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# Effect of Rambusa Fruit Extract (Passiflora Foetida) On the Growth of Streptococcus Pyogenes Bacteria

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**Abstract**

**Background:** *Streptococcus pyogenes* is a bacterium have ability to attack the epidermis and upper respiratory tract of humans, such as pharyngitis and impetigo. The Rambusa plant (*Passiflora foetida L*) is famous for containing larvicide bioactive compounds. It also contains alkaloids, tannins, coumarins, procyanidins, triterpenoids, flavonoids, citric acid, acetogens, polyphenols, and saponins. A minority of individuals continue to employ this herb sporadically as an antibacterial agent. Intrigued by this phenomenon, this study aimed to examine the effect of rambusa fruit (*Passiflora foetida*) administration on the *Streptococcus pyogenes* bacterium development inhibitory zone. **Methods:** This research uses Only Post Test Control Group Design, exclusively experimental. The population for this study was *Streptococcus pyogenes* bacteria obtained from the microbiology laboratory of the Faculty of Medicine, Wijaya Kusuma University, Surabaya. There were 24 samples in this study, with four repetitions per sample and consisting of 6 groups, including a treatment group with 4 subgroups (10%, 20%, 40%, and 80%), as well as two control groups. **Results:** The Mann-Whitney U test showed that a concentration of 80% in the *Passiflora foetida* fruit extract treatment group resulted in an inhibitory zone diameter of 8.5 mm, which was statistically significant (P<0.05). **Conclusion:** The conclusion is that administration of rambusa fruit extract (*Passiflora foetida*) can inhibit bacterial growth of *Streptococcus pyogenes*, with the average highest inhibitory zone diameter being 8.5 mm.

**Keywords:** Growth, Rambusa Fruit (*Passiflora foetida), Streptococcus pyogenes*

***Original Research Article***

## INTRODUCTION

Streptococcus pyogenes is a gram-positive, human-specific pathogen that asymptomatically colonizes the human respiratory tract (Banerji and Saroj, 2021). Gram-positive cocci Streptococcus pyogenes are characterized by a chain-like shape, a size range of 0.5 to 11 µm, and qualitative anaerobic properties. It grows with a pH range of 7.4 to 7.6 at an optimal temperature of 37 °C, and its growth slows down significantly when the temperature is increased to 40 °C (Milah Nihayatul, 2017). These bacteria attack using sophisticated virulence mechanisms to fight the host's immune system. The toxins and immunological response caused by *Streptococcus pyogenes* can result in systemic surface infections. Pharyngitis and impetigo are diseases caused by this microorganism (Suhartati, 2017). *Streptococcus pyogenes* is a major contributor to the 10 highest death rates associated with infectious diseases in humans worldwide (Jespersen *et al.*, 2020). Several epidemiological studies have found that the

prevalence of *Streptococcus pyogenes* infection is higher in men than in women. The highest incidence of *Streptococcus pyogenes* infection occurs in the elderly population, with the second highest prevalence in infants (Androulla Efstratiou, 2016).

*Passiflora foetida L.* commonly known as the rambusa plant, is underutilized by the public as an antibacterial agent, and this plant is only known for its sweet and small fruit. This plant is often used as a remedy for cough and fever as well as an insomnia remedy (Lamato, 2017). Rambusa usually grows as a wild herbaceous plant in various locations. This location can be a forest area, a coastal area, rice fields, or unmaintained open land that is fully lit. Even so, this plant likes to live in moist soil. The ripe fruit of the wild plant known as rambusa has a pleasant taste and aroma, and the plant itself is edible (Silfi Roihanah, 2020) An herbaceous plant that can grow between 1.5 and 5 meters long and has coils that look like a spiral. Inside the fruit, there are many seeds, while the outside of the fruit is covered with many hair fibers (Andini and Rahayu, 2020).

The Rambusa fruit is a good source of antioxidants. In addition, Rambusa is an excellent source of vitamin C and fiber (Irawati, 2020). The rambusa fruit is a type of plant species that is recognized for its presence of larvasides, which are bioactive chemicals. The compound contained in it includes acetogenethanol, proanthocyanidins, coumarins, tannins, citric acid, polyphenols, triterpenoids, flavonoids, and saponins (Olla, Hasan, and Rupidara, 2020).

The benefits of rambusa fruit are not widely known to the public, so researchers are interested in studying the effect of giving rambusa fruit (*Passiflora foetida*) on the inhibition zone on the growth of *Streptococcus pyogenes* bacteria in the bacterial growth barrier zone *Streptococcus pyogenes. T*his research can inform the public about using rambusa fruit as an alternative to prevent inflammation and the formation of abscesses caused by the bacteria *Streptococcus pyogenes*. Therefore, studying how rambusa fruit (*Passiflora foetida*) affects the *Streptococcus pyogenes* bacterial growth inhibition zone is an interesting topic for researchers.

## MATERIALS AND METHODS

### Sample Size

This study used a Post-Test Only Control Group using a simple random sampling method. Sampling in this study was calculated using the Federer formula. In this study, there were 6 groups of 4 treatment groups (10%, 20%, 40%, 80%) and 2 control groups. So, the total number of samples used was 24 because each sample had 4 repetitions**.** This study has obtained ethical approval from Faculty Medicine Universitas Wijaya Kusuma Surabaya with Certificate Number 7/SLE/FK/UWKS/2023.

### Materials Preparation

Sterile distilled water, suspension *Streptococcus pyogenes*, rambusa fruit extract (*Passiflora foetida)* with concentrations of 10%, 20%, 40%, 80%, *Mueller Hinton Agar*, bacitracin antibiotic.

### Equipment Preparation

Petri dishes, test tubes, filter paper, hole punch, glue, tube needle, spirit lamp, tweezers, measuring cup, autoclave, incubator, disposable spray, sterile cotton swab, ruler, knife, and cutting board. Disc diameter 0.5 cm, handscoon. Sterilize all tools to be used at 121$^{o}$C with an autoclave for 15 minutes.

### Extraction of Rambusa Fruit

100 grams of rambusa fruit powder, use a spatula to transfer it to a maceration container (Erlenmeyer flask) and pour in 96% ethanol gradually until the powder is completely covered with liquid. The samples were then stored undisturbed for 24 hours in a dark, tightly closed container with a spatula shaking occasionally. After waiting 24 hours, filter paper is used to transfer the sample to a storage container. Processed goods are stored in cold storage. A rotary evaporator is used to condense the filtered liquid into a thick extract

**Preparation of Rambusa Fruit Extract Solution**

This research, obtained rambusa fruit extract concentrations at 10%, 20%, 40%, and 80% through dilution with 100% rambusa fruit extract and adding distilled water. The 10% test solution concentration was obtained by taking 0.5 ml of 100% test solution plus 4.5 ml of distilled water. The 20% test solution concentration is obtained by taking 1 ml of 100% test solution plus 4 ml of distilled water. The 40% test solution concentration was obtained by taking 2 ml of 100% test solution plus 3 ml of distilled water. A test solution concentration of 80% is obtained by taking 4 ml of 100% test solution plus 1 ml of distilled water.

**Preparation of *Mueller Hinton Agar***

In an *Erlenmeyer*, dissolve 3.8 grams of medium *Mueller Hinton Agar* in 100 milliliters of distilled water, then heat the above solution on a hot plate. The media was sterilized for 15 minutes in an *autoclave* at a temperature of 121$^{o}$C. The sterilized loop is then placed on the media by scraping it with a loop needle so that it is tilted. The samples were then incubated at 37$^{o}$ C for 24 hours.

**Procedure**

Rambusa fruit extract (*Passiflora foetida)* in concentrations of 10%, 20%, 40%, and 80% were divided into 4 groups (P3, P4, P5, P6) with 2 control groups (P1 and P2) using sterile distilled water and bacitracin. Each concentration group was placed in 5 petri dishes so that there were 30 petri dishes containing rambusa fruit extract with various concentrations and a control solution. Place the blank disk in the petri dish for 15-30 minutes. Later, the blank disk, which has been soaked for 15-30 minutes in rambusa fruit extract and the control solution is placed on top of *Mueller Hinton Agar* containing bacterial cultures *Streptococcus pyogenes.*

Next, the plate was incubated in an incubator at 37$^{0}$C for 24 hours. Then, the *disk* will diffuse in the medium *Mueller Hinton Agar*. The clear area indicates that the growth of microorganisms on the surface of the *Mueller Hinton Agar* media is inhibited. Then, the diameter of the inhibition zone formed on the MHA was measured by *Streptococcus pyogenes* and entered into statistical tests, after obtaining the data conclusions were drawn.

**Statistical Analysis**

The results of the data were analyzed for normality using the Kolmogorov-Smirnov test. Levene's test was carried out to determine the homogeneity of the data ($p>0.05$). The one-way ANOVA test is performed if the data is normal and homogeneous. LSD (Least Significant Difference) test with a degree of significance of $\alpha$=0,05. If the data is not obtained normally distributed or homogeneous, then proceed with the Kruskal Wallis nonparametric statistical test.

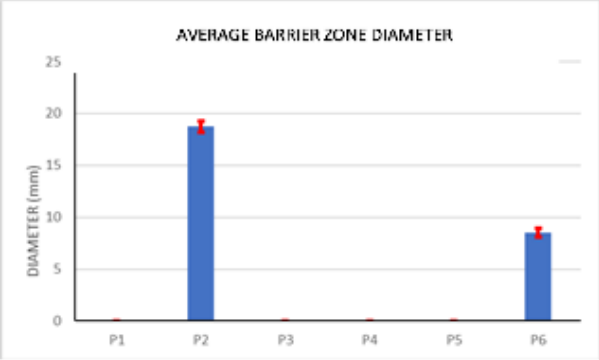
## RESULTS

**Table 1.** Average group barrier zone

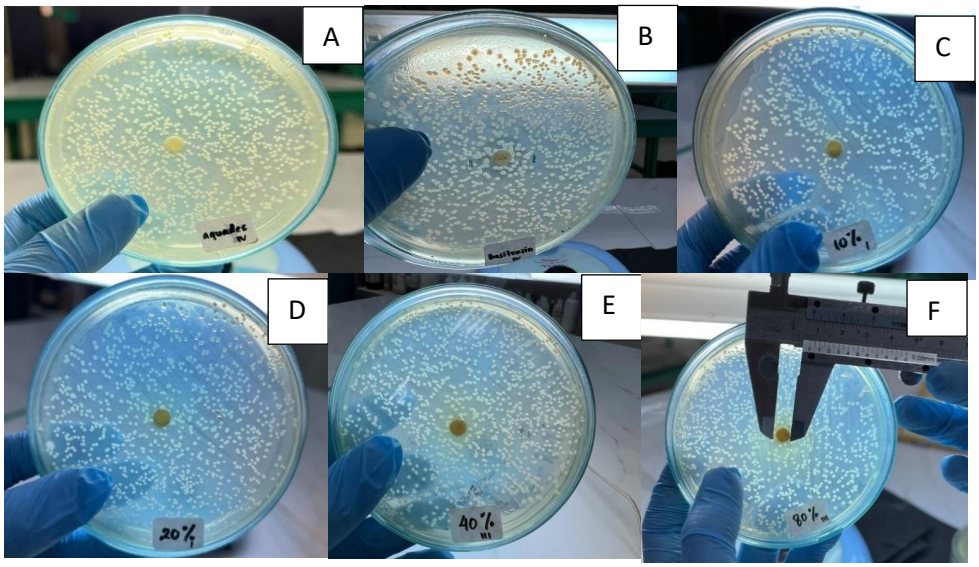
| Barrier Zone | N | Mean (mm) | Std. Deviation |
|---|---|---|---|
| P1 (aquadest) | 4 | 0.00 | 0.00 |
| P2 (bacitracin) | 4 | 18.73 | 0.54 |
| P3 (10%) | 4 | 0.00 | 0.00 |
| P4 (20%) | 4 | 0.00 | 0.00 |
| P5 (40%) | 4 | 0.00 | 0.00 |
| P6 (80%) | 4 | 8.5 | 0.41 |

**Source:** Results of 2023

The average barrier zone diameter in group P2 (the antibiotic Bacitracin treatment group) was the highest group i.e 18.73 mm, whereas the negative control group with aquadest and extract solution 10%, 20%, 40% had the lowest average diameter of 0.00 mm. As shown in the Figure 1.




**Source:** Results of 2023
**Figure 1.** Diagram of Average Barrier Zone Diameter




**Source:** Results of 2023
**Figure 2.** Measurement of Barrier Zone Diameter of Negative Control Group/ P1 (A); Positive Control Group/P2 (B); 10% Rombusa Extract/ P3 (C); 20% Rombusa Extract/ P4 (D); 40% Rombusa Extract/ P5 (E); 80% Rombusa Extract/ P6 (F)

**Table 2.** Kruskal Wallis Intergroup Test

| Variable Research | *p-value* | Identifying |
|---|---|---|
| **Diameter of the barrier zone** | 0.000 | There is difference |

**Source:** Results of 2023

Based on the aforementioned findings, the p-value significance is 0.000 ($p < 0.05$), therefore indicating a statistically significant difference between basitration as a positive control, distilled water as a negative control, and rambusa fruit extract with concentrations of 10%, 20%, 40%, 80% against *streptococcus pyogenes.*

**Tabel 3.** Post-Hoc tests with the Mann-Whitney U test

| | | Sig. | Identifying |
|---|---|---|---|
| **P1** | P2 | 0.014 | There is difference |
| | P3 | 1.000 | There is no difference |
| | P4 | 1.000 | There is no difference |
| | P5 | 1.000 | There is no difference |
| | P6 | 0.014 | There is difference |
| **P2** | P3 | 0.014 | There is difference |
| | P4 | 0.014 | There is difference |
| | P5 | 0.014 | There is difference |
| | P6 | 0.021 | There is difference |
| **P3** | P4 | 1.000 | There is no difference |
| | P5 | 1.000 | There is no difference |
| | P6 | 0.021 | There is difference |
| **P4** | P5 | 1.000 | There is no difference |
| | P6 | 0.021 | There is difference |
| **P5** | P6 | 0.021 | There is difference |

**Source:** Results of 2023

Concentrations of rambusa extract 80% (P6) have a greater inhibitory to the growth of the bacterium *Streptococcus pyogenes* compared to other concentrations but not greater than its positive control, namely bacitracin (P2). From the concentrations of 10% (P3), 20% (P4), and 40% (P5) it can be seen that no barrier zone is equal to aquadest as negative control (P1). Based on the results of statistical tests obtained, comparison results of the group Bacitracin with the group aquades, rambusa extract with concentrations of 10%, 20%, 40%, and 80% have significant differences with the intention indeed there is a significant difference from the concentration of the smallest to the largest rambusa extract, although of the 80% concentration rambusa extract is still not able to better its effectiveness compared to Bacitrazin.

The group of aquadest compared to the group of rambusa extracts with concentrations of 10%, 20%, and 40% had no significant difference, so the effectiveness was the same as aquadest at such rates. The concentration is 80% more effective than aquadest due to chemical content in rambusa extract that can inhibit the growth of bacteria, such as tannins, flavonoids, saponins, and citric acids. In the group of rambusa extract with a concentration of 80% compared to the concentrations of 10%, 20%, and 40% have significant differences, so at concentration 80% have better effectiveness than the other concentrations.

The data is said to mean there is a difference when $p < 0.05$. Rambusa extracts at 80% concentration have the largest barrier zone area compared to other smaller concentrations, this is due to the chemical compound content of the pineapple extract, such as tannins, flavonoids, and essential oils at a greater or greater concentration at 80% compared with other small concentrations. In the manufacture of a less concentrated test solution, the addition of aquadest to the rambusa extract is greater. Statistical research data obtained in table 5, can be concluded H0 rejected because there are differences from 6 treatment groups (minimum of a pair). The formation of a barrier zone in the growth of the bacteria *Streptococcus pyogenes* using the MHA medium means that the extract of rambusa (*Passiflora fortida L.*) has antibacterial power in terms of inhibiting the growth of *Streptococcus Pyogenes*, so the conclusion H1 was accepted.

## DISCUSSION

*Streptococcus pyogenes* is a bacterial pathogen, that has a wide spectrum of clinical presentations, including benign local infections and potentially fatal invasive infections (Ibrahim J. et al., 2016). *Streptococcus pyogenes* also known as Group A *Streptococcus* (GAS). The infections can be superficial or systemic. Streptococcal toxic shock syndrome, impetigo, acute pharyngitis, necrotizing fasciitis, sepsis, dengue fever, and acute rheumatic fever are illnesses caused by *Streptococcus pyogenes* (Sanyahumbi et al., 2016; (Walker et al, 2014).

*Streptococcus pyogenes* is responsible for acute pharyngitis. Pharyngitis is the inflammation of the mucous membranes in the oropharynx and posterior pharynx, including the tonsils. It is characterized by symptoms such as a sore throat, sudden fever, redness in the throat, swollen tonsils, yellow or blood-tinged discharge, and little red spots called petechiae (Jr. Hurst et al., 2018). Previous studies showed treatment for infection of *Streptococcus* remained fail, in penicillin resistance increase almost 40% in many countries (Cattoir, 2016)

Traditional medicine from Indonesia called Markisa or Rambusa fruit, has long been used for various treatments or therapies (Wijayanti et al, 2022). Rambusa fruit contain high of antioxidant activities especially from center Kalimantan (Mulia et al, 2019). This study providing rambusa fruit (*Passiflora foetida*) is capable of inhibiting the growth of *Streptococcus pyogenes*, because rambusa fruit contains many secondary metabolite compounds, such as acetogenethanol, proanthocyanidins, coumarins, tannins, citric acid, polyphenols, triterpenoids, flavonoids, and saponins (Olla, Hasan and Rupidara, 2020). Several compounds contained in these plants can be used as antibacterial agents (Lamato, 2017*)*. Rambusa mini passion fruit is a plant that looks like a soft liana and is commonly found on the edges of ponds (Marpaung, Aa et al., 2021). The leaves, fruit, roots, and flowers of the rambusa plant are used as medicine. Rambusa fruit has antibacterial and larvicide characteristics, making it an alternate treatment for a variety of upper respiratory tract disorders (Astuti, Md., 2017).

According to the results, the inhibitory zone diameter in the group treated with 80% rambusa fruit extract (*Passiflora foetida*) was 8.5 mm. The group P2, which received the antibiotic bacitracin, exhibited the largest average diameter of the inhibition zone at 18.73 mm. In contrast, the groups P1, P3, and P4 contained the smallest average diameters of the inhibition zones. Group P5, which served as the negative control consisting of distilled water and extract solutions of 10%, 20%, and 40%, measured 0.00 mm. Furthermore, at a concentration of 80% (P6), an inhibitory zone measuring 8.5 mm was formed. Based on the results of statistical tests obtained comparison results of the group Bacitracin (P2) with the group aquades (P1), rambusa extract with concentrations of 10% (P3), 20% (P4), 40% (P5), and 80% (P6) have significant differences with P value <0,05, which means that there is indeed a significant difference from the concentration of the smallest to the largest rambusa extract, although of the 80% (P6) concentration rambusa extract is still not able to better its effectiveness compared to Bacitrazin.

According to Mohd Nazriet al. (2011), The amount of antibacterial potency is measured by measuring the diameter of the inhibition zone. None (0 mm), mild (0-9 mm), moderate (10-14 mm), and robust (15-20 mm) are the criteria. *Passiflora foetida* fruit extract at an 80% concentration is thought to have mild growth-inhibitory power since it only stops growth in an 8.5 mm zone.

The categorization of bacterial development phases, according to Mahjani, and Putri (2020), encompasses four distinct stages: lag phase, log phase, stationary phase, and death phase. The lag phase is influenced by various factors including the composition of the media, its pH level, temperature, aeration, the initial quantity of cells in the inoculum, and the physiological characteristics of the microorganisms that previously inhabited the media. Microbial growth kinetics can be used to identify the best environmental conditions. Development kinetics describes the pattern of microbial development as well as the amount of time required to grow and adapt (Wya Saraswati et al., 2021).

Several prior investigations that utilized extracts derived from various components of the rambusa plant to determine the inhibition zone of gram-positive bacteria yielded nearly identical findings. Giving *Staphylococcus aureus* rambusa stem and leaf extract at 15%, 30%, and 45%

concentrations did not make a statistically significant difference in the average result (-8 mm), according to a study by Jufriet et al. (2020). Noviyanti, P, and Tarigan (2014) say that *Staphylococcus aureus* grew when a 20% concentration of an ethanol-water extract of rambusa leaves was used to make it grow. The resultant average yield was 12 mm.

This might be because the rambusa fruit (*Passiflora foetida*) was not extracted properly, so it could not stop the growth of *Streptococcus pyogenes*. Groups of some bacterial cells obtained from susceptible population develop mutations that influence the activity of the drug, following bacterial survival and resistance to antimicrobial agents (Munita et al., 2016). The integrity of rambusa fruit extract (*Passiflora foetida*), which is subject to chemical and biological influences, might also impact this. Biological aspects include the plant species itself, the region of origin, harvest time, raw material storage, age, and plant parts used in the study, whereas chemical factors comprise external and internal elements. External parameters include filter size, heavy metal, pesticide content, and the extraction process utilized, whereas internal aspects include active chemical type, composition, and content. According to phytochemical testing, the active constituents are saponins, phenols, steroids, terpenoids, glycosides, alkaloids, flavonoids, and tannins (Brigita et al., 2021).

## CONCLUSION

Growth of *Streptococcus pyogenes* inhibited by Rambusa fruit extract (*Passiflora foetida*)., especially in concentration of 80%. Inhibitory zone diameter of *Streptococcus pyogenes* wa 8.5 mm and showed significantly different with the others groups (P<0.000) with Kruskal-Wallis test.

The effectiveness of Rambusa fruit extract as an antibiotic is known to be able to inhibit the growth of *Streptococcus pyogenes*, so that it can be an alternative herbal treatment for diseases such as acute pharyngitis, necrotizing fasciitis, sepsis, dengue fever, and acute rheumatic fever. Further research to determine the effect of consuming Rambusa (*Passiflora foetida*) fruit extract on the growth of various types of pathogenic bacteria should be explored, moreover to its killing activity.

## CONFLICT OF INTEREST

The authors affirm that there are no conflicts of interest in this study.

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