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# Strobilanthes crispus antibacterial against Aggregatibacter actinomycetecomitans

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**Abstract**

Aggregatibacter actinomycetecomitans is a bacteria that causes periodontitis. One of the plants that has antibiotic properties is the shard (Strobilanthes crispus). This study aims to determine the effect of the ethanol extract of Strobilanthes crispus leaves on the growth of Aggregatibacter actinomycetecomitans bacteria. This research is a quantitative study using the experimental laboratory method. The research stages included the selection of Strobilanthes crispus leaves as herbal ingredients, manufacture of leaf simplicia, ethanol extraction of Strobilanthes crispus leaves using the maceration method, and testing the effect of ethanol extract of Strobilanthes crispus leaves on the growth of Aggregatibacter actinomycetemcomitans bacteria using the well diffusion method with three each. Repeat times, measurement of inhibition zone diameter, and MIC determination with chlorhexidin 0.2% positive control. The measurement results of the average diameter of the inhibition zone of Strobilanthes crispus extract against Aggregatibacter actinomycetecomitans bacteria at a concentration of 25%, 50%, 75% were 10 mm, respectively, and at a concentration of 100% was 16.67 mm. The mean of inhibition zone for positive control against Aggregatibacter actinomycetecomitans bacteria was 16.33 mm. Based on the inhibition zone diameter, the MIC against Aggregatibacter actinomycetecomitans was 10 mm, namely at a concentration of 25%. Strobilanthes crispus extract is effective for Aggregatibacter actinomycetecomitans bacteria.

**Keywords: Antibacterial, Agregatibacter actinomycetecomitans, Strobilanthes crispus.**

## 1. INTRODUCTION

Treatment of infectious diseases using a combination therapy of various kinds of antibiotics can also cause new problems, namely the resistance of these bacteria to antibiotics ( Negara,2014). The number of deaths caused by antibiotic resistance until 2014 is 700,000 per year. Therefore, in fact, herbal medicine is now in great demand by the public. This is because it is believed that herbal remedies are less likely to cause adverse effects (Wantenia,et.al, 2020).

Knowledge about the efficacy and safety in the use of medicinal plants in Indonesia is usually obtained only based on empirical experiences that have been passed down from generation to generation by our parents. The use of natural substances / phytochemicals / plant secondary metabolites that contain antimicrobial effects will be the basis for the discovery of new antibiotics used in the treatment of infections caused by bacteria (Nugrahani,2012).

One plant that has antibacterial properties and can be developed as an alternative antibiotic is *Strobilanthes crispus* (kejibeling). Kejibeling leaf extract had potential inhibition against bacterial growth with an average inhibition zone of the kejibeling leaf extract of 1.6 mm, positive control for amoxicillin of 2.72 mm, and distilled water of 0 mm (Rawung et, al. 2019). The high antibacterial activity of *Strobilanthes crispus* leaf extract is due to the presence of natural chemical compounds such as sodium, alkaloids, polyphenols, potassium, calcium, flavonoids, silicic acid, and saponins (Nurraihana and Norfarizan-Hanoon,2013).

*Strobilanthes crispus* (kejibeling) is usually widely used by people as a hedge plant because of its beautiful flowers. The stem has a shrub with a shrub, has a height of approximately 1-2 m, the leaves are single leaves, the leaves sit side-by-side, the leaves are lanceolate or oval, the edges of the leaves are tipped, with the tip of the leaf and the base of the leaves are pointed, the length of the leaves is approximately 9- 18cm, leaf width ranges from 3-8cm, short-stemmed leaves, pinnate leaves, and green color. This plant is also a wild herbal plant, which lives chronically and has many benefits for human health in healing several diseases (Trubus,2012). The use of natural ingredients as traditional medicine today has progressed rapidly. This is because these natural ingredients have bacteriostatic properties that can inhibit growth certain bacteria. Knowledge about the efficacy and safety of medicinal plants in Indonesia is usually only based on empirical experience which is usually passed down from generation to generation and has not been scientifically tested. For this reason, research on traditional medicine is needed, so that later the drug can be used

safely and effectively. About 80% of individuals from developing countries use traditional medicine with ingredients derived from medicinal plants. The use of plant extracts and phytochemicals that contain antimicrobial properties can be the basis for the discovery of new antibiotics in the treatment of cases of bacterial infection (Nugrahani,2012).

Infectious disease is still a type of disease that often occurs in developing countries, including Indonesia. One of the causes of infectious disease is bacteria (Radji,2011). Aggressive periodontitis is a progressive tissue disorder in healthy young adults whose cause is dominated by the bacteria *Aggregatibacter actinomycetemcomitans* (Afrina et. al, 2016). *Aggregatibacter actinomycetemcomitans* (A. *actinomycetemcomitans*) is a gram-negative bacterium in the form of cocobacil with a size of 0.4–0.5 µm x 1.0–1.5 µm, non-motile and facultative anaerobic ( Henderson,et.al,2010). *Aggregatibacter actinomycetemcomitans* which is a gram-negative cocobacil which can be found in the oral cavity and gingival infection and periodontitis (Chauhan, et.al, 2012).

Kejibeling can be used as medicine because these plants contain substances or chemicals such as silicic acid, calcium, saponins, alkaloids, potassium, polyphenols, flavonoids and calcium. Compounds such as flavonoids and alkaloids are compounds that have potential as antioxidants and inhibit the growth of cancer cells (Komariah et.al ,2011). Kejibeling (*Strobilanthes crispus*) has phenolic compounds which have antibacterial properties. Potassium and silicate content help treat hemorrhoids and dysentery. The content of vitamins C, B1, B2, and catechins makes kejibeling potential as antioxidants

The content of catechins which are compounds of the flavonoid class apart from being antioxidants also have other effects, namely as antibacterial, antiviral, oral antiseptic, anti-diarrheal, anticancer, for cardiovascular disease and anti-inflammatory (Amalia,et.al). Based on the content of catechins, which are flavonoid group compounds in the *Strobilanthes crispus* plant which function as oral antiseptics, the researchers wanted to find out whether *Strobilanthes crispus* had oral antiseptic activity caused by *Aggregatibacter actinomycetecomitans* bacteria.

## 2. METHOD

This research was conducted in February - May 2018 at the Biology Laboratory of the PGRI Madiun University, the Chemical Analyst Laboratory of SMKN 3 Madiun, and the Microbiology Laboratory of the Faculty of Veterinary Medicine, Gadja Mada University

Yogyakarta. This research is a laboratory experimental study using the well diffusion method with three replications

## 3. TOOLS AND MATERIALS

The tools used in this research are those in our laboratory which consist of: petri dishes, filter paper, measuring cups, spritus lamps, ose needles, cotton sticks, incubators, tweezers, knives, rulers, disposable syringes, autoclaves, a series of tools. distillation, hot plate, Buchner funnel, cork punch, Erlenmeyer, technical balance, mixer (stirrer), blender, label paper, stationery.

The materials used in this study were fresh kejibeling leaves (*Strobilanthes crispus*), 96% ethanol solution, 10% DMSO, 0.2% Chlorhexidin, *Aggregatibacter actinomycetecomitans* bacteria, and Mueller Hinton Agar (MHA) media obtained from the Laboratory of Microbiology, Faculty of Veterinary Medicine, University. Gadjah Mada Yogyakarta.

## 4. WORK PROCEDURES

### *4.1. Sampling*

The leaves of the kejibeling (Strobilanthes crispus) used in this study were old, fresh, and free pests which were taken directly from the house yard in Sidorejo village, Wungu district, Madiun district.

### *4.2. Sample Preparation*

The selected kejibeling leaves (Strobilanthes crispus) were weighed as much as 1,500 g, washed, chopped or cut into small pieces and dried for about 14 days until completely dry with a weight of 1,083.38 grams. The dried leaves are mashed in a blender to form 1,051.85 grams of simplicia.

### *4.3. Making Extract By Maceration*

The manufacture of kejibeling leaf extract (*Strobilanthes crispus*) by maceration using 96% ethanol. 1.051.85gr Strobilanthes crispus simplicia powder was put into a maceration container, then 2.7 liters of 96% ethanol were added, soaked for 24 hours, stirring occasionally, then filtered. The maserate was separated and the process was repeated 3 x 24 hours with the amount of solvent 2.5 liters on the second day and 1.8 liters on the third day.

All macerate was collected and evaporated using a distillation device at 35ºC until a thick extract was obtained. The thick extract of *Strobilanthes crispus* leaves obtained was 39.90 gr.

### *4.4. Preparation of Sample Test Solutions*

a. Making the concentration of Strobilanthes crispus extract 75% is by dissolving 0.75 g of Strobilanthes crispus extract plus 10% to 1 ml of DMSO and shaking until homogeneous.
b. Making the concentration of Strobilanthes crispus extract 50% is by dissolving 0.50 grams of Strobilanthes crispus extract plus 10% DMSO to 1 ml and shaking until homogeneous.
c. The concentration of 25% Strobilanthes crispus leaf extract is made by dissolving 0.25 grams of Strobilanthes crispus extract added with 10% to 1 ml DMSO and shaking until homogeneous.
d. Positive control used chlorhexidin 0.2% as much as 50 µg.
e. The test solution for the ethanol extract of Strobilanthes crispus leaves and 0.2% chlorhexidin as a positive control is ready to be dropped on the well.

### *4.5. Antibacterial Activity Test*

This test is carried out by diffusion, namely to find out how large the zone of inhibition is formed around the well. In each petri dish containing Mueller Hinton Agar media evenly suspended the *Aggregatibacter actinomycetemcomitans* bacteria. Hollowed out with a cork hole with a diameter of 6 mm on the surface of the MHA media. Dropped with the ethanol extract of 96% *Strobilanthes crispus* leaves with a concentration of 25%, 50%, 75%, and 100% as much as 50 µl on the well that was in a petri dish that had been suspended by the *Aggregatibacter actinomycetecomitans* bacteria. As a positive control, 50 µg chlorhexidin 0.2% was used. This antibacterial test was carried out in three repetitions. Then all treatments were put into an incubator at 37 ° C for 24-48 hours.

### *4.6. Data analysis*

The antibacterial effect is positive if the inhibition zone (clear zone) is seen around the well. The zone of inhibition is measured using a ruler in mm.

The drag zone measurement formula is:

$X = (Z1 + Z2 + Z3 \ldots + ZN) / n$

Information :
X = average resistance (mm)
Z = diameter of the zone of inhibition (mm)
n = number of treatments

The research data were analyzed by visual observation and measurement of the average diameter of the bacterial inhibition zone around the well that had been dripped with the test solution against *Aggregatibacter actinomycetecomitans* bacteria.

### *4.7. Minimum Inhibitory Level Test (KHM)*

This test is carried out by diffusion to determine the lowest concentration of *Strobilanthes crispus* (kejibeling) leaf extract which is able to inhibit the growth of the *Aggreagatibacter actinomycetecomitans* bacteria. The minimum inhibitory level can be determined by measuring the smallest diameter of the clear zone (inhibition) formed around the well that has been dripped with the test solution against the bacteria *Aggregatibacter actinomycetecomitans*. MIC measurements were carried out in conjunction with the antibacterial activity test.

## 5. RESULT

In this antibacterial effectiveness test study, 96% ethanol solvent was used for the extraction of natural ingredients from *Strobilanthes crispus*. Ethanol solvent is a solvent that can dissolve all active ingredients contained in a natural material, both polar, semipolar and non-polar active ingredients. The extraction process is carried out by the remaceration method, namely the method of filtering the powder soaked in a solvent until it absorbs and softens the cells, so that the soluble substances will dissolve. Soaking was carried out for 3 days with daily solvent replacement.

In this study, it was found that the leaf extract of Keji Beling (*Strobilanthes crispus*) had activity in inhibiting the growth of *Aggregatibacter actinomycetecomitans* bacteria. . *Strobilanthes crispus* plants contain chemical substances including: potassium, sodium, calcium, silicic acid, alkaline, saponins, flavonoids, and polyphenols. According to the results of research (Mohamad,et.al, 2015) states that compounds such as flavonoids and alkaloids are proven to be compounds that have potential as antioxidants and also inhibit the growth of cancer cells. The content of catechins in the kejibeling plant, which is a compound in the flavonoid class apart from being an antioxidant, also has other effects, namely as an

antibacterial, antiviral, and oral antiseptic (Amalia, et.al ,2015). The results of measuring the diameter of the inhibition zone *Strobilanthes crispus* against the *Aggregatibacter actinomycetecomitans* bacteria can be seen in Table 1.

**Table 1. Measurement results of the inhibition zone *Strobilanthes crispus* against the growth of *Aggregatibacter actinomycetecomitans* bacteria.**

| No. | Concentration (%) | Mean diameter of inhibition zone for bacterial growth (mm) *Aggregatibacter actinomycetecomitans* |
|---|---|---|
| **1.** | 25 | 10 |
| **2.** | 50 | 10 |
| **3.** | 75 | 10 |
| **4.** | 100 | 16,67 |
| **5.** | Positive control | 16,33 |

MIC test was carried out by well diffusion, where the measurement was carried out in conjunction with the antibacterial effectiveness test. KHM test is intended to determine the lowest concentration of leaf extract of vicious beling (Strobilanthes crispus) which is able to inhibit the growth of the Aggregatibacter actinomycetecomitans bacteria can be seen in Table 1. Based on Table 1., it can be seen that the Minimum Inhibitory Concentration of Strobilanthes crispus leaf extract with the Aggregatibacter actinomycetecomitans test bacteria is found at a concentration of 25% with an average inhibition zone of 10 mm each.

## 6. DISCUSSION

Table 1 shows that the concentration of the test extract of 25% with three repetitions has shown a clear zone with an average diameter of 10 mm around the good wells in the Aggregatibacter actinomycetecomitans bacteria. This shows that at a concentration of 25% the Strobilanthes crispus extract was able to inhibit the growth of the Aggregatibacter actinomycetecomitans bacteria. At 50% and 75% test extract concentrations from three repetitions of Srtobilanthes crispus extract also showed an inhibition zone of 10 mm on the growth of Aggregatibacter actinomycetecomitans bacteria. The amount of clear zone formed at a concentration of 100% for Aggregatibacter actinomycetecomitans was 16.67 mm.

The zone of inhibition is indicated by the clear zone around the well. The clear zone begins to form at a concentration of 25%, which means that at this concentration it has shown an inhibitory response of Strobilanthes crispus extract to the growth of Aggregatibacter actinomycetecomitans bacteria. This inhibitory response is due to the fact that at this concentration the active substance that acts as an antibacterial is able to inhibit bacterial growth. The zone of inhibition (clear area) around the age (well) shows the inhibition of Strobilanthes crispus extract against the growth of Aggregatibacter actinomycetecomitans bacteria. The inhibition of Strobilanthes crispus against the growth of Aggregatibacter actinomycetecomitans bacteria colonies is due to the damage that occurs in the structural components of the bacterial cell membrane. The damage to the membrane structure of the bacterial cell will be able to disrupt the continuity of the nutrient transport process, so that in the end the bacterial cell will experience a lack of nutrients that are needed in the process of growth.

In addition, the flavonoid compounds contained in Strobianthes crispus have antibacterial activity through three mechanisms, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism. Flavonoids inhibit the function of the bacterial cell membrane through complex bonds with extracellular proteins that are soluble so that they can interfere with the integrity of the bacterial cell membrane (**Jannah, 2017**). In addition, the inhibition of bacterial energy metabolism by flavonoids is carried out by inhibiting the process of bacterial respiration so that the energy being inhibited will have an effect on the activity of metabolite absorption and bacterial macromolecular biosynthesis (**Rahman and, Haniastuti,2017**).

The criteria for the strength of the antibacterial power are as follows: the inhibition zone diameter of less than 5 mm is categorized as weak, the inhibition zone diameter of 5-10 mm is categorized as moderate, the inhibition zone diameter of 10-20 mm is categorized as strong and the diameter of the inhibition zone more than 20 mm is categorized as very strong ( 12). Based on the diameter of the inhibition zone, Strobilanthes crispus extract at a concentration of 25%, 50%, and 75% was categorized as moderate. At 100% concentration 16.67 mm for Aggregatibacter actinomycetecomitans was interpreted as strong. This is because at high concentrations, active substances that act as antibacterials such as alkaloids, flavonoids, tannins, catechins, and saponins are increasing in number, so that their ability to inhibit bacterial growth is also getting bigger, which is marked by the formation of a wider clear zone around well

Chlorhexidine was chosen as a positive control because it is an antiseptic and disinfectant that has bactericidal and bacteriostatic activity against gram-positive and gram-negative bacteria. In the positive control the inhibition zone formed was smaller than the inhibition zone formed in the Strobilanthes crispus extract test at a concentration of 100% for Aggregatibacter actinomycetecomitans was 16.33 mm. This shows that Strobilanthes crispus extract can be used as an oral antiseptic to replace chlorhexidine.

This is possibly due, among other things, when the remaseration process has been maximized so that many natural ingredients (secondary metabolites) in Strobilanthes crispus have been dissolved in ethanol and the diffusion rate of the active ingredients in the MHA medium is adequate, so that at a concentration of 25% it is able to inhibit bacterial growth. . This is in accordance with the opinion (**Salni, 2013**) which states that antibacterials are said to have high activity against bacteria, if the minimum concentration value is low but has a large inhibitory power. The difference in the size of the inhibition area for each concentration can be caused, among other things, the difference in the concentration or size of the antibacterial active substance contained in the extract, the diffusion rate of the antibacterial material into the medium, the sensitivity of bacterial / fungal growth, the reaction between the active ingredient and the Incubation medium and temperature, environmental pH, media components, incubation time, and metabolic activity of microorganisms.

The ethanol extract of Strobilanthes crispus leaves has antibacterial activity against Aggregatibacter actinomycetecomitans bacteria. The minimum inhibitory concentration of the ethanol extract of Strobilanthes crispus leaves against Aggregatibacter actinomycetecomitans is 25%

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