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Inhibitory Effect of Insulin Leaf (*Smallanthus sonchifolius*) Extract on the Growth of *Staphylococcus aureus* in Vitro

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ABSTRACT

Staphylococcus aureus infection is a serious health problem, especially amid increasing resistance to antibiotic use. One alternative treatment is the use of natural ingredients such as insulin leaves (*Smallanthus sonchifolius*), which are known to contain active compounds with potential antibacterial properties. This study aims to determine the ability of insulin leaf extract (*Smallanthus sonchifolius*) to inhibit the growth of *Staphylococcus aureus in vitro*, as well as to determine the optimal concentration for maximum inhibitory activity. This study is an experimental laboratory study using a posttest control group design with the well diffusion method on MHA medium at extract concentrations of 25%, 50%, and 75%. The negative control used DMSO, and the positive control used *clindamycin*. The results showed that insulin leaf extract (*Smallanthus sonchifolius*) was able to inhibit the growth of *Staphylococcus aureus*, with the inhibition zone diameter increasing with increasing concentration, where the 75% concentration produced the greatest inhibitory effect. ANOVA analysis showed significant differences between treatments ($p < 0.05$). The insulin leaf extract (*Smallanthus sonchifolius*) was able to inhibit the growth of *Staphylococcus aureus In Vitro*, with the greatest inhibitory effect at a 75% extract concentration.

Keywords: antibacterial, inhibition zone, insulin leaves, smallanthus sonchifolius and staphylococcus aureus

BACKGROUND

Bacterial infectious diseases are a serious health problem worldwide. Infection occurs when pathogenic microorganisms enter the body and cause disease. One type of microorganism that can cause infection is Staphylococcus aureus, a widespread infectious pathogen and a global health threat. Staphylococcus aureus is commonly found on human skin and mucous membranes. The World Health Organization (WHO) recently classified it as a high-grade pathogen II (Putri et al., 2022).

Staphylococcus aureus is an opportunistic pathogenic bacteria that can cause various diseases in humans and animals. Some types of infections caused by Staphylococcus aureus include skin infections such as boils, impetigo, furuncles, and wound infections. Staphylococcus aureus has typical symptoms such as inflammation, necrosis, and abscess formation. Infections can appear as small pimples on the skin or develop into severe, life-threatening infections (Handayani et al., 2020).

Antibiotics are the primary method of treating infectious diseases. Antibiotics can be produced naturally by microorganisms or through chemical synthesis. The therapeutic effect of an antibiotic by inhibiting the growth of microorganisms is called bacteriostatic, while one that kills microorganisms is called bactericidal (Dewi, 2019).

Lack of public knowledge about inappropriate (irrational) antibiotic use, such as using it too short, using it too low a dose, making an incorrect initial diagnosis, and using antibiotics without a prescription, can contribute to problems such as antibiotic resistance. Bacterial resistance to antibiotics makes treatment less effective. This is caused by the lack of antibiotics available to the bacteria, which leads to the bacteria becoming increasingly resistant. This results in increased morbidity and mortality, as well as increased healthcare costs (Sukertiasih et al., 2021).

In ß-lactam antibiotics, ß-lactamase enzymes can destroy the amide bond, rendering the drug ineffective. Meanwhile, resistance rates were 22.2% for chloramphenicol, 44.4% for amoxicillin, and 11.1% for ciprofloxacin. Staphylococcus aureus also showed resistance to clindamycin, with inhibition zones of 6.5 mm and 12.1 mm, respectively, which are considered resistant by the CLSI (Septiana et al., 2024).

Acne treatment in dermatology clinics typically uses antibiotics that inhibit inflammation and kill bacteria, such as tetracycline, erythromycin, doxycycline, and clindamycin. However, these drugs have side effects, including irritation, when used as anti-acne agents. Long-term antibiotic use can lead to resistance, organ damage, and immunohypersensitivity (Wardania et al., 2020). Synthetic chemicals can actually have negative health effects. One option is to use active antibacterial ingredients found in medicinal plants (Priamsari et al., 2020).

The use of natural ingredients is considered to have fewer side effects than chemical-derived drugs, in addition to being more affordable. Many plants contain secondary metabolites such as phenolics, flavonoids, and other beneficial compounds. These compounds have pharmacological effects that can be useful as antidiabetic and antibacterial drugs. One such plant is the insulin leaf plant (Smallanthus sonchifolius), which is known to have various health benefits (Saputri et al., 2023). This study aims to determine the ability of insulin leaf extract to inhibit the growth of Staphylococcus aureus in vitro.

METHODS

This research is an experimental laboratory study with a post-test control group design. This design was used to determine the inhibition zones formed around the wells in the growing medium after Staphylococcus aureus in the growing medium was treated with insulin leaf extract at various concentrations of 75%, 50%, and 25%, as well as an untreated control group. The test was replicated five times using the Federer formula to produce reliable and consistent data, ensuring that the results obtained were not due to chance factors but rather to the influence of the treatment. Data were collected and presented in tabular form, averaged, and categorized as inhibition zones for information that will be subsequently reported as research results. The tabulated data were then analyzed using SPSS software. Determination tests, extraction processes, and phytochemical screening will be conducted at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, and the Phytochemistry Laboratory, Faculty of Pharmacy, Padjadjaran University. Antibacterial inhibition tests will be conducted at the Bacteriology Laboratory, Institut Kesehatan Rajawali. The research will be conducted in December 2024.

RESULTS

The inhibitory activity of insulin leaf extract (Smallanthus sonchifolius) on the growth of Staphylococcus aureus was tested using the well diffusion method with varying extract concentrations of 25%, 50%, and 75%. Clindamycin was used as the K (+), and 1% DMSO was used as the K (-). The results of the inhibition zone diameter measurements for each treatment are shown in Table 1.

Table 1. Inhibitory Activity Test of Insulin Leaf Extract on the Growth of Staphylococcus aureus

Treatment	Inhibition Zone Diameter (mm)					Mean	Inhibition Category
	1	2	3	4	5		
K (-)	0	0	0	0	0	0	None
K (+)	29,5	29,5	29,7	29,5	29,7	29,58	Very strong
25%	20.4	20,5	20,3	20.5	20,4	20,42	Strong
50%	22,7	22,5	22,6	22,6	22,7	22,62	Very strong
75%	24,9	25,0	24,8	24,9	24,9	24,9	Very strong

Description:
K (-): DMSO 1%
K (+): Clindamycin 0.03%

Table 2. Normality Test Results

Concentration	Kolmogrof-Smirnov			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
25%	.231	5	.200*	.881	5	.314
50%	.241	5	.200*	.881	5	.314
75%	.241	5	.161*	.883	5	.325

In the Normality Test, because the number of samples in each concentration group was <50, the Shapiro-Wilk test was performed, which is more appropriate for small sample sizes. The results of the Shapiro-Wilk normality test, as seen in Table 2, obtained significance values (p > 0.05) for all concentration groups: 0.314 for the 25% and 50% concentrations, and 0.325 for the 75% concentration. The significance values for all three groups were normally distributed (p > 0.05).

Table3. Homogeneity Test Results

Levene statistic	Df1	Df2	Sig
.426	2	12	.663

The Levene's test for homogeneity showed a variance between groups with a significance of 0.663 (p > 0.05), as shown in Table 3. Therefore, it can be concluded that the diameter data in all three concentration groups were normally and homogeneously distributed, thus meeting the requirements for further parametric testing.

Table 4. ANOVA Test Results

	Sum of Square	df	Mean Square	f	Sig
Between groups	50.181	2	25.091	3961.684	.000
Within groups	.076	12	.006		
Total	50.257	14			

Based on the results of the ANOVA test on the diameter of the inhibition zone at various extract concentrations (25%, 50%, and 75%), a significance value (p-value) of 0.000 (p <0.05) was obtained, which can be seen in Table 4. This shows that there is a significant difference between treatment groups in the diameter of the bacterial inhibition zone. The mean square value between groups is 25.091, while the mean square within the group is only 0.006. This difference in value indicates that the variation between groups is greater than the variation within the group, so that the effect of treatment (extract concentration) on the diameter of the inhibition zone is real and not caused by random variation.

Table 5. Post Hoc Test Results

(I) Concentration	(J) Concentration	Mean Difference (I - J)	Std. Error	Sig	95% Confidence Interval	
					Lower Bound	Upper Bound
25%	50%	-2.20000*	.05033	.000	-2.3097	-2.0903
	75%	-4.48000*	.05033	.000	-4.5897	-4.3703
50%	25%	2.20000*	.05033	.000	2.0903	2.3097
	75%	-2.28000*	.05033	.000	-2.3897	-2.1703
75%	25%	4.48000*	.05033	.000	4.3703	4.5897
	50%	2.28000*	.05033	.000	2.1703	2.3897

*. The mean difference is significant at the 0.05 level

Based on the results of the Post Hoc Tests with the LSD method can be seen in Table 5, a significance value ($p < 0.05$) was obtained in all comparison pairs, namely between concentrations of 25% and 50%, 25% and 75%, and 50% and 75%. The 50% concentration showed a significantly larger inhibition zone diameter than 25% ($p = 0.000$), and the 75% concentration had a significantly larger inhibition zone diameter than 25% ($p = 0.000$) and 50% ($p = 0.000$).

DISCUSSION

In this study, insulin leaves were extracted using a maceration method in 96% ethanol to obtain a thick extract. The maceration method was chosen because it is a simple extraction technique without direct heating and is effective in extracting secondary metabolites such as flavonoids, alkaloids, quinones, steroids, triterpenoids, and other phenolic compounds effectively without degradation due to excessive heating (Suhendar et al., 2020).

Research by Putri et al. (2022) showed that insulin leaf extract contains flavonoids, alkaloids, phenolics, saponins, and tannins. In this study, phytochemical screening of the insulin leaf ethanol extract revealed the presence of alkaloids, quinones, steroids, and triterpenoids. Differences in phytochemical test results can be caused by several factors, such as the environmental conditions where the plants grow, variations in secondary metabolite levels, the type of solvent used, and the extraction method applied (Fitriani et al., 2021).

The medium used in the antibacterial inhibition test in this study was Mueller Hinton Agar (MHA) because it has a balanced nutrient composition and supports the growth of various types of bacteria. It is non-selective and non-differential, making it very suitable for general antibacterial inhibition tests, especially against pathogenic bacteria such as Staphylococcus aureus. Mueller Hinton Agar (MHA) is also a standard medium recommended by WHO and the Clinical and Laboratory Standards Institute (CLSI) in antibiotic susceptibility testing (Marliana et al., 2022). The inhibition test in this study was conducted using the well method. The well method is known to produce a wider inhibition zone than the disc method. This is due to the method of applying the test compound directly into the well, resulting in a more even and efficient diffusion of the antibacterial compound (Hayati et al., 2022).

Clindamycin was chosen as a positive control because it is known to be effective in inhibiting the growth of various types of gram-positive bacteria, including Staphylococcus aureus. Thus, clindamycin provides a benchmark for the extent to which the test extract inhibits bacterial growth (Fitriani et al., 2021).

The formation of an inhibition zone in the medium inoculated with Staphylococcus aureus indicates that insulin leaf extract (Smallanthus sonchifolius) has the ability to inhibit bacterial growth. This inhibitory effect is closely related to the active compounds in the insulin leaf extract, namely alkaloids, quinones, steroids, and triterpenoids, each of which has its own mechanism of action in inhibiting bacterial growth. These active compounds work synergistically to disrupt the bacterial defense and metabolism systems, thereby inhibiting the growth of Staphylococcus aureus. Clindamycin, a positive control, showed a larger inhibition zone compared to insulin leaf extract, a natural antibacterial. This is due to clindamycin's specific mechanism of action, which inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit, in contrast to insulin leaf extract, a natural antibacterial, which acts nonspecifically (Ramonah et al., 2020).

Statistical test results showed that the data were normally distributed (Shapiro-Wilk, $p > 0.05$), homogeneous (Levene's Test, $p > 0.05$), and there were significant differences between treatment groups (ANOVA, $p = 0.000$). A post hoc Tukey test showed that all pairs of concentrations of 25%, 50%, and 75% had significant differences in the inhibition zone diameter ($p < 0.05$). This significant difference indicates that the effect of extract concentration on inhibition is significant and not caused by random factors.

CONCLUSION

Insulin leaf extract (Smallanthus sonchifolius) at concentrations of 25%, 50%, and 75% has been shown to inhibit the growth of Staphylococcus aureus in vitro, with 75% being the most effective. The formation of inhibition zones is known to originate from the active compounds contained in the extract.

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