Antibacterial Activity Test of Gotu Kola Leaf Extract (*Centella asiatica*) Against *Staphylococcus aureus* Bacteria Isolated from Wounds of Diabetes Mellitus Patients

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Abstract: *Staphylococcus aureus*, a gram-positive bacterium, is the causative agent of diabetic foot. Traditional medicine using plants needs much further research, especially for wound healing in diabetes mellitus patients. This study is an experimental laboratory investigation on the efficacy of Gotu kola (Centella asiatica) leaf extract against wound-isolated *Staphylococcus aureus*. Gotu kola leaf extract (12.5%, 25%, 50%, 75%, and 100%), cefixime suspension (positive control), and sterile distilled water were tested. The results of the research showed the formation of an inhibitory zone from testing Gotu kola leaf extract against *Staphylococcus aureus* bacteria, where the average inhibitory zone was at concentrations of 12.5% (7.2 mm), 25% (11 mm), 50% (13.7 mm), 75% (14 .3 mm), and 100% (19.5 mm). The higher the concentration used, the larger the inhibition zone formed. Research shows that Gotu kola leaves' active substances inhibit energy formation, nucleic acid formation, cell membrane permeability, structure and function, and membrane protein lysis and damage.

Keywords: Diabetes mellitus; Gotu kola leaves; *Staphylococcus aureus*; Wounds

Introduction

Hyperglycemia caused by insulin secretion, action, or both characterizes diabetes mellitus (American Diabetes Association, 2010; Bhatti et al., 2022; Perkeni, 2021; Pfeifer et al., 1981). At least 463 million persons aged 20–79 had diabetes mellitus in 2019, representing 9.3% of the global population, according to the International Diabetes Federation (IDF) (IDF Committee, 2019). Patients with diabetes mellitus often lose control of their blood sugar levels when sick (Stachelek et al., 2021; Varela et al., 2021). High blood glucose levels increase infection risk and make it more challenging to treat existing illnesses. Soft tissue and skin infections that diabetes patients typically experience are furuncles, abscesses, and gangrene. Gram-positive aerobic bacteria are the typical culprits in acute skin infections, including cellulitis and abscesses (Perkeni, 2021).

Gram-positive *Staphylococcus aureus* causes diabetic foot (Stachelek et al., 2021; Varela et al., 2021). The most common microorganisms in the researcher's investigation were *Staphylococcus sp*. *Staphylococcus aureus* was the most common bacteria in ulcers (97%), *Staphylococcus aureus* is the most frequent pathogenic bacteria in diabetic feet, and 50% of isolated cases are methicillin-resistant (MRSA) (Anafo et al., 2021; Chiţă et al., 2013; Mamdoh et al., 2023; Taki et al., 2023). High antibiotic use generates difficulties and threatens world health, including bacterial resistance (Stachelek et al., 2021; Varela et al., 2021). Because of its high frequency, Methicillin Resistant *Staphylococcus aureus* has become a significant concern. This incidence affects the world, particularly Indonesia because many organisms are methicillin-resistant and may be resistant to other drugs (Nuryah et al., 2019).

Indonesians have used plant medicine for years (Rahayu et al., 2020). Medical use is trending "back to

nature" and is in demand by various groups, especially urban communities, and has become a global concern, as WHO estimates that 80% of the world's population relies on herbal medicine (Ahmad et al., 2023). WHO promotes traditional medicines, including herbal medicines, for public health, illness prevention, and treatment (Chung et al., 2021). Traditional herb Gotu kola leaves (*Centella asiatica* (L) urban) grow vines and blossom year-round. Gotu kola's bioactive components can treat disease in numerous ways. Saponins, tannins, and flavonoids in Gotu kola are antimicrobial (Dirjen Kefarmasian dan Alat Kesehatan Kemenkes RI, 2017; Duke et al., 2022).

In several studies, Flavonoids found in Gotu kola leaves have been shown in multiple trials to reduce acne-causing bacteria (Sari et al., 2022). As a natural disinfectant, a mixture of 1:1 Gotu Kola extract and Aloe Vera can be effective (Mayefis et al., 2023a). Gotu Kola herb extract combined with aloe vera extract has antibacterial effects against Pseudomonas aeruginosa (Mayefis et al., 2023b). There are hints from related studies that Gotu kola leaves may have antimicrobial properties; hence, more study into this area is warranted. This study will be the first to examine the potential antibacterial effects of Gotu kola leaves on *Staphylococcus aureus* germs isolated from diabetic wounds, with the purpose of research to investigate the antibacterial activity of Gotu kola leaf extract (*Centella asiatica*) in suppressing the development of *Staphylococcus aureus* bacteria at various extract concentrations (12.5, 25, 50, 75, and 100%).

Method

Experimental laboratory research, authentic experimentation, or study center research is conducted in a laboratory, study center, or artificial environment (Notoatmodjo, 2018). Extracts of *Centella asiatica* (also known as Gotu kola) leaves were tested under controlled laboratory conditions for their ability to kill *Staphylococcus aureus* germs isolated from wounds. Laboratory testing is conducted at the Herbarium of the Faculty of Mathematics and Natural Sciences, University of Lampung.

The study used Gotu kola (*Centella asiatica*) leaves from Pringsewu Regency, Lampung. The sample was 500 grams of sun-dried Gotu kola leaves. Research hypotheses (temporary results or conjectures) are tested with independent and dependent variables to examine if they match theories and empirical realities (Suwarno & Nugroho, 20 23). The tools used in this research are gloves, masks, bunsens, aluminum foil, laminar air flow, test tubes, paper discs, coolers, tweezers, matches, micropipettes, autoclaves, tubes, scales, alcohol, rulers, incubator, petri dish, knife, vortex, beaker, thermometer, blender, rotary evaporator, cotton swab transporter. The materials used in this research were Gotu kola leaves (*Centella asiatica*), *Staphylococcus aureus* bacterial culture, 96% ethanol, distilled water, nutrient agar, nutrient broth, spirit, positive control (cefixime antibiotic), MSA, 3% hydrogen peroxide, gentian violet, blood agar media, MHA.

The research began with planning and testing. Starting from Equipment sterilization, Test Material preparation, Plant identification, Simplicia making, Gotu Kola Leaf Extract concentration, and subject repetitions. Then identify *Staphylococcus aureus* bacteria (taking samples from diabetes mellitus patient wounds, gram staining, culturing on blood agar media, culturing on MSA media, e catalase test and coagulase test), then conduct a sensitivity test (making nutrient broth media, Mueller Hinton Agar Media, Planting Bacteria, and Planting Discs), then measure the inhibition zone. If data distribution is abnormal, the non-parametric Kruskall-Wallis statistical test is used.

Result and Discussion

Result

This study used diabetes mellitus wound-isolated *Staphylococcus aureus* germs. Gotu kola (*Centella asiatica*) leaf extract was tested for its ability to suppress *Staphylococcus aureus* growth. The investigation began with diabetes mellitus wound samples extracted and identified as bacteria. Next, gotu kola leaf extract at 12.5, 25, 50, 75, and 100% concentrations was tested for antibacterial activity. The disc's clear zone and diameter reveal the inhibition zone's results. Gotu kola (*Centella asiatica*) leaf extract contains saponins, steroids, tannins, alkaloids, and flavonoids, according to phytochemical testing—antibacterial chemical against *Staphylococcus aureus*. Gotu kola leaves inhibit energy formation, nucleic acid formation, cell membrane permeability, structure and function, and membrane protein lysis and damage.

Tannin inhibits reverse transcriptase and DNA to prevent bacterial growth (Tong et al., 2022). Flavonoids create compounds with extracellular proteins that harm bacterial cell membranes, destroy cell components, and limit energy synthesis (Mutha et al., 2021). Saponins promote cell permeability, causing intracellular compound lysis (Zhou et al., 2023). Next, diabetes wound bacteria were identified, and Vitek 2 identified *Staphylococcus aureus* germs (Joseph et al., 2013; Tentolouris et al., 1999; Thanganadar Appapalam et al., 2021). Antibacterial experiments were performed on these bacteria using gotu kola (*Centella asiatica*) leaf extract at 12.5, 25, 50, 75, and 100%, and positive and

negative controls. The clean zone around the extract-soaked disk was observed to evaluate the outcomes.

Table 1. Bacterial Inhibition Zone Results (mm)

Concenters	Repetition 1	Repetition 2	Repetition 3
12.5%	6	8	7.50
25%	11	11.50	11
50%	14.60	14.50	12
75%	14	16	13
100%	18	20	20.50
Control +	24.50	25	25.50
Control -	-	-	-

The mean length of pockmarked wounds in white mice was measured daily from the first day to the 14th day to assess healing. Table 2 shows in each PRP and salmon serum DNA group that white Wistar rats repair cuts. The two groups had different healing rates. Pocked wounds in the PRP group healed 100% on the 10th day and in the salmon DNA serum group on the 14th. The PRP group healed faster than the salmon DNA serum group. The Shapiro-Wilk normality test was used because this study had fewer than 50 samples. Data is normally distributed if the p-value is > 0.05 and not normally distributed if p < 0.05 (Ghozali, 2018).

Table 2. Normality Test

	Shapiro-Wilk			
	Group	Statistic	df	Sig.
	12.5%	.923	3	.463
	25%	.964	3	.637
Obstacles zone	50%	.779	3	.065
	75%	.964	3	.637
	100%	.893	3	.363
	Control +	1.000	3	1.000

*. This is a lower bound of the true significance.

a. Inhibition_zone is constant when extract_concentration = control -. It has been omitted.

The normality test (Table 2) shows significant findings in the 12.5% extract concentration group of 0.463, 25% concentration of 0.637, 50% concentration of 0.65, 75% concentration of 0.637, 100% concentration of 0.363, and control group positive by 1,000. All groups had significance values > 0.05, indicating normal distribution. So, homogeneity testing can proceed.

Table 3. Homogeneity Test

Test of Homogeneity of Variances				
	Levene Statistic	df1	df2	Sig.
Based on Mean	2.838	6	14	.051

Homogeneity tests use the Levene test with 5% significance. Data is considered homogeneous if the significance value is > 0.05 and not homogeneous if < 0.05 (Ghozali, 2018). Table 3 shows that all treatment

groups have the same variance or are homogenous data groups because the SPSS homogeneity test yielded a significance value 0.051.

Table 4. One-Way ANOVA Test

	Amount	Df	Mean square	F	Sig
Between groups	1188.627	6	198.104	169.735	.000
In group	16.340	14	1.167		
Total	1204.967	20			

Table 5. Post Hoc Bonferroni Test Results

(I) Extract Concentration	(J) Extract Concentration	Mean Difference (I-J)	Std. Error	Sig
12.5%	25%	-3.66667*	.88210	.001
	50%	-6.53333*	.88210	.000
	75%	-7.16667*	.88210	.000
	100%	-12.33333*	.88210	.000
	Control +	-17.83333*	.88210	.000
	Control -	7.16667*	.88210	.000
25%	12.5%	3.66667*	.88210	.001
	50%	-2.86667*	.88210	.006
	75%	-3.50000*	.88210	.001
	100%	-8.66667*	.88210	.000
	Control +	-14.16667*	.88210	.000
	Control -	10.83333*	.88210	.000
50%	12.5%	6.53333*	.88210	.000
	25%	2.86667*	.88210	.006
	75%	-.63333	.88210	.485
	100%	-5.80000*	.88210	.000
	Control +	-11.30000*	.88210	.000
	Control -	13.70000*	.88210	.000
75%	12.5%	7.16667*	.88210	.000
	25%	3.50000*	.88210	.001
	50%	.63333	.88210	.485
	100%	-5.16667*	.88210	.000
	Control +	-10.66667*	.88210	.000
	Control -	14.33333*	.88210	.000
100%	12.5%	12.33333*	.88210	.000
	25%	8.66667*	.88210	.000
	50%	5.80000*	.88210	.000
	75%	5.16667*	.88210	.000
	Control +	-5.50000*	.88210	.000
	Control -	19.50000*	.88210	.000
Control +	12.5%	17.83333*	.88210	.000
	25%	14.16667*	.88210	.000
	50%	11.30000*	.88210	.000
	75%	10.66667*	.88210	.000
	100%	5.50000*	.88210	.000
	Control -	25.00000*	.88210	.000
Control -	12.5%	-7.16667*	.88210	.000
	25%	-10.83333*	.88210	.000
	50%	-13.70000*	.88210	.000
	75%	-14.33333*	.88210	.000
	100%	-19.50000*	.88210	.000
	Control +	-25.00000*	.88210	.000

*. The mean difference is significant at the 0.05 level.

The results of the one-way ANOVA test (Table 4) show a significance value of 0.000 (p < 0.05), so it can be

concluded that there are significant differences between treatment groups. After the One-Way ANOVA Test, the Post Hoc Test was performed to determine treatment group differences. Further test results showed (Table 5) that all groups showed differences in the average inhibition zone of *Staphylococcus aureus* bacteria by Gotu kola (*Centella asiatica*) leaf extract.

Discussion

The purpose of this research is to determine whether or not an extract of the leaves of the *Centella asiatica* plant can prevent the growth of *Staphylococcus aureus* bacteria isolated from the wounds of people with diabetes. Bacteria are identified with a Vitek 2 instrument and grown in MHA media. Gotu kola (*Centella asiatica*) leaf extract was used in an antibacterial test, with varying concentrations (12.5, 25, 50, 75, and 100%), as well as a positive control and a negative control, on the study's human participants. Gotu kola leaf extract is obtained by the maceration of 500 grams of dried simplicia, which is then soaked using 96% ethanol and followed by evaporation using a Rotary evaporator to obtain a thick extract. The extract was then prepared in a range of strengths using sterile distilled water as a solvent: 12.5, 25, 50, 75, and 100%.

Phytochemical analysis revealed the presence of many secondary metabolite chemicals, including saponins, steroids, tannins, alkaloids, and flavonoids, in the Gotu kola (*Centella asiatica*) leaf extract. This is consistent with prior studies that isolated several metabolite chemicals from Gotu kola leaves, such as tannins, alkaloids, flavonoids, saponins, and tannins, but failed to isolate steroids except for those isolated from in vitro cultured gotu kola leaves. Gotu kola leaves contain chemicals with antibacterial characteristics. Hence, they may be able to prevent the growth of *Staphylococcus aureus*. Inhibiting energy production and nucleic acid synthesis are just two of the many goals pursued by the active substances in Gotu kola leaves. These substances can also alter the structure and function of cell membranes, leading to lysis and damage to membrane proteins.

Tannin has antibacterial effects by preventing the formation of bacterial cells by interfering with the reverse transcriptase enzyme and DNA (Akiyama et al., 2001; Kaczmarek, 2020). Bacterial cell membranes are damaged when flavonoids combine with external proteins to create molecules, which are then lysed within the cell, preventing energy synthesis (Zhang et al., 2005). Saponins raise permeability, which causes cells to leak and intracellular chemicals to be lysed (Johnson et al., 1986; Stockdale et al., 2019). The disc method was employed to test for antibacterial efficacy, and the process was repeated three times with 12, 25, 50, and 100% concentrations, as well as sterile water control and positive control. The test results demonstrated that except for the negative control group, all concentrations tested displayed antibacterial inhibitory power against *Staphylococcus aureus* bacteria. Therefore, the inhibition zone formed is unaffected by using sterile distilled water in diluting the extract to make the test concentration.

The average size of the inhibition zone generated in the test, as measured from the clear zone formed around the disc, is the biggest in the 100% concentration group at 19.5 mm and most minor in the 12.5% concentration group at 7.17 mm. The size of the inhibitory zone is proportional to the extract concentration. In both treatment groups, the Shapiro-Wilk normality test demonstrated that the zone of inhibition followed a normal distribution ($p > 0.05$). When the homogeneity test was continued, it demonstrated that all treatment groups had homogeneous variances at the 0.05 significance level or higher. All treatment groups demonstrated statistically significant differences in the bacterial inhibition zones they produced, as determined first by the One-way Annova test and subsequently by the LSD follow-up test. These findings suggest that the antibacterial properties of *Centella asiatica* (gotu kola) leaf extract vary depending on the concentration.

Conclusion

Flavonoids, tannins, saponins, steroids, and alkaloids were identified as metabolite compounds in the Gotu kola (*Centella asiatica*) leaves studied. The Gotu kola (*Centella asiatica*) leaf extract contains secondary metabolite chemicals having antibacterial properties with diverse modes of action/targets. The results of this study show that at concentrations of 12.5% (7.2 mm), 25% (11 mm), 50% (13.7 mm), 75% (14.3 mm), and 100% (19.5 mm), Gotu kola leaf extract inhibits the growth of *Staphylococcus aureus* bacteria. The size of the inhibition zone depends on the concentration utilized. For future studies, it is suggested that Gotu kola leaf extract be tested for its antibacterial activity at lower concentrations than those used here and that its ability to inhibit the growth of *Staphylococcus aureus* bacteria in vivo be investigated.

Author Contributions
Fioni and Debby Heldayani conceptualized the research idea, designed of methodology, management and coordination responsibility; Gilbert Lister analyzed data, conducted a research and investigation process; Andika Zayani Tambunan conducted literature review and provided critical feedback on the manuscript.

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