ACTIVITY TEST OF LEMONGRASS LEAF EXTRACT (CYMBOPOGON CITRATUS) AGAINST STREPTOCOCCUS MUTANS BACTERIA AND ESCHERCHIA COLI

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ABSTRACT

This study aims to determine the antibacterial activity of lemongrass leaf extract (*Cymbopogon citratus*) against *Streptococcus mutans* and *Escherchia coli bacteria.* This research method uses a Complete Randomized Design (RAL) consisting of 7 treatments and 4 repeats. The treatments are: P1 (negative control), P2 (10% concentration), P3 (20% concentration), P4 (30% concentration), P5 (40% concentration), P6 (50% concentration) and P7 (Amoxicillin positive control). The results showed that the concentration of lemongrass leaf extract (*Cymbopogon citratus)* has activity to inhibit the growth of *Streptococcus mutans* and *Escherchia coli bacteria.* The results of the statistical analysis *of the Kruskall Wallis* test showed a significant value of 0.008 or <0.05, so it can be said that there is a dose of lemongrass leaves (*Cymbopogon citratus*) against the growth of *Streptococcus mutans* bacteria. The processed data using the *Kruskall Wallis* test on *Escherchia coli bacteria showed a significant value of 0.002 or <0.05, so it can be said that there is a dose of lemongrass leaves (*Cymbopogon citratus*) against* Escherchia coli *bacteria.* Further tests (*Duncan* Test) found that there was no significant difference between one concentration and another, so it can be said that the optimum concentration of lemongrass leaf extract treatment (*Cymbopogon citratus*) in inhibiting the growth of *Streptococcus mutans* and *Escherchia coli bacteria* is at a concentration of 50%

Keywords: Lemongrass Leaf Extract (*Cymbopogon citratus*), Antibacterial Activity, *Streptococcus mutans bacteria.* and *Escherchia coli*

INTRODUCTION

Infectious disease is a condition caused by pathogenic microorganisms with or without clinical symptoms. In some developing countries such as Indonesia, infectious diseases are one of the most important health problems. Infectious diseases are a contributor to death in children aged 29 days to 11 months. Infections are caused by microorganisms such as bacteria, viruses, fungi, or parasites [15].

The infectious process is the interaction of pathogenic microorganisms with macroorganisms under certain environmental and social conditions. The concept of "infectious disease" is a disorder caused by

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microorganisms such as bacteria, viruses, fungi, or parasites. Infectious diseases are generally caused by pathogenic microorganisms. This disease can spread directly or indirectly. One of the microoganisms that cause infectious diseases is bacteria [15]. Bacteria can be harmful to their host cells, if the bacteria are pathogenic. There are several bacteria that can infect the host's body, including *streptococcus mutans* and *Escherichia coli*.

Streptococcus mutans *bacteria* are the bacteria that play the most dominant role in the formation of caries and dental plaque. The negative impact of caries that can occur if not treated is in the form of pain in the mouth, bad breath, abscesses, difficulty speaking and swallowing which will ultimately reduce physical health and interfere with aesthetics causing lack of confidence in sufferers [6].

Escherichia coli bacteria are gram-negative bacteria that normally live in the digestive tract but will be pathogenic if they leave their habitat. [14]. *Escherchia coli* ranks first in the *Enterobacteriaceae* group and is therefore responsible for increased treatment costs, morbidity, and mortality [20].

Giving antibiotics can overcome this problem, but antibiotics are known to have side effects and can cause bacterial resistance if used with improper doses, so new antibiotic products are needed that resemble the potential of antibiotics from herbal plants to overcome this problem. The use of medicinal plants as traditional medicine is believed to be quite effective and safe because it rarely causes side effects and the price is relatively cheaper [9]. One plant that can be used as a medicinal plant is lemongrass plant (*Cymbopogon citratus*) [27

Lemongrass leaves (*Cymbopogon citratus*) are plants that contain alkaloids, flavonoids, and several monoterpenes. The substances contained in lemongrass leaves are useful as anti-protozoal, anti-inflammatory, anti-microbial, anti-bacterial, anti-diabetic, anti-cholinesterase, mollusscidal, and antifugal. Lemongrass is also very easy to be developed by the wider community. Lemongrass leaves also contain essential oils composed of moterpene compounds such as citrate and graniol. Essential oil contains anti-bacterial and antifungal, so it is used as a treatment of bacteria *Staphylococcus aureus, Salmonella typhimurium*. Based on previous research showed lemongrass leaves were able to inhibit the activity of *Propionabacterium acnes bacteria* with the largest inhibitory zone diameter at a concentration of 80%, which is 16.35 mm [27].

Based on the description above, the author is interested in testing the antibacterial activity of lemongrass leaf extract (*Cymbopogon citratus*) against the growth of *Streptococcus mutans* and *Escherchia coli bacteria.*

RESEARCH METHODS

2.1 Tools and materials

The tools used in this study were ovens (Memmert), *autoclaves* (Gemmy), incubators (Memmert*), Laminar Air Flow, calipers, measuring pipettes, Erlenmeyer* (pyrex), Petri dishes, sterile ose needles, shakers, micro pipettes, analytical balances, hot plates, stirers, spectrophotometers, cotton, test tubes *(pyrex), measuring cups (*pyrex*) and other glassware and glass jars.*

The materials used in this study were lemongrass leaf extract (*Cymbopogon citrus),* Streptococcus mutans bacterial culture, Escherichia coli *bacterial culture*, magnesium (Mg) and hydrochloric acid (concentrated HCl 2N), dragendrof reagent FeCl3, *Muller Hinton Agar* (MHA), 0.9% NaCl, alcohol, sterile aquades, disc paper, 96% ethanol and amoxicillin with a concentration of 25 mcg as a positive control.

2.2 How it Works

2.2.1 Sample Setup

a. Sampling

The part of the lemongrass plant (*Cymbopogon citratus*) taken is the fresh leaves.[9]

b. Simplisia Processing

The processing technique of lemongrass leaf simplisia (*Cymbopogon citratus*) consists of wet sorting, washing, drying, dry sorting and storage. Then the simplisia is weighed wet weight and dry rice and then the moisture content of the simplisia is calculated using the following formula.

$$\%\text{Moisture content} = \frac{simplisia\ basah - simplisia\ kering}{wsimplisia\ basah} \times 100\%$$

c. Extraction

Lemongrass leaf powder (*Cymbopogon citratus*) weighed 500 grams, put in a maceration container, soaked with a 96% ethanol filter solution as much as 3 liters. Then the maceration container is closed with a container cover covered with *aluminum foil* and allowed to stand for 3x24 hours at room temperature without being exposed to direct sunlight, (while stirring using *a digital overhead stirrer*) so that an extract is still mixed with solvent. Next, the extract is filtered using filter paper which produces mafiber I and residue. Then the residue is added with 2 L of solvent and simmered for 3x24 hours, then filtered with filter paper and produces maserat II. Maserat I and II are mixed and filtered with a strained rice. The extract obtained is then concentrated using a *shaker* to remove the solvent contained in the extract so that a thick extract is obtained.

Then the extract is weighed and then the yield is calculated with the following formula [12]:

$$\%\text{Yield} = \frac{jumlah\ berat\ ekstrak\ kental\ (g)}{jumlah\ berat\ kering\ (g)} \times 100\%$$

d. Phytochemical Screening

Phytochemical screening testing was carried out on 4 compounds, namely flavonoid compounds, saponins, tannins, alkaloids and essential oils.

Flavonoid testing was carried out by reacting a solution of lemongrass leaf extract (*Cymbopogon citratus*) with concentrated magnesium and HCl powder reagents. If red, yellow, orange colors are formed in the mixture indicates the presence of flavonoid compounds [10].

Saponin testing was carried out by reacting a solution of lemongrass leaf extract (*Cymbopogon citratus*) with hot aquades that were strongly coated for 10 seconds. If foam forms that do not disappear for 10 minutes, it indicates the presence of saponin compounds [24].

Tannin testing was carried out by reacting a solution of lemongrass leaf extract (*Cymbopogon citratus*) with FeCl3 reagent. If there is a change in color to a blackish-green color, it positively contains tannin compounds [20].

Alkaloid testing was carried out by reacting a solution of lemongrass leaf extract (*Cymbopogon citratus*) with Drendof's Reagent. If there is a red or orange precipitate, it is positive to contain alkaloids [16].

Essential oil testing is done by reacting lemongrass leaf extract solution then or heated in a hotplate on a watch glass until residue is obtained. The positive results of essential oils are characterized by the characteristic odor produced by such residues [2].

e. Determination of Lemongrass Leaf Extract Concentration 10%, 20%, 30%, 40% and 50%

The use of this concentration is based on the fact that it still uses viscous extracts. Making a concentration of 10% was carried out weighing 1 g of lemongrass leaf extract dissolved with 10 mL of aquades. For a concentration of 20%, weighing 2 g of lemongrass leaf extract dissolved with 10 mL of aquade. A concentration of 30% weigh 3 g of lemongrass leaf extract dissolved with 10 mL of aquades. A concentration of 40% weighed 4 g of lemongrass leaf extract dissolved with 10 mL of aquade. While the concentration of 50% weigh 5 g of lemongrass leaf extract dissolved with 10 mL of aquades.

2.2.2 Antibacterial Activity Test

a. Sterilization of tools and materials

The tools to be used are first washed with detergent and then rinsed with aquades. Tools made of glass are wrapped in white hvs paper and sterilized using an oven with a temperature of 170 ° C for 1 hour. Ose needles and tweezers are sterilized using a spritus lamp for 30 seconds. Rubber and plastic tools (not resistant to high-temperature heating) were sterilized by autoclave at 121°C for 15 minutes. Laminar Air Flow (LAF) is cleaned of dust then sprayed with 70% ethanol, left for 15 minutes and sterilized with a UV lamp for 5 minutes before use.

b. Manufacture of Bacterial Suspension

The test bacteria are rejuvenated first by being taken using an ose needle, then implanted in the media to tilt it by scratching in a zigzag manner. Then it is opened at a temperature of 37ºC for 18-24 hours. The rejuvenated test bacteria were each specimated in a sterile 0.9% w/v NaCl solution. Bacterial suspensions are measured for transmittance value or *Optical Density* (OD) using a spectrophotometer. Optical density measurement using a spectrophotometer aims to calculate the population density of Escherichia coli bacteria *or to determine the number of* surviving Escherichia coli *bacteria* , based on the number of colonies that grow. The bacterial culture is inserted into the cuvette to the limit mark and inserted into the spectro then measured the transmittance value using a spectrophotometer at 580 nm until a 25% transmittance is obtained equal to 1x106 (1,000,000) bacterial colonies.

c. MHA Media Creation

Weighing media as much as 4.56 gr then poured into Erlenmeyer 200 ml and dissolved with sterile aqueous as much as 120 ml then cooked media using a hot plate at a temperature of 200. Sterilized media that has been cooked in an autoclave for 30 minutes°

d. Antibacterial Testing

This antibacterial test uses the pour plate method. The activity testing using the pour plate method is as follows.

1) Prepared tools and materials to be used

2) *The prepared paper disc* is soaked into lemongrass leaf extract with concentration variations of 10%, 20%, 30%, 40% and 50%.
3) Pour bacteria into a saucer as much as 1 ml = 1 micropipette using a micropipette and spread to all sides
4) Put in the already warm substrate and let stand for 5-10 minutes
5) Inserted disc paper that has been soaked in each concentration
6) Then each petri dish is incubated for 24 hours at 37°C.
7) After that, the average diameter of the resulting inhibitory zone is measured using a caliper.

1.3.3 Data Analysis

Data analysis in this study used the SPSS Statistical test. If the data meets the requirements of the parametric test, the *One Way Anova test is used*. If the data does not meet the parametric requirements (homogeneous and normally distributed) then proceed with a non-parametric alternative test, namely *Kruskal Wallis.*

RESEARCH RESULTS

3.1 Descriptive Analysis

The results of the research that has been carried out will be described as follows.

a. Simplisia Water Content Results

The moisture content results from weighing the wet weight and dry weight of lemongrass leaf *simplisia (Cymbopogon citratus*) obtained percentage results that can be seen in Table 1

Table 1. Water Content Results of Citronella Leaf Simplisia (*Cymbopogon citratus*)

Sample	Wet weigh t (g)	Dry weight (g)	Power water(%)
Lemon grass leaf extract	550 g	500 g	9%

Source : Data processed, 2023

Based on table 1, the moisture content of lemongrass leaf simplisia is obtained at 9% so as to produce good water content.

b. Extract Yield Results

The extraction process of lemongrass leaf extract (*Cymbopogon citratus*) is carried out using maceration method. The maceration results are then calculated the yield value to determine the percentage of the remaining amount of material. The yield of lemongrass leaf *ethanol extract (Cymbopogon citratus*) can be seen in Table 2

Table 2. Yield Results of Lemongrass Leaf Extract

Sample	Heavy dry (g)	Weight Extract (g)	Yield (%)
Lemon grass leaf extract	500 grams	98.3 grams	19,66%

Source : Data processed, 2023

Based on table 2 The yield of lemongrass leaf extract (*Cymbopogon citratus*) obtained a thick extract of 98.3 grams resulting in a good yield of 19.66%

c. Phytochemical Screening Results

Phytochemical screening of lemongrass leaf extract (*Cymbopogon citratus*) was tested on 5 secondary metabolite compounds using appropriate reagents. The results of

phytochemical screening can be seen in Table 3

Table 3. Phytochemical Screening Results of Lemongrass Leaf Extract (*Cymbopogon citratus*)

	Compound	Reagent	Result	Information
1.	Flavonoids	Mg and concentrated HCL	+	Reddish-yellow color
2.	Saponin	Aquadest	+	Formed stable foam
3.	Tannin	FeCl3 10%	+	Blackish-green color
4.	Alkaloid	Dragendrof Reagents	+	Formed sediment
5.	Essential oils	Aquadest	+	Causes a characteristic odor

Source: Processed Data, 2023

Table 3 shows testing of 4 secondary metabolite compounds as antibacterial from lemongrass *leaf extract (Cymbopogon*

Source: Processed Data, 2023

Table 4 shows lemongrass *leaf extract*

Treatment	Average diameter of the inhibitory zone (mm)			
	S. mutans	Criterion	*E. coli*	Criterion
10%	6,37	Keep	5,07	Keep
20%	7,92	Keep	6,14	Keep
30%	9,87	Keep	7,43	Keep
40%	10,06	strong	8,58	Keep
50%	10,52	Strong	11,36	Strong
K(+)	9,79	keep	18,24	Strong
K(-)	-	-	-	-

(Cymbopogon citratus) against the growth of S. mutanss *bacteria and* E. coli *can be seen in the average diameter ofthe* inhibitory zone formed. In this study consisted of 7 *citratus*) including flavonoids, saponins, tannins and alkaloids and essential oils. Testing each secondary metabolitt compound using the appropriate reagents as shown in the table. From the four tests, positive results were obtained which were characterized by the occurrence of reactions in each test of metabolite compounds.

d. Antibacterial Test Results

Observation of the activity of lemongrass leaf ethanol extract (*Cymbopogon citratus*) against *Streptococcus mutans* bacteria was carried out by measuring each clear zone formed in each treatment using a caliper with the results obtained can be seen in Table 4

Table 4. Results of Measurement of the Inhibition Zone of Lemongrass *Leaf Extract (Cymbopogon citratus*) Against the Growth of *S. mutanss* and *E. Coli Bacteria*

treatments including negarif control (Aquades), concentration of 10%, 20%, 30%, 40%, 50% and positive control (*Amoxicilin).* The average value of diameter is categorized based on the criteria of inhibition zones namely weak, medium and strong

The results obtained in measuring the diameter of the inhibitory zone of each control solution and the concentration of lemongrass *leaf extract (Cymbopogon citratus*) in *Streptococcus mutans* bacteria showed an average value at a concentration

of 10% of 6.37 mm (medium), 20% of 7.92 (medium), 30% of 9.87mm (medium), 40% of 10.06 mm (strong), 50% of 10.52 mm (strong) and positive control *(Amoxicilin*) of 9.79 mm (medium). While in the negative control using Aquades no clear zone was formed.

The results obtained in measuring the diameter of the inhibitory zone of each control solution and the concentration of lemongrass *leaf extract (Cymbopogon citratus*) in *Escherichia coli* bacteria showed an average value at a concentration of 10% of 5.07 mm (medium), 20% of 6.14 (medium), 30% of 7.43 mm (medium), 40% of 8.58 mm (medium), 50% of 11.36 mm (strong) and positive control *(Amoxicilin*) of 18.24 mm (strong). While in the negative control using Aquades no clear zone was formed.

3.2 Statistical Analysis

Based on non-parametric statistical tests using *wallis crucials* with a level of 5% or 0.05, it was found that hypothesis 1 was accepted, namely the presence of a dose of lemongrass leaf extract (*Cymbopogon citratus*) against the large inhibitory zone in *Streptococcus mutans* and *Escherchia coli* bacteria , then further tests were carried out using *Duncan's analysis test*. The results obtained from the *Kruskal Wallis* test on *Streptococcus mutans* bacteria obtained asymp values. Pig 0.008 or <0.05, it can be said that there is a dose of lemongrass leaf ethanol extract (*Cymbopogon citratus*) against the large inhibitory zone in *Streptococcus mutans bacteria* or which means that the conclusion of the hypothesis, namely H0 is rejected and H1 is accepted.

The results obtained from the *Kruskal Wallis* test on *Escherchia coli* bacteria obtained asymp values. Pig 0.002 or <0.05, it can be said that there is a dose of lemongrass leaf ethanol extract (*Cymbopogon citratus*) against the large inhibitory zone in *Escherichia coli bacteria* or which means that the conclusion of the hypothesis, namely H0 is rejected and H1 is accepted. Furthermore, *Duncan's test was carried out* with a level of 5% or 0.05 to see the effective concentration of lemongrass leaf ethanol extract (*Cymbopogon citratus*) against the growth of *Streptococcus mutans* and *Escherichia coli bacteria*. The results of *Duncan*'s analysis test can be seen in Table 5. and Table 6

Table 5. Duncan test results *of* lemongrass leaf extract (*Cymbopogon citratus*) against the growth of *Streptococcus mutans bacteria*

Treatment Group	N	1	2	3
Control Negative	4	,0000		
P1	4		6,3700	
P2	4		7,9200	7,9200
P3	4			9,8750
P4	4			10,3025
P5	4			10,5875
Positive control	4			9,7950

Source : Data processed, 2023

Table 6. Duncan test results *of* lemongrass leaf extract (*Cymbopogon citratus*) against the growth of *Escherchia Coli bacteria*

Treatment groups	N	1	2	3
Control Negative	4	,0000		
P1	4		6,3700	
P2	4		7,9200	7,9200
P3	4			9,8750
P4	4			10,3025
P5	4			10,5875
Positive control	4			9,7950
Itself.		1,000	,277	,098

Source : Data processed, 2023

Ket. The numbers followed by the same letter in the same column are not markedly different, on the contrary, different letters in different columns show a noticeable difference according to the test *Duncan* at a confidence level of 5% or 0.05.

Based on the results of *Duncan's test analysis on* Streptococcus mutans *bacteria on* negative controls differed markedly with concentrations of lemongrass leaf ethanol extract (*Cymbopogon citratus*) concentrations of 10%, 20%, 30%, 40%, 50% and positive controls. The concentration of 10% did not differ markedly from the concentration of 20%, 30%, 40%, 50% and positive control . While the results *of Duncan*'s test analysis on *Escherchia coli bacteria* in negative controls did not differ markedly at concentrations of 10%, 20% and 30% but differed markedly with concentrations of 40%, 50% and positive controls. 50% concentration and positive control did not differ markedly.

2. DISCUSSION

Based on the results of the study, the moisture content value was obtained at 9% as intended at Tabel 1. This percentage indicates that the moisture content in lemongrass leaf simplisia (*Cymbopogon citratus*) has met the minimum requirements set by SNI. According to the provisions of the requirements, the allowable water content of SNI is 10%. Water content can affect physical properties (hardness and dryness) and physico-chemical properties, chemical changes (browning of enzymes, microbiological damage, and enzymatic changes) [25].

Based on research, the yield of lemongrass leaf extract was obtained by 19.66% as shown in Table 2. This result is in accordance with the requirements of the Indonesian Herbal Pharmacopoeia, namely the yield of lemongrass leaf extract is not less than 9.2% [17]. Yield is a comparison between the results of many metabolites obtained after the extraction process with the weight of the sample which is said to be good if the value is more than 10%.

Based on the results of the phytochemical screening test of lemongrass leaf extract

showed the presence of flavonoid compounds, saponins, tannins, alkaloids and essential oils as addressed Table 3 This result is in line with previous research on phytochemical screening tests of lemongrass leaf extract showed positive results containing flavonoid compounds, saponins, tannins, alkaloids and essential oils [22]. The reason for only testing these 5 compounds is because in previous studies lemongrass leaves did not contain phenol compounds [20]. And lemongrass leaves do not contain steroid compounds [8] so in phytochemical screening studies tested only flavonoids, saponins, tannins, alkaloids and essential oils. Lemongrass leaves contain flavonoids of 2.866% and essential oil of 0.4% which can function as antibacterial [27].

Based on the results of antibacterial activity tests of lemongrass leaf extract (*Cymbopogon citratus*) on the growth of *Streptococcus mutans* bacteria with variations in concentration of 10% = 6.37 mm , 20% = 7.92 mm, 30% = 9.87 mm so that the inhibitory strength is categorized as medium and at concentrations of 40% = 10.06 mm and 50% = 10.58 mm so that the inhibitory strength is categorized as strong as shown in Table 4.

Based on the results of antibacterial activity tests of lemongrass leaf extract (*Cymbopogon citratus*) on the growth of *Escherchia coli* bacteria with variations in concentration of 10% = 5.07 mm, 20% = 6.14 mm , 30% = 7.43 mm, 40% = 8.58 mm so that the inhibitory strength is categorized as medium and at concentration and 50% = 11.36 mm so that the inhibitory strength is categorized as strong as shown in Table 4.

Based on the results described above, it can be seen that the average diameter of the inhibitory zone in each treatment increases in accordance with the increase in concentration given, so it can be said that the higher the concentration, the greater the inhibitory power. The higher the concentration of the extract, the more secondary metabolite compounds contained, so as to inhibit bacterial growth characterized by the formation of a clear zone around the disc [11].

The difference in the size of the inhibitory zone formed can also be caused by the content of secondary metabolite compounds in lemongrass leaf plants in the phytochemical screening test of lemongrass leaf extract showed positive results containing flavonoids, saponins, tannins, and essential oils [22].

The mechanism of flavonoid compounds as antibacterial is through interaction with several vital enzymes and inhibition of nucleic acid synthesis and cytoplasmic membrane function [1]. The mechanism of saponin compounds as antibacterial is by causing leakage of proteins and enzymes from within bacterial cells. Tannins as antibacterial are able to inhibit bacterial cell wall synthesis and protein synthesis in germ cells both in gram positive and gram negative [18].

According to (Alfi Amalia, et al 2018) Alkaloids work as antibacterial by disrupting the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed intact and causes cell death. [22].

The mechanism of action of essential oils includes cell wall degradation, damaging cytoplasmic membranes, cytoplasmic coagulation, damaging membrane proteins, increasing permeability leading to leakage of cell contents, reducing proton motive strength, reducing intracellular ATP through

decreased synthesis and hydrolysis and reducing membrane potential through increased membrane permeability [25].

In the treatment given using lemongrass leaf extract, it can be seen that the inhibitory zone formed in *Streptococcus mutans* bacteria is larger than *Escherichia coli* bacteria. This can be caused by differences in the cell wall structure of gram-negative and gram-positive bacteria where *S. mutans* bacteria are gram-positive bacteria and *E.coli* bacteria are gram-negative bacteria. Gram-positive bacteria cell walls are composed of PG (Peptidoglycan) there is a compound called teicoic acid. Gram-negative bacteria contain PG (Peptidoglycan) in much smaller quantities, but on the outside of PG there is an outer membrane composed of lipoproteins and phospholipids, and contains lipopolysaccharides. Due to this difference in cell wall composition, gram-positive bacteria and gram-negative bacteria have different resistance. Gram-positive bacteria are more susceptible to penicillin antibiotics, as these antibiotics are capable of damaging PG. Because PG is larger, gram-positive bacteria are usually more susceptible to mechanical damage. [23]. Differences in bacterial cell wall structure are one of the factors that cause differences in inhibitory zones formed between *S. mutans* and *E. coli* bacteria.

Positive control in research using the antibiotic amoxicilin inhibitory zone formed in *S. Mutans* bacteria is 8.91 mm so it is categorized as medium because it ranges from 5-10 mm. This can happen because the range of inhibitory zones of amoxicillin against bacteria is 7-33 mm [3].The inhibition zone of the antibiotic is also due to the availability of pure antibiotic discs that do not exist so that researchers do their own formulations so that the inhibition of antibiotics is partial. While *the inhibitory zone E. coli* bacteria formed is 11.36 mm so it is categorized as very strong because it ranges from 10-20 mm. Antibiotics Amoxicillin belongs to the class of beta-lactam antimicrobials. Beta-lactam acts by binding to penicillin-binding proteins that inhibit a process called transpeptidation (the process of crosslinking in cell wall synthesis), which causes activation of autolytic enzymes in the bacterial cell wall. This process causes lysis of the cell wall, thereby destroying the bacterial cell. This type of activity is referred to as bactericidal killing [4]. While negative control using sterile aquades does not form an inhibition zone because sterile aquades do not have antibacterial substances so they cannot inhibit bacterial growth and testing using aquades aims as a sample thinner, and it is known that aquadest has no activity against test bacteria so there is no influence between the solvent and the test bacteria used. [7].

Based on the processed data using the *Kruskall Wallis* test showing a significant value of 0.008 or <0.05, it can be said that there is a dose of lemongrass leaf (*Cymbopogon citratus*) and the use of negative and positive controls on the size of the inhibitory zone in *Streptococcus mutans bacteria* or which means the conclusion of the hypothesis, namely H0 is rejected and H1 is accepted. Based on the processed data using *the Kruskall Wallis* test on Escherchia coli bacteria showed a significant value of 0.002 or <0.05, it can be said that there is a dose of lemongrass leaves (*Cymbopogon citratus*) and the use of negative and positive controls on the size of the inhibitory zone in *Escherchia coli* bacteria or which means the

conclusion of the hypothesis that H0 is rejected and H1 is accepted.

Further tests were carried out, namely *the Duncan* test which obtained results on *Streptococcus mutans* bacteria in negative controls in real contrast with the concentration of lemongrass leaf ethanol extract (*Cymbopogon citratus*) concentrations of 10%, 20%, 30%, 40%, 50% and positive controls. The concentration of 10% did not differ markedly from the concentration of 20%, 30%, 40%, 50% and positive control. While the results *of Duncan*'s test analysis on *Escherchia coli bacteria* in negative controls did not differ markedly at concentrations of 10%, 20% and 30% but differed markedly with concentrations of 40%, 50% and positive controls. 50% concentration and positive control did not differ markedly.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that:

1. Lemongrass leaf extract (*Cymbopogon citratus*) has antibacterial activity against *Streptococcus mutans* and *Escherchia coli bacteria*
2. The optimum concentration of lemongrass leaf extract (*Cymbopogon citratus)* which has antibacterial activity against *Streptococcus mutans* and *Escherchia coli bacteria* is at a concentration of 50%

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