Effect of Piper Betle Linn Extract on the Growth of Staphylococcus Aureus ATCC 25923

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Abstract

Piper betle Linn or betel is a plant that is known to have antiseptic properties. The purpose of this study was to determine the effect of betel leaf extract (Piper betle Linn.) on Staphylococcus aureus ATCC 25923. The method used was an experimental method with a completely randomized design experimental design (CRD) using 2 methods, namely reflux and maceration methods, respectively. Each concentration of betel leaf extract (Piper betle L) which consists of one factor, namely the concentration of betel leaf extract (Piper betle L) with a variable factor level of betel leaf extract preparations of six treatments, positive, negative control and treatment 1%, 2%, 4% and 8%. By using betel leaf extract, 70% Methanol, distilled water and antibiotics. The results of the study the value of 1.650 > 0.

I. Introduction

In 2020, countries in parts of the world are hit by the Covid-19 virus, which is spreading so rapidly. This has a negative impact on human life, because the Covid-19 virus can cause death for humans if they are infected with the virus. That is why the Indonesian government provides directions for the community to live clean, so they can avoid the corona virus. Our first hygiene is recommended to always wash our hands so that it is the first countermeasure for infection with the Covid-19 virus. However, there are still many people who are lazy to do it. From this, ”Ferzt” comes with a hand sanitizer solution in the form of a hand sanitizer in the form of a gel containing active ingredients from betel leaf extract which acts as an antibacterial agent that effectively kills pathogenic bacteria.

Piper betle Linn or betel is a plant that is known to have antiseptic properties. Traditional usage is usually by boiling the betel leaf then boiled water is used to rinse or clean other parts of the body, or crushed betel leaf and then placed on the wound (Mardisiswojo & Rajak, 1985). It is known that the content of betel leaf is an essential oil that contains hydroxycyclic compounds, kavikol, cavibetol, estradiol, eugenol, metal-eugenol, carvacrol, terpeneba, sesquiterpene, phenyl propan, and tannins (Moeljanto, 2003). Phenolic derivatives, namely eugenol and kavikol, have antiseptic properties and especially kavikol are known to have five times the power to kill bacteria than phenol (Sastrohamidjojo, 2004). The development of an antiseptic gel preparation formula for betel leaf extract that has been carried out has not been known about the effectiveness of the antiseptic preparation of the preparation (Sari, et al., 2012). Betel leaf (Piper betle L.) provides inhibition against Staphylococcus aureus where the higher the concentration given, the greater the inhibitory power is formed (Rahmawati, 2014).
Betel leaf (Piper betle L.) is a type of medicinal plant that is often used for a long time to maintain health, prevention and treatment of various diseases. (Anonymous, 2002). Betel leaf contains various chemical compounds which have medicinal properties, namely essential oils consisting of phenols and their derivative compounds such as kavikol. The essential oil content of betel leaf has a function to cure sore throat (pharyngitis) caused by bacteria (Dakunil, 2007). Triono in Maimunah (2019) stated that betel leaf extract is able suppress the intensity of anthracnose. Betel without fractionation and betel fractionation with water solvents is comparable to the ability of probineb fungicides to suppress anthracnose disease intensity.

The phenol and kavikol content of betel leaf can cure sore throat (pharyngitis), sore throat accompanied by fever and cough are symptoms of pharyngitis. Pharyngitis is an infection of the throat caused by viruses or bacteria (Heyne, 1987). Bacteria found in the throat and cause pharyngitis include pathogenic Staphylococcus aureus (causing infection) 5. Pharyngitis can usually be treated using antibiotics (Gupte, 1990).

The antiseptic power of an antiseptic preparation is influenced by, among others: the levels of the active ingredients and ingredients contained in the preparation formula. Cleaning hands with antiseptic agents has been known since the early 19th century. The development of modern society requires humans to move fast and use time as efficiently as possible. Green betel extract fractions (Piper betle L.) have strong inhibitory activity against food pathogenic bacteria (Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cerius and Listeria monocytogenes) where the GC-MS analysis results kavikol, dosecanoic acid, myristic, palmitic and oleic acid (Suliantari et al, 2012). Meanwhile, the research results of Poeloengan et al. 2005 gave results that in vivo betel leaf extract was able to reduce the number of germs in cow’s milk with subclinical mastitis. Jayalakshmi’s (2015) research informs that methanol and chloroform extracts, apart from having significant antibacterial activity, also have antioxidant activity.

The demands of this age require humans to maintain their health in order to avoid diseases that can hinder movement and reduce time efficiency (Wahyono, 2010). Regarding behavior, factors that can affect the health status of the community are inseparable from the culture and habits of the family and the daily environment of the community (Notoatmojo in Fahmi, 2019). Poor environmental sanitation, personal hygiene and bad behavior such as bowel habits, population density, poor use of water in washing cooking utensils and cutlery or use of water for drinking, not using footwear when outside activities a house that is directly in contact with the ground, the habit of children playing outside the house signs using footwear is very supportive of the breeding of parasites in Indonesia (Natadisastra in Aswita (2020)).

The use of hand antiseptics in the form of gel preparations in the middle to upper class society has become a lifestyle. Several hand sanitizer preparations can be found on the market and usually contain lots of alcohol. How to use it by dropping it on the palm of the hand, then smoothing it on the surface of the hand (Retnosari, 2006). Hand sanitizer is a gel preparation with various ingredients that quickly kill organisms in the skin of the hands (Benjamin, 2010). According to the FDA, hand sanitizers can kill germs relatively quickly.

Based on the explanation regarding betel leaf extract for the manufacture of hand sanitizers, this research will first carry out extract testing in the USU laboratory. The research activity aims to determine the effect of the betel leaf extract (piper betle linn.) As a hand sanitizer. We know that during the corona-19 pandemic the government recommended that we always use hand sanitizers to be useful to prevent the spread of the spread of corona-19. This is the basis for the research team to conduct research activities.
II. Research Methods

This research is a laboratory experiment conducted in the microbiology laboratory of the Biology Study Program and the Chemistry Study Program at USU Medan. Testing of betel leaf ethanol extract as an antiseptic was carried out by the phenol coefficient method using S. aureus ATCC 25923.

This study will use a completely randomized design (CRD) with five treatments: negative control (distilled water), positive control (erythromycin as an antibiotic), the test solution was betel leaf ethanol extract with a concentration of 70%, betel leaf methanol extract, and betel leaf methanol extract. Each treatment was repeated 3 times.

Tools and Materials

The ingredients in the process of activities in this study were Nutrient Agar (E. Merck) betel leaf extract, distilled water, 70% alcohol, and antibiotics. While the tools in the study were petri dishes, mortar and stampers, beaker glass, water bath, gram and milligram scales, measuring cups, glass stirrers, watch glasses, glass objects, drop pipettes, pH meters. The process of making the extract can be explained in Figure 1 below.

III. Result and Discussion

This research was conducted using six betel leaf extract dosage variables with positive, negative control treatments and 1%, 2%, 4% and 8% treatments. By using betel leaf extract, 70% Methanol, distilled water and antibiotics.

![Figure 1. Extract Making Process](image-url)
**Figure 2.** Diameter of betel leaf extract (Piper betle L.) inhibition zone at concentrations of A: 1%, B: 2%, and C: 4% and D: 8%

Information:
- a. Obstacles zone
- b. Bacterial colonies
- c. Discs given betel leaf extract

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Deuteronomy</th>
<th>Amount</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>K (+)</td>
<td>26.2</td>
<td>26.5</td>
<td>26.8</td>
</tr>
<tr>
<td>K (-)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P1</td>
<td>6.1</td>
<td>6.1</td>
<td>6.8</td>
</tr>
<tr>
<td>P2</td>
<td>7.2</td>
<td>6.6</td>
<td>7.5</td>
</tr>
<tr>
<td>P3</td>
<td>8</td>
<td>8.6</td>
<td>10.2</td>
</tr>
<tr>
<td>P4</td>
<td>12.5</td>
<td>14.7</td>
<td>16.9</td>
</tr>
</tbody>
</table>

**Table 1.** Extract Treatment Process

Based on the explanation in the table above, the process of research activities regarding the treatment of betel leaf extract will be carried out in the Microbiology Study Program of Biology and Chemistry Study Program at USU Medan.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Clear Zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K (+)</td>
<td>26.5</td>
</tr>
<tr>
<td>K (-)</td>
<td>0</td>
</tr>
<tr>
<td>P1 (1%)</td>
<td>6.33</td>
</tr>
<tr>
<td>P2 (2%)</td>
<td>7.1</td>
</tr>
<tr>
<td>P3 (4%)</td>
<td>8.93</td>
</tr>
<tr>
<td>P4 (8%)</td>
<td>14.7</td>
</tr>
</tbody>
</table>

**Table 2.** Clear Zone Treatment

The results of the clear zone average consisting of six treatments can be specified, namely positive control with a value of 26.5%, negative control 0%, treatment 1 with a value of 6.33%, treatment 2 with a value of 7.1%, treatment 3 with a value value 4% and treatment 4 with a value of 14.7.
Figure 3. Average Clear Zone

Based on Figure 1 shows the research results obtained can be treated betel leaf extract has an inhibition zone against growth bacteria *Staphylococcus aureus* that is, the zone of inhibition is the highest shown at a concentration of 8% at 14.7 mm and the lowest zone at a concentration of 4% betel leaf extract is 8.93 mm, while the one that does not show an inhibition zone is at a concentration of 0%.

The process of research results obtained in research by conducting experimental methods, with data analysis with the ANOVA formula, the results of this study are shown in the table below.

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>1764.476</td>
<td>5</td>
<td>352,895</td>
<td>1.650</td>
<td>.221</td>
</tr>
<tr>
<td>Within Groups</td>
<td>2567.273</td>
<td>12</td>
<td>213,939</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4331.749</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This is consistent with the statement of Jawetz et al (2001), that an antimicrobial substance becomes effective when it is influenced by the concentration of the substance. The higher the concentration of betel leaf extract also causes the active ingredient content that functions as an antimicrobial ingredient to increase, so that its ability to inhibit microbial growth is also greater.

Betel leaf extract contains alkaloid compounds, flavonoids, steroids and saponins with standard levels. These compounds are thought to work together to strengthen each other, among these compounds steroids are the more common compounds, while alkaloid compounds in only one reagent are stated to be positive in the extract. A small amount of alkaloid compounds also acts as an antimicrobial through its mechanism of action which is related to the ability to interact with bacterial DNA (Harborne, 1987).

Betel leaf has an antibacterial effect against *Staphylococcus aureus* (Mahendra, 2005) and as an eye cleanser, removes body odor, nosebleeds, mouth sores, bleeding gums, coughs, bronchitis, and vaginal discharge. Betel leaf boiled water can get rid of bad breath by gargling and smoothing the facial skin (Muhlisah, 2010). In addition, the interaction of aloe vera leaf extract (*Aloe vera* L.) and betel leaf (*Piper betle* L.) provides inhibition against
Staphylococcus aureus where the higher the concentration given the greater the inhibition formed (Rahmawati, 2014).

Meanwhile, the results of research by Poeloengan et al., 2005 showed that in vivo betel leaf extract was able to reduce the number of germs in cow's milk with subclinical mastitis. Jayalakshmi's (2015) research informs that methanol and chloroform extracts, apart from having significant antibacterial activity, also have antioxidant activity. The results of phytochemical analysis contain phenolic compounds, tannins, flavonoids, glycosants and proteins. Streptococcus mutans and Staphylococcus aureus are normal flora in the oral cavity. However, both can turn into pathogens if the oral cavity is not maintained. Dental caries is a disease that arises from poor oral hygiene. The ethanol extract of betel leaf (Piper betle L.) provides the largest inhibition zone for Streptococcus mutans, namely 24.5 mm at a concentration of 100% while for Staphylococcus aureus the inhibition zone is 27.5 mm at a concentration of 100% (Saanin and Lidya, 2014). The provision of essential oil from betel leaf (Piper betle L.) with different concentrations affects the inhibition zone of Staphylococcus aureus Rosebanch bacteria at a concentration of 3% with a diameter of 2.1 mm (Simanjuntak & Risita, 2014).

IV. Conclusion

Based on the results contained in table 3. By using the anava formula using the SPSS application Fcount > Ftable with a value of 1.650 > 0.221 with a significant level of 5%, it can be stated that the result is that Fcount is greater than Ftable there is an effect of betel leaf extract on the growth of Staphylococcus aureus ATCC 25923.

References

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