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Effectiveness of Lemongrass Leaf Ethanol Extract Spray Preparation in Inhibiting the Growth of Diabetic Ulcer Isolate MRSA Bacteria

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ABSTRACT

Lemongrass (*Cymbopogon nardus*) is a type of grass from the order Graminales which is typical of tropical areas of Asia. Compounds contained in lemongrass leaves can inhibit the growth of Methicillin Resistant Staphylococcus aureus (MRSA) bacteria. This research aims to determine the effectiveness of spray preparations of lemongrass leaf extract in inhibiting the growth of MRSA bacterial isolates from diabetic ulcers.

The research design of this study is quasi experimental. Data obtained from primary data by testing the inhibitory power of spray preparations against MRSA bacteria. The results obtained were analyzed using the One Way Anova statistical test.

The diameter of the inhibition zone for MRSA bacteria in the 25% concentration ethanol extract spray preparation was 4 mm, the 35% concentration was 7 mm, the 45% concentration was 11 mm and the antibiotic Vancomycin was 12 mm. There are differences in the inhibition zone of spray preparations of ethanol extract of lemongrass leaves with concentrations of 25%, 35%, 45% and the antibiotic disc vancomycin with p value = 0.00 < α 0.05.

Spray preparations of ethanol extract of lemongrass leaves with concentrations of 25%, 35% and 45% are not effective in inhibiting the growth of Methicillin Resistant Staphylococcus aureus (MRSA) bacteria, however, the average zone of inhibition for spray preparations with ethanol extract of lemongrass leaves with a concentration of 45% is against MRSA bacteria. 11 mm is included in the strong category.

Keywords: *Lemongrass Leaves, MRSA Bacteria, Spray preparation.*

INTRODUCTION

Diabetes mellitus is a condition of chronically increased blood sugar levels as a result of disturbances in carbohydrate, fat and protein metabolism due to a lack of the hormone insulin.[1] The main problem in diabetes mellitus sufferers is complications, one of which is diabetic ulcers.[2] The number of diabetes mellitus sufferers continues to increase every year, based on data from the Pontianak City Health Service In 2018, there were 44,003 people suffering from diabetes mellitus in the city of Pontianak with the number of sufferers experiencing complications of diabetic ulcers as many as 3,334 people. bottom.[3.4]

The main problem in treating diabetic ulcers to date is resistance to antibiotics. Antibiotic resistance causes bacteria to become resistant to antibiotics. Consequence If there is antibiotic resistance, it is necessary to use natural ingredients as an alternative, such as lemongrass.[5] Lemongrass leaves contain secondary metabolite compounds saponins, flavonoids, polyphenols and alkaloids.[6] The

saponins, flavonoids, polyphenols and alkaloids found in lemongrass leaves have the ability to inhibit bacterial growth. especially MRSA. Based on the research results of Sarlina et al (2017), lemongrass leaf extract gel with a concentration of 20% can inhibit the growth of Staphylococcus aureus bacteria with an inhibition zone of 13.5 mm.[7]

In order for the bioactive compounds contained in lemongrass leaves to be applied to diabetic ulcer infections, they need to be processed into a herbal product, one of which is a spray. The spray preparation contains secondary metabolite compounds, containing propylene glycol which functions as a solvent and methyl paraben which functions as a preservative for the spray preparation.[8]

LITERATURE REVIEW

Diabetes mellitus is a series of chronic metabolic disorders caused by the pancreas not producing enough insulin, causing both absolute and relative insulin deficiency, resulting in an increase in glucose concentration in the blood. One of the complications of diabetes mellitus is ulcers, namely superficial infections on the sufferer's skin caused by bacteria. Patients with diabetes mellitus who have open wounds will be more susceptible to infection because they have a weak immune system and the presence of high blood sugar provides nutrition and a place for bacterial growth. [9] One of the bacteria that can infect diabetic wounds is Staphylococcus aureus which can cause damage. tissue accompanied by purulent abscesses. The type of bacteria most commonly found in diabetic ulcer pus is Staphylococcus sp (92.9%). [10]

S. aureus bacteria is one of the bacteria that can cause infections in various body tissues in diabetic ulcer sufferers. Stapylococcus infections can be transmitted from person to person. S. aureus bacteria that have become resistant to antibiotics are known as MRSA (Methicillin-resistant Staphylococcus aureus). Mieke (2008) stated that resistance to a bacteria can occur due to administration of antibiotics that are not in the right dosage, wrong diagnosis, and not targeting the bacteria that cause it properly. MRSA bacteria will produce transmembrane proteins known as efflux proteins and plasmids that code for resistance genes to an antibiotic. MRSA bacteria experience antibiotic resistance due to genetic changes caused by irrational antibiotic therapy.[11] Transmission of bacteria moves from one patient to another through medical equipment whose sterility is not respected, through the air or hospital facilities. MRSA infections have become a big problem for hospital clinicians for many years as a cause of nosocomial infections whose incidence rates increase by 10-20% every year. [12] Since the emergence of cases of resistance to the antibiotic methicillin, MRSA bacteria are widely known as a cause of bacteremia, pneumonia, postoperative infections and other nosocomial infections. MRSA bacteria can cause infections of the skin, bones, lungs, heart or systemic infections. MRSA infections can only be treated with certain antibiotics. If the antibiotics given are not able to kill MRSA, the infection is not resolved and spreads widely and endangers the sufferer's life. 11 Infections by MRSA have also begun to spread in hospitals, such as MRSA infections in surgical wounds. With the spread of MRSA infections, MRSA-specific early diagnostic alternatives (Anti-MRSA) are urgently needed. One fast and accurate diagnostic technique is to use PCR (Polymerase Chain Reaction) to detect specific MRSA genes, namely the mecA gene.[1]

MATERIALS & METHODS

Types of research

This research is an experimental study to analyze the antibacterial effectiveness of a spray preparation of ethanol extract of lemongrass leaves against Methicillin Resistant Staphylococcus aureus (MRSA) bacteria.

Sample Collection and Processing

7 kg of lemongrass leaves were obtained from Rasau Jaya Village, Kubu Raya District, then washed clean and dried naturally in the air without direct sunlight.

Making Lemongrass Leaf Extract

4900 grams of pollinated lemongrass leaves were obtained, then put into a maceration jar, then added with 96% ethanol solvent, covered and left for 24 hours. The resulting extract was then filtered using squeezed chili filter paper, the same procedure was repeated until the ethanol solution was relatively clear. The maserate obtained was evaporated using a rotary evaporator until a thick extract was obtained.

Preparation of Lemongrass Leaf Ethanol Extract Spray Preparation

The ethanol extract of lemongrass leaves is weighed at 5 grams for a concentration of 25%, 7 grams for a concentration of 35% and 9 grams for a concentration of 45%, then each concentration is added with 1 gram of propylene glycol and 0.4 grams of methyl paraben, then the entire concentration is mined with distilled water. add 20 ml.

The Making of Mueller Hinton Agar Media

38 grams of MHA media was weighed, dissolved in 1000 ml of distilled water, then heated while stirring on a hot plate until homogeneous. Sterilize the media solution in an autoclave for 15 minutes at 121°C. The sterile MHA media is left to cool slightly at around 40-45oC. Then pour 15 ml of the finished product into a sterile petri dish.

Making Methicillin Resistant Staphylococcus aureus (MRSA) Suspension

Put 10 ml of sterile NaCl solution into a sterile test tube. Then take the MRSA bacterial colony from the pure culture with a sterile round tube, put it in a test tube containing sterile NaCl solution, homogenize it. View and compare the turbidity with the Mc standard. Farland.

Resistance Testing

Dip a sterile swab into the MRSA bacterial suspension, press the cotton swab against the side of the tube to drain. The sterile swab that has been drained is inoculated onto the surface of the media evenly until all surfaces are covered, let stand for 3-5 minutes. With sterile tweezers, take a paper disc that has been soaked in a spray preparation of ethanol extract of lemongrass leaves for 15 minutes (Dewi Hartini Oktovia, 2017), place it on the surface of the media that has been inoculated with MRSA bacteria. Press the paper disc so that it sticks to the media, each petri dish contains one paper disc, then incubate at a temperature of 37^{o} C for 24 hours in an incubator. Growth results are observed by looking at the size of the inhibition zone.[14]

RESULT

Table 1. Inhibitory power of spray preparations of ethanol extract of lemongrass leaves and vancomycin antibiotic disc against MRSA bacteria

Replication	Inhibition Zone (mm)				
	25%	35%	45%	Vancomycin	base control
1	4	8	9	11	0
2	3	5.5	12	13.5	0
3	4	5	13.5	11.5	0
4	5	6.5	11.5	12	0
5	3.5	6.5	10.5	11.5	0
6	4.5	7	10	11.5	0
7	4.5	7.5	11	13	0
8	5	7.5	11	13.5	0
9	4	9	10	12.5	0
Average	4	7	11	12	0

Table 2. One way ANOVA statistical analysis of differences in inhibition zones for spray preparations with concentrations of 25%, 35%, 45% and vancomycin

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	369,076	3	123,025	107,858	**,000**
Within Groups	36,500	32	1,141		
Total	405,576	35			

Table 3. Post-Hoc statistical analysis of differences in inhibition zones for spray preparations with concentrations of 25%, 35%, 45% and vancomycin

(i) Formulas	(J) Formula	Mean Difference (IJ)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Concentration 25%	Concentration 35%	-2.77778*	.50346	,000	-4.1937	-1.3619
Concentration 25%	Concentration 45%	-6.77778*	.50346	,000	-8.1937	-5.3619
Concentration 35%	Concentration 45%	-4.00000*	.50346	,000	-5.4159	-2.5841
Vancomycin	Concentration 25%	8.05556*	.50346	,000	6.6397	9.4715
Vancomycin	Concentration 35%	5.27778*	.50346	,000	3.8619	6.6937
Vancomycin	Concentration 45%	1.27778	.50346	,097	-.1381	2.6937

DISCUSSION

The research used samples of lemongrass leaf ethanol extract spray preparations in concentrations of 25%, 35% and 45%. The wet weight of lemongrass leaves was obtained as much as 7 kg, then dried and made into simplicia powder as much as 4.9 kg with a water content of 3.881%. After that, it was extracted and a thick extract of 274 grams was obtained with a drying loss of 2.889%. The ethanol extract spray preparation of lemongrass leaves has a brown color, a characteristic odor of lemongrass leaves and is in liquid form. Based on analysis of the diameter of the inhibitory zone of spray preparations of ethanol extract of lemongrass leaves with a concentration of 25%, the smallest is 3 mm, the largest is 5 mm and the average diameter of the inhibition zone of 9 spray preparations of ethanol extract of lemongrass leaves against MRSA bacteria is 4 mm. The diameter of the inhibition zone for spray preparations of ethanol extract of lemongrass leaves with a concentration of 35% is at least 5 mm, the maximum is 9 mm and the average diameter of the inhibition zone for 9 spray preparations of ethanol extract of lemongrass leaves against MRSA bacteria is 7 mm. The diameter of the inhibition zone for spray preparations of ethanol extract of lemongrass leaves with a concentration of 45% is the smallest 9 mm, the largest is 13.5 mm and the average diameter of the inhibition zone of 9 spray preparations of ethanol extract of lemongrass leaves against MRSA bacteria is 11 mm.

The smallest diameter of the inhibition zone of the vancomycin antibiotic disc is 11 mm, the largest is 13.5 mm and the average diameter of the inhibition zone of 9 spray preparations of ethanol extract of lemongrass leaves against MRSA bacteria is 12 mm. These results are in line with the results of research conducted by Sarlina et al (2017) which stated that lemongrass leaf extract gel with a concentration of 20% could inhibit the growth of Staphylococcus aureus bacteria with an inhibition zone of 13.5 mm. The size of the inhibition zone in this study is smaller than the inhibition zone in the study conducted by Sarlina et al (2017) because this study used Staphylococcus aureus bacteria isolated from diabetic ulcers which were resistant to the antibiotic Methicillin.[7] The results of this study are also in line with the results of this study. Ariffialdi et al (2022) stated that the antibiotic Vancomycin is sensitive to MRSA bacteria isolated from diabetic ulcers.[15]

Based on the analysis of the differences in the inhibition zone of ethanol extract spray preparations of lemongrass leaves with concentrations of 25%, 35%, 45% and the antibiotic disc vancomycin on the growth of MRSA bacteria using the one way anova statistical test with p value = 0.00 < α 0.05 so that there are differences in the zone of inhibition of the spray extract preparations Lemongrass leaf ethanol concentrations of 25%, 35%, 45% and vancomycin antibiotic

disc. Analysis of differences in inhibition zones of spray preparations of ethanol extract of lemongrass leaves at concentrations of 25% and 35%, concentrations of 25% and 45%, concentrations of 35% and 45%, concentrations of 25% with vancomycin and concentrations of 35% with vancomycin showed p value = 0.00 < α 0.05 so there are differences in inhibition zones. Analysis of differences in the zone of inhibition of spray preparations of ethanol extract of lemongrass leaves with a concentration of 45% and vancomycin with p value = 0.097 > α 0.05

Based on the results above, it can be said that there is no difference in the zone of inhibition between the spray preparation of ethanol extract of lemongrass leaves at a concentration of 45% and the antibiotic vancomycin on the growth of MRSA bacteria, so that at a concentration of 45% the spray preparation of ethanol extract of lemongrass leaves is effective in inhibiting the growth of MRSA bacteria. Based on Davis and Stout (1971), the inhibitory zone measurement parameters of 9 spray preparations of lemongrass leaf ethanol extract with a concentration of 45% with an average inhibitory zone of 11 mm are included in the strong category.[16]

CONCLUSION

The spray preparation of ethanol extract of lemongrass leaves with a concentration of 45% is effective in inhibiting the growth of Methicillin Resistant Staphylococcus aureus (MRSA) bacteria and the average zone of inhibition of the spray preparation with ethanol extract of lemongrass leaves with a concentration of 45% against MRSA bacteria is 11 mm in the strong category.

Declaration by Authors

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