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# The Test of Antibacterial Activity of Bangle Rhizome (Zingiber montanum) against the Growth of Streptococcus mutans, Porphyromonas gingivalis, and Enterococcus faecalis bacteria

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**Keywords**
Disk Diffusion, Antibacterial Activity, Inhibitory Property, Zingiber montanum, Streptococcus mutans, Porphyromonas gingivalis,

**Abstract**
Various therapeutic agents can be used to help maintain a neutral condition in the oral cavity, one of which is mouthwash. However, mouthwash uses synthetic active chemical ingredients which, in the long term, may pose risks to health; in certain levels, it has been reported to cause side effects and is toxic. Bangle rhizome (Zingiber montanum) is a natural ingredient that contains various phytochemicals and has antibacterial properties. This research aims to observe the extent of inhibitory property of bangle rhizome against the growth of Streptococcus mutans, Porphyromonas gingivalis and Enterococcus faecalis. The ethanol extract of bangle rhizome was divided into 5 concentrations (60% w/v, 70% w/v, 80% w/v, 90% w/v, and 100% w/v). The inhibitory property test was performed using the Kirby-Bauer disk diffusion test with 3 replications. Chlorhexidine 0.2% was used as positive control and DMSO 10% was used as negative control. The results of this research showed that the ethanol extract of bangle rhizome could inhibit the growth of Streptococcus mutans, Porphyromonas gingivalis and Enterococcus faecalis bacteria in all concentrations.

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## INTRODUCTION

Based on data quoted from the WHO Global Oral Health Status Report (2022), it is estimated that oral diseases affect almost 3.5 billion people worldwide. Research conducted by the WHO Global Oral Health Status Report also determined five main oral diseases and conditions (hereinafter referred to as oral diseases) with the highest prevalence and most commonly experienced, namely untreated caries in primary and permanent teeth, severe periodontal disease, edentulism (total loss of teeth) and cancer of the lips and oral cavity. Quoting the results of the Indonesian Ministry of Health's Basic Health Research (Riskesdas) in 2018, the proportion of dental and oral problems in the Indonesian population was 57.6% and only around 10.2% had received medical services. For dental and oral problems, the population in East Kalimantan Province is quite high with a proportion of 61.52% and Samarinda City is 61.73%. This shows that more than half of the population of Samarinda City complains or has problems with their dental and oral health.

Dental caries is the most common multifactorial disease and its prevalence in a population is influenced by a number of factors, one of which is bacterial activity. The frequency of the presence of Streptococcus mutans is considered to be much higher in the group with active caries than in the caries-free group, so that S. mutans is believed to be the main pathogen causing dental caries. (Babaeekhou, Mehrizi, & Ghane, 2020) . Untreated dental caries can cause disease in the dental pulp which ultimately requires root canal treatment, but in practice, root canal treatment is not always successful. The main reason for failure of root canal treatment is the presence of several bacterial species in the root canal

system such as Enterococcus faecalis which are resistant to disinfectant agents thereby causing persistent intra-radicular or extra-radicular infections. (Alghamdi & Shakir, 2020) .

Apart from dental caries, the prevalence of periodontal disease in Indonesia ranks second after caries, reaching 96.58%. (Duwisda, Rusminah, & Susanto, 2016) . Research on the incidence of periodontal disease has shown various evidence regarding the contribution of the anaerobic bacteria Porphyromonas gingivalis to the development of periodontal disease. P. gingivalis is one of the main etiological agents in the pathogenesis and development of inflammatory periodontal disease (How, Song, & Chan, 2016) .

Many therapeutic agents can be used to help maintain a neutral condition of the oral cavity. Therapeutic agents that are often used in the field of dentistry contain synthetic chemical active ingredients which in the long term pose risks to health and in certain levels have been reported to cause side effects and are toxic. Therefore, it is necessary to develop therapeutic agents made from herbal ingredients with minimal side effects. Several studies have shown the feasibility of using medicinal plants as therapeutic agents in preventing oral diseases (Hasan, Danishuddin, & Khan, 2015) .

East Kalimantan has a tropical rainforest type with enormous biodiversity. Among the biodiversity of tropical forests, there are various types of plants that have the potential to be used as medicinal plants. Bangle rhizome is a plant that is often used as herbal medicine by the community. This plant is easy to find and cultivate, so it has enough potential to explore the benefits it contains (Buldani et al., 2017).

Previous research has shown that bangle rhizomes contain various active compounds such as saponins, flavonoids, essential oils, alkaloids, tannins and glycosides (Padmasari et al., 2013). Based on research conducted by Pardosi et al., (2022) it shows that bangle rhizome is effective in reducing the growth of Streptococcus mutans bacteria in the weak category. Based on research by Astuti et al. (2023) stated that the essential oil content of bangle rhizome is effective in reducing the growth of Porphyromonas gingivalis bacteria by forming an inhibition zone in the weak category. The results of other studies show that essential oils from the Zingiberaceae family show bioactivity against several gram-positive bacteria and gram-negative Enterococcus faecalis (ATCC 2921) and Escherichia coli (ATCC 25922) (Abobakr, Tawfick, Ibrahim, & Abdulall, 2022) . The use of antibacterial compounds from bangle rhizomes on oral bacteria still needs further research. Based on this background, researchers are interested in researching and testing the potential antibacterial activity of bangle rhizome extract (Zingiber montanum) on the growth of oral bacteria.

The aim of this research was to determine the antibacterial activity of bangle rhizome extract (Zingiber montanum) against the bacteria Streptococcus mutans, Porphyromonas gingivalis and Enterococcus faecalis. Apart from that, this research also aims to determine the diameter of the antibacterial inhibition zone of bangle rhizome extract against these bacteria. It is hoped that the results of this research will provide benefits to science and technology, health institutions, society and researchers. For science and technology, this research can add insight into the antibacterial activity of bangle rhizome extract and has the potential for the development of natural therapies and further research. For health institutions, these results can be used as input to maximize the use of bangle rhizomes in health promotion activities. For the public, this research can provide information regarding the potential use of bangle rhizomes as a natural alternative in overcoming dental and oral health problems. For researchers, this research can increase scientific insight and knowledge regarding the potential antibacterial activity of bangle rhizome extract.

## RESEARCH METHODS

This research is a type of pure experimental research (true experimental) with a post test only control group design. This experimental research was carried out using the in vitro disc diffusion method. The test group in this study was a group consisting of 7 treatments with 2 different groups of Streptococcus mutans, Porphyromonas gingivalis and Enterococcus faecalis bacteria. The first group was the test group which was given bangle rhizome extract (Zingiber montanum) in concentrations of 60% w/v, 70% w/v, 80% w/v,

90% w/v and 100% w/v. Manufacturing concentration is calculated using the percent weight/volume equation:

$$\frac{\text{grams dissolved substances } (weight\ solute)}{\text{mL solution } (volume\ solution)} \times 100$$

The second group is the control group which in this study used a positive control in the form of Chlorhexidine 0.2% and a negative control in the form of DMSO (Dymethyl Sulfoxide) 10% (Rohama et al., 2023). This research was carried out with 3 repetitions (triplo) in the test group and control group. This research was carried out at the Pharmacology Laboratory of the Faculty of Medicine, Mulawarman University for the process of extracting bangle rhizomes and making extract concentrations, and the UPTD Regional Health Laboratory of East Kalimantan Province to test the antibacterial activity of bangle rhizome extract against the bacteria Streptococcus mutans, Porphyromonas gingivalis and Enterococcus faecalis using the disc diffusion method. . The research was carried out in February - May 2024. The test bacterial subjects used were Streptococcus mutans ATCC 25175, Porphyromonas gingivalis ATCC 33277, and Enterococcus faecalis ATCC 29212 which were obtained from the UPTD Regional Health Laboratory of East Kalimantan Province. Meanwhile, the subject plant used was bangle rhizome (Zingiber montanum) obtained from Tanjung Warat Village, Sambaliung District, Berau Regency, East Kalimantan at one year old. The tools used in this research include simplicia ovens, simplicia grinders, digital scales, analytical scales; extract bowls, oses, maceration jars, autoclaves, rotary vacuum evaporators, and others. The materials used are ethanol extract of bangle rhizome (Z. montanum), 96% ethanol solvent, distilled water, 10% DMSO, 0.2% chlorhexidine, paper discs, bacterial culture, Mueller Hinton Agar (MHA)+5% sheep blood, blood agar and Nutrient Agar (NA).

## RESULTS AND DISCUSSION

### Results of Antibacterial Activity Test of Bangle Rhizome Extract (Zingiber montanum) against Streptococcus mutans Bacteria

The results of the antibacterial activity test of bangle rhizome extract against Streptococcus mutans bacteria showed that there was a clear zone at all test concentrations and the positive control was 0.2% chlorhexidine. This can be seen in each repetition which shows the formation of an antibacterial inhibition zone.

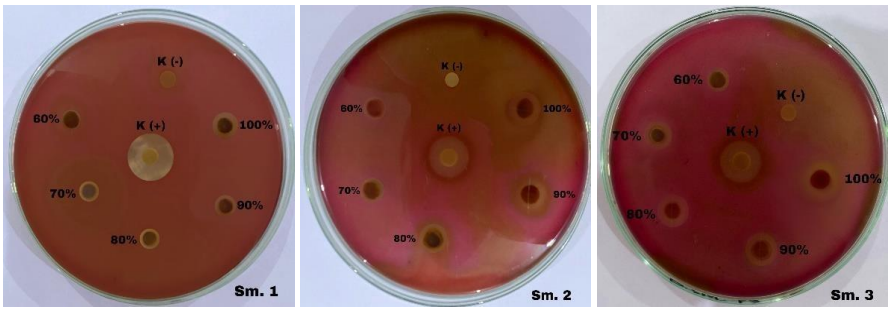


**Figure 1 Antibacterial inhibition zone of Bangle rhizome extract against bacteria S. mutans**

Based on Figure 1, it can be seen that the inhibition zone produced by bangle rhizome extract in S. mutans bacteria shows an increase in the diameter of the inhibition zone as the extract concentration increases.

**Table 1 Diameter of the zone of inhibition of bangle rhizome extract against S. mutans bacteria**

| Group treatment | Diameter zone resistor (mm) | | | Average (mm) ± S.E | *p* |
|---|---|---|---|---|---|
| | P1 | P2 | P3 | | |
| 60% w/v | 1.00 | 1.00 | 1.70 | 1.23±0.23 | |
| 70% w/v | 1.40 | 2.00 | 2.00 | 1.80±0.20 | |
| 80% w/v | 1.45 | 2.80 | 3.40 | 2.55±0.57 | |
| 90% w/v | 2.15 | 3.45 | 4.50 | 3.36±0.67 | 0,000 |
| 100% w/v | 3.20 | 4.50 | 4.65 | 4.11±0.46 | |
| CHX 0.2% (K+) | 7.15 | 7.20 | 7.05 | 7.13±0.44 | |
| DMSO 10% (K-) | 0 | 0 | 0 | - | - |

Ket. : One Way Anova, p<0.01
CHX=Chlorhexidine ; DMSO=Dimethyl Sulfoxide

In table 1, the results of the average diameter of the inhibition zone (mm) of bangle rhizome extract against S. mutans bacteria obtained from the treatment of each extract concentration and also the control group with three repetitions were presented and reduced by the diameter of the paper disc, namely 6 mm. At extract concentrations of 60% w/v, 70% w/v, 80% w/v, 90% w/v, and 100% w/v, the average size of the inhibition zone was 1.23 ± 0.23 mm, 1.80 respectively. ± 0.20 mm, 2.55 ± 0.57 mm, 3.36 ± 0.67 mm and 4.11 ± 0.46. Meanwhile, the positive control using 0.2% chlorhexidine produced the largest inhibitory zone diameter compared to the extract group, namely 7.13 ± 0.44 mm and for the negative control using 10% DMSO, the inhibition zone formed was 0 mm or no inhibition zone was formed in the treatment.

The Shapiro-Wilk normality test and Lavene's test of homogeneity show that Streptococcus mutans bacteria have a significant value of p>0.05, so in this case the research data is said to be normally distributed and homogeneous so that it meets the requirements for carrying out the One Way ANOVA test. The p value in the Sig column. Shows a value of 0.000 which indicates that there is a difference between each treatment group in the results of bangle rhizome extract, so the next test is to see which groups are different using a further test (Post Hoc test).

Based on the results of the Test of Homogeneity of Variances, showing the same variance or homogeneous data distribution, the further test used is the Bonferroni test.

**Table 2 Bonferroni's follow-up (Post-Hoc) test results against S. mutans bacteria**

| *Bonferroni test* | 60% w/v | 70% w/v | 80% w/v | 90% w/v | 100% w/v | K+ |
|---|---|---|---|---|---|---|
| **60% w/v** | | 1,000 | 0.760 | 0.064 | 0.007* | 0,000* |
| **70% w/v** | 1,000 | | 1,000 | 0.359 | 0.037* | 0,000* |
| **80% w/v** | 0.760 | 1,000 | | 1,000 | 0.359 | 0,000* |
| **90% w/v** | 0.064 | 0.359 | 1,000 | | 1,000 | 0.001* |
| **100% w/v** | 0.007* | 0.037* | 0.359 | 1,000 | | 0.005* |
| **K+** | 0,000* | 0,000* | 0,000* | 0.001* | 0.005* | |

Note: * = has a significant difference (p<0.05)

Bonferroni Post-Hoc Test was carried out to find out which groups had significant differences. Significant differences are marked with a p value <0.05. Table 5.2 shows that the positive control 0.2% chlorhexidine had a significant difference with all treatments of bangle rhizome extract concentration. Apart from that, concentrations of 60% w/v and 70% w/v were significantly different from bangle rhizome extract with a concentration of 100% w/v.

**Results of Antibacterial Activity Test of Bangle Rhizome Extract (Zingiber montanum) against Porphyromonas gingivalis Bacteria**

The results of the antibacterial activity test of bangle rhizome extract against Porphyromonas gingivalis bacteria showed that there was a clear zone in each concentration group and the positive control was 0.2% chlorhexidine. This can be seen in each repetition which shows the formation of an antibacterial inhibition zone.

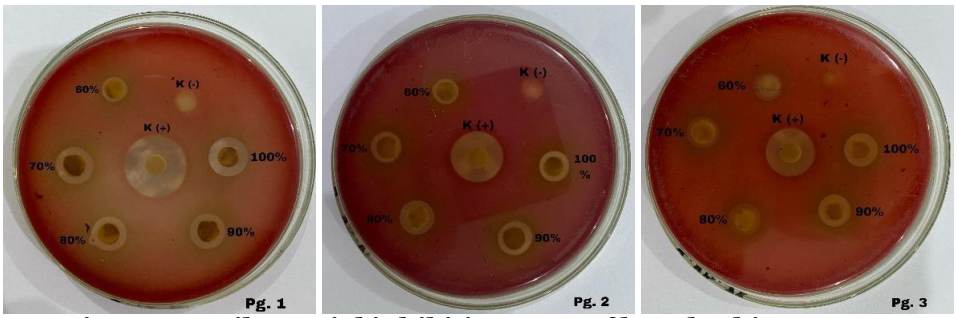


**Figure 2 Antibacterial inhibition zone of bangle rhizome extract against P. Gingivalis bacteria**

Based on Figure 2, it can be seen that the inhibition zone produced by bangle rhizome extract on P. gingivalis bacteria shows an increase in the diameter of the inhibition zone as the extract concentration increases.

**Table 3 Diameter of inhibition zone of Bangle rhizome extract against P. gingivalis bacteria**

| Group treatment | Diameter zone resistor (mm) | | | Average (mm) ± S.E | *p* |
|---|---|---|---|---|---|
| | P1 | P2 | P3 | | |
| 60% w/v | 2.00 | 2.55 | 1.10 | 1.88±0.42 | |
| 70% w/v | 4.30 | 3.00 | 3.00 | 3.43±0.43 | |
| 80% w/v | 4.40 | 3.20 | 3.20 | 3.60±0.40 | |
| 90% w/v | 4.40 | 3.70 | 3.20 | 3.76±0.34 | 0,000 |
| 100% w/v | 5.55 | 3.80 | 4.00 | 4.45±0.55 | |
| CHX 0.2% (K+) | 9.30 | 11.40 | 9.50 | 10.06±0.66 | |
| DMSO 10% (K-) | 0 | 0 | 0 | - | - |

Ket. : One Way Anova, p<0.01
CHX=Chlorhexidine ; DMSO=Dimethyl Sulfoxide

In table 3, the results of the average diameter of the inhibition zone (mm) of bangle rhizome extract against P. gingivalis bacteria obtained from treatment with each extract concentration and also the control group are presented. At extract concentrations of 60%, 70%, 80%, 90% and 100% the average inhibitory zone sizes were 1.88 ± 0.42 mm, 3.43 ± 0.43 mm, 3.60 ± 0.40 mm, 3.76 ± 0.34 mm and 4.45 respectively. ± 0.55. Meanwhile, the positive control using 0.2% chlorhexidine produced the largest inhibitory zone diameter compared to the extract group, namely 10.06 ± 0.66 mm and for the negative control using 10% DMSO no inhibition zone was formed in the treatment.

The Shapiro-Wilk normality test and Lavene's test of homogeneity show that Porphyromonas gingivalis bacteria have a significant value of p>0.05, so in this case the research data is said to be normally distributed and homogeneous so that it meets the requirements for carrying out the One Way ANOVA test. The p value in the Sig column. shows a value of 0.000 which indicates that there is a difference between each treatment group in the results of bangle rhizome extract, so the next test is to see which groups are different using a further test (Post Hoc test).

Based on the results of the Test of Homogeneity of Variances, showing the same variance or homogeneous data distribution, the further test used is the Bonferroni test.

**Table 4 Bonferroni follow-up (Post-Hoc) test results against P bacteria. gingivalis**

| *Bonferroni test* | 60% w/v | 70% w/v | 80% w/v | 90% w/v | 100% w/v | K+ |
|---|---|---|---|---|---|---|
| **60% w/v** | | 0.639 | 0.410 | 0.261 | 0.041* | 0,000* |
| **70% w/v** | 0.639 | | 1,000 | 1,000 | 1,000 | 0,000* |
| **80% w/v** | 0.410 | 1,000 | | 1,000 | 1,000 | 0,000* |
| **90% w/v** | 0.261 | 1,000 | 1,000 | | 1,000 | 0,000* |
| **100% w/v** | 0.041* | 1,000 | 1,000 | 1,000 | | 0,000* |
| **K+** | 0.013* | 0.053 | 0.170 | 0.562 | 1,000 | |

Note: * = has a significant difference (p<0.05)

Bonferroni Post-Hoc Test was carried out to find out which groups had significant differences. Significant differences are marked with a p value <0.05. Table 5.4 shows that the positive control 0.2% chlorhexidine had a significant difference with all treatments of bangle rhizome extract concentration. Apart from that, the concentration of 60% w/v was significantly different from the bangle rhizome extract with a concentration of 100% w/v.

**Results of Antibacterial Activity Test of Bangle Rhizome Extract (Zingiber montanum) against Enterococcus faecalis Bacteria**

The results of the antibacterial activity test of Bangle rhizome extract against Entercococcus faecalis bacteria showed that there was a clear zone in each concentration group and the positive control was 0.2% chlorhexidine. This can be seen in each repetition which shows the formation of an antibacterial inhibition zone.

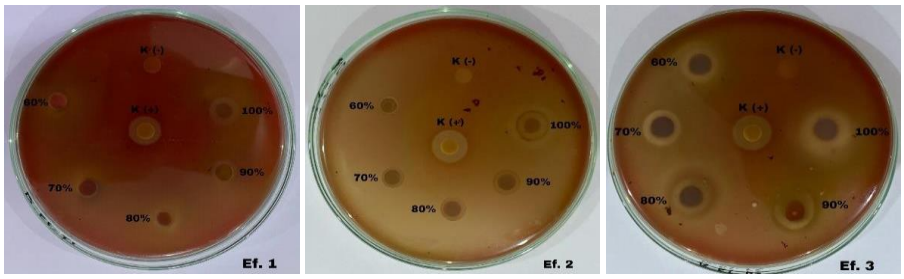


**Figure 3 Antibacterial inhibition zone of bangle rhizome extract To E. faecalis bacteria**

Based on Figure 3, it can be seen that the inhibition zone produced by bangle rhizome extract in S. mutans bacteria shows an increase in the diameter of the inhibition zone as the extract concentration increases.

**Table 5 Antibacterial activity test results for treatment groups against the bacteria E. faecalis**

| Group treatment | Diameter zone resistor (mm) | | | Average (mm) ± S.E | *p* |
|---|---|---|---|---|---|
| | P1 | P2 | P3 | | |
| 60% w/v | 0.90 | 1.10 | 3.25 | 1.75±0.75 | |
| 70% w/v | 1.75 | 1.70 | 4.00 | 2.48±0.75 | |
| 80% w/v | 2.00 | 2.70 | 4.50 | 3.06±0.74 | |
| 90% w/v | 2.55 | 3.70 | 4.75 | 3.66±0.63 | 0,000 |
| 100% w/v | 3.45 | 4.40 | 5.60 | 4.48±0.62 | |
| CHX 0.2% (K+) | 5.40 | 5.70 | 6.40 | 5.83±0.29 | |
| DMSO 10% (K-) | 0 | 0 | 0 | - | - |

Ket. : One Way Anova, p<0.01
CHX=Chlorhexidine ; DMSO=Dimethyl Sulfoxide

In table 5, the results of the average diameter of the inhibition zone (mm) of Bangle rhizome extract against E. faecalis bacteria obtained from treatment with each extract concentration and also the control group are presented in table 5. At extract concentrations of 60%, 70%, 80%, 90% and 100% the average inhibitory zone sizes were 1.75 ± 0.752 mm, 2.48 ± 0.758 mm, 3.06 ± 0.744 mm, 3.66 ± 0.635 mm and 4.48 respectively. ± 0.622. Meanwhile, the positive control using 0.2% chlorhexidine produced the largest inhibitory zone diameter compared to the extract group, namely 5.83 ± 0.296 mm and for the negative control using 10% DMSO no inhibition zone was formed in the treatment.

The Shapiro-Wilk normality test and Lavene's test of homogeneity show that Enterococcus faecalis bacteria have a significant value of p>0.05, so in this case the research data is said to be normally distributed and homogeneous so that it meets the requirements for carrying out the One Way ANOVA test. The p value in the Sig column. shows a value of 0.000 which indicates that there is a difference between each treatment group in the results of bangle rhizome extract, so the next test is to see which groups are different using a further test (Post Hoc test).

Based on the results of the Test of Homogeneity of Variances, showing the same variance or homogeneous data distribution, the further test used is the Bonferroni test.

**Table 6 Bonferroni follow-up (Post-Hoc) test results against E. faecalis bacteria**

| Bonferroni *test* | 60% w/v | 70% w/v | 80% w/v | 90% w/v | 100% w/v | K+ |
|---|---|---|---|---|---|---|
| **60% w/v** | | 1,000 | 1,000 | 0.912 | 0.182 | 0.013* |
| **70% w/v** | 1,000 | | 1,000 | 1,000 | 0.777 | 0.053 |
| **80% w/v** | 1,000 | 1,000 | | 1,000 | 1,000 | 0.170 |
| **90% w/v** | 0.912 | 1,000 | 1,000 | | 1,000 | 0.562 |
| **100% w/v** | 0.182 | 0.777 | 1,000 | 1,000 | | 1,000 |
| **K+** | 0.013* | 0.053 | 0.170 | 0.562 | 1,000 | |

Note: * = has a significant difference (p<0.05)

Bonferroni Post-Hoc Test (appendix 12) was carried out to find out which groups had significant differences. Significant differences are marked with a significance value of p<0.05. Table 5.6 shows that the positive control chlorhexidine 0.2% has a significant difference from bangle rhizome extract at a concentration of 60% w/v. This is different from the test results on the bacteria Streptococcus mutans and Porphyromonas gingivalis.

## Discussion

### Interpretation of Results and Discussion

Test of the antibacterial activity of bangle rhizome extract (Zingiber montanum) with concentrations of 60%, 70%, 80%, 90% and 100% against the bacteria Streptococcus mutas, Porphyromonas gingivalis and Enterococcus faecalis which had been incubated for 24 hours showed a clear zone around the paper disc. This shows that bangle rhizome extract (Zingiber montanum) has antibacterial activity.

Chlorhexidine 0.2% as a positive control showed a clear zone around the paper disc, indicating that Chlorhexidine 0.2% had antibacterial activity, while DMSO 10% which was used as a negative control in this study did not show the formation of a clear zone. This shows that 10% DMSO does not have antibacterial activity.

The antibacterial activity of bangle rhizome extract (Zingiber montanum) against the bacteria Streptococcus mutas, Porphyromonas gingivalis, and Enterococcus faecalis can be influenced by several factors including technical factors and biological factors. Technical factors are factors that can be controlled by researchers, but biological factors cannot be controlled by researchers. Technical factors consist of inoculum density, incubation time, environmental temperature and media selection.

The inoculum density used has been adjusted to the 0.5 McFarland standard or the equivalent of 1x108 bacteria/mL which has been confirmed using spectrophotometry. The media used to test antibacterial activity was Mueller Hinton agar with the addition of 5% sheep blood for S. mutans and E. faecalis bacteria and blood agar for the growth of P.

gingivalis bacteria. The use of Muller Hinton agar with the addition of 5% sheep blood for S. mutans and E. faecalis bacteria is because Mueller Hinton agar with 5% sheep blood is the medium recommended by the Clinical and Laboratory Standards Institute (CLSI) for disc diffusion testing. antimicrobial against bacteria. Performance standards for antimicrobial disc susceptibility tests provide procedures for testing aerobic and facultative anaerobic bacteria that include members

Enterobacteriaceae, Staphylococcus spp., Enterococcus spp., Pseudomonas spp., Acinetobacter spp., and Streptococcus spp (Nobrega et al., 2021) . The use of blood agar as a growth medium for P. gingivalis bacteria is because Porphyromonas gingivalis requires the availability of heme (iron) and vitamin K as a source of nutrition in its environment. P.gingivalis forms black pigmented colonies on blood agar plates which is related to the aggregation of heme on the cell surface because this bacterium requires iron as a nutrient (Septiwidyati & Bachtiar, 2020) . P. gingivalis is also an asaccharolytic organism that depends on nitrogenous substrates for energy. Therefore, blood agar is an optimal medium for the growth of P. gingivalis bacteria.

The incubation time in this study was 24 hours at a temperature of 37°C. All technical factors in this research can be controlled by the researcher. Biological factors consist of persistence and resistance. Persistence comes from cells that are dormant or replicate slowly so they cannot be killed by antibacterial agents. The persistence factor can be controlled by using an inoculum that does not exceed 24 hours or an inoculum in the logarithmic phase (Huemer, Mairpady Shambat, Brugger, & Zinkernagel, 2020) . Resistance cannot be controlled in research because it is an adaptation of bacteria to survive. Resistance in this study did not occur because there was an antibacterial inhibition zone in the extract treatment group and the positive control. The higher the concentration of the extract used, the greater the amount of dissolved antibacterial compounds, making it easier for antibacterial compounds to penetrate into bacterial cells and the larger the inhibition zone that will be formed.

The results of this study indicate the antibacterial ability of ethanol extract of bangle rhizome which is also related to the active compound content in secondary metabolites of bangle rhizome. Based on phytochemical screening carried out by Padmasari et al (2013), bangle rhizomes contain secondary metabolites in the form of tannins, flavonoids, saponins, alkaloids and essential oil compounds.

Tannin is an antibacterial compound that works by binding to protein, thereby inhibiting the formation of bacterial cell walls. Tannins have antibacterial effects through reactions with cell membranes, inactivation of enzymes, and inactivation of the function of genetic material. Tannins have the ability to inactivate microbial cell adhesins, inactivate enzymes, and disrupt protein transport in the inner layers of cells. Tannins also have a specific target action on cell wall polypeptides so that the formation of bacterial cell walls is less than perfect. These various mechanisms of action of tannins can cause bacterial cells to lyse due to osmotic or physical pressure so that the bacterial cells will die (Sarijowan, Bodhi, Lebang, & Abdullah, 2022) .

Flavonoids have antibacterial properties that can inhibit bacterial motility by releasing transduction energy against the bacterial cytoplasmic membrane. Flavonoids also contain hydroxyl groups which can cause changes in organic components and nutrient transport which ultimately have toxic effects on bacteria. The mechanism of action of flavonoids functions as an antibacterial by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes. The mechanism of action is by denaturing bacterial cell proteins and damaging the cell membrane beyond repair (Fatmawati, Hikmawanti, Fadillah, & Putri, 2022) .

Saponin is able to penetrate the cell membrane of gram-negative bacteria as a result of the reaction of saponin with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming strong polymer bonds, resulting in damage to the porins. This results in the permeability of the bacterial cell membrane being reduced which will result in the bacterial cells becoming deficient in nutrition, so that bacterial growth is hampered or dies (Wahyuni & Karim, 2020) .

Another antibacterial compound contained in bangle rhizomes is alkaloids. The mechanism of action of alkaloids as antibacterials is by disrupting the peptidoglycan components in bacterial cells so that the cell wall layer is difficult or not even fully formed and causes cell death. In addition, the alkaloid component is known to be a DNA interchelator and inhibits the topoisomerase enzyme in bacterial cells (Wiart, 2021) . The mechanism of action of flavonoids as antimicrobials can be divided into 3, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism (Sari & Yowani, 2022) .

Essential oil is an important ingredient in bangle rhizomes. Essential oils are known for several antibacterial mechanisms, including lysing cell membranes by dissolving phospholipids, causing cell membranes to be in a hypertonic environment thereby inhibiting cell wall formation and interacting hydroxyl groups with carbonyl groups with bacterial cell membrane proteins so that these proteins lose their function (Halimathussadiah, Rahmawati, & Indriyanti, 2021) .

The content of secondary metabolites in a plant is influenced by several factors, both internal and external. Internal factors such as genes and external factors include location, light, temperature, humidity, pH and nutrient content in the soil. Different locations will produce different temperatures. Location is one of the factors that influences the growth of a plant. A series of metabolic processes in plants will depend on the fertility conditions in each region. Apart from location, the quality of secondary metabolites is also influenced by plant age. These factors can influence the amount of antibacterial substances contained in the sample and have the possibility of producing different antibacterial strengths on the same type of plant. (Nabila, Purnamasari, & Alhawaris, 2021) .

The maceration method was chosen to extract bangle rhizomes because it is the simplest method that does not involve a heating process with the aim of avoiding damage to compounds that are not resistant to heating. Maceration allows many compounds to be extracted, although some compounds have limited solubility at room temperature. In the extraction process, the choice of organic solvent is important to be able to dissolve secondary metabolites optimally. The solvent chosen in this study was 96% ethanol. 96% ethanol was chosen as the solvent in this extraction because according to research conducted by Yuswi (2017), it was stated that the best maceration extraction treatment test results were obtained in the solvent type treatment using 96% ethanol (Yuswi, 2017) .

**Antibacterial Activity of Bangle (Zingiber montanum) Rhizome Extract against Streptococcus mutans Bacteria**

The results of the antibacterial activity test of bangle rhizome extract were proven to inhibit the growth of S. mutans by forming an inhibitory zone diameter around the disc. This research is in accordance with previous research conducted by Pardosi et al., 2022 that the essential oil of bangle rhizome is able to provide antibacterial activity on Streptococcus mutans bacteria (Pardosi, Purnamasari, Paramita, Astuti, & Arung, 2022) .

The results of the concentration treatment of 60% w/v, 70% w/v, 80% w/v, 90% w/v, and 100% w/v respectively showed the results of large inhibition zones, namely 1.23 ± 0.233 mm, 1.80 ± 0.200 mm, 2.55 ± 0.576 mm, 3.36 ± 0.679 mm and 4.11 ± 0.460. The measurement results were then interpreted based on the criteria of Davis & Stout (1971) and it was found that bangle rhizome extract had antibacterial activity in the weak category.

The results of the diameter of the inhibition zone showed concentrations of 60% w/v, 70% w/v, 80% w/v, 90% w/v, and 100% w/v with a p value <0.05 which indicates the presence of bacterial growth inhibitory activity at this concentration. extract. In the inhibition zone, the positive control showed significant differences with all extract concentration treatments. This shows a significant difference in the strength of inhibiting bacterial growth in 0.2% chlorhexidine compared to all treatment concentrations of bangle rhizome extract. Apart from that, the extract with a concentration of 60% w/v and 70% w/v also had a significant difference in the zone of inhibition compared to the bangle rhizome extract with a concentration of 100% w/v.

Based on the results of research and evaluation of the antibacterial inhibition zone formed in Streptococcus mutans bacteria, it shows that the inhibition zone of bangle rhizome extract (Zingiber montanum) increases along with increasing concentrations of the bangle rhizome extract tested. The increase in the diameter of the inhibition zone along with the increase in the concentration of the extract tested shows that the higher the extract concentration, the stronger the antibacterial inhibitory power of Bangle rhizome extract on the growth of Streptococcus mutans bacteria. This is supported by the statement of Sarijowan et al., 2022 in their research that the higher the concentration of an antibacterial ingredient, the stronger the antibacterial activity (Sarijowan et al., 2022) . This is also in accordance with research by Roslizawaty et al., 2015 that the effectiveness of an antibacterial substance is influenced by the concentration of the substance. Increasing the concentration of substances causes an increase in the content of active compounds which function as antibacterials, so that the ability to kill bacteria is also greater.

This research is in line with research conducted by Buldani et al., 2017 and Iswantini et al., 2011 that bangle rhizomes (Zingiber montanum) contain a number of active compounds which act as antibacterial compounds (Buldani, Yulianti, & Soedomo, 2017) . This research is also in line with that carried out by Pardosi et al., 2022 which showed that bangle rhizome essential oil was able to inhibit the growth of S. mutans bacteria in the weak category. (Pardosi et al., 2022) .

The structure of bacterial cell walls can determine the penetration of a substance, binding and activity of antibacterial compounds. Streptococcus mutans bacteria are gram-positive bacteria that have a cell wall structure with more peptidoglycan, less pleated and contain polysaccharides (teichoic acid). Teichoic acid is a water-soluble polymer, which functions as a transporter of positive ions in and out of substances. This water-soluble nature shows that the cell walls of gram-positive bacteria are more polar (Abobakr et al., 2022) . Bangle rhizome contains flavonoid compounds which are polar in nature so that it is easier to penetrate the peptidoglycan layer which is polar in the bacterial cell wall. The incoming antibacterial compounds will cause greater osmotic pressure in the cells, thereby causing lysis (Kováč et al., 2022) .

**Antibacterial Activity of Bangle (Zingiber montanum) Rhizome Extract against Porphyromonas gingivalis Bacteria**

The results of the antibacterial activity test of Bangle rhizome extract against Porphyromonas gingivalis bacteria showed the formation of a diameter of inhibition zone around the disc, thus proving that Bangle rhizome extract has antibacterial activity against P. gingivalis bacteria. This research is in accordance with previous research conducted by Astuti et al. (2023) that Bangle rhizome essential oil through the dilution method is able to provide antibacterial activity on P. gingivalis bacteria with a maximum concentration of 50% and a weak inhibitory category (Astuti, Asfirizal, Utami, Listyawati, & Fabiola, 2023) .

The results of treatment concentrations of 60%, 70%, 80%, 90%, and 100% respectively showed large inhibition zone results, namely 1.88 ± 0.422 mm, 3.43 ± 0.433 mm, 3.60 ± 0.400 mm, 3.76 ± 0.348 mm and 4.45 ± 0.553. Meanwhile, the positive control using 0.2% chlorhexidine produced the largest inhibitory zone diameter compared to the extract group, namely 10.06 ± 0.669 mm and for the negative control using 10% DMSO no inhibition zone was formed in the treatment. The measurement results were then interpreted based on the criteria of Davis & Stout (1971) and it was found that bangle rhizome extract had antibacterial activity in the weak category.

In the results of the multiple comparison test (appendix 11), the positive control inhibition zone showed a significant difference with all extract concentration treatments. This shows a significant difference in the strength of inhibiting bacterial growth in 0.2% chlorhexidine compared to all treatment concentrations of bangle rhizome extract. Apart from that, the extract with a concentration of 60% w/v also had a significant difference in the zone of inhibition compared to the bangle rhizome extract with a concentration of 100% w/v.

This research is in line with research conducted by Astuti et al., 2023 that the rhizome of bangle (Zingiber montanum) contains essential oils which are effective in reducing the growth of P.gingivalis bacteria as seen by the formation of an inhibitory zone in the weak category and the inhibitory power formed increases as increasing concentration of essential oils (Astuti et al., 2023) . Based on the results of research and evaluation of the antibacterial inhibition zone formed in Porphyromonas gingivalis bacteria, the research results showed that the inhibition zone of bangle rhizome extract (zingiber montanum) increased along with increasing concentrations of the bangle rhizome extract tested even though it was still classified as a weak antibacterial category.

One of the ingredients in bangle rhizomes which is thought to have antibacterial activity is the essential oil component of tropolone-derived monoterpenoids which are found naturally in bangle plants so that they can inhibit the growth of oral bacteria, such as S. mutans, S. sobrinus, and P. gingivalis in vitro (Rezaei et al., 2023) .

From the results of research on bangle rhizome extract using 96% ethanol solvent, the antibacterial inhibition zone produced was still relatively low even though it was at the optimum concentration. Differences in the sensitivity of pathogenic bacteria to antibacterials can be caused by different cell wall structures. Gram-negative bacteria have a thicker cell wall structure, high lipid content, and a single peptidoglycan (Duwisda et al., 2016) . As for this study, the inhibition zone produced by Porphyromonas gingivalis bacteria produced the greatest inhibitory power compared to Streptococcus mutans and Enterococcus faecalis bacteria. This research is in line with research conducted by (Sidauruk, Sari, Diharmi, & Arif, 2021) which shows that antibacterial inhibition of gram-negative bacteria is greater than gram-positive bacteria. This shows that gram-negative bacteria are more susceptible to the antibacterial active compounds of bangle rhizome extract than gram-positive bacteria. This is thought to be because gram-negative bacteria have protein groups that are hydrophilic and can be easily penetrated by polar compounds in the ethanol extract of bangle rhizomes (Rachmawati, Rabbani, Rumidatul, Fadhila, & Maryana, 2020) . This research is not in line with research conducted by (Nurbianti, Alhawaris, & Yani, 2021) which states that growth inhibition in P. gingivalis bacteria is less than in S. mutans and E. faecalis, because the components are more complex in gram-negative bacteria.

The inhibition zone formed can be caused by the essential oil content in bangle rhizome extract which contains compounds in the form of 4-terpineol (Fitriana, Fatimah, & Fitri, 2020) and flavonoids (Mardianingrum, 2019) . Terpene compounds such as 4-terpineol have hydrophobic properties which can disrupt bacterial cell growth by reducing intracellular ATP reserves, reducing bacterial membrane potential, and lowering intracellular pH. Apart from that, terpenoid compounds also have the ability to damage bacterial cell walls through their lipophilic groups (Halimathussadiah et al., 2021) . Phenolic compounds, namely flavonoids, found in bangle rhizomes, have the ability to denature bacterial cells so they can stop bacterial cell activity. This causes the permeability function of bacterial cells to be disrupted and bacterial cells will experience lysis which results in bacterial cell death (Permatasari & Saputri, 2023) .

**Antibacterial Activity of Bangle (Zingiber montanum) Rhizome Extract against Enterococcus faecalis Bacteria**

The results of the antibacterial effectiveness test of Bangle rhizome extract were proven to inhibit the growth of Enterococcus faecalis by forming an inhibitory zone diameter around the disc. At extract concentrations of 60%, 70%, 80%, 90% and 100%, the average inhibitory zone sizes were 1.75 ± 0.752 mm, 2.48 ± 0.758 mm, 3.06 ± 0.744 mm, 3.66 ± 0.635 mm and 4.48 respectively. ± 0.622. Meanwhile, the positive control using 0.2% chlorhexidine produced the largest inhibitory zone diameter compared to the extract group, namely 5.83 ± 0.296 mm and for the negative control using 10% DMSO no inhibition zone was formed in the treatment. The measurement results were then interpreted based on the criteria of Davis & Stout (1971) and it was found that bangle rhizome extract had antibacterial activity in the weak category.

In Table 6 it can be seen that a significant difference is only shown by the comparison of the positive control 0.2% chlorhexidine with the 60% w/v concentration extract. This shows that 0.2% chlorhexidine has a significant difference in the strength of inhibiting bacterial growth in bangle rhizome extract with a concentration of 60% w/v.

Based on the results of research and evaluation of the antibacterial inhibition zone formed in E. faecalis bacteria, the research results showed that the inhibition zone of bangle rhizome extract (zingiber montanum) increased along with increasing concentrations of the bangle rhizome extract tested even though it was still classified as a weak anibacterial. The increase in the diameter of the inhibition zone along with the increase in the concentration of the extract tested shows that the higher the extract concentration, the stronger the antibacterial inhibitory power of Bangle rhizome extract on the growth of E. faecalis bacteria. The higher the concentration of the extract, the higher the active compound content.

The results of this research are in line with the antibacterial activity tests that have been carried out on the antioxidant essential oil and methanol extract of Zingiber montanum, that the extract has potential inhibitory power against Bacillus subtilis, Staphylococcus aureus, S. epidermidis, Escherichia coli and Enterococcus faecalis bacteria. (Permatasari & Saputri, 2023) .

The structure of bacterial cell walls can determine the penetration of a substance, binding and activity of antibacterial compounds. Enterococcus faecalis bacteria are gram-positive bacteria that have a cell wall structure with more peptidoglycan, less pleated and contain polysaccharides (teichoic acid). Teichoic acid is a water-soluble polymer, which functions as a transporter of positive ions in and out of substances. This water-soluble nature shows that the cell walls of gram-positive bacteria are more polar (Abobakr et al., 2022) . Bangle rhizome contains flavonoid compounds which are polar in nature so that it is easier to penetrate the peptidoglycan layer which is polar in the bacterial cell wall. The incoming antibacterial compounds will cause greater osmotic pressure in the cells, thereby causing lysis (Kováč et al., 2022) .

In this research there are several limitations. First, the bangle rhizomes taken as samples were done randomly, so the specific age, planting and harvest time of the plants tested were not known. Second, in this study no qualitative phytochemical screening of active compounds was carried out, so the amount of active substances in the ethanol extract could not be measured. This is a limitation because it cannot be known exactly what active compounds are contained in bangle rhizome extract and their role in antibacterial activity.

## CONCLUSION

Bangle rhizome ethanol extract has inhibitory power against the bacteria Streptococcus mutans, Porphyromonas gingivalis and Enterococcus faecalis at all concentrations. There are several suggestions that the researcher would like to put forward based on the results of the research that has been carried out. First, it is necessary to screen the phytochemical compounds for the active compounds of bangle rhizomes so that we can find out the types of phytochemicals that have the most potential in producing antibacterials. Second, further research needs to be done regarding the type of solvent that can be used to extract more phytochemicals from bangle rhizomes so that they are more effective in the extraction process. Third, further research needs to be carried out to determine the minimum inhibitory concentration (MIC) of bangle (Zingiber montanum) rhizome extract against the bacteria S. mutans, P. gingivalis and E. faecalis. Fourth, further research is needed to determine the optimum dose and risk of toxicity of bangle rhizome extract (Zingiber montanum). Fifth, further research needs to be carried out on testing the antibacterial activity of bangle rhizome extract (Zingiber montanum) against different bacteria. Sixth, further research needs to be carried out regarding the ability of bangle rhizome extract when applied as a material used in dental practice.

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