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THE ETHANOL EXTRACT OF JUKUT PENDUL (KYLLINGA NEMORALIS) EXHIBITS INHIBITORY ACTIVITY AGAINST THE GROWTH OF STAPHYLOCOCCUS AUREUS

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Abstract

Background*: Staphylococcus aureus is a pathogenic-bacteria that is often found to have resistance to antibiotics. Jukut pendul (Kyllinga nemoralis) extract is known to have antibacterial compounds such as flavonoids, tannins, saponins, and steroids.* ***Aims****: The purpose of this study was to determine the inhibitory ability of jukut pendul ethanol extract against Staphylococcus aureus bacteria.* ***Method****: This research used the Kirby-Bauer disc diffusion method with five treatments within five repetitions. Data analysis was carried out using One Way Anova variance analysis. The variation in extract concentration tested was 5, 10, 20, and 40% with 96% ethanol as the control group and Ciprofloxacin as the work control.* ***Result****: The results showed that extract concentrations of 5, 10, 20, and 40% were able to inhibit Staphylococcus aureus bacteria with an average inhibitory zone of 9,36; 10,85; 11,64; and 12.15 mm, respectively.* ***Conclusion****: The inhibitory zone formed indicates that jukut pendul ethanol extract has moderate to strong inhibitory ability in inhibiting the growth of Staphylococcus aureus bacteria.*

Keywords*:* Kyllinga nemoralis extract, inhibition zone, Staphylococcus aureus

1. Introduction

Staphylococcus aureus is one example of pathogenic bacteria that often infects the skin, mucous membrane of the anterior nares, gastrointestinal tract, pharynx, genitourinary tract, and perineum (1). When compared to other Staphylococcus species, this bacterium has the highest pathogenicity with varying levels of severity, starting from minor infections such as skin abscesses, urinary tract infections, respiratory tract infections, to serious infections, such as bacteremia due to food poisoning, necrotic pneumonia in children, endocarditis, and infections of the central nervous system (2).

Infection caused by the bacterium Staphylococcus aureus is compounded by the ability of these bacteria to be resistant to various types of antibiotics (Multi-Drug Resistance or MDR). This is because these bacteria have a great adaptability to rapidly respond to each new antibiotic with the development of resistance mechanisms, starting with penicillin and methicillin, to the most recent resistance to linezolid and daptomycin. Mechanisms of resistance in these

bacteria can include enzymatic inactivation of antibiotics, changes in target structure (which causes a decrease in antibiotic affinity), antibiotic trapping, and efflux pumps (3). One of the strains of Staphylococcus aureus, which is often found to be resistant to many antibiotics, is Methicillin resistant Staphylococcus aureus (MRSA) (3). MRSA is known to be resistant to a class of antibiotics called β-lactams, including penicillinase resistant penicillins, cephalosporins, and carbapenems (4).

With resistance to various antibiotics, the selection of the right antibiotic becomes more challenging, which can impact the effectiveness of disease treatment. One strategy that has been developed is to research and develop technology utilizing natural ingredients from various herbal plants with the potential to act as antibacterials.

One of the plants with the potential to act as an antibacterial agent is the Jukut pendul plant (Kyllinga nemoralis). This plant is readily accessible and recognized as a wild species within the local community. Nevertheless, limited awareness exists regarding the constituents and properties that can be derived from this botanical specimen.

Jukut pendul as a herbal plant is believed to offer several health benefits. Its leaves have traditionally been used to alleviate shivering caused by malaria, skin pruritus, diabetes, and thirst resulting from fever. Additionally, the rhizomes of K. nemoralis are traditionally employed as antidiarrheals, expectorants, treatments for worm infections, and diuretics (5). The health benefits of this plant are further supported by the discovery of secondary metabolite compounds, including terpenoids, saponins, and phenolic compounds in the methanol extract of the leaves, as well as flavonoids, triterpenoids, and glycosides in the ethanol extract of the rhizome (6). Extracts of K. nemoralis with methanol and water solvents have demonstrated antibacterial activity against bacteria such as Staphylococcus aureus and MRSA. Moreover, essential oil extracts from K. nemoralis have shown antimicrobial, antimalarial, and anticancer activities (5). The aim of this research is to assess the inhibitory potential of Jukut pendul (Kyllinga nemoralis) ethanol extract against the growth of Staphylococcus aureus using the disc diffusion method.

2. Method

The type of research carried out is a true-experimental research with a Posttest Only Control Group Design plan. This research was conducted at the Bacteriology Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Denpasar in January-April 2023.

The samples used in this research were ethanol extracts from the stems, leaves, and flowers of Jukut pendul taken from the Wanasari Village area, Tabanan. In this study, there were experimental groups, including varying extract concentrations of 5,

10, 20, and 40%, a control group with 96% ethanol, and a positive control with the antibiotic Ciprofloxacin 5 µg, with five repetitions based on the Federer formula.

Jukut pendul Extraction (Kyllinga nemoralis)

Jukut pendul plant samples were collected from rice fields located in the Wanasari Village area of Tabanan. Jukut pendul plants grow in clusters as wild vegetation around rice fields. The author obtained a certain number of Jukut pendul plant samples, which then underwent a sorting process. Subsequently, the sorted samples underwent a drying stage that lasted for seven days to produce dry samples. The next step involved dry sorting and grinding the dried samples to create simplicia in the form of fine powder. The simplicia obtained was extracted using the maceration method with 96% ethanol as the solvent, at a sample-to-solvent ratio of 1:5 for three cycles of 24 hours each, with remaceration done twice. In the extraction process, 96% ethanol is preferred because this solvent has high absorption and extraction abilities to extract compounds that are non-polar, semi-polar, and polar. When compared to low-concentration ethanol solvents, ethanol is 96% easier to penetrate into the cell wall so that active substance compounds can be extracted effectively to produce concentrated extracts (7).

The macerate obtained from this process was evaporated to obtain a concentrated ethanol extract. The concentrated extract was then diluted with 96% ethanol to create varying concentrations of Jukut pendul ethanol extract at 5%, 10%, 20%, and 40%, which were used as samples for antibacterial inhibition tests.

Phytochemical screening

Phytochemical screening tests include the testing of alkaloids, flavonoids, tannins, saponins, and steroids (8).

a. Alkaloid test

A total of 3 ml was mixed with three drops of 2 N hydrochloric acid. The mixture was then divided into two tubes. In the first tube, 1 ml of Dragendroff's reagent was added, while in the second tube, 1 ml of Mayer Wagner's reagent was added. A positive result is indicated by the formation of an orange-red precipitate in the first tube and a white precipitate in the second tube.

b. Uji flavonoid

A total of 1 ml of the sample was mixed with approximately 0.1 g of Mg (equivalent to the amount on the tip of a spatula), and then 10 drops of concentrated HCl reagent are added. Positive results for flavonoids are indicated by the formation of an orange color.

c. Uji tannin

A total of 2 ml of sample was mixed with 1-2 drops of FeCl3 reagent 1%. A positive tannin result is indicated by a color change to blackish blue or blackish green.

d. Uji saponin

A total of 1 ml of the sample was mixed with 10 ml of hot water and 1 drop of 2 N hydrochloric acid, then shaken vigorously for 10 seconds. If foam forms as high as 1-10 cm and remains stable for no less than 10 minutes, this indicates the presence of saponin compounds.

e. Test for steroids and terpenoids

A total of 3 ml of the sample was mixed with concentrated H2SO4 (sulfuric acid), then shaken. The presence of steroids and flavonoids is indicated by the formation of a red color.

Antibacterial Inhibition Test

Antibacterial inhibitory testing of Jukut pendul ethanol extract was conducted in vitro against Staphylococcus aureus ATCC bacteria. The method used in this test is the Kirby-Bauer disk diffusion method, based on standard procedures outlined by the Clinical and Laboratory Standards Institute (CLSI). A bacterial suspension of Staphylococcus aureus was prepared by homogenizing 24-hour-old bacterial colonies in sterile 0.9% NaCl solution and measuring turbidity with a MacFarland Densitometer to obtain a MacFarland number of 0.5, equivalent to 1.5 x 108 CFU/ml. Subsequently, the bacterial suspension was inoculated evenly on Mueller-Hinton agar (MHA), covering the entire surface of the

agar. Discs saturated with 5%, 10%, 20%, and 40% extract solutions, along with 96% ethanol and Ciprofloxacin discs, were then placed on the agar surface, with a minimum distance of 24 mm between them. The media were incubated at 37°C for 18-24 hours. The diameter of the inhibition zone was measured using a caliper, calculated from end to end of the inhibition zone.

Data analysis

The inhibition zone diameter data obtained were analyzed using a statistical test, specifically One-way ANOVA, to determine whether significant differences exist between the groups. If a significant difference is indicated by $p \leq 0.05$, further analysis will be conducted.

3. Result and Discussion

Phytochemical screening

The results of the phytochemical screening test for the ethanol extract of Jukut pendul indicated the presence of secondary metabolite compounds, including flavonoids, tannins, and saponins. These results are presented in Table 1

Table 1. Phytochemical Screening Test Results

Phytochemical test	Reagent	Results
Alkaloid	Dragendrof	Negative (-)
	Mayer	Negative (-)
	Wagner	Negative (-)
Flavonoid	Concentrated HCl and Mg	Positive (+)
Tannin	$FeCl_3$	Positive (+)
Saponin	Hot water and HCl 2N	Positive (+)
Steroid	H_2SO_4	Negative (-)

Inhibition zone diameter

In the research that has been conducted, data were obtained from testing the antibacterial inhibitory effect of Jukut pendul ethanol extract on the growth of *Staphylococcus aureus*. The series of extract concentrations used included 5%, 10%, 20%, and 40%. The data resulting from the measurements of the inhibitory effect of Jukut pendul ethanol extract against *S. aureus* bacteria are presented in Table 2.

Table 2. Diameter of Inhibition Zone at Each Extract Concentration, Ethanol 96%, and Control

Repetition	Etanol 96% (mm)	Positive control (mm)	Diameter of inhibition zone for each extract concentration (mm)			
			5%	**10%**	**20%**	**40%**
I	6,44	25,4	8,4	10,2	11,3	12,6
II	6,22	26,9	10,7	10,3	11,4	12,28
III	6,35	25,2	8,2	11,58	11,6	12,2
IV	6,24	26,68	9,72	11,8	11,2	12,2
V	6,20	26,9	9,82	10,4	12,7	11,5
Mean	6,29	26,21	9,36	10,85	11,64	12,15

From the statistical analysis test One-Way ANOVA, a significance value of <0.05 was obtained, indicating that there were significant differences between the research groups. These differences were further elucidated by the test results after this, using Games-Howell, which showed that the 96% ethanol control group had a significance of <0.05 at each extract concentration. However, significant differences between concentration variations were only observed at the 5% concentration, with no significant differences in the inhibition zones produced at concentrations of 10%, 20%, and 40%.

Phytochemical screening

The phytochemical screening carried out in this study included testing for alkaloids, flavonoids, tannins, saponins, and steroids. Based on the test results, the ethanol extract of Jukut pendul was found to contain flavonoids, tannins, and saponins. Flavonoids and tannins are secondary metabolite compounds, both belonging to the group of phenolic compounds.

These compounds are characterized by the presence of a phenol group, which is a hydroxyl functional group. Structurally, flavonoids consist of a fifteen-carbon framework composed of two benzene rings connected via a heterocyclic pyrene ring (C). Most flavonoid compounds exhibit antioxiandt, anti-inflammatory, and antitumor activities. Additionally, flavonoids have shown antibacterial activity as well (9).

Tannin is a polyphenol macromolecule that exists in two compound forms: hydrolyzed tannin and condensed tannin. Tannins possess the ability to inhibit the growth of various types of microbes, including both Gram-positive and Gram-negative bacteria, fungi, and yeast. On the other hand, saponin is a compound with a structure comprising sugar (sugar chain) and non-sugar parts (glycosides) linked by glycosidic bonds. Saponins exhibit various biological activities, such as antibacterial, anti-inflammatory, antifungal, and antiviral properties, which are attributed to their chemical structure.

The inhibitory power of the ethanolic extract of jukut pendul

Based on the antibacterial inhibition test conducted, it's evident that the ethanol extract of jukut pendul exhibits antibacterial activity, as indicated by the formation of an inhibitory zone around the disc. The data on inhibition zone diameters reveal differences among the four concentration variations, with the zone size increasing as the concentration increases. To assess the significance of these differences between groups, the inhibition zone data were collected and analyzed using a One-Way ANOVA statistical test.

The data analysis reveals significant differences between the control group and the experimental group at each concentration variation, as indicated by a p-value <0.05. Subsequently, to determine the differences within each extract concentration, a Games-Howell test was conducted. This method was chosen because the tested data did not exhibit the same or homogeneous variance. The results of this post-hoc test indicate a significant difference between the control group and all concentration variations of Jukut pendul ethanol extract, with a p-value <0.05. This underscores that the majority of the inhibitory power generated by Jukut pendul ethanol extract is attributable to the antibacterial compounds it contains. Furthermore, the average inhibition zone for the 5% extract concentration measured at 9.36 mm ± 1.04, significantly differs from both the control group (96% ethanol) and the 20% and 40% extract concentrations. The 10% extract concentration, with an average inhibition zone of 10.85 mm ± 0.76, only differs significantly from the control group (96% ethanol). Meanwhile, the 20% extract concentration, which yields an average inhibition zone of 11.64 mm ± 0.61, shows a significant difference compared to the control group and the 5% extract concentration. Lastly, the 40% extract concentration, with an average inhibition zone of 12.15 mm ± 0.4, demonstrates a significant difference from both

the control group and the 5% concentration. From these results, it is apparent that there is no significant difference in the inhibition zones produced at concentrations of 10%, 20%, and 40%. The significant difference between the 5% concentration and the 20% and 40% concentrations suggests that the higher extract concentrations (20% and 40%) exhibit more effective antibacterial performance due to a higher concentration of active compounds. Meanwhile, the insignificant differences among the concentration variations of 10%, 20%, and 40% indicate that the amount of antibacterial compounds in these three concentrations can inhibit bacterial growth with nearly the same strength. In other words, an extract concentration of 10% exhibits the same robust inhibitory ability as extract concentrations of 20% and 40%.

Based on the obtained results, the antibacterial inhibitory power of each extract concentration can be determined by matching the diameter of the inhibition zone obtained with predefined inhibition zone categories. The inhibitory power of an antibacterial compound can be categorized into four categories: weak inhibitory power if the inhibitory zone diameter is <5 mm, moderate if the inhibitory zone diameter is 5-10 mm, strong if the inhibitory zone diameter is 10-20 mm, and very strong if the diameter of the inhibition zone is >20 mm (10). Based on this classification, it is evident that the antibacterial inhibitory power exhibited at an extract concentration of 5% (9.36 mm) falls into the moderate category, while concentrations of 10% (10.85 mm), 20% (11.64 mm), and 40% (12.14 mm) are categorized as strong.

The antibacterial ability exhibited by a plant extract can be attributed to the presence of secondary metabolite compounds within the extract, which contribute to inhibiting the growth of the test bacteria (11). The quantity of secondary metabolite compounds present in the extract directly influences its effectiveness in combating the test bacteria (12). Among the various phytochemical compounds found in plants, there are three primary classes: terpenoids, phenolic metabolites, and alkaloids. Among these, phenolics represent the most crucial group of compounds in a plant's defense system against pests and diseases. Phenolic compounds can be further categorized into phenolic acids (hydroxybenzoic and hydroxycinnamic acids), polyphenols (hydrolyzable and condensed tannins), and flavonoids (13).

In this study, the inhibitory ability demonstrated by the ethanol extract of jukut pendul against *Staphylococcus aureus* bacteria can be attributed to the presence of phenolic compounds and saponin content within the extract. This is supported by the results of phytochemical screening tests conducted by researchers, which reveal that the active compounds present in the Jukut pendul ethanol extract include flavonoids, tannins, and saponins. These three compounds are known to possess antibacterial capabilities, each with its own mechanism.

The concentration of phenolic compounds is known to exhibit a positive correlation with the antibacterial activity of the extract. Flavonoids and tannins, as polyphenolic compounds, are recognized for their antibacterial activity, each with distinct mechanisms. The antibacterial action of flavonoids and tannins can be both bactericidal and bacteriostatic. Flavonoids exert their antibacterial effects by forming bonds to create protein complexes through nonspecific forces such as hydrogen bonds and hydrophobic effects, as well as through covalent bond formation. This ability allows these compounds to deactivate adhesins, enzyme systems, transport proteins in cell membranes, and other cellular components (14). Additionally, flavonoid compounds can interfere with bacterial cell membranes due to their lipophilic nature (15).

Similarly, tannin compounds possess the ability to bind to proteins and form protein complexes. They can also interact with polysaccharides in bacterial cell walls, leading to the disintegration of the membrane and bacterial cell wall function, ultimately impacting the growth and multiplication of bacterial cells. This is supported by research conducted by Sindhu (2014), which found that the methanol extract of *K. nemoralis* contains significant amounts of total phenolic compounds, flavonoids, flavonols, and tannins (16). These findings were substantiated by testing antimicrobial activity against five species of bacteria that are human pathogens, including *S. pneumonia, S. saprophyticus, S. aureus, S. mutans,* and *E. faecalis*. In addition to phenolic compounds, jukut pendul extract also contains saponins. The antibacterial mechanism of saponins is similar to that of cephalosporins and is related to the bacterial cell membrane system (17). Saponin compounds can penetrate the lipid bilayer and bind with cholesterol, forming a cholesterol-saponin complex that ultimately lyses bacterial cells (18).

The composition and quantity of phytochemical compounds present in an extract depend on several factors, including the geographical conditions of the plant samples, the simplicia preparation technique (which involves wet sorting, drying, and dry sorting stages), the type of extraction solvent used, and the chosen extraction method. The choice of solvent in the extraction process is a key factor influencing the composition and quantity of active compounds in the extract (5).

For extracting polyphenolic compounds from plant matrices, polar solvents are typically preferred. Various polar solvents are commonly used to dissolve these compounds, such as mixtures of distilled water with ethanol, methanol, acetone, and ethyl acetate. Ethanol is known for its effectiveness in extracting polyphenolic compounds and is considered safe for consumption. Methanol, on the other hand, is more efficient in extracting polyphenolic compounds with smaller molecular weights, while acetone is better suited for dissolving flavanol compounds with larger molecular

weights. When it comes to extracting flavonoids from tea, ethanol outperforms methanol and acetone in a water solvent (13).

Research conducted by Supriyanto (2017) demonstrated that both ethanol and methanol extracts of neem plants yielded positive results in phytochemical tests, indicating the presence of flavonoids, tannins, terpenoids, and saponins in both extracts (19). These results suggest that ethanol and methanol extracts have similar capabilities in extracting the same phytochemical compounds.

Based on the research conducted, several weaknesses in the resistance test results have been identified. These weaknesses become evident from the results obtained in the control group, which unexpectedly showed the formation of an inhibitory zone around the disc. Ideally, the control group should exhibit no inhibitory effect on bacterial growth, indicating that the ethanol solvent alone does not affect it. To understand the cause of this unexpected result, further investigations are necessary.

Additionally, limitations in this research are also observed in the interpretation of the inhibitory ability demonstrated by the extract. Researchers cannot conclusively determine whether the antibacterial compounds in the extract are bacteriostatic or bactericidal. This uncertainty arises because the measurement of extract inhibition zones was only carried out for a single day, typically after 18-24 hours of incubation. Extending the measurement to two days would enable researchers to discern whether the extract tends to be bacteriostatic (characterized by growth in the inhibition zone on the second day) or bactericidal (indicated by the absence of growth in the inhibition zone). Alternatively, researchers can perform a time-kill test or Minimal Bactericidal Concentration (MBC) test to determine the antibacterial properties of the extract. The time-kill test assesses the effect of a specific antibacterial compound over time and at various concentrations.

Furthermore, weaknesses were identified in the resistance testing method used, namely the disc diffusion method. This method has limitations in diffusing high or concentrated extracts onto agar media. It is unable to absorb extracts with thicker consistency, which hinders proper diffusion into the media and introduces bias in the inhibition zone measurements. Therefore, it is essential to carefully consider the choice of antibacterial testing method in conjunction with the consistency and concentration of the extract to be used.

4. Conclusion

Based on the results of the research testing the antibacterial inhibitory power of jukut pendul ethanol extract on the growth of *Staphylococcus aureus* bacteria, it can be concluded that the ethanol extract of jukut pendul contains secondary metabolite compounds, namely flavonoids, tannins, and saponins. The antibacterial inhibitory power at an extract concentration of 5% is moderate, while at concentrations of 10%, 20%, and 40%, it is classified as strong.

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