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Antibacterial Effects of Methanol Extract from Gletang (*Tridax procumbens L.*) on *Streptococcus mutans* Growth

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Abstract:

- ***Background:***

Dental caries is a form of bacterial infection of the hard tissues of teeth caused by the activity of *Streptococcus mutans bacteria* in dental plaque. Caries treatment must be comprehensive, encompassing preventive, curative, rehabilitative, and promotive efforts. The use of chemical antibacterials such as *chlorhexidine* has side effects, so alternatives from natural ingredients are needed. The use of natural ingredients as antibacterials can inhibit bacterial growth, one of which is the gletang plant (*Tridax procumbens L.).* The purpose of this study was to determine the antibacterial activity of the methanol fraction of the gletang plant (*Tridax procumbens L.)* with concentrations of 2%, 4%, 6% and 8% against *Streptococcus mutans bacteria.* This type of research was conducted *in vitro* with *a post-test only control group design.* Testing the antibacterial activity of the methanol fraction of the gletang plant (*Tridax procumbens L.)* used concentrations of 2%, 4%, 6% and 8, *chlorhexidine* as k (+) and methanol as k (-). The results showed that the methanol fraction of the gletang plant (*Tridax procumbens L.)* produced an inhibition zone with the average diameter of the inhibition zone increasing with increasing concentration. The highest concentration (8%) produced an inhibition zone of 4.1 mm, while 0.2% *chlorhexidine* produced an inhibition zone of 5.1 mm. Statistical analysis using the *Kruskal-Wallis test* showed that there was a significant difference between concentrations on the inhibition zone (p <0.05), this means that the methanol fraction of the gletang plant (*Tridax procumbens L.)* with concentrations of 2%, 4%, 6% and 8% has the potential as an antibacterial against *Streptococcus mutans* . The conclusion in this study is that there is antibacterial activity of the methanol fraction of the gletang plant (*Tridac procumbens L.)* against the growth of Streptococcus mutans bacteria.

Keywords: *Streptococcus mutans, Gletang plant, Tridax procumbens L., Inhibition Zone.*

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I. INTRODUCTION

Oral and dental health is one of the most important indicators that can determine a person's health status. *The World Health Organization* (WHO) in 2019 defined oral and dental health as a state free from chronic mouth and facial pain, mouth and throat cancer, mouth infections and sores, periodontal (gum) disease, tooth decay, tooth loss, and other conditions that can impede a person's ability to bite, chew, smile, speak, and psychosocial well-being [1].

Tooth tissue that is damaged is called caries, starting from the surface area of the tooth such as pits, fissures, interproximal areas and then can spread to the pulp area [2]. Caries is caused by a combination of factors, namely host, substrate, time and microorganisms [3]. The main cause of caries is microorganisms that form dental plaque which acts as *dental* plaque (*biofilm)* and becomes a habitat for microorganisms to survive. 4 The main microorganism that causes caries is *Streptococcus mutans* [5].

Streptococcus mutans is a Gram-positive facultative anaerobic bacterium in the oral cavity that is involved in the development of caries, both directly and indirectly [6,7]. *Streptococcus mutans* is usually found primarily in plaque attached to the tooth surface. This bacterium has a good ability

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to adapt to environmental changes in dental plaque by forming choline, thus facilitating the growth of other bacteria [8]. *Streptococcus mutans* can grow perfectly in an acidic environment and is able to produce acid as a result of carbohydrate fermentation [9]. This acid plays a role in tooth demineralization. The growth of *Streptococcus mutans bacteria* can be controlled with antibacterial agents [5].

Indonesia is known as *a Megadiversity Country*, meaning it is rich in biodiversity because it is located along the equator and has a tropical climate. Indonesia has around 9,600 species of medicinal plants. Based on the results of Novita's previous research in 2016, the antibacterial activity test of betel leaf fractions (*Piper betle L)* was carried out with n-Hexane, ethyl acetate and methanol fractions with a concentration of 10 mg/ml, the results showed that the n-Hexane fraction had the highest activity against the growth *of Streptococcus* mutans [10]. Betel leaves contain secondary metabolites such as essential oils, flavonoids, alkaloids and phenolic compounds that can inhibit the growth of *Streptococcus mutans* [5]. In addition to these plants, other plants that have antibacterial potential are gletang plants (*Tridax procumbens L.)* [11].

Gletang (*Tridax procumbens L.)* is a weed found along roadsides, vacant lots, waste areas, and grasslands [12]. Gletang, also known as "clothes button," is a plant from the *Asteraceae family* native to South and Central America [13]. Its use in therapy is due to its content such as alkaloids, carotenoids, dexamethasone, luteolin, flavonoids, tannins, titerpenoids, and sitosterol, which have antimicrobial properties. Alkaloids, flavonoids, and titerpenoids act as antibacterials [14].

Previous research conducted by Kumar in 2018 used methanol solvent to test the antibacterial activity *of Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Bacillus subtilis* and *Staphylococcus aureus* using gletang plant extract (*Tridax procumbens L.)*. The results showed that Gram-positive bacteria, namely *Bacillus subtilis* and *Staphylococcus aureus*, showed higher susceptibility to flower, leaf and whole plant extracts. *Staphylococcus aureus bacteria* showed maximum inhibitory power against the extract, while negative bacteria, namely *Escherichia coli, Klebsiella pneumoniae* and *Proteus vulgaris* showed the lowest inhibitory power against flower, leaf and whole plant extracts. Methanol solvent showed stronger activity against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*. It can be concluded from the results of Kumar's research that leaves and whole plant extraction " *Tridax procumbens L."* have great potential as antibacterial agents [15].

Fractionation is a method for separating and grouping chemical components in an extract based on their polarity. This process involves two solvents that do not mix with each other and have different levels of polarity [16]. In a study by Putri et al. in 2023, it was stated that polar compounds will dissolve in polar solvents, semi-polar compounds will dissolve in polar and non-polar solvents, and non-polar compounds will dissolve in non-polar solvents. Fractionation can be carried out with three solvents that have varying polarities, namely n-Hexane which is non-polar, ethyl acetate which is semi-polar, and methanol which is polar [17].

To date, there has been no previous research that has conducted research on the antibacterial activity test of the gletang plant fraction (*Tridax procumbens L.)* using methanol solvent. Therefore, researchers are interested in conducting research with the title of antibacterial activity test of the methanol fraction of the gletang plant (*Tridax procumbens L.)* against the growth of *Streptococcus mutans bacteria*. This initial fractionation research used small concentrations.

This study aims to determine the antibacterial activity of the methanol fraction of the gletang plant (*Tridax procumbens L.)*.

II. MATERIALS AND METHODS

The research conducted was an *in vitro* laboratory *experiment*. Using *a post-test Only Control Group Design research design* which aims to determine the antibacterial activity of the methanol fraction of the gletang plant (*Tridax procumbens L.) against Streptococcus mutans* bacteria. This research was conducted from June 2024 to December 2024. The location of this research was carried out at the Inter-University Research Laboratory (PAU) of Gajah Mada University, Yogyakarta. The population in this study was *Streptococcus mutans bacteria* obtained from the Inter-University Research Laboratory (PAU) of Gajah Mada University, Yogyakarta.

The samples in this study were methanol fraction bacteria from gletang plants (*Tridax procumbens L.)* with various concentrations, namely 2%, 4%, 6% and 8%.

➢ *The Samples Used were Divided into Several Groups, as Follows:*

- Group I: Methanol fraction of gletang plant (*Tridax procumbens L.)* with a concentration of 2%.
- Group II: Methanol fraction of gletang plant (*Tridax procumbens L.)* with a concentration of 4%.
- Group III: Methanol fraction of gletang plant (*Tridax procumbens L.)* with a concentration of 6%.
- Group IV: Methanol fraction of gletang plant (*Tridax procumbens L.)* with a concentration of 8%.
- Group (+): *Chlorhexidine* (CHX) 0.2%

- *Group (-): Methanol*

The sample size in this study used the Freeder formula: This study used four repetitions, therefore, there were many repetitions in this study. Based on the calculation results using the formula, there were four repetitions for each group. A total of six groups were used, resulting in a sample size of 24.

➢ *Research Procedures*

The working procedure in this research has several stages as follows:

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- Preparation of test material for gletang plants (*Tridax procumbens L.)*
- The process of making gletang plant extract (*Tridax procumbens L.)*
- The process of making fractionation of gletang plants (*Tridax procumbens L.)*
- *Streptococcus mutans* bacterial suspension
- Antibacterial activity testing process (inhibition zone)

➢ *Preparation of Gletang Plant Test Materials (Tridax procumbens L.)*

The test material preparation process is carried out in the following stages:

- Sampling of gletang plants (*Tridax procumbens L.)* as test material in Aie Pacah, Koto Tangah, Padang City, West Sumatra.
- The gletang plants are washed clean using running water, then drained and dried at room temperature without exposure to sunlight for 14 days on a clean white cloth.
- After drying, the gletang is cut into small pieces, then blended until smooth, so that 2 kg is obtained.

➢ *Preparation of Gletang Plant Extract (Tridax proccumbens L.)*

This extraction uses the maceration method and the solvent used in this study is 96% methanol. The stages of making gletang plant extract (*Tridax procumbens L.)* are as follows:

- Put the finely ground gletang plant (*Tridax procumbens L.) into a tube and soak the gletang plant (Tridax procumbens L.)* in methanol solvent. Then stir periodically and let it sit for 3 x 24 hours at room temperature.
- After 3 x 24 hours of soaking the gletang plant (*Tridax procumbens L.)* it was filtered using a plastic funnel and *Whatman filter paper* into an *Erlenmeyer flask* until the dregs separated so that the color of the mixture became slightly faded to obtain residue (results that were not used) and filtrate (results that were used).
- After that, it is put into a flask tube to be evaporated with *a vacuum evaporator* at a temperature of 60°C until a thick extract is obtained and put into a glass jar.
- Make the extract concentration using methanol solvent according to the concentration groups of 2%, 4%, 6% and 8%.

➢ *Preparation of Gletang Plant Fraction (Tridax procumbens L.)*

How to create a fraction:[18]

- Fractions were prepared using column chromatography, using silica gel G60 as the stationary phase and stirring methanol until thoroughly mixed. This mixture was poured into the column using a separating funnel and gently tapped to distribute the silica gel evenly throughout the column.
- Next, 30 grams of concentrated gletang extract was slowly introduced into the column using a dropper. It was then eluted with solvents of varying polarity, including non-polar, semi-polar, and polar solvents.
- The silica gel inserted into the first column was eluted with a non-polar solvent, n-hexane. 500 ml of n-hexane was added to the column. The resulting solution was collected in a vial labeled with the code GN, yielding the n-hexane fraction.
- Then, the second eluent, ethyl acetate, which is semi-polar, was inserted into the column as much as 500 ml and then collected in a vial tube labeled with the GE code. The ethyl acetate fraction was also obtained.
- Next, the final eluent that was put into the column, namely 500 ml of polar methanol solvent, was put in and collected in a vial tube coded GM and the methanol fraction was obtained.
- After obtaining the three fractions, they were then evaporated using *a vacuum rotary evaporator*. For n-hexane and ethyl acetate, the temperature was 40°C, and for methanol, the temperature was 45°C to obtain the concentrated fractions used for antibacterial activity testing.
- Then make variations of the test concentration solution with concentrations of 2%, 4%, 6% and 8%.

Table 1 Preparation of Concentrated Solution of Gletang Plant Extract (*Tridax procumbens L).*

2%	**4%**	**6%**	**8%**
0.2 gr extract + 10 ml solution	0.4 gr extract + 10 ml solution	0.6 gr extract + 10ml solution	0.8 gr extract + 10 ml solution

➢ *Streptococcus mutans Bacterial Suspension*

Streptococcus mutans bacterial culture from pure culture then *streaked on blood agar* culture media and incubated at 37°C for 24 hours. *Streptococcus mutans test bacteria* were taken using a sterile *loop needle* from the bacterial culture suspended in a test tube containing 5 ml of 0.9% NaCl solution then diluted, then homogenized using *a vortex* for 10 minutes. *Streptococcus mutans bacterial suspension* was applied using a sterile *cotton swab* that had been drained on the edge of the test tube wall then *streaked* evenly on the upper surface of the *Mueller Hinton agar* (MHA) media finally closed the media and let it sit for 5 minutes in *the bio safety cabinet* [19,20]

➢ *Antibacterial Activity Test*

Testing the inhibitory power of the methanol fraction of the gletang plant (*Tridax procumbens L.)* using the agar diffusion method with several stages as follows: [21]

- The tools used were washed clean and dried before conducting the test, petri dishes, test tubes, *Erlenmeyer flasks*, measuring cups, covered with cotton, wrapped in *aluminum foil*. Then sterilized in *an autoclave* at a temperature of 127°C then prepare the bacterial suspension that has been made.

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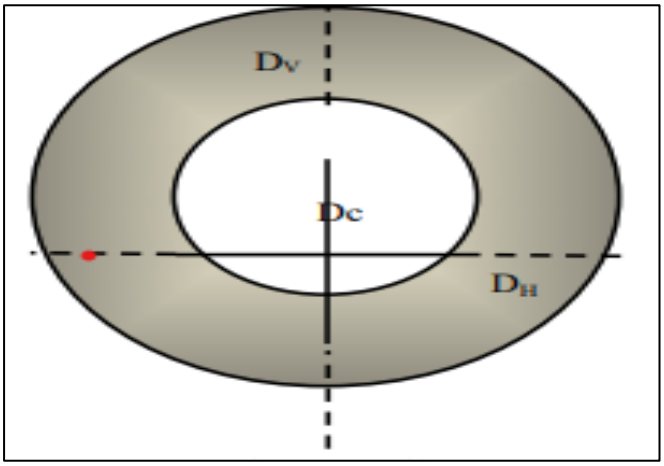
- Label the petri dishes using the respective codes for each concentration, positive control and negative control.
- Take a sterile cotton swab, dip it in *Streptococcus mutans bacteria*, apply it and spread it slowly onto *Nutrient Agar* (NA) on all sides.
- Next, drop all concentrations of the gletang *(Tridax procumbens L.) fraction* onto *the paper disc, along with a* 0.2% *chlorhexidine* positive control and a 96% methanol negative control. Then, place the paper disc on *Nutrient Agar* (NA) containing *Streptococcus mutans bacteria*. Then, cover the petri dish with paper film.
- Place the petri dish into *the anaerobic jar*, then incubate for 24 hours in an incubator at 37°C. After 24 hours, the clear area formed is observed and measured using a vernier caliper as the inhibition zone with the following formula:

- *Formula for Measuring the Spherical Inhibition Zone [22]*

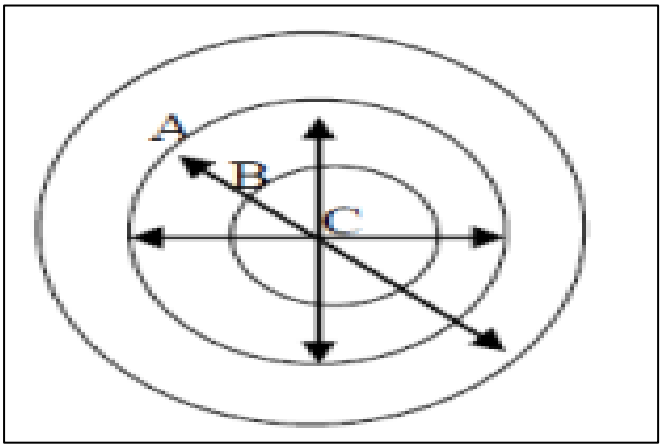


$$D = \frac{(Dv-Dc)+(Dh-Dc)}{2}$$

- *Information:*

✓ Dv: Vertical diameter
✓ Dh: Horizontal diameter
✓ Dc: Disc diameter

- *The Formula for Measuring the Inhibition Zone for Non-Round or Irregular Shapes, with the Formula:*



$$d = \frac{d_1 + d_2 + d_3 + \ldots + d_n)}{n}$$

- *Information:*

✓ *d* = Diameter of inhibition zone
✓ *d1* = Diameter of inhibition zone 1
✓ *d2* = Diameter of inhibition zone 2
✓ *n* = Number of measurements

Data analysis was carried out to determine the antibacterial activity of the methanol fraction of the gletang plant (*Tridax procumbens L.)* against the growth of *Streptococcus mutans bacteria.* and to determine the differences in antibacterial activity of the methanol fraction of the gletang plant (*Tridax procumbens L.)* with concentrations of 2%, 4%, 6% and 8% against *Streptococcus mutans bacteria*. The data was entered into *Microsoft Word* in the form of a table to see the results of the inhibition zone measurements.

The data obtained will be tested for normality using the *Shapiro-Wilk test.* Data analysis used to see the relationship between treatment groups is *the One Way ANOVA test* if the data distribution is normal and *the Kruskall-Wallis test* if the data distribution is not normal. *Post-hoc tests* are used to see the differences between the treatment group and the positive control group.

III. RESULTS

➢ *Antibacterial Effects of Methanol Extract from Gletang (Tridax procumbens L.) on Streptococcus mutans Growth*

Determination of antibacterial activity of methanol fraction of gletang plant against the growth of *Streptococcus mutans bacteria* using *Chlorhexidine* 0.2% as a positive control, because it is one of the best and most effective mouthwashes that are often used. The solvent used in this negative control is used for the preparation of gletang plant fraction (*Tridax procumbens L.)* is 96% methanol, to determine the effect of the solvent on the growth of *Streptococcus mutans bacteria*.

Testing of gletang plant fractions (*Tridax procumbens L.)* was done with concentrations of 2%, 4%, 6%, and 8%. The parameter used was the diameter of the inhibition zone around *the paper disk*. The diameter of the inhibition zone caused by antibacterial activity was measured with *a caliper*, then compared with 0.2% *Chlorhexidine* as a positive control and 96% methanol as a negative control. The following image shows the presence of an inhibition zone in this study:

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- *The Reesult Anibacterial Acitivity Test:*

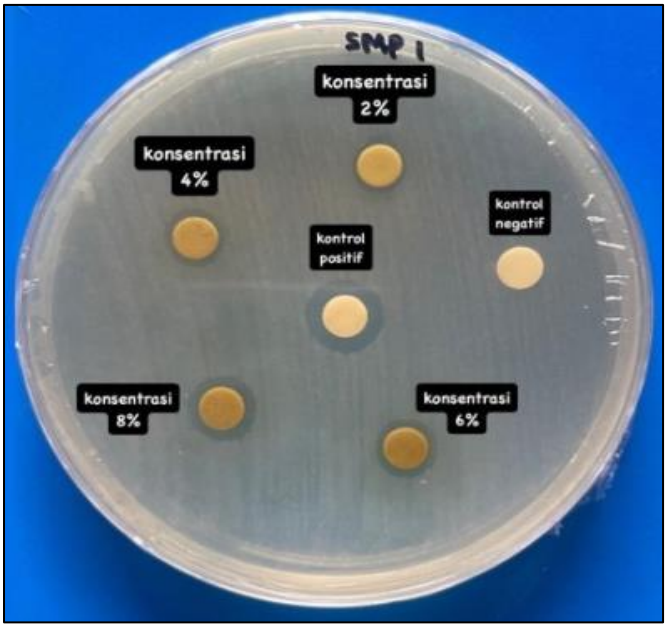

Fig 1 Antibacterial Activity Test of Methanol Fraction of Gletang Plant Against the Growth of *Streptococcus mutans Bacteria.*

Results of testing and observation of the antibacterial activity of gletang plant fractions on bacterial growth *Streptococcus mutans* is listed in Table 2 as follows:

Inhibition zone samples (mm) Mean + SD

Table 2 Antibacterial Activity Test Results of Methanol Fraction of Gletang Plant (Tridax procumbens L.) Against the Growth of Streptococcus mutans Bacteria.

Repetition	**Concentration**					
	2%	**4%**	**6%**	**8%**	***Chlorhexidine* 0.2% (+)**	**Methanol 96% (-)**
1	3.8	3.8	3.8	4.3	4.8	0
2	3.1	3.6	3.8	4.8	4.9	0
3	3.7	3.8	3.8	3.9	5.1	0
4	2.7	3.7	3.8	3.8	5.4	0
Average	3.3	3.7	3.8	4.2	5.1	0

Based on table 2 shows that the average diameter of the inhibition zone at a concentration of 2% (3.3 mm), a concentration of 4% (3.7 mm), a concentration of 6% (3.8 mm), a concentration of 8% (4.2 mm), while 0.2% *chlorhexidine* as a positive control is 5.1 mm and 96% methanol as a negative control is 0 mm. The average diameter of the largest inhibition zone in the positive control of 0.2% *chlorhexidine* is 5.1 mm with a medium category due to the results of measuring the area of the inhibition zone of 5-10 mm.

The inhibition zone of the methanol fraction of the gletang plant (*Tridax procumbens L.)* against the growth of *Streptococcus mutans bacteria* was seen to increase with increasing concentration. The higher the concentration of the methanol fraction of the gletang plant (*Tridax procumbens L.)*, the stronger its inhibitory power against the growth of *Streptococcus mutans bacteria*.

➢ *Data Analysis*

The Shapiro-Wilk normality test used because the sample size was less than 50.

➢ *Normality Test*

The data normality test aims to determine whether the data are normally distributed. This study used the *Shapiro-Wilk test* as a normality test because the sample size was less than 50. The results of the normality test are as follows:

Table 3 *Shapiro-Wilk* Test Results

Group	**Sig Value**
2%	0.734
4%	0.006
6%	0.005
8%	0.043
K (+)	0.042

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The results of the data normality test in Table 3 obtained a sig value in the 2% group is > 0.05 while the 4%, 6%, 8% and K (+) groups are <0.05. Thus, it can be concluded that the data distribution is not normally distributed. Therefore, the test is continued with *Kruskal Wallis* with the provision that if the sig value <0.05 means Ha is accepted or Ho is rejected.

➢ *Kruskal Wallis Test*

The Kruskal Wallis data test is a non-parametric statistical test used to test whether there is a statistically significant difference between two or more groups of independent variables and their dependent variables.

Table 4 *Kruskal Wallis* Test Results

Variables	Sig	sig limit	Information
Inhibition Zone	0.001	0.05	Ha accepted

The Kruskal Wallis test obtained a sig value of 0.001 < 0.05, this means that the tested treatment has a significant effect on the inhibition zone of *Streptococcus mutans* bacteria in the methanol fraction of the gletang plant (*Tridax procumbens L.)*. Based on the research hypothesis, H0 is rejected and Ha is accepted, which means that there is an inhibition zone of the methanol fraction of the gletang plant (*Tridax procumbens L.) against Streptococcus mutans* bacteria.

To find out more, a *Mann Whitney* post hoc test was conducted to determine the difference in the effect between each treatment group on the diameter of the inhibition zone of the methanol fraction of the gletang plant (*Tridax procumbens L.) against Streptococcus mutans* bacteria. with the criteria that if the Sig value <0.05 it is concluded that there is a significant difference.

➢ *Mann-Whitney Test*

Mann-Whitney data test was used to determine the magnitude of the differences in each treatment group.

Table 5 *Mann-Whitney* Test Results

Treatment	Comparison of Concentrations Between Treatments	Sig
2%	4%	0.081*
	6%	0.018
	8%	0.015
	K (+)	0.008
4%	6%	0.059
	8%	0.034
	K (+)	0.007
6%	8%	0.502*
	K (+)	0.007
8%	K (+)	0.011

* = Significant Difference (p< 0.05).

- *Information*

Mann-Whitney test , it shows that in the measurement group of the inhibition zone of the methanol fraction of the gletang plant (*Ttriax procumbens L.) against Streptococcus mutans* bacteria , there is a significant difference between the concentration of 2% with a concentration of 6% and 8%, a concentration of 4% with a concentration of 6% and 8%, a concentration of 6% with 8%, as well as the control (+) with all concentration groups because the sig value <0.05 means that there is a difference in the antibacterial activity of the methanol fraction of the gletang plant (*Tridax procumbent L.)*. Furthermore, at a concentration of 2% with 4%, a concentration of 6% with 8% there is no significant difference because the sig value is> 0.05, meaning that there is no difference in the antibacterial activity of the methanol fraction of the gletang plant (*Tridax procumbent L.)*.

IV. DISCUSSION

This study was conducted to determine the antibacterial activity of the methanol fraction of the gletang plant (*Tridax procumbens L.)* against the growth of *Streptococcus* mutans bacteria. The treatment in this study used a methanol solvent group at a concentration of 2%, 4%, 6% and 8%, a positive control of 0.2% *chlorhexidine* and a negative control of 96% methanol. These results indicate the antibacterial activity of the methanol fraction against the growth of *Streptococcus mutans bacteria* with a weak category. This study used the agar diffusion method to see the antibacterial activity and column chromatography as a method for separating the mixture.

➢ *Antibacterial Effects of Methanol Extract from Gletang (Tridax procumbens L.) on Streptococcus mutans Growth*

The antibacterial activity of the methanol fraction of the gletang plant (*Tridax procumbens L.)* against the growth of *Streptococcus mutans bacteria* was seen from the presence or absence of an inhibition zone formed in the form of a clear area around the disc paper measured in millimeters (mm) using a caliper [24]. The results showed that the average diameter of the inhibition zone at a concentration of 2% had antibacterial activity (3.3 mm) which was weak, a concentration of 4% (3.7 mm) was weak, a concentration of 6% (3.8 mm) was weak, and a concentration of 8% (4.2 mm) was weak. According to Davis and Stout (1971) the strength of bacterial inhibition was categorized as follows: Very strong (clear zone >20 mm),

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strong (clear zone 11-20 mm), moderate (clear zone 5-10 mm) and weak (<5 mm). The concentrations used in this study were 2%, 4%, 6% and 8% at these concentrations antibacterial activity was included in the weak category. All concentrations used in this study had antibacterial activity. The extent of the ability to inhibit the growth of the tested bacteria is influenced by the concentration of a material that functions as an antibacterial [25]. Judging from the results of the study, it was found that the greater the concentration of the methanol fraction of the gletang plant (*Tridax procumbens L.)* added, the greater the inhibition zone obtained. According to the theory of Imansyah, et al. in 2022, it was stated that the difference in the diameter of the inhibition zone of each concentration was due to the difference in the amount of active substances contained in the concentration, the greater the concentration, the greater the compound that acts as an antibacterial in the gletang plant fraction.

Methanol fractionation of the gletang plant (*Tridax procumbens L.)* showed a higher inhibitory capacity against *Streptococcus mutans bacteria at a concentration of 8%. This is due to the content of bioactive compounds sufficient to provide a synergistic effect in the antibacterial mechanism of action.* At low concentrations, the active compound content is not sufficient to effectively inhibit bacterial growth, while at high concentrations, the metabolites reach an effective level capable of producing significant inhibition against microorganisms .

Based on the results in Table 5, a significant effect of 0.003 <0.05 was obtained, meaning that the growth of *Stretptococcus mutans bacteria* was influenced by the methanol fraction of the gletang plant (*Tridax procumbens L.)* with concentrations of 2%, 4%, 6% and 8%. This was due to the influence of several factors, including the results of the secondary metabolite compound test on the ethyl acetate fraction, which showed that the gletang plant contained compounds of the alkaloid, flavonoid, saponin and tannin groups which had antibacterial properties.

The small inhibition zone results are caused by several factors such as the type of bacteria used, the method tested, the content of active compounds in the test fraction and the concentration of the test fraction used. The factor that causes the small inhibition zone results is because *Streptococcus mutans bacteria* are a type of Gram-positive bacteria that have a cell wall structure, namely the cell wall consists of a thick peptidoglycan layer while the other layer is teichoic acid. This is what is thought to cause the cell wall of Gram-positive bacteria to be difficult to penetrate by antibacterial compounds from the gletang plant fraction (*Tridax procumbens L* .).26 Differences in media thickness affect the diffusion of the test substance into the agar, thus affecting the diameter of the inhibition zone. The normal media thickness used in the inhibition zone test is around 4 mm. The thicker the media used, the smaller the inhibition zone that occurs. This is caused by the increased distance that the active substance must travel to reach the bacteria [27]. Small inhibition zones occur because differences in the pH of the media used can cause differences in the amount of test substance that diffuses, pH also determines the number of test substance molecules. The ideal pH for agar media usually ranges from 6.5 to 7.5.28].

Positive control in this study used 0.2% *chlorhexidine* as an antibacterial agent and *gold standard* for chemical plaque control [29]. *Chlorhexidine* can reduce the amount of plaque on teeth because it has bactericidal and bacteriostatic properties that work by inhibiting bacterial metabolism in plaque, thereby causing death of the bacteria [30]. The average diameter of the inhibition zone obtained from 0.2% *chlorhexidine against the growth of Streptococcus mutans* bacteria was 5.1 mm with a moderate category. 0.2% *chlorhexidine* when compared with the gletang plant fraction (*Tridax procumbens L.), the antibacterial activity of 0.2% chlorhexidine* in inhibiting the growth of *Streptococcus mutans bacteria* was higher.

This shows that the gletang plant fraction (*Tridax procumbens L.)* is less able to inhibit the growth of *Streptococcus mutans bacteria* compared to 0.2% *chlorhexidine. The 0.2% chlorhexidine molecule* has a positive charge (cation) and most of the bacterial molecule has a negative charge (anion). This causes strong binding *of chlorhexidine to the bacterial cell membrane. Chlorhexidine* will cause changes in the permeability of the bacterial cell membrane, causing the cell cytoplasm and low molecular weight cell components to penetrate from within the cell through the cell membrane, causing bacterial death [31].

The negative control was 96% methanol, also used as a solvent. 96% methanol had no bacterial activity, producing a 0 mm zone of inhibition. This demonstrates that the 96% methanol solvent used as a negative control does not contribute to antibacterial activity.

- *Differences in Antibacterial Activity of Methanol Fraction of Gletang Plant (Tridax Procumbens L.) Against the Growth of Streptococcus mutans Bacteria*

Man Whitney test shows that there is a significant difference between the concentration of 2% with the concentration of 6% and 8%, the concentration of 4% with the concentration of 6% and 8%, the concentration of 6% with 8%, as well as the positive control (+) with all concentration groups. There are several things that cause significant differences, namely differences in the bacterial inhibition mechanism, differences in the compounds contained in the methanol fraction of the gletang plant and differences in the concentration of the methanol fraction of the gletang plant. In addition, there are differences in the amount of active substances contained in these concentrations, the greater the concentration, the greater the content of compounds that act as antibacterials in the gletang plant fraction (*Tridax procumbens L.)* [32].

The inhibitory power test of the gletang plant fraction (*Tridax procumbens L.)* can inhibit the growth of *Streptococcus mutans bacteria* because the gletang plant contains secondary metabolite compounds in the methanol fraction of the gletang plant (*Tridax procumbens L.),* possibly contributing to antibacterial activity. Based on phytochemical screening conducted by Bhagyasri *et al* ., in 2017 on the

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gletang plant (*Tridax procumbens L.)* using methanol solvent containing alkaloids, flavonoids, saponins and tannins which have antibacterial properties [33].

The mechanism of alkaloids as antibacterial activity is by disrupting the components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed completely and causes cell death [34]. The mechanism of action of flavonoids as antibacterial compounds works to damage cell walls because they bind to proteins and lipids, coagulate proteins, damage cell walls and cause cell lysis to death [35]. Saponin compounds are substances that when they interact with bacterial walls, the walls will break. Saponins will disrupt the surface tension of the cell wall, so when the surface tension is disrupted, antibacterial substances will be able to easily enter the cell and will disrupt metabolism until finally bacterial death occurs. 36 The mechanism of tannins has a role as antibacterials by binding proteins so that cell wall formation will be inhibited [37].

Based on the results of the research that has been done, it can be concluded that: there is antibacterial activity of the methanol fraction of the gletang plant (*Tridax procumbensL* .) with concentrations of 2%, 4%, 6% and 8% against *Streptococcus mutans bacteria* with a weak category. There are differences in the antibacterial activity of the methanol fraction with concentrations of 2%, 4%, 6% and 8% against *Streptococcus mutans bacteria*.

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