



The Effectiveness Test of Bangun-Bangun Leaves Extract (*Plectranthus Amboinicus*) Against *Streptococcus Pyogenes* and *Salmonella Typhi* Bacteria

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ABSTRACT

Background Bangun-bangun leaves (*Plectranthus amboinicus*) are part of the Lamiaceae family. (1) (1) Bangun-bangun leaves have a characteristic aromatic leaves aroma used as a medicinal plant (2). According to research conducted by (Dalimunthe et al., 2016) to find the use of this plant as an antibacterial because it contains compounds such as flavonoids, tannins, tripenoids, saponins, and essential oils. On the bangun-bangun leaves there is carvacrol, which is an essential component that has antibacterial activity in positive or negative gram bacteria. *streptococcus pyogenes* bacteria are vectors of suppurative infectious diseases and other diseases such as pharyngitis. While *salmonella typhi* bacteria is a vector of the disease which is usually called typhus and the medical language is typhoid fever. **The purpose** of this study was to determine that the bacteria *salmonella typhi* and *streptococcus pyogenes* can be inhibited by the leaves extract. The benefit of this research is to increase public understanding and knowledge in the use of bangun-bangun leaves. **The method** used in this study is an experimental method and post test only controlled group design. For the concentration used, namely 5%, 10%, 15%, 20%, 25% and this strength test is done by inserting the extract into a petri dish that has a bacterial culture. Data processing uses one-way ANOVA with the aim of comparing each variable. To see the extract against bacteria. **The results** showed that there were variations in the diameter of the inhibition zone formed by the concentration of the extract. And the most effective concentration to inhibit *streptococcus pyogenes* bacteria was a concentration of 25%, the inhibition zone diameter was 19.5mm, while *salmonella typhi* bacteria had a concentration of 25% and the inhibition zone diameter was 37.7m.

1. Introduction

Streptococcus β hemolyticus group A or can be called *streptococcus pyogenes* which is included in coccus-shaped bacteria with a chain-like and non-motile shape and has a diameter of 0.6 - 1.0 micrometers and is facultative anerobic, and includes⁽³⁾. Metabolism of *streptococcus* which is fermentative and requires media that usually uses blood agar media but can also be used MHA media, these bacteria can live well at optimum temperatures, namely temperatures of 37°C and pH of 7.4-7.6 and including bacteria have capsules containing hyaluronic acid.

In general, bacteria can infect humans that can cause suppurative diseases such as pharyngitis, impetigo, erysipelas, and scarlet fever, there were 9000-11500 cases of *streptococcus pyogenes* and the mortality rate from these cases was 1000-1800 deaths from all cases United States of America

There is a case of *streptococcus pyogenes* which is a very important case in Indonesia for the treatment used is a ampicillin but there are many resistant cases during treatment and there are side effects that trigger other cases so alternative treatment is needed to

minimize side effects and reduce resistance rates when treatment is carried out.⁽⁴⁾

Salmonella typhi is a disease vector of typhoid fever or commonly referred to as typhoid, salmonella typhi is included in the enterobacteriaceae family which is rod-shaped and belongs to the gram-negative group and has flagella which are facultative anaerobes. As well as these bacteria have varying levels of invasion and can usually infect humans from minor infections, for example, the disease caused is diarrhea and severe infections which are usually caused by typhoid fever ⁽⁵⁾.

Typhoid fever or can be called typhus abdominalis which is an acute disease that infects the small intestine but can also occur in the bloodstream caused by salmonella typhi. Clinical symptoms that often occur when infected with these bacteria are high fever, abdominal pain, red and red spots and weakness, lethargy and many other clinical symptoms that arise when infected by these bacteria. Usually the spread of bacteria or the affected person because it is contaminated through food or drink which causes clinical symptoms of acute typhoid fever infection. And there are several factors that influence the incidence of typhoid fever, namely the lack of cleanliness by an individual, a dense home or living environment and also insufficient water availability and a decline in the system. immunity or a person's immunity.

In medicinal plants, the bangun-bangun leaves (*Plectranthus ambonicus*) belong to the lamiaceae family. Woke leaves is a traditional plant that has a distinctive aroma and is found in tropical areas and is known as an aromatic plant which can also be useful as a traditional medicine. The bangun-bangun leaves of these shapes are found in many Asian and African countries around 80%. The bangun-bangun leaves of these shapes are usually used to increase breast milk during breastfeeding but apart from that, they can be consumed as food ingredients and can also be used as antibacterials which can inhibit the growth of bacteria or kill bacteria that cause infection.⁽²⁾

According to research conducted by (Dalimunthe *et al.*, 2016) which indicates the use of antibacterial properties in the extract of the *Bangun-Bangun leaves*

and in his research found metabolite compounds such as flavonoids which can be useful for destroying bacterial cells and disrupting the metabolism of bacterial cells, tannins which are useful for inhibiting DNA synthesis / RNA, a tripenoid which is useful as an inhibitor of the process of cell wall formation and saponins as an inhibitor of the stability of the cytoplasmic membrane. suppresses antibacterial activity which is included in gram positive and negative but can also be used as an antifungal.

And for the purpose of being used to prove the effect of *Bangun-Bangun leaves* on the growth of *Streptococcus* and *Salmonella* bacteria, while the other objective is to determine the level of influence of the leaves extract on *Streptococcus* and *Salmonella typhi* bacteria which will be carried out in vitro. For the benefits of this research is to be able to utilize natural ingredients and increase public understanding and knowledge of the benefits of the leaves of the wakes apart from being a food ingredient.

2. Research Methods

This study used the post test only controlled group design research method as its design plan. where this design is used because the sample was selected without any pretest treatment first.

Location and time

The location used in the research was in the Biomolecular Laboratory at the Medical Faculty of the Prima Indonesia University on the path of pot No.1 Ayahanda, Medan Utara, which was chosen as a good and ideal place for the extraction process in September 2020 which was then carried out by testing the extract as antibacterial from *Streptococcus pyogenens* and *Salmonella* bacteria. Typhi, which is located in the Biomolecular Laboratory of Prima Indonesia University in September 2020.

Research Samples

In this study we used the research sample that was used at the time of the study was the *bangun-bangun*

leaves (*plencatrathus ambonicus*) which came from the wake-up plant found in the Lubuk Pakam area, North Sumatra.

Method of collecting data

Data obtained from *Bangun-bangun leaves extracts* (*plecthranthus ambonicus*) obtained during research conducted in the laboratory, which used primary data during the test of the effectiveness of the leaves extracts in inhibiting *streptococcus pyogenes* and *salmonella typhi* bacteria.

Research Instruments

During the research, the equipment indispensable in terms of research in this research are glass beakers, test tubes, volumetric flasks, ose, bunsen lamps, petri dishes, erlen mayer tubes, rotary evaporators and others.

Meanwhile, the materials used at the time of the research were the bangun-bangun leaves, *streptococcus pyogenens*, *salmonella typhi* and MHA media as well as positive control (aztromycin) and negative control (aquadest).

Extraction Method

On the *bangun-bangun leaves* of the shapes that were given the extract treatment of this research using the maceration method. For the solvent to be used in this study is 96% ethanol as a solvent. The *bangun-bangun leaves* are collected as much as 10 kg after drying into a drying place to be 5 kg then grinding using a blender as much as 600 grams then the maceration process is carried out using 96% ethanol solvent which is needed as much as 600 ml mixed with and left to stand for 72 hours at room temperature.

The solution is filtered and then a *rotary evaporator* is used to evaporate the *solvent*. Then the extract is placed in a water bath until the extract is thick and good for use, then proceed by dividing into 5 concentrations, namely:

1. 5% concentration: the concentration of 5% bangun-bangun leaves extract is taken as much as 10 ml mixed with 100 ml of aquabidest.
2. 10% concentration: The 10% concentration of 10% of the bangun-bangun leaves extract is taken as much as 10ml mixed with 100ml of aquabidest.
3. Concentration of bangun-bangun leaves extract 15%: The concentration of 15% of bangun-bangun leaves extract is taken as much as 10 ml mixed with 100 ml of aquabidest.
4. 20% concentration: The concentration of 20% of the bangun-bangun leaves extract is taken as much as 10 ml mixed with 100 ml of aquabidest.
5. 25% concentration: The concentration of 25% of bangun-bangun leaves extract is taken as much as 10 ml mixed with 100 ml of aquabidest.

Test the effectiveness of the bangun-bangun leaves extract

The Biomolecular Laboratory of Prima Indonesia University was chosen as the place to carry out the effectiveness test of the leaves of the shapes. The test of the effectiveness of the leaves extract as antibacterial was carried out *in vitro* which will be used for the samples in this research.

1. In supensi bacteria *streptococcus pyogenens* and *salmonella typhi* which has been smeared.
2. A cotton stick is used to apply to MHA media.
3. Then the disc paper that has been provided that has been dripped by the leaves extract concentration rises for 15 minutes.
4. Next, it will be wrapped in plastic wrap and followed by wrapping it with aluminum foil
5. Then incubate in an incubator with a temperature of 37°C for 24 hours.
6. Furthermore, the incubation is complete, followed by measuring the diameter of the inhibition zone

Data analysis

After obtaining the inhibition zone diameter data from the antibacterial test of the *bangun-bangun leaves* extracts of the *streptococcus pyogenens* and *salmonella*

typhi bacteria, then processing the data to be tested through the one way ANOVA test via SPSS software.

3. Results

The research was carried out at the Prima Indonesia Biomolecular Laboratory on July 11-20 October 2020. By utilizing leaves from plants as antibacterial agents from *Streptococcus pyogenes* and *Salmonella typhi*.

Based on the table of results from the screening test phytochemicals in **table 1** can be seen that extract of the bangun-bangun leaves (*Plectranthus amboinicus*) contains compounds chemicals in the form of alkaloids, saponins, flavonoids, tannins, triterpenes / steroids, and polyphenols

Comparison of Inhibition Zone Diameter from bangun-bangun Leaves Extract (*Plectranthus Amboinicus*) Against Growth of *Streptococcus pyogenens* Bacteria.

In the bangun-bangun leaves extract (*plectharanthus ambonicus*) on the growth of the *Streptococcus pyogenens* bacteria, which in this study used the disc diffusion method, it was shown that the inhibition zone was measured using a caliper. The results of the study used a concentration of 5%, 10%, 15%, 15%, 20%, 25%, the inhibition zones were obtained which are listed in the following figure and figure:

In this study we will perform a statistical test using the *one way ANOVA* test, but provided that the data for each variable in the study must be normally distributed and homogeneous. So from the normality test of the inhibition zone diameter data on the *Streptococcus pyogenens* bacteria, it was obtained that it was normally distributed and the homogeneity test obtained the results of $\text{sig} > \alpha$ ($0.75 > 0.05$) so that the data variants were homogeneous, then the data analysis test was carried out using one way ANOVA

Based on the results of the statistical analysis test with the *One Way Anova* test, the significance value was obtained = 0.00. In accordance with the provisions, if the significance value is $\alpha = 0.05$, it can be stated

that the various concentrations of the bangun-bangun leaves extract (*Plectranthus amboinicus*) have an influence on the growth of *Streptococcus pyogenes* bacteria. And based on these results, it can be concluded that h_0 is rejected and h_a is accepted. "There is an effect of the bangun-bangun leaves extract (*Plectranthus amboinicus*) on the growth of *Streptococcus pyogenes* bacteria.

Comparison of Inhibition Zone Diameter from Bangun Bangun Leaves Extract (*Plectranthus amboinicus*) on Growth of *Salmonella typhi* Bacteria

In the bangun-bangun leaves extract (*plectharanthus ambonicus*) on the growth of the *Streptococcus pyogenens* bacteria, which in this study used the disc diffusion method, it was shown that the inhibition zone was measured using a caliper. The results of the study used a concentration of 5%, 10%, 15%, 15%, 20%, 25%, the inhibition zones were obtained which are listed in the following figure:

In this study we will perform a statistical test using the *one way ANOVA* test, but provided that the data for each variable in the study must be normally distributed and homogeneous. So from the normality test of the inhibition zone diameter data on the *salmonella typhi* bacteria, it was obtained that the distribution was normal and the homogeneity test obtained the results of $\text{sig} > \alpha$ ($0.406 > 0.05$) so that the data variants were homogeneous, then the data analysis test was carried out using *one way ANOVA*.

The results of statistical analysis tests using the One Way Anova test where the significance value = 0.00. From the specified significance value if, the value $\alpha = 0.05$ can be expressed by a different concentration of the extract produced and it affects the growth of *Salmonella typhi* bacteria. it can be concluded that h_0 is rejected and h_a is accepted. "There is an effect of the bangun-bangun leaves extract (*Plectranthus amboinicus*) on the growth of *Salmonella typhi* bacteria.



Figure 1. Concentration of the leaf extract

Table 1. Results of the phytochemical screening test

No	Compound Group	Reagent Name	Formed Colors	Results
1	Alkaloids	Meyer	Yellow	-
		Dragendroff	End. Yellow orange	+
		Bouchart	End. Red Brown	+
2	Tannins	Hot water + FeCl ₃ 10%	Bluish green	+
3	Saponins	Hot water + HCL 2N	A stable foam is formed	+
4	Flavonoids	Concentrated HCL + Mg powder	Yellow	+
5	Triterpenes / steroids	Lieberman – burchat	Bluish green	+
6	Sugar glycosides	LP molish	No purple rings were formed	-
7	Non Sugar Glycosides	Lieberman Burchad	Clear white	-
8	Anthraquinone Glycosides	CCl ₄ + dilute ammonia	The ammonia layer is brown	-
9	Polyphenols	FeCl ₃ 1%	Blackish Green	+

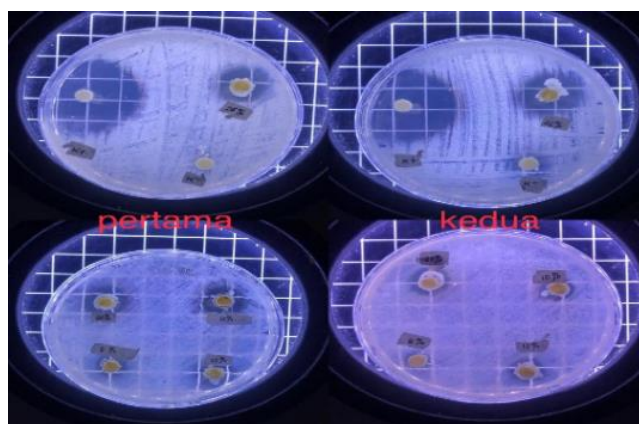


Figure 2. Inhibition zone in streotococcus pyogenes

Table 2. Diameter of the inhibition zone of *Streptococcus pyogenes*

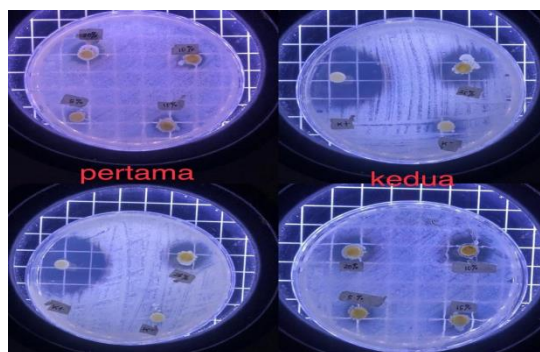
Concentration	Inhibition Zone Diameter (mm)		Average
	1 st Petri	2 nd Petri	
5 %	10.2	11.9	11.1
10 %	11.5	14.5	13.0
15 %	13.2	15.0	14.1
20 %	14.2	17.4	15.8
25 %	18.2	20.8	19.5
K ⁺	36.7	37.7	37.2
K ⁻	0	0	0

ANOVA

Streptococcus Pyogenes Zone of Inhibition

Table 3.Anova test on *Streptococcus pyogenes*

	Sum Of Squares	Df	Mean Square	F	Sig.
Between Groups	1.389.216	5	277.843	201.275	.000
Within Groups	16.565	12	1.380		
Total	1.405.781	17			

**Figure 3.** Inhibition zone in *Salmonella Typhi***Table 3.** Diameter of the inhibition zone of *salmonella typhi* bacteria

Concentration	Inhibition Zone Diameter (mm)		Average
	1 st Petri	2 nd Petri	
5 %	10.2	11.9	11.1
10 %	11.5	14.5	13.0
15 %	13.2	15.0	14.1
20 %	14.2	17.4	15.8
25 %	18.2	20.8	19.5
K ⁺	36.7	37.7	37.2
K ⁻	0	0	0

ANOVA

Salmonella typhi barrier zone

Table 4. One way ANOVA test on *salmonella typhi*

	Sum Of Squares	Df	Mean Square	F	Sig.
Between Groups	1411.399	6	235.23	21.982	.000
Within Groups	149.813	14	10.701		
Total	1561.212	20			

4. Discussion

Bangun-bangun Leaves extract activity as antibacterial by measuring the diameter of the inhibition zone formed in MHA media. Based on the data obtained, it is known that the concentration of the bangun-bangun leaves extract (*plecthranthus ambonicus*) has the ability to reduce the number of bacteria such as *streptococcus pyogenes* and *salmonella typhi* by inhibiting growth rather than bacterial growth.

Based on the results of the study, there was a variation in the diameter of the inhibition zone formed due to differences in concentration, which was determined by the researcher where it was found that the greater the concentration carried out, the greater the diameter of the inhibition zone obtained when conducting antibacterial effectiveness test.

The bangun-bangun leaves have a content that is rich in compounds that act as antibacterial, where in the results of our photochemical screening, we have found that the leaves of the bangun-bangun leaves contains saponins, flavonoids, polyphenols, tannins, tripenoids, and 0.2% essential oil. and carvacrol and other compounds that can exhibit antibacterial suppressive activity.

Where previous research has been carried out by Estherina et al. 2017 in the form of gel preparations with 70% ethanol solvent leaves, this bangun-bangun leaves extract can inhibit the bacteria *staphylococcus aureus* and *pseudomonas auruginosa* and the effective concentrations used in this study are concentrations II and III with clear zones of 17, 64 and 18, the stphlycoccus aureus bacteria were 17.87 and 20.67. (6)

Research Advantages

In this study we can find out the benefits of the *bangun-bangun* leaves extract in terms of inhibiting the growth of the *Streptococcus pyogenes* bacteria, which are gram-positive bacteria and *salmonella typhi* which are gram-negative bacteria. and in this study using equipment that is relatively simple and easy to do.

Research Weaknesses

In this study, we obtained the results that the bangun-bangun leaves extract was able to inhibit the growth of *streptococcus* and *salmonella typhi* bacteria. However, the inhibition zone diameter did not exceed the zone diameter from the positive control and the maceration process took a long time to work.

5. Conclusion

1. Based on the above results, it shows that the increase in the inhibition zone against the growth of *Streptococcus pyogenes* bacteria in the leaves of the *bangun-bangun* leaves extract (*Plectranthus amboinicus*) from a concentration of 5% (11.1 mm), 10% (13.0 mm), 15% 1 mm), 20% (15.8mm), 25% (19.5 mm), K + (37.2 mm), and K- (0).
2. Based on the above results indicate that the increase in the inhibition zone against the growth of *Salmonella typhi* bacteria with the extract of bangun-bangun leaves (*Plectranthus amboinicus*) 5% (20.7 mm), 10% (24.8 mm), 15% (28.9 mm)), 20% (34.9), 25% (37.7 mm), K + (45.3 mm), and K- (0).

3. Based on the results of the *One Way Anova* test, the *significance value* is 0.00. Then the effect of the bangun-bangun leaves extracts (*Plectranthus amboinicus*) is found on the growth of *Streptococcus pyogenes* bacteria.
4. Based on the results of the *One Way Anova* test, the *significance value* is 0.00. Obtained the effect of the bangun-bangun leaves extract (*Plectranthus amboinicus*) on the growth of *Salmonella typhi* bacteria.
5. Based on the results of research on the bangun-bangun leaves extract (*Plectranthus amboinicus*). more effective, in inhibiting the growth of *Salmonella typhi* bacteria than *Streptococcus pyogenes* bacteria. This is due to the inhibition zone produced in *salmonella typhi* with a concentration of 25% (37.7mm) while in *Streptococcus pyogenes* with a concentration of 25% (19.5mm) but does not exceed the inhibition zone formed by the diameter of the K + inhibition zone.

6. Suggestion

1. It is recommended that the next research be more careful in culturing bacteria.
2. To carry out further research in seeing the effects of leaves or other fruits that can inhibit the growth of *Salmonella typhi* and *Streptococcus pyogenes* bacteria.
3. It is suggested to the next researchers to be careful in keeping the equipment sterile.

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