# SCREENING OF PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITY OF DADANGKAK ROOTS EXTRACTS *(Hydrolea spinosa L.)* AGAINST *Streptococcus mutans*

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**Abstract**

Infectious disease is the highest problem in the world, especially in developing countries, such as Indonesia. One of the infection causes is the inappropriate use of antibiotics, which lead to resistance. Exploration of dadangak plant (*Hydrolea spinosa L.)* which was expected has antibacterial effectiveness due to it contains of secondary metabolites in the form of alkaloids, flavonoids, saponins, and tannins. To determine the antibacterial activity in the extracts of dadangak root *(Hydrolea spnosa L.)* against *Sterptococcus mutans* by disc diffusion and dilution methods to determine the values of MIC *(Minimum Inhibitory Concentration)* and MBC *(Minimum Bactericidal Concentration).* Extraction using maceration method and antibacterial activity testing using disc diffusion and dilution methods with concentrations of 20%, 40%, 60%, 80%, and 100%. Data analysis used qualitative and quantitative descriptive. Secondary metabolites produced by dadangakak root extract were alkaloids, flavonoids, tannins, and saponins which have antibacterial activity. antibacterial activity of extract of dadangak root *(Hydrolea spinosa L.)* against *Streptococcus mutans* bacteria by diffusion test resulted in an average inhibition zone of 8.22 mm and using the dilution method to observe MIC which was found at a concentration of 40%. Meanwhile, MBC was not found in all concentrations. Dadangkak root extract has secondary metabolites, namely: alkaloids, flavonoids, tannins, and saponins. The antibacterial activity test using the disc diffusion method showed that there was an inhibition zone, the extract of dadangak root *(Hydrolea spinosa L.)* had a MIC value at a concentration of 40% against *Streptococcus mutans* bacteria by the dilution method.

**Keywords:** Antibacterial, Root Extract, Dadangkak, Diffusion, Dilution

## Introduction

Infectious disease is the highest problem in the world, especially in developing countries such as Indonesia. Infection is a disease caused by a microbe that enters, reproduces and forms a broad group consisting of many cells such as fungi, bacteria, viruses, and parasites (Novard et al., 2019). Dental caries is one of the infectious diseases that often occurs in the Indonesian population, the factors that cause dental caries are the presence of microorganisms, tooth shape, lack of saliva, and the environment such as cleaning teeth using unclean water. According to a journal researched by (Anggraini, 2018), said that *Streptococcus mutans* bacteria had resistance to chlorampenicol antibiotics by 100%, cefotaxime 50%, levofloxacin 50%, and to tetracycline 50%. The results of several studies also showed that every year there is an increase in *Streptococcus mutans* resistance to

these antibiotics. The results of several studies also show that every year there is an increase in *Streptococcus mutans* resistance to these antibiotics. To overcome this, new efforts are needed to explore natural plants in Indonesia in order to create a therapy made from plants as well as research related to plants that have the potential for antibacterial activity (Trisia et al., 2018).

Indonesia has an abundant natural wealth of flora and fauna diversity. It is estimated that 100 to 150 types of plants can be used as herbal remedies for most communities. According to (Yassir & Asnah, 2019), the benefits of herbal medicine are proven to be safe, effective, efficient, and economical in healing a disease. South Kalimantan which has dadangkak plants *(Hydrolea spinosa L.)* or it can also be called jeruju. People in South Kalimantan use the leaves and roots of the dadangak plant *(Hydrolea spinosa L.)* to lowering blood sugar levels, lowering fever, and antimalarial (Forestryana & Yunus, 2018).

Research on leaves has been conducted by (Darsono & Fajriannor, 2020), and the results showed that the leaves of dadangak *(Hydrolea spinosa L.)* had secondary metabolites of flavonoids, triterpenes, saponins and tannins that have antibacterial activity. Based on another study conducted by (Darsono & Kuntorini, 2012), revealed that the leaves and stems of the dadangkak plant *(Hydrolea spinosa L.)* had antioxidant activity. Research had also been carried out on the stems of dadangak *(Hydrolea spinosa L.)* (Andryanie, 2020), and the results it had secondary metabolites in the form of alkaloids, flavonoids, triterpenoids, saponins, and tannins which have antibacterial activity. Meanwhile, on the roots of the dadangak plant *(Hydrolea spinosa L.)* research has been carried out by (Zaini et al., 2017) and found secondary metabolites, namely tannins and saponins which are useful as antidiabetic. however, no research has been conducted on the roots of dental caries infection caused by *Streptococcus mutans* bacteria. According to (Made et al., 2015), said that herbal treatment in plants is mostly in the bark, leaves, flowers, seeds, and roots.

Research on the roots of dadangak *(Hydrolea spinosa L.)* was conducted to find secondary metabolites that have the potential as antibacterial by using diffusion and dilution methods. And this study used Streptococcus mutans bacteria. *Streptococcus mutans* bacteria are gram-positive bacteria, shaped like chains, which play a role in the metabolism of sucrose into lactic acid, this lactic acid causes tooth enamel demineralization and tooth decay which is the main cause of infection in teeth or commonly called dental caries. 2016) (Bontjura et al., 2015). This study used *Streptococcus mutans* with diffusion and dilution methods.

## Material and Methdos

The materials used in this research were 96% ethanol, H2SO4 P, 2N HCl, 1% FeCl3, Amoxicillin Disk, DMSO, Sodium Agar (NA) media, Nutrien Broth (NB) media, Muller-Hinton Agar (MHA)

media, Dragendorf reagent, Mg powder, aquadest, HCl P, chloroform, anhydrous acetic acid, dadangak root *(Hydrolea spinosa L.)* as sample test material, *Streptococcus mutans.*

**Sample Preparation**

The preparation process of the dadangak root sample were collecting materials, wet sorting, washing, chopping, drying, sifting, milling, and packing.

**Identification of Secondary Metabolites With Chemical Reagent.**

Alkaloid test, was conducted by giving 2-3 drops of Dragendroff reagent, then it showed the presence of brown precipitate (Noval et al., 2019). Tannin test, added 2-3 drops of 1% FeCl3, then it showed blue or blackish green color (Jannah et al., 2017). The triterpenoid test was conducted by dripping with 1 ml of Lieberman-Burchard reagent, then blackish green or dark green was appeared (Riana Ningsih et al., 2016). The flavonoid test, was conducted by adding Mg powder, 3-5 drops of concentrated HCL, then an orange or yellow color was appeared (Noval et al., 2019). The saponin test, was conducted by adding aquadest in a test tube, then heated for 2-3 minutes, after being slightly cold, shake vigorously, after 1-2 cm high foam was formed that lasts for 30 seconds, it means that the sample shows positive saponins (Noval et al., 2019).

**Bacterial Rejuvenation**

Take as much as 1 ose and scratched or transferred to the slanted surface of the NA media. Afterwards, incubated in an incubator for 1x24 hours at $37^0$C (Rosmania & Yanti, 2020).

**Bacterial Inoculation**

Took 1 needle of a suspension of Streptococcus mutans from an oblique agar (NA) medium and then immersed in 10 ml of liquid medium (NB). Then incubated in an incubator for 1x24 hours at $37^0$C (Jannah et al., 2017).

**Antibacterial Activity Test**

1. Difusion

   Activity testing was carried out using the disc diffusion method by placing a paper disc with a diameter of 6 mm that had been immersed in an extract solution, 1 ml of DMSO as a negative control, and 25 mcg of amoxicillin as a positive control. The testing process was performed in the BSC *(Bio Safety Cabinet)*, then the test bacteria were taken and poured on the MHA media while flattening using an L rod. Then the soaked disc paper was placed on the MHA media. The media that had been inputted with disc paper were incubated in an incubator at a temperature of $37^0$C for 1x24 hours (Darsono & Fajriannor, 2020). Finally, the inhibition zone was measured after the incubation was completed, which was marked by the formation of a clear zone around the paper disc
2. Dilusion

The antibacterial activity of dadangak root extract was tested using liquid dilution method. The first step, each tube was filled with 1 ml of NB media, then in the same tube added a bacterial solution that had been equated with Mc Farland as much as 1 ml. Next, a solution of extract of dadangak root was added in each tube, which had been adjusted to a concentration of 20%, 40%, 60%, 80%, and 100%. Comparative test bacteria solution as a negative control and antibiotics (amoxicillin) as a positive control. Then, it was incubated for 18-24 hours at a temperature of 35-37$^0$C. Observe the clarity that occurs in each tube and compare it with the positive control (amoxicilin) and the negative control. The MIC value was determined at the lowest concentration that can inhibit bacterial growth through its clarity (Sariadji et al., 2019).

The next step was to determine the MBC, by pouring the tube from the MIC test results and spreading evenly over the entire surface of MHA media, then incubated for 18-24 hours at a temperature of 35-37$^0$C. The lowest concentration which showed the presence of bacteria colonies on MHA media was the MBC (Sariadji et al., 2019)

**Result and Discusion**

Phytochemical screening is a qualitative analysis of compounds that have secondary metabolites. Plants produce a secondary metabolite to protect themselves from attacks in their surrounding environment such as bacteria, fungi, insect attacks, and other pathogens. Based on Darsono's research (2020), leaf extracts from the dadangak plant contain phytochemicals, including: flavonoids, tannins, alkaloids, and steroids. Meanwhile, in Andryanie's (2020) study, extracts from dadak stem contained secondary metabolite compounds, namely: flavonoids, alkaloids, saponins, tannins, and triterpenoids.

In contrast to the secondary metabolites produced by extracts from this dadangak root, which contain alkaloids, flavonoids, tannins, and saponins. According to (Sholekah, 2017), the factors that influence a plant are internal or external factors from the plants. Internal factors such as genes and external factors such as temperature, humidity, light, pH, nutrients contained in the soil surrounding the plant.

*Table 1 Phytochemical Screening Results*

| No. | Compound | Reagent | Result | Description |
|---|---|---|---|---|
| 1. | Alkaloids | Dragendroft's | Sediment chocolate | Positive |
| 2. | Tannins | $Fecl_3$ 1% | Dark green | Positive |
| 3. | Triterpenoids | Sulfuric acid+ chloroform | Golden yellow | Negative |
| 4. | Flavonoids | Mg powder + concentrated Hcl | Yellow | Positive |
| 5. | Saponins | Aquadest | High foam 1cm | Positive |

Testing of inhibition zone or clear area around the discs that have been carried out shows the results in table 2. The results of this test by using the extract, obtained the average diameter of inhibition zone of 8.22 mm. According to the category in (CLSI, 2019), it could be said as sensitive if the results showed

a diameter of inhibition zone 24 mm and there was no information for intermediate or resistant category. It can be concluded that the extract obtained could not be categorized as sensitive, intermediate, or resistant. The inhibitory power produced by the extract of dadangak root against Streptococcus mutans bacteria with a moderate strength category. *Streptococcus mutans* is a gram-positive bacteria that has the ability to very easily absorb a solution, making it easier for solutes to enter the cell wall from the bacteria itself. However, the peptidoglycan in the cell walls of these gram-negative bacteria is not easily destroyed by solvents from the extract. According to (Ernawati & Sari, 2015), the greater the solute, the easier it is for bacteria to die by the substance itself. Meanwhile, according to (Sari et al., 2017), gram-positive bacteria are more sensitive to antibacterial compounds because the cell wall structure of gram-positive bacteria is simpler than gram-negative bacteria, making it easier for an antibacterial compound to enter the cells of gram-positive bacteria. Therefore, the inhibition zone of the extract of dadangak root against *Streptococcus mutans* bacteria was smaller.

*Tabel 2 Test Result Of Inhibition Zone*

| No. | Compound | Diameter (mm) | | | Average Diameter (mm) |
|---|---|---|---|---|---|
| | | R1 | R2 | R3 | |
| 1. | Control (-) | 0 | 0 | 0 | 0 |
| 2. | Control (+) | 0 | 22,42 | 14,75 | 12,39 |
| 3. | Dadangkak Root Extract | 0 | 16,26 | 8,42 | 8,22 |

MIC is one of the tests used to determine the sensitivity of microbes to bioactive substances. Dilution method is a test of antibacterial strength in a liquid medium that had been given an antibacterial agent and incubated for 1x24 hours. Dadadak root extract used several concentrations, namely 20%, 40%, 60%, 80%, 100% which were made in 10 ml stock solution and dissolved with DMSO at each concentration. According to (Sariadji et al., 2019), in determining the value of MIC, the lowest concentration which showed clarity was chosen since the ability to inhibit bacterial growth.

Table 3 shows that there was bacterial growth in the tube that contained negative control, which means that DMSO did not have antibacterial activity. The positive control tube containing the antibiotic amoxicillin did not show any bacteria growing in the tube. It can be concluded that the antibiotic amoxicillin had antibacterial activity against *Streptococcus mutans*. In the tube containing the extract of dadangak root with a concentration of 20%, 40%, 60%, 80%, and 100%, it was found that the antibacterial activity of the dadangak root extract obtained a MIC value *(Minimum Inhibitory Concentration)* at a concentration of 40% which indicated by clarity.

*Table 3 Test Results of Minimum Inhibitory Concentration (MIC) Dadangkak Root Extract (Hydrolea spinosa L.) Against Streptococcus Mutans Bacteria*

| No. | Compound | Replication | | |
|---|---|---|---|---|
| | | I | II | III |

| | | | | |
|---|---|---|---|---|
| 1. | Nagative control (DMSO) | | + | |
| 2. | Positive control (Amoxicillin) | | - | |
| 3. | Concentration 20% | + | + | + |
| 4. | Concentration 40% (KHM Result) | - | - | - |
| 5. | Concentration 60% | - | - | - |
| 6. | Concentration 80% | - | - | - |
| 7. | Concentration 100% | - | - | - |

This related with the secondary metabolites contained in the extract, such as alkaloids, flavonoids, tannins, and saponins. These secondary metabolites are known to have antibacterial properties in plants.

Based on the research results obtained from the extract of dadangak root, there was no value of MBC *(Minimum Killing Concentration)* in *Streptococcus mutans* bacteria after being incubated for 1x24 hours, indicated by the growth of bacteria that filled MHA solid media. Table 4 describes that there was bacterial growth in all concentrations, which indicate that it might have MBC value if the concentration of extract was more than 100%, which according to (Jatmiko, 2020) the greater concentration of the extract cause the less bacteria will grow. Based on the results of this study, it can be concluded that the extract from the dadangak root had antibacterial ability which was indicated by the presence of MKC which can inhibit the growth of a bacterium.

*Table 4 Test Results of Minimum Bactericidal Concentration (MBC) Extract of Dadangkak Root (Hydrolea spinosa L.) Against Streptococcus Mutans*

| **No.** | **Compound** | **Replicatoin** | | |
|---|---|---|---|---|
| | | **I** | **II** | **III** |
| 1. | Nagative control (DMSO) | | + | |
| 2. | Positive control (Amoxicillin) | | - | |
| 3. | Concentration 20% | + | + | + |
| 4. | Concentration 40% | + | + | + |
| 5. | Concentration 60% | + | + | + |
| 6. | Concentration 80% | + | + | + |
| 7. | Concentration 100% | + | + | + |

## Conclusion

Based on the results of this study, it can be concluded that the extract of dadangak root contains secondary metabolites that contained antibacterial activity, such as alkaloids, flavonoids, tannins, and saponins. Dadangkak root extract also has antibacterial ability against *Streptococcus mutans* bacteria with MIC value was medium category. The value of *minimum inhibitory concentration* (MIC) in the extract of dadangak root was found at 40% concentration, Meanwhile, there was no MBC at all concentrations, namely: 20%, 40%, 60%, 80%, and 100%.

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