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Potential Anti-Bacterial Extract of Red Belt (Piper Crocatum Ruiz & Pav.) Against Staphylococcus Epidermidis

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Abstract
Introduction: *Red betel leaf is a plant native to Indonesia which is used as an antiseptic, anti-cancer, and infection treatment. The ethanol content of red betel leaf extract is known to have antibacterial activity. Among them there are alkaloids, flavonoids, essential oils, tannins, and saponins.*
Objective*: to determine the potential of red betel leaf extract (Piper crocatum Ruiz & Pav.) in inhibiting the growth of Staphylococcus epidermidis bacteria.*
Methods*: Staphylococcus epidermidis bacteria were divided into 5 groups, PEG 5% as negative control, clindamycin 30µg/disk as positive control, extract 20%, 40%, 80% as treatment group. Measuring the zone of inhibition using a ruler.*
Result*: Staphylococcus epidermidis inhibition zone was formed at concentrations of 20%, 40%, 80%. Kruskal Wallis analysis showed significant results of p = 0.000 for bacteria.*
Conclusion*: Red betel leaf extract (Piper crocatum Ruiz & Pav.) has significant antibacterial activity against Staphylococcus epidermidis. The higher the concentration, the larger the diameter of the inhibition zone.*

Keywords: Betel Leaf Extract; Bacteria; Staphylococcus Epidermidis;

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Introduction

The ethanol content of red betel leaf extract is known to have antibacterial activity (Cahyono & Indrayudha, 2013). This is due to the presence of alkaloids, flavonoids, essential oils, tannins, and saponins in red betel leaf extract (Piper crocatum Ruiz & Pav.) which act as antibacterial (Candrasari et al., 2011). The activity of these compounds can damage the cell membrane or cell wall of bacteria. These compounds are mostly contained in the red leaves, but under certain conditions red betel leaves can change color (Afiff & Amilah, 2017). Leaves that are initially red at the bottom will change color to green and the silvery pattern will disappear (Rachmawaty et al., 2018)

Staphylococcus epidermidis is a normal flora on the skin and is not pathogenic, but if there is a change in skin condition, the bacteria can become invasive (Lutpiatina, 2017). Diseases that are often caused by these bacteria include acne (Acne vulgaris), bullous impetigo, furunculosis, folliculitis and urinary tract infections (UTI) (Wirawan, n.d.). The results of research by (Wardania et al., 2020) stated that the test of antibacterial activity that causes acne from ashitaba leaf extract with the well method using Mueller Hinton Agar (MHA) media showed an inhibitory effect on the development of Staphylococcus epidermidis bacteria due to the activity of flavonoid compounds in inhibiting metabolism. energy in bacteria.

Based on the description above, Staphylococcus is a normal flora on human skin, but if there is a change in the condition of the skin, the bacteria will be invasive and become the main cause of infection on human skin, especially in medical personnel in hospitals (Brown & Horswill, 2020). In another study, it was also stated that Staphylococcus epidermidis bacteria were resistant to several antibiotics (Negara, 2016). So, the authors are interested in conducting research to test the antibacterial effectiveness of red betel leaf extract against Staphylococcus epidermidis.

Method

This study is an experimental study using the posttest only control group design method to test the concentration of red betel leaf extract (Piper crocatum Ruiz & Pav.) on the growth of Staphylococcus epidermidis bacteria (Septiani, 2017). Determination of bacterial activity using the disk diffusion method with Mueller Hinton Agar media based on the inhibition zone generated around the disk.

The experimental group of red betel leaf extract with concentrations of 20%, 40%, 80%, 5% PEG group as negative control, and clindamycin 30µg/disk group as positive control.

Research Result

1. Determination Plant

Determination of red betel leaf (Piper crocatum Ruiz & Pav.) was carried out at the Biology Laboratory, Faculty of Teacher Training and Education, University of Muhammadiyah Surakarta, the results were Piper betle L. var. rubrum with the synonym Piper crocatum Ruiz & Pav.

2. Growth of Staphylococcus epidermidis bacteria.

This research was conducted at the Pharmacology Laboratory, and the Microbiology Laboratory, Faculty of Medicine, University of Muhammadiyah Surakarta from July to September 2021. There were 5 treatment groups, namely:

a) Red betel leaf extract 20% concentration
b) Red betel leaf extract concentration 40%
c) Red betel leaf extract 80% concentration
d) PEG 5% as negative control
e) Clindamycin 30 micrograms/disk as positive control

Table 1
Table of Results of Measurement of Staphylococcus epidermidis Inhibitory Zone

Replikasi *Staphylococcus epidermidis*	20%	40%	80%	*Clindamycin K+*	PEG 5% K-
1	9	10	10	25	6
2	7	9	12	24	6
3	8	10	11	24	6
4	7	10	12	23	6
5	8	10	10	25	6
Rerata	7,8	9,8	11	24,2	6

Results of normality test

The sample used in this study was less than 50 samples so that the normality test used was Shapiro-wilk. The results of the normality test for the diameter of the Staphylococcus epidermidis inhibition zone are shown in the table below.

Table 2
Normality Test Results for Staphylococcus epidermidis

Group	Value *p*	Interpretation
20%	0.314	Normal Distribution
40%	0.000	Abnormal Distribution
80%	0.119	Normal Distribution
5% K-	-	-
Clindamycin K+	0.314	Normal Distribution

1. Homogeneity test results

In this study using the Levene test homogeneity test can be displayed from the independent sample t-test if there are two research groups or from One-way Anova if there are more than two research groups. In this study, five research groups were used so that the Levene test was chosen from the One-way Anova. Based on the results of the homogeneity test of the diameter of the inhibition zone of Staphylococcus epidermidis bacteria, p value = 0.016, indicating that the p value <0.05, which means that the diameter of the inhibition zone has inhomogeneous data variants. The results of the homogeneity test were used to determine the post-hoc analysis method to be used.

2. The results of the analysis using the Kruskall-Walli's method.

The results of the normality test of the data showed that in this study the data were not normally distributed, so the statistical test used was the Kruskall-Walli's test. This

test was chosen because of the numerical scale variables, more than two treatment groups, not normally distributed, unpaired groups. The results of the Kruskal-Walli's inhibition zone test are shown in the table below.

The results of the Kruskal Wallis test showed that the p value was 0.000 <0.05. This means that there is a significant difference between the average diameter of the Staphylococcus epidermidis inhibition zone in each treatment group. The existence of differences in each group indicates the need for post-hoc testing.

3. Post-Hoc

Testing the results of the homogeneity test on Staphylococcus epidermidis bacteria did not show a p value <0.05, which means the data was not homogeneous. Therefore, the post-hoc test used was the Mann Whitney test. The post-hoc test results are shown in the table below.

Table 4
Post-Hoc Test Results for Staphylococcus epidermidis

Group	Mean Inhibitory Zone Diameter (mm)	Value *P*	Interpretation
20% vs 40%	2	0.009	Significant
20% vs 80%	3.2	0.008	Significant
20% vs K+	16.4	0.008	Significant
20% vs K-	1.8	0.005	Significant
40% vs 80%	1.2	0.045	Significant
40% vs K+	14.4	0.007	Significant
40% vs K-	3.8	0.004	Significant
80% vs K+	13.2	0.008	Significant
80% vs K-	5	0.005	Significant

Discussion

The results of this study showed that red betel leaf extract (Piper crocatum Ruiz & Pav.) at concentrations of 20%, 40%, and 80% was more effective in inhibiting the growth of Staphylococcus epidermidis bacteria compared to negative control using 5% PEG. However, the three concentrations in forming the inhibition zone were not as good as the positive control, namely clindamycin.

Red betel leaf extract (Piper crocatum Ruiz & Pav.) is able to inhibit the growth of Staphylococcus epidermidis bacteria because it contains flavonoids, tannins, alkaloids, and essential oils. Flavonoid compounds as antibacterial act by forming complex compounds against extracellular proteins that damage bacterial cell membranes. Alkaloids are able to reduce the integrity of the peptidoglycan constituent particles of bacterial cells. Tannins can destroy bacterial cell membranes. Essential oils can interfere with the formation of bacterial cell membranes and walls and can prevent them from

forming completely. Tannins have an effect on the toxic mechanism of tannins that can interfere with the permeability of bacterial cell membranes.

The results of the inhibition zone measurements showed that the higher the concentration of red betel leaf extract (Piper crocatum Ruiz & Pav.) the greater the inhibition zone formed and the more antibacterial content. This is because the amount of concentration affects the growth of bacteria. According to Afifi et al., (2018), the higher the concentration, the greater the bacterial inhibition process. The higher the concentration, the more concentrated the solution used and the more antibacterial potential it contains.

The results of the post hoc analysis using the Mann-Whitney method showed that each concentration group had a significant value ($p < 0.05$). The research hypothesis was accepted that red betel leaf extract (Piper crocatum Ruiz & Pav.) had potential as an antibacterial against Staphylococcus epidermidis.

Conclusion

Red betel leaf extract (Piper crocatum Ruiz & Pav.) in this study was effective in suppressing the growth of Staphylococcus epidermidis bacteria. Concentrations of 20%, 40%, and 80% in red betel leaf extract (Piper crocatum Ruiz & Pav.) have antibacterial effectiveness against the growth of Staphylococcus epidermidis, and the higher the concentration, the higher the effectiveness value.

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