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The Antibacterial Effectiveness of Red Ginger (*Zingiber Officinale Roscoe*) Essential Oil in Inhibiting The Growth of *Staphylococcus Aureus* and *Streptococcus Mutans*

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ABSTRACT

Red ginger extract has a category strong antibacterial effect on Staphylococcus aureus and Streptococcus mutans. Red ginger essential oil has the potential for stronger inhibition. This study aims to compare the antibacterial effectiveness of red ginger essential oil against Staphylococcus aureus and Streptococcus mutans. The design of this study was a laboratory experimental design with a factorial completely randomized design. The red ginger used in this study was proven to be a species of Zingiber officinale Roscoe. The production of essential oils in this study uses the steam distillation method. The content of secondary metabolites in red ginger was tested quantitatively by the GC-MS method. Determination of antibacterial activity using the disc diffusion method. The data were processed using the SPSS 21.0 program. The normality of data distribution was tested with the Shapiro-Wilk test, followed by one-way ANOVA, Levene's test, and the Tukey HSD Post Hoc Test. The results of the antibacterial test of red ginger essential oil against Staphylococcus aureus (21.21mm ± 0.315) and Streptoococcus mutans (23.43mm ± 0.189) proved that the inhibition power of the category was very strong at a concentration of 75%.

1. Introduction

Periodontal disease and caries are common in the oral cavity. In Indonesia, the prevalence of periodontal disease is 96.58%, while the problem of caries is 25.9%.[1,2] The most common periodontal diseases are gingivitis, periodontitis, and gingival abscess.[3,4] The main cause of gingival abscess is Gram-positive bacteria. Staphylococcus aureus.[5] Dental caries occurs due to Gram-positive bacteria Streptococcus mutans fermenting carbohydrates from food.[6,7]

The main principle in the treatment of periodontal problems and dental caries is to eliminate the causes of disease, either through mechanical plaque removal or the use of chemicals. Mechanically, it can be done scaling, root planning and, brushing the teeth.[8,9] Antibiotics are chemicals used to kill bacteria that cause abscesses in the oral cavity. However, its use can cause bacterial resistance and eliminate other useful bacteria in the oral cavity.[10,11] The use of chlorhexidine mouthwash is effective in inhibiting bacterial growth and preventing the formation of dental plaque.[12,13] While the inhibition of chlorhexidine against Staphycoccus aureus ranges from 15, 80 mm to 27.32 mm.[14,15,16] Various studies on the effectiveness of

chlorhexidine mouthwash against Streptococcus mutans showed an inhibition zone between 14.15mm to 21.39 mm.[13,17,18]

Various studies using natural ingredients were carried out to find antibacterial alternatives against Staphylococcus aureus and Streptococcus mutans. One plant that has the potential as a natural antibacterial agent is red ginger or Zingiber officinale Roscoe. Widiastuti and Pramestuti (2018) obtained an inhibition zone from red ginger extract against Staphylococcus aureus of 12.54 ± 0.76 mm.[19] Meanwhile, research by Hendrastuti et al. (2018) showed the antibacterial potential of 100% red ginger extract against S. mutans. with an inhibition zone of 15.92 mm[2] The research conducted showed the antibacterial effectiveness of red ginger extract on S. aureus and S. mutans bacteria with a strong category of inhibition zone.[20,21] Phytochemical content of flavonoids, phenols, essential oils, and tannins. has antibacterial properties. [22]

Research on natural ingredients of red ginger uses more extract form. Meanwhile, red ginger essential oil has stronger antibacterial potential than the extract. This study aims to compare the antibacterial effectiveness of red ginger essential oil against Staphylococcus aureus and Streptococcus mutans. The results of this study are expected to add to the list of potential natural ingredients in the treatment of gingival abscess and dental caries.

2. Method

This research is a laboratory experimental in vitro. The research design was factorial completely randomized design. The sampling method was done by using purposive sampling technique.

Time and place of research

This research was conducted from February to March 2021. Plant taxonomic identification was carried out at the Herbarium Medanense Laboratory, and essential oil distillation was carried out at the Organic Chemistry Laboratory - University of North Sumatra. Quantitative phytochemical screening at the North Sumatra Customs Laboratory. Testing for bacterial inhibition zones was carried out at the Microbiology Laboratory of the Faculty of Dentistry - Universitas Prima Indonesia.

Research samples

The sample used in this study was red ginger, which was taken from a family plantation in Kabanjahe, North Sumatra. Red ginger essential oil used in the study was divided into four concentrations, namely: 100%, 75%, 50% and 25%. 0.2% chlorhexidine was used as a positive control, and DMSO as a negative control. Staphylococcus aureus and Streptococcus mutans isolates were provided by the Microbiology Laboratory of the Faculty of Dentistry - Prima Indonesia University.

Research tools

The tools used in this study were white tips, aluminum foil, cotton, tissue, gloves, cotton swabs, label paper, disc paper, masks, stahl flasks, oil heaters, 5mL syringes, stahl distillation tools, plastic wrap, GC-tools. MS, and calipers / rulers.

Research materials

In this study, using red ginger, Mueller Hinton Agar (MHA), Na_2SO_4, aquadest, spirits, 96% alcohol, 0.2% chlorhexidine, Dimethyl Sulfoxide (DMSO).[21]

Method of collecting data

How to make red ginger essential oil

The production of essential oils in this study uses the steam distillation method. A total of twenty five (25) kg of red ginger is thinly sliced. Then the slices are put into a Stahl flask and mixed with aquadest with a ratio of 1 : 3. After the

sample and aquadest are mixed, the Stahl flask is put into the Stahl distillation tool. Then carried out distillation for 5-6 hours with a temperature of about 100-105 ^{o}C. The distillate obtained is put into the vial bottle and add Na_2SO_4 to separate the water in the oil. Then 5.23 grams of red ginger essential oil was transferred to a new vial and closed tightly with aluminum foil.[7]

Identification of essential oil content with gas chormatogrphy-mass spectrometry (GC-MS)

Essential oils were analyzed using GC-MS to determine their phytochemical components quantitatively. Dilute three (3) drops of red ginger essential oil with 3 drops of n-hexane, and stir until homogeneous. Then 1 µL of red ginger essential oil was put into the column type Hp-5 ms on the GC-MS 7890-5977 device. The sample that has been entered will be carried by the carrier gas supply through the preheated column. The components contained in the essential oil will be read by a detector and recorded. The results obtained are in the form of a peak area that comes from the reading of the graph at a certain time. Then the analysis results from the GC-MS tool were matched with the literature. [23]

Antibacterial activity testing

Preparation of various concentrations of red ginger essential oil using the calculation formula N1 x V1 = N2 x V2.[24] Red ginger essential oil samples were diluted with DMSO. The concentrations of red ginger essential oil used in this study were 100%, 75%, 50% and 25%.

In this study, the determination of antibacterial activity used the disc diffusion method. Muller Hinton Agar (MHA) media was put into a petri dish and planted with different bacteria on each plate. Then the discs were soaked evenly in the sample solution of red ginger essential oil with various concentrations, chlorhexidine 0.2% (positive control), and DMSO (negative control). After that, the discs were placed in a previously prepared petri dish. Petri dishes were put into an incubator at 37^{o}C for 24 hours. After 24 hours, the diameter of the inhibition zone of the red ginger essential oil was measured using a caliper. The inhibition zone that is formed is clear in color around the disc paper.[25,26]

Data analysis

Data obtained from the measurement of inhibition zone diameter of red ginger essential oil against Staphylococcus aureus and Streptococcus mutans were recorded in the logbook. The data were processed using the SPSS 21.0 program. The normality of the data distribution was tested by using the Shapiro-Wilk test. If the data is not normally distributed, then a non-parametric test is performed using the Kruskal-Wallis test. In normally distributed data, continued testing with one-way ANOVA to analyze the significance of the difference in mean effectiveness between treatment groups.[27,28] Then analyzed the homogeneity of the studied variables with Levene's test, and the significance test with the Tukey HSD Post Hoc Test.

3. Result

Results of taxonomic identification of red ginger essential oil

The results of plant identification, at the Herbarium Medanense, University of North Sumatra, showed that the type of red ginger used in this study was Zingiber officinale Roscoe (identification number 5637 / MEDA / 2021).

Results of GCMS identification of red ginger essential oil

The results of GC-MS analysis of red ginger essential oil showed the ten largest phytochemical components detected (Table 1). The essential oil of red ginger (Zingiber officinale Roscoe) used in this study was shown to contain nine (9) characteristics of secondary metabolites in large quantities, namely: ar-curcumene, zingiberene,

cedrelanol, geraniol, selina-6-en-4o, geranyl acetate, nonifenol, trans-sesquisabinene hydrate, and citral.

Antibacterial activity test results

The results of the antibacterial test of red ginger essential oil against Staphylococcus aureus and Streptoococcus mutans (table 2) showed an increase in the zone of inhibition from a concentration of 25% to 75%. Of the four (4) concentrations studied (100%, 75%, 50%, and 25%), the inhibition zone of red ginger essential oil was in the very strong category21 at a concentration of 75% both against Staphylococcus aureus (21.21mm ± 0.315) and Streptococcus mutans (23.43mm ± 0.189). Inhibition produced by red ginger essential oil was more effective in inhibiting Streptococcus mutans bacteria compared to Staphylococcus aureus at all concentrations studied (Figures 1 and 2). In this study, it was seen that the inhibition power produced by red ginger essential oil was higher than 0.2% chlorhexidine.

In statistical analysis using the SPSS 21.0 program, the results of the data normality test for each group showed a p value> 0.05. The normally distributed data were analyzed using the one-way ANOVA test to determine the relationship between red ginger essential oil and the inhibition of growth of Staphylococcus aureus and Streptococcus mutans bacteria during a 24 hour period (table 3). The results showed a significant relationship between red ginger essential oil in inhibiting Staphylococcus aureus bacteria (p value = 0.042), and Streptococcus mutans (p value = 0.028).

Table 1. Identification results GC-MS red ginger essential oil

Compound Name	RT (retention time, minutes	Area	Score	Compound Formulas
1-(1,5-DIMETHYL-4-HEXENYL)-4-METHYLBENZENE (ar-Curcumene)	7.553	100	89.85	$C_{15}H_{22}$
1H-3a.7-Methanoazulene. octahydro3,8,8-trimethyl-6-methylene (Zingiberene)	7.92	30.21	97.37	$C_{15}H_{24}$
7-epi-cis-sesquisabinene hydrate (Cedrelanol)	8.139	25.25	87.2	$C_{15}H_{26}O$
Geraniol	3.417	25.16	93.89	$C_{10}H_{18}O$
Selina-6-en-4-o	9.018	21.64	8491	$C_{15}H_{26}O$
Geranyl acetate	6.602	16.73	99.09	$C_{12}H_{20}O_2$
Longipinocarveol, trans- (Nonilfenol)	9.274	14.77	85.02	$C_{15}H_{24}O$

trans-Sesquisabinene hydrate	8.615	9.16	88.25	$C_{15}H_{26}O$
trans-Sesquisabinene hydrate	8.432	8.4	82.51	$C_{15}H_{26}O$
2,6-Octadienal, 3,7-dimethyl *(citral)*	5.613	7.99	94.75	$C_{10}H_{16}O$

Table 2. Measurement results of inhibition zone diameter of red ginger essential oil against S. aureus and S.mutans bacteria

	Inhibition Zone Diameter (mm)	
	S. aureus	**S. mutans**
red ginger 100 %	18.61 ± 0.263	21.33 ± 0.452
red ginger 75%	21.21 ± 0.315	23.43 ± 0.189
red ginger 50%	16.84 ± 0.048	15.96 ± 0.256
red ginger 25%	14.79 ± 0.175	14.56 ± 0.229
chlorhexidine 0.2%	14.43 ± 0.150	15.33 ± 0.087
DMSO	0	0

Table 3.Relation between red ginger essential oil extract and growth inhibition of staphylococcus aureus and streptococcus mutans bacteria during a 24 hour period

Extract	**P value**
Red Ginger Extract * S.Aureus 24 Hours	0.042
Red Ginger Extract * S.Mutans 24 Hours	0.028

Explanation:
The category of bacterial inhibition zone according to Davis Stout (Ni made et al, 2016):
Constraint Zone Diameter > 20mm = Very strong
Inhibition Zone Diameter 10-2mm = Strong
Inhibition Zone Diameter 5-10mm = Medium
Obstacle Zone Diameter <5mm = Weak

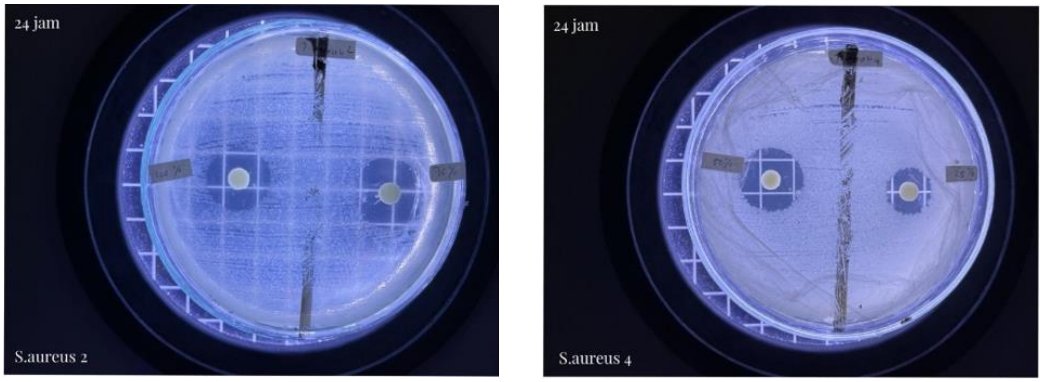

Figure 1. Inhibition of red ginger essential oil against staphylococcus aureus at concentrations of 100%, 75%, 50% and 25%.

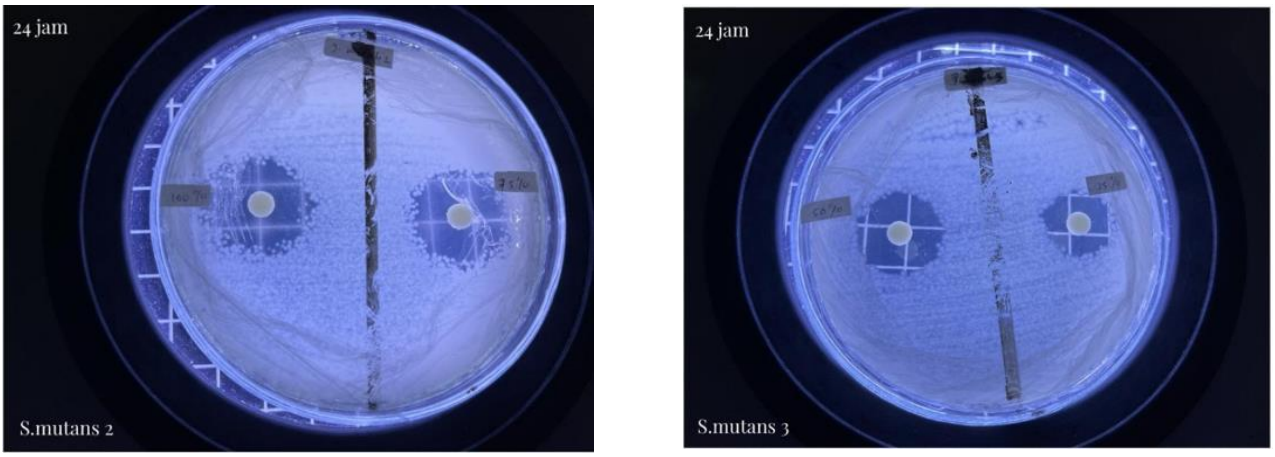

Figure 2. Inhibition of red ginger essential oil against streptococcus mutans at concentrations of 100%, 75%, 50% and 25%.

4. Discussion

The results of this study prove that red ginger essential oil is very effective in inhibiting Gram-positive bacteria, especially Streptococcus mutans and Staphylococcus aureus. In this study, the inhibition zone of red ginger essential oil was very strong at a concentration of 75% both against Staphylococcus aureus (21.21mm ± 0.315) and Streptococcus mutans (23.43mm ± 0.189). Research by Lely et al. (2016) showed that red ginger essential oil was effective against S. aureus bacteria at a concentration of 20% with an inhibition zone diameter of 20.1 ± 0.6 mm.[29] Febriyosa and Rahayuningsih (2021) explained that the content of white ginger essential oil in their research contained curcumin, antimicrobial substances that kill bacteria by leaking bacterial cell membranes.[30]

The red ginger essential oil samples examined by GC-MS proved the presence of ar-curcumene, zingiberene, cedrelanol, geraniol, selina-6-en-4o, geranyl acetate, nonifenol, trans-sesquisabinene hydrate, and citral compounds. Sekarini et al. (2020) stated that curcumin (ar-Curcumene), the largest content in red ginger essential oil, shows antibacterial effectiveness against Gram-positive and Gram-negative bacteria.[31] Other studies have stated that zingiberene, zingiberol, geraniol, citral and citronellol in essential oils Red ginger is an active compound with antimicrobial properties.[19,21,23,32,33], The content of geraniol and citral of red ginger essential oil has antibacterial activity.[29] Geraniol is a monoterpen compound in the form of alcohol which works by denaturing protein. Terpenes have a toxic effect on the function and structure of bacterial membranes.[34] Citral is bactericid by damaging the permeability of bacterial cell walls and causing disturbed supply of nutrients, ions and water resulting in bacterial cell death.[35]

5. Conclusion

The results of this study detected a very strong antibacterial effect on the essential oil of red ginger against Gram-positive bacteria. The content of secondary metabolites in red ginger essential oil containing ar-curcumene, zingiberene, geraniol, and citral plays a role in inhibiting the growth of Staphylococcus aureus and Streptococcus mutans bacteria.

Researchers suggest that further research is carried out to test the effectiveness of the inhibition of red ginger essential oil on other types of bacteria.

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