Original Research Article

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Antibacterial activities of combination of ethanol extract of Delima fruit (Punica granatum L.) and Sereh stem (Cymbopogon citratus) on Staphylococcus aureus

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ABSTRACT

Background: Pomegranates contain chemical saponins and flavonoids while lemongrass stems have flavonoid chemical compounds. Substances that can inhibit the linking of bacteria are saponins and flavonoids. The most common microorganism found in ulcus diabetikum (ulcer diabetes) is *Staphylococcus aureus*. The purpose of this study was to determine the antibacterial activity of a combination of pomegranate ethanol extract and ethanol extract of lemongrass stems against *S. aureus* bacteria.

Methods: This study used an experimental design with a complete randomized design study divided into 5 groups: groups 1 (75:25), 2 (50:50), 3 (25:75), positive control (tetracycline) and negative control. Manufacture of pomegranate peel extract and lemongrass stems was done by maceration method for further rotary, after the extract was obtained tested for antibacterial activity by diffusion method using a cylinder. With the test used analysis of variance one way.

Results: The results of this study showed that the combination of pomegranate ethanol extract and citronella stem ethanol extract showed activity against *S. aureus* with a ratio of 15 ul:5 ul, 15 ul:15 ul, 5 ul:15 ul with inhibitory diameter of respectively 13 mm, 11 mm, 8 mm. Whereas for positive control with chloramphenicol, the inhibition area is 9 mm.

Conclusions: Statistically the combination of pomegranate ethanol extract and lemongrass stems has antibacterial power which uses p=0.005 and p<0.05. The combination of pomegranate ethanol extract and lemongrass stems has very strong antibacterial activity against *S. aureus* due to inhibition zones of 10-20 mm.

Keywords: Pomegranate, Lemongrass, Staphylococcus aureus

INTRODUCTION

Diabetes mellitus is a global endemic disease, and diabetic ulcers are a serious complication and expensive medical expenses. Diabetic foot according to the World Health Organization (WHO) is a foot in diabetics who are at risk for infection, ulceration and/or tissue destruction associated with neurological and vascular abnormalities in the lower extremities. This infection occurs in patients with diabetic foot ulcers of about 58%.

Based on the results of the 2015-2018 Medan Kuman Map at the Padang Regional Hospital in Padang, the most bacteria found were *Staphylococcus aureus*. While the results of a preliminary study at Derah Padang General Hospital, the bacteria found in diabetic ulcers were *Staphylococcus aureus*, *Acinetobacter baumannii*, *Klebsiella pneumonia*, and *Proteus mirabillis*.

Management of infections in diabetic ulcers is generally done by debridement and wound care accompanied by antimicrobial treatment.³ The administration of antibiotics is determined by the results of bacterial sensitivity tests which depend on the pattern of bacteria found. Irrational antibiotics can trigger bacterial resistance. Bacteria that are resistant to antibiotics are also caused by the widespread u2010;1:12-35se of antibiotics. Therefore, the presence of antibacterial resistant bacteria will encourage the importance of extracting sources of antimicrobial drugs from natural materials.⁴

Plants that can be used as traditional medicines as antibacterial are lemongrass (*Cymbopogon citratus*) and pomegranate (*Punica granatum L.*). Lemongrass (*Cymbopogon citratus*) contain compounds consisting of citral, citronellol, geranial, neral, mirsen, terpene, cinnamaldehyde, linalool, citral, citronellal, eugenol, and phenols which are antibacterial.⁵

While pomegranate skin contains alkaloids, pelletierin, granatin, betulic acid, ursolic acid, isokuersertin, elagitanin, triterpenoid, calcium oxalate, and starch.⁶ Elagitanin is an ingredient that is suspected to have antibacterial activity. In previous studies, pomegranate peel methanol extract has been shown to have activity against the bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.⁷ Pomegranate rind extract and ethanol extract of lemongrass stems were also reported to have decreased blood sugar activity. So that its use other than as an antibiotic can also be a decrease in blood sugar.⁸

The purpose of this study was to determine the antibacterial activity of a combination of pomegranate ethanol extract and citronella stem ethanol extract against *Staphylococcus aureus* bacteria.

METHODS

This research is a quantitative study using a quassi experiment design with an experimental design with a randomized study design divided into 5 groups: groups 1 (75:25), 2 (50:50), (25:75), positive control (tetracycline), negative control. This research was conducted at the Regional Health Laboratory of West Sumatra Province. The was held on 2nd May 2019 to 5th July 2019. With the inclusion criteria of ripe pomegranate skin and fresh lemongrass stems. Lemongrass is obtained from the depths of minturun, Indonesian desert, pomegranate is obtained from Lembang, West Java in Indonesia. Staphylococcus aureus is a standard bacterium from the regional health laboratory. The study was divided into 5 groups, negative controls with distilled water, positive controls with chloramphenicol 15 ul, groups 1 ((15 ul:5 ul), 2 (15 ul:15 ul, 3 (5 ul:15 ul)) respectively for lemongrass: pomegranate, the positive control used in this study was chloramphenicol at a dose of 15 ul. Data collection was carried out by making pomegranate peel extract and lemongrass stems carried out by maceration method then rotated, after extracting it was tested for antibacterial activity by diffusion method using a cylinder. Data was presented in tabular form and narrative text using analysis of variance (ANOVA) one way statistical test.

RESULTS

The results of the study in Table 1 obtained calculations that have been done, the largest mean diameter of the inhibitory zone is in group K (+), which is 9.3 mm, then followed by group 1 by 13.3 mm, group 2 is 11, 6 mm, group 3 was 11.6 mm, and the negative control group did not have inhibition because the mean diameter of the inhibition zone was 0.00 mm.

Table 1: Diameter of inhibition zone of pomegranate ethanol extract and citronella stem ethanol extract against *Staphylococcus aureus* bacteria by diffusion method.

Treatment	Deuteronomy (mm)			Mean
	1	2	3	(mm)
Positive control	11	8	9	9,3
Negative control	0	0	0	0
1 (15 ul:5 ul)	15	12	13	13,3
2 (15 ul:15 ul)	13	11	11	11.6
3 (5 ul:15 ul)	8	9	7	8

Data on mean diameter of inhibition zones in each sample group were then analyzed statistically to find out whether the data in each sample group was normally distributed or not by using the Kolmogorov-Smirnov normality test. Kolmogorov-Smirnov test results obtained significance value (p) greater than 0.05 so it can be concluded that the data are normally distributed. After the data is known to be normally distributed, it is followed by a homogeneity test using the Levene's test. Levene's test results show a significance value (p) greater than 0.05 so that it can be concluded that the data is homogeneous. Based on the Kolmogorov Smirnov test results and the Levene's test, the research results obtained are normally distributed and homogeneous so that it is continued with the One Way ANOVA parametric statistical test. One way ANOVA test results obtained significance value (p) is smaller than 0.05. This shows that there are differences in all study groups. The statistical test was continued with the LSD (least significant difference) test to find out if there were significant differences between the study groups. LSD test results showed that p<0.05.

DISCUSSION

The results showed that the average value of the inhibition zone diameter of the positive control group was 9.3 mm, group one was 13.3 mm, group two was 11.6, group three was 8 mm, whereas the negative control group did not have inhibitory zones from the results of the study It is seen that the higher the inhibition

concentration of pomegranates the greater the diameter of the inhibition zone formed. $^{9\text{-}11}$

Antibacterial properties can be distinguished by its strength. 12-16 According to Davis and Stout, the antibacterial power strength criteria are divided into four groups, namely inhibition zone diameters of 5 mm or less are categorized as weak, diameter inhibition zones 5-10 mm are categorized as medium, diameter inhibition zones 10-20 mm are categorized as strong and inhibitory zones are 20 mm or more categorized as very strong (Jannah, 2014). 17-25

According to the general standards issued by the National Committee for Clinical Laboratory Standards (NCCLS), disistasi from Tambekar and Dahikar (2010) i.e., bacteria are declared sensitive to antibacterial originating from plants if they have a diameter of inhibition diameter greater than 12 mm.²⁶ And supported by Putra's research, it is stated that *S. aureus* is sensitive to pomegranate ethanol extract and citronella stem ethanol extract.²⁷ And also supported by Tabekar's research, it means that *S. aureus* is sensitive to pomegranate ethanol extract and citronella stem ethanol extract and citronella stem ethanol extract.²⁸

Based on research data the inhibition zone diameter of the combination group of pomegranate extract and ethanol extract of citronella stem 15 ul:5 ul, 15 ul:15 ul, 5 ul:15 ul were 13.3 mm, 11, respectively 6 mm, 8 mm so that it is included in the strong category. Group K (+) is included in the medium category because the inhibition zone diameter is 9 mm, while group K (-) has no antibacterial power. Based on these categories, it can be seen that the extract of red pomegranate with a concentration of 15 ul:5 ul has a good ability to inhibit the growth of *S. aureus* with a strong category.

After the one way ANOVA test, it was continued with the Least significant difference (LSD) test to find out if there were significant differences between the study groups. LSD test results showed that there were significant differences between the study groups marked by a significance value (p) smaller than 0.05, except in group 2 with group 3. This showed that the red pomegranate extract concentration of 75% had almost the same potential with red pomegranate extract concentration of 100% in inhibiting the growth of S. aureus. This is because at 100% concentration the consistency of the extract material is almost dense so that the active substance contained in the concentration is not effective to diffuse into the disk and so that the bacterial inhibition results are not much different from the extract concentration of 75%.29

Inhibition zones formed around the disk which had been dripped with red pomegranate extract showed that the extract contained active compounds that were as antibacterial. The antibacterial content in red pomegranates is polyphenols (flavonoids, anthocyanins,

and tannins including ellagic acid, ellagitannins and punicalgin).³⁰ Each of these active substances has different mechanisms as antibacterial. The mechanism of action of flavonoids as antibacterial compounds is divided into three, namely inhibiting the synthesis of nucleic acids, inhibiting the function of cell membranes, and inhibiting energy metabolism (Hendra, 2011).

The mechanism in inhibiting nucleic acid synthesis is by inhibiting the formation of DNA and RNA through rings A and B which play a role in hydrogen bonds. This causes a buildup of nucleic acid bases, and damage to the permeability of bacterial cell walls, lysosomes, and microsomes. The mechanism in inhibiting the function of cell membranes is to form complex compounds with extracellular and dissolved proteins that cause damage to bacterial cell membranes and followed by the release of intracellular compounds. While the mechanism of flavonoids in inhibiting energy metabolism is by inhibiting cytochrome C reductase and inhibiting the use of oxygen in bacteria. Even though energy is needed by bacteria in doing macromolecular biosynthesis. The series of the control of the property of the pr

Alkaloids are heterocyclic nitrogen compounds that contain at least one nitrogen atom and are basic. These base groups will react with acidic compounds present in bacterial cells such as DNA which is the main constituent of cell nuclei. With the disruption of DNA, the synthesis of protein and nucleic acids in cells will be disrupted.³²

Apart from the ethanol extract of pomegranates the antibacterial activity also comes from the ethanol extract of lemongrass stems. The content of chemical compounds found in lemongrass stems such as saponins, flavonoids, alkaloids, tannins, terponoids and essential oils can completely inhibit the growth of *S. aureus*. Saponin mechanism interacts with sterols in cell membranes, causing leakage of certain proteins and enzymes. Flavonoids function as antioxidants and antimicrobials can prevent lipid oxidation in *S. aureus* cell walls. Besides tannins also function to damage the cell walls of *S. aureus*. This is what causes the formation of inhibitory zones on media. ³³

Chloramphenicol is an antibiotic that can be used for the treatment of *S. aureus* infections.³⁴ The increasingly widespread use of antibiotics has led to the emergence of antibiotic-resistant bacteria that has prompted the importance of extracting sources of antimicrobial drugs from natural materials. one natural substance is believed to have antibacterial activity, one of which is pomegranate which contains elagitanin which is thought to have antibacterial activity.³⁵ The lemongrass contains essential oils which have antibacterial activity.

CONCLUSION

Statistically, the combination of pomegranate ethanol extract and lemongrass stems has antibacterial power

which uses p=0.005 and <0.05. The combination of pomegranate ethanol extract and lemongrass stems has very strong antibacterial activity against *S. aureus* due to inhibition zones of 10-20 mm.

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