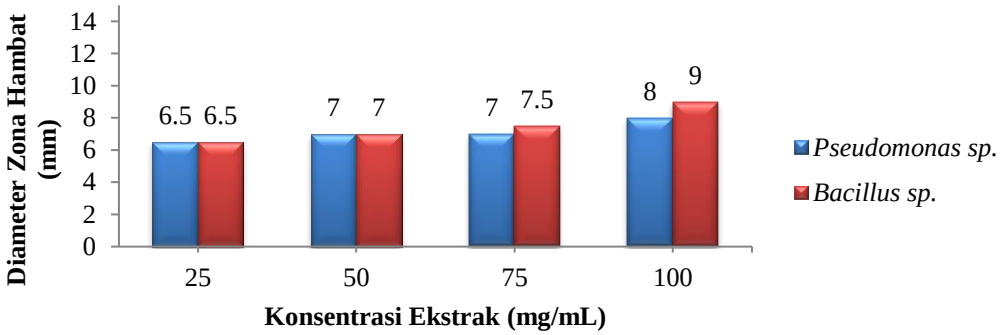

Figure 2. Diameter of inhibition zone of red ginger rhizome ethanol extract on growth*Pseudomonas*sp. and Bacillus sp.

Figure 1 and Figure 2 show that the higher the concentration of the ethanol extract of Dayak onion bulbs and the ethanol extract of red ginger rhizome as antibacterial agents, the greater the inhibition zone formed in the medium According to (Ajizah, 2004), the higher the extract concentration causes the formation of a larger clear zone. The more concentrated the concentration of an extract, the more active compounds contained in the extract, thus affecting the diameter of the clear zone formed.

The results showed that the ethanol extract of Dayak onion bulbs and red ginger rhizome had antibacterial activity against the growth of Gram-negative (Pseudomonas sp.) and Gram-positive (Bacillus sp.) bacteria. Antibacterial activity of extracts of Dayak onion bulbs and red ginger rhizome on the growth of Pseudomonas sp. and Bacillus sp. said to be an antibacterial that is moderate to strong, because according to(Davis & Stout, 1971), the determination of the strength of a test substance against bacteria if the diameter of the inhibition zone is 20 mm or more is referred to as very strong, if the diameter of the inhibition zone is 10-20 mm strong, 5-10 mm is medium, and below 5 mm is weak.

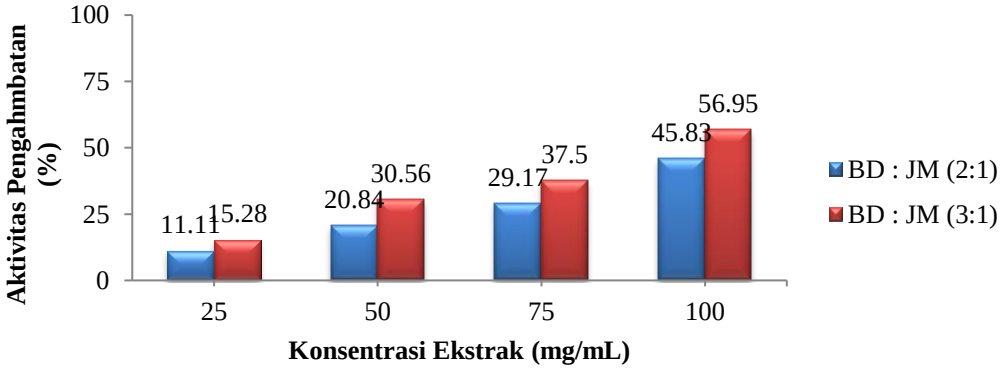

Figure 3. Inhibition Activity of the Combination of Ethanol Extract of Dayak Onion Bulbs and Red Ginger Rhizomes on Growth*Pseudomonas*sp.

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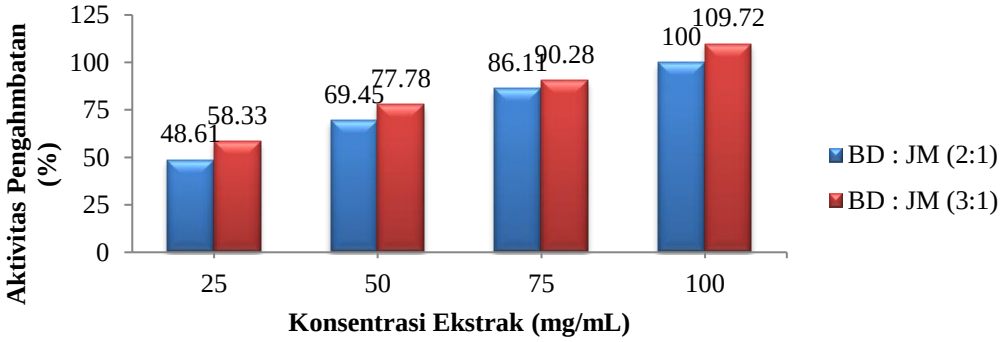

Figure 4. Inhibition Activity of the Combination of Ethanol Extract of Dayak Onion Bulbs and Red Ginger Rhizomes on Growth *Bacillus* sp.

Figure 3 and Figure 4 show the antibacterial activity of the combination of ethanol extract of Dayak onion bulbs and red ginger rhizome on the growth of Pseudomonas sp. and Bacillus sp. The results showed that the combination of ethanol extract of Dayak onion bulbs and red ginger rhizome with a combination of 2:1 and 3:1 at different concentrations had antibacterial activity against the growth of Pseudomonas sp. and Bacillus sp. The greatest inhibitory activity on the growth of Pseudomonas sp. is at a concentration of 100 mg/mL with a ratio of 3:1 which is equal to 56.95%; while Bacillus sp. at a concentration of 100 mg/mL with a ratio of 3:1 that is equal to 109.72%.

Statistical tests carried out on the antibacterial activity of the combination of ethanol extract of Dayak onion bulbs and red ginger rhizome using ANOVA showed that variations in extract concentrations had a very significant effect ($p>0.05$) on inhibiting the growth of Pseudomonas sp. and Bacillus sp. The results of the 5% DMRT follow-up test showed that the higher the concentration of the combined ethanol extract of Dayak onion bulbs and red ginger rhizome, the greater the inhibition zone formed.

(Jawetz et al., 1996)stated that the antibacterial activity of a compound was influenced by several factors including the content of the antibacterial compound, the diffusivity of the extract, the type of bacteria being inhibited and the concentration of the extract. Based on the research results, it was proven that increasing concentrations of the ethanol extract of Dayak onion bulbs, the ethanol extract of red ginger rhizome, and the combination of the two extracts had a significant effect on the inhibitory activity.

Antibacterial activity test results also showed greater inhibition of Gram-positive bacteria (Bacillus sp.) than Gram-negative bacteria (Pseudomonas sp.). These results indicate that Bacillus sp. more sensitive to both types of extract than other test bacteria. Differences in the sensitivity of bacteria to antibacterials are influenced by the structure of the bacterial cell wall. The cell wall of Gram-negative bacteria only contains a small amount of peptidoglycan and does not contain teichoic acid, so that the cell wall of Gram-negative bacteria is relatively more resistant to mechanical damage.(Pratiwi, 2008).

The different compound content between the ethanol extract of Dayak onion bulbs and red ginger rhizome can have an effect on the inhibition produced in each combination. The compounds contained in each extract are different, namely, Dayak onions contain flavonoids, terpenoids, alkaloids, tannins and steroids.(Harlita et al., 2018), while red ginger extract contains essential oils, oleoresin, and flavonoids as antibacterial compounds(Setyawan, 2015).This class of compounds has been widely studied and proven to have antibacterial effects.

Negative control and positive control were used as a comparison in determining the antibacterial effectiveness of the combination of extracts of Dayak onion bulbs and red ginger rhizome. The results showed that sterile aquadest as a negative control did not show inhibition of bacterial growth, this proved that the solvent had no effect on antibacterial activity, so the activity only came from the active compounds contained in the extract of Dayak onion bulbs and red ginger rhizome, not from the solvent used. .

The diameter of the inhibition zone and the antibacterial effectiveness of the combination of Dayak onion bulbs and red ginger rhizome were smaller than the diameter of the inhibition zone of the positive control chloramphenicol 30 µg/mL. The results showed that the diameter of the inhibition zone formed by using 30 µg/mL chloramphenicol against Pseudomonas sp. of 14 mm and Bacillus sp. by 27mm. Antibacterial effectiveness was obtained by comparing the best treatment with a positive control. Chloramphenicol 30 µg/mL obtained an effectiveness of 67.21% in inhibiting the growth of Pseudomonas sp. and 46.59% in inhibiting the growth of Bacillus sp. This shows that the combination of extracts of Dayak onion bulbs and red ginger rhizome has potential as an antibacterial, but its potential is still below that of 30 µg/mL chloramphenicol.

CONCLUSION

The ethanolic extracts of Dayak onion (E. palmifolia (L.) Merr) and red ginger (Z. officinale Rosc. Var. Rubrum) bulbs were shown to have antibacterial activity in inhibiting the growth of Pseudomonas sp. and Bacillus sp. either alone or with various combinations of extracts. The combination of ethanol extract of Dayak onion bulbs and red ginger rhizome had the best antibacterial activity at a 3:1 combination of 56.95% on the growth of Pseudomonas sp. and 109.72% on the growth of Bacillus sp in vitro. The inhibition effectiveness of Pseudomonas sp. the combination of extracts to chloramphenicol 30 µg/mL was 67.21% while for Bacillus sp. of 46.59%. This test can be continued with various variations of different combinations of extracts on various bacterial species, both Gram positive and Gram negative, and also against fungi.The community can also utilize Dayak onion bulbs and red ginger rhizome as an alternative ingredient for natural traditional treatment of bacterial gastrointestinal diseases through simple methods such as by boiling or infusion.

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