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Antibacterial activity of bangle rhizome essential oil (zingiber montanum) against streptococcus mutans

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Abstract

Objective: This research was conducted to determine the potential of bangle rhizome essential oil (zingiber montanum) as an antibacterial against the growth of streptococcus mutans.

Method: Tests are carried out using the diffusion method for the well's technique. In this study, eugenol was used as a positive control. Essential oil was diluted with acetone and obtained essential oil of bangle rhizome with a concentration of 3.12%, 6.25%, 12.5%, 25%, and 50%.

Results: That bangle rhizome essential oil succeeded in inhibiting the growth of streptococcus mutans with the minimum inhibitory concentration 3.12% and with the optimal inhibitory concentration 50%.

Conclusion: That essential oil from bangle rhizome is a strong, new natural antibacterial agent for streptococcus mutans. There was a slight difference of antibacterial effectiveness between eugenol, as positive control, and essential oil from bangle rhizome on streptococcus mutans bacterial growth observed from their inhibition zones.

Keywords: Antibacterial, Bangle rhizome, Caries, Essential oils, Streptococcus mutans

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Introduction

Basic Health Research in 2018 showed that Indonesian suffer caries reached 45.3% around 48% in the province of East Kalimantan.[1] Dental caries itself is an oral infection of demineralized tooth enamel.[2] Caries begins with the formation of biofilm and plaque.[3] The biofilm will provide a place for bacteria to adhere and colonize to grow. The main bacteria that cause caries in teeth is streptococcus mutans.[4] This is due to the ability of this bacteria to colonize and form biofilms to adhere to the tooth surface. Attempts to prevent dental caries can be done to remove plaque, which is also called plaque control. [5] Plaque control therapy ingredients from natural products have been shown to be the source for the development of new drugs throughout human history. Several studies have shown the feasibility of using medicinal plants as a source of therapeutic agents for the prevention of oral diseases.[6] One of the medicinal plants used is bangle (zingiber montanum). Bangle has been reported that it can be an antiulcer, anti-inflammatory and anti-inflammatory properties.[7,8] One of the active ingredients of bangle rhizome is essential oil. The phytochemicals contained in bangle essential oil with various pharmacological properties, including anti-inflammatory, antifungal, and antibacterial effects.[9] Therefore, the researchers tested the essential oil antibacterial activity of the rhizome of zingiber montanum against the growth of streptococcus mutans.

Material and Methods

Material: bangle rhizome, acetone, eugenol, media nutrient agar, weight scaler, essential oil steam distillation equipment, glass bottles, storage cabinets, handscoons, masks, petridishes, autoclaves, test tubes, micropipettes, incubators, cotton swabs, analytical scales, ose needles / inoculation needles, and cork borer.

Methods: Steam distillation method; Bangle rhizome as much as six and a half kilos collected and brushed to clean. Bangle rhizome is sliced 3-5 mm thick for essential oil distillation and processing. Bangle rhizome that has been sliced is put in a distiller, and begins to be distilled by the steaming method. After four hours, a distillate containing essential oil and water was obtained. Furthermore, oil and water are separated using a separating funnel. After obtaining the essential oil, it is necessary to make sure that the water has separated completely. Therefore, Na_2SO_4 is added to unify the water contained in essential oils. Furthermore, the essential oil is taken using a pipette, then stored in a closed container.

Dilution of essential oil, bangle essential oil is diluted with acetone and the obtained with concentrations of 3.12%, 6.25%, 12.5%, 25% and

Table 1 The diameter of the inhibition zone of essensial oil from bangle rhizome on the growth of streptococcus mutans

Samples	Concentration (%)	Mean (mm)
Essential Oil from Bangle Rhizome	3.12	9.53
	6.25	12.00
	12.5	14.33
	25	17.03
	50	20.12
Positive Control (Eugenol)	3.12	19.37
	6.25	25.63
	12.5	26.13
	25	28.97
	50	24.90
Negative Control (Acetone)		0.00

Table 2 Antibacterial category according to greenwood

Clear zone diameter	Growth inhibitory response
>20 mm	Very strong
10-20 mm	Strong
5-10 mm	Moderate
< 5 mm	Weak

50%; Microbial culture; Nutrient agar media was weighed with the composition of nutrient broth (13gr / 1000 ml), glucose (2gr and agar (20gr/1000ml). Furthermore, it is dissolved using aquadest, homogenized and brought to a boil. The media and tools used were sterilized using an autoclave at a temperature of 121°C for 15 minutes. Microbial culture is carried out by regrowing the microbes that have grown previously on the old media into new media. The microbes were taken 1 ose and inoculated on new media. Incubation for 24 hours at 32°C: Antibacterial test; Pour the sterile medium into a petri dish and compress it. Next, make a bacterial suspension by dissolving the bacteria that were taken using a loop needle and homogenized in a sterile aquadest. The suspension is adjusted to the standard Mc. Farland. Pour 25 of suspension onto the agar surface and smear it using a sterile cotton swab 3 times. Make a hole in the media using a sterile cork borer. The sample is inserted into a hole with a diameter of 6 mm which has been made as much as 20 . Each test was repeated 3 times, and incubated for 24 hours at 37°C.

Measurement of the inhibition zone of the inhibition zone by calculating the size of the inhibition zone then averaged and calculated into mm.

The statistical analysis used in the study are test of normality using One-Sample Kolmogorov-Smirnov Test, test of homogeneity using Levene's Test and parametric test using One Way Annova.

Results

Data from the table 1 shows the diameter of the inhibition zone (in mm) formed after 24 hours incubation at 37°C. One of the requirements for using the One-way Annova parametric test, the data must have a normal distribution and the same variance (homogeneous). The data distribution was carried out by the Kolmogorov-Smirnov Test for normality. Based on the normality test, the value of p>0.05 was obtained. Shows that the data distribution is normal. Furthermore, the homogeneity test was carried out. Based on the homogeneity test, the data obtained has a homogeneous variant because the significance value is >0.05. Thus, the One-way Annova parametric test can be used in this study. The results of the One-way Annova statistical test showed p value: 0.00 (p<0.05). This shows that there are significant differences in the inhibition zone.

The results of the research on the antibacterial activity of bangle rhizome essential oil (zingiber montanum) showed that the essential oil had antibacterial activity against the growth of streptococcus mutans. The results of this study were proven by the formation of a clear zone. The category of antibacterial ability based on the diameter of the inhibition zone formed according to Greenwood can be classified as follows table 2.

Discussion

The results of the interpretation according to the criteria of greenwood conducted in this study found that the essential oil of bangle rhizome (zingiber montanum) with a concentration of 3.12% had moderate antibacterial activity. Bangle rhizome essential oil with a concentration of 6.25%, 12.5%, and 25% has strong antibacterial activity against the growth of streptococcus mutans. Meanwhile, bangle rhizome essential oil with a concentration of 50% has very strong antibacterial properties against the growth of streptococcus mutans. This statement is also supported by previous research that the essential oil of bangle rhizome (zingiber montanum) has antibacterial properties.[9,10] The diameter of the

bangle rhizome essential oil inhibition zone (zingiber montanum) increased along with the increase in the concentration of the essential oil tested. The increase in the diameter of the inhibition zone along with the increase in the concentration of essential oils tested indicated that the higher the concentration of essential oils, the stronger the antibacterial inhibition of bangle essential oil against the growth of streptococcus mutans. The higher the bangle rhizome essential oil concentration, the more active compound content will be.

The chemical active compounds contained in bangle rhizome essential oil have been studied in previous studies, including sabinene, gamma-terpinene, alpha-terpinene, terpinene-4-ol, and (E) -1- (3,4 dimethoxyphenyl) butadiene (DMPBD).[9] According to previous research, sabinene is the dominant compound contained in essential oils.[11,12] The sabinene content in bangle rhizome essential oil reaches 36-53%.[9] Sabinene is antibacterial because these compounds show anti-inflammatory activity by inhibiting the production of nitric oxide in bacterial lipopolysaccharides (LPS), one of which is streptococcus mutans.[11] Terpinene-4-ol is one of the active compounds contained in the essential oil of bangle rhizome (zingiber montanum) with a percentage of 21-29%.[9] Terpinene-4-ol is able to reduce the viability of bacterial strains, one of which is streptococcus mutans.[13] Terpinene-4-ol was reported to be able to destroy biofilms formed by oral pathogens such as Porphyromonas gingivalis, fusobacterium nucleatum,[14] streptococcus mutans, and Lactobacillus acidophilus.[15] Gamma terpinene is a natural compound found in essential oils from various types of plants.[16] Gamma terpinene is one of the active chemical compounds contained in bangle rhizome essential oil with a percentage of 6-7%.[9] Gamma terpinene is antibacterial, both in gram-positive and gram-negative bacteria. These compounds are bactericidal because they damage the lipid layer of the outer membrane of bacteria with a phenolic structure. The hydrophobic nature of essential oils results in the compounds contained in them partitioning the lipids of cell membranes and bacterial mitochondria, damaging the structure of bacterial cells, and causing these cells to become more permeable.[17] The antibacterial test of bangle rhizome essential oil against streptococcus mutans resulted in gamma terpinene damaging the membrane layer of streptococcus mutans and causing the cell structure of streptococcus mutans to be more permeable and easily damaged.

Each active compound contained in essential oils has a different effect in inhibiting bacterial activity.[18] This study proves that the active compounds contained in the essential oil of bangle rhizome (zingiber montanum) have antibacterial properties against the growth of streptococcus mutans. The measurement results of the average diameter of the inhibition zone by bangle rhizome essential oil and eugenol as a positive control were different. Along with the increase in the concentration of the bangle rhizome essential oil (zingiber montanum), the diameter of the inhibition zone that was formed was getting bigger. However, the highest inhibition zone formed by eugenol was at a concentration of 25% and decreased at its highest concentration. These results indicate that the increase in eugenol concentration is not directly proportional to the increase in the inhibition zone for the growth of streptococcus mutans bacteria. The same thing also happened to the results of the inhibition zone test the diameter of the inhibition zone does not always increase in proportion to the increase in the concentration of the test material. This can occur due to differences in diffusion rates of antibacterial compounds on agar media. Different types and concentrations of antibacterial compounds provide different inhibition zone diameters for a certain length of time.[19] Eugenol at the highest concentration, namely 50%, decreased the ability and speed to diffuse in agar. The diffusion process of antibacterial compounds can be influenced by the dilution factor. The higher the concentration of the compound, the lower the solubility, so that it can slow down the diffusion of the compound into the media. This results in a lack of ability of eugenol with high concentrations to inhibit the growth of streptococcus mutans.[20]

Conclusion

In this study, the rhizome of zingiber montanum can produce the essential oil with the strong anti-microbial activities against streptococcus mutans. It is concluded from this research that essential oil from bangle rhizome is a strong, new natural antibacterial agent for streptococcus mutans. There was a slight difference of antibacterial effectiveness between eugenol, as positive control, and essential oil from bangle rhizome on streptococcus mutans bacterial growth observed from their inhibition zones.

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Conflict of Interest

The authors report no conflict of interest.

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