ANTIBACTERIAL ACTIVITIES TEST OF RED GINGER (*Zingiber officinale var. rubrum)* ETHANOL EXTRACT ON *STAPHYLOCOCCUS EPIDERMIDIS*

Andrea Budiyanto[1)], Mulya Dinata[2)], Endang Isbandiati[3)]

Correspondent Email : andrea.budianto@gmail.com

DOI: https://doi.org/10.33508/jwmj.v4i2.3791

ABSTRACT

Introduction: Antibiotic is a drug often used against infections caused by bacteria; however, irrational use of antibiotics can cause multidrug resistance (MDR). *Staphylococcus epidermidis* is a bacteria that has developed an MDR characteristic against antibiotics. *Staphylococcus epidermidis* is a normal opportunistic flora. Said bacteria can produce a biofilm that enables the bacteria to adhere to prosthetic devices and enter the bloodstream. Red ginger (*Zingiber officinale var. rubrum*) is a medicinal plant considered antibacterial for its secondary metabolites.
Purpose: This study aims to determine the antibacterial effect of red ginger (*Zingiber officinale var. rubrum*) ethanol extract against *Streptococcus epidermidis* using *a non-equivalent control group design study*.
Method: Red ginger (*Zingiber officinale var. rubrum*) ethanol extract was made with the maceration method. This study uses a microdilution test on 96 well plates. The absorbance value was read using a spectrophotometer to determine the Minimum Inhibitory Concentration (MIC) value, and streaking agar was used to find the Minimum Bactericidal Concentration (MBC) value.
Result: The result shows that the red ginger (*Zingiber officinale var. rubrum*) ethanol extract at a concentration of 62,5 µg/mL inhibits 75% of bacteria growth, at 125 µg/mL inhibits 60% of bacteria growth, at 250 µg/mL inhibits 45% of bacteria growth, at 500µg/mL inhibits 65% of bacteria growth and at 1.000µg/mL inhibits 50% of bacteria growth. The streaking agar test result shows bacteria growth on every concentration.
Conclusion: Red ginger (*Zingiber officinale var. rubrum*) ethanol extract in the concentration range of 62,5-1.000 µg/mL have no MIC and MBC value against *Staphylococcus epidermidis.*

Keyword: *Antibiotic, Multidrug-resistant, Staphylococcus epidermidis, Zingiber officinale var. rubrum*

[1)] *Faculty of Medicine, Widya Mandala Catholic University Surabaya*
E-mail : andrea.budianto@gmail.com
[2)] *Department of Clinical Pathology, Faculty of Medicine, Widya Mandala Surabaya Catholic University*
[3)] *Department of Pharmacology, Faculty of Medicine, Widya Mandala Surabaya Catholic University*

INTRODUCTION

Antibiotic is a type of drug often used against infections caused by bacteria (tuberculosis, typhus, diphtheria, and others); however, irrational use of antibiotics (excessive use, antibiotics sold over-the-counter) and poor hygiene can cause *antimicrobial resistance* (AMR). AMR poses a threat to human and environmental health as it could cause the emergence of *multidrug-resistant* (MDR) bacteria[1]. MDR bacteria is a type of organism that has developed resistance against an antibiotic agent from three different groups of antimicrobial drugs[2]. *Staphylococcus epidermidis* is a type of bacteria that has developed an MDR characteristic.

Staphylococcus epidermidis is a coagulase-negative and Gram-positive bacteria[3]. *Staphylococcus epidermidis* is an opportunistic normal flora[3]. Most *coagulase-negative staphylococci* infections and bacteremia cases in the implanted medical device (IMD) are caused by *Staphylococcus epidermidis*[3].

Staphylococcus epidermidis can form a biofilm, allowing bacteria accumulation on prosthetic devices and insertion into the bloodstream[3]. Biofilm is an extracellular layer that creates bacteria protection against antibiotics and hosts defense mechanism[4]. In 2010 as many as ten general education hospitals in Indonesia recorded the incidence of nosocomial infections by 6-16%[5].

An alternative drug using natural ingredients that can be easily found and are well known to Indonesian people need to be developed to combat the decrease of antibiotics effectiveness caused by the increase of resistance. Ginger (*zingiber Officinale)* is one of many plants in Indonesia that is often used to cure illness. Like medicine, the secondary metabolites of ginger, namely flavonoids, phenols, terpenoids, and essential oils, can inhibit the growth of pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus,* dan *Penicillium sp*. [6,7]. One type of ginger that is often used as medicine is red ginger (*zingiber officinale var. rubrum)* due to the high content of red ginger essential oil, which is 2,58-3,72% of its dry weight, and the oleoresin content, which reaches up to 3% of its dry weight[7].

METHOD

This research uses red ginger powder acquired from Materia Medica, Batu, Malang. The powder is then extracted by Laboratorium Penelitian Fakultas Farmasi Universitas Katolik Widya Mandala Surabaya using maceration method for a week, and the *Staphylococcus epidermidis* bacteria suspension is acquired from Balai Besar Laboratorium Kesehatan (BBLK) Surabaya. The samples were divided into control and treatment groups. The control group (K1-K5) contained *Mueller Hinton Broth* + red ginger ethanol extract (*Zingiber officinale var. rubrum*) concentration of 62,5 µg/mL (K1), 125 µg/mL (K2), 250 µg/mL (K3), 500 µg/mL (K4), and 1.000 µg/mL (K5). The treatment group (P1-P5) contained *Mueller Hinton Broth + S. epidermidis* suspension + red ginger ethanol extract (*Zingiber officinale var. rubrum*) concentration of 62,5 µg/mL (P1), 125 µg/mL (P2), 250 µg/mL (P3), 500 µg/mL (P4), and 1.000 µg/mL (P5). Positive control (K6) contained *Muller Hinton Broth + Staphylococcus epidermidis+ Penicillin*, and negative control (K7) contained *Mueller Hinton Broth + Staphylococcus epidermidis + dimethyl sulfoxide.*

This research uses the microdilution method to determine the value of Minimum Inhibitory Concentration (MIC) and streaking agar test to determine the Minimum Bactericidal Concentration (MBC) value. The microdilution result is incubated for 24 hours, followed by optical density reading using spectrophotometry at 600 *nm*. *Mueller Hinton Agar* is used as a streaking media to determine the MBC value by visual observation after incubation for 24 hours.

RESULTS

The inhibitory percentage graph in **figure 1** shows that the red ginger (*Zingiber officinale var. rubrum*) ethanol extract at a concentration of 62,5 µg/mL inhibits 75% of bacteria growth, at 125 µg/mL inhibits 60% of bacteria growth, at 250 µg/mL inhibits 45% of bacteria growth, at 500µg/mL inhibits 65% of bacteria growth and at 1.000µg/mL inhibits 50% of bacteria growth. In conclusion, the MIC value of red ginger ethanol extract is not in the concentration range of 62,5 µg/mL-1.000 µg/mL.

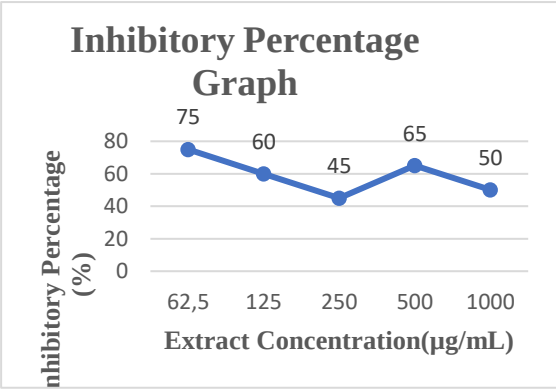

Figure 1. Inhibitory Percentage Graph

The observation result of treatment group P1 with an extract concentration of 62,5 µg/mL shows a looser bacteria colony (white speckles) compared to the other treatment groups, indicating *zingiber Officinale var. rubrum* ethanol extract inhibits *Staphylococcus epidermidis* growth. Treatment group P2 (125 µg/mL) shows a denser bacteria colony than P1. Treatment group P3 (250 µg/mL) shows looser bacteria growth compared to P2. Treatment group P4 (500 µg/mL) shows looser bacteria growth compared to P2 and P3. Treatment group P5 (1.000 µg/mL) shows looser bacteria growth compared to P2.

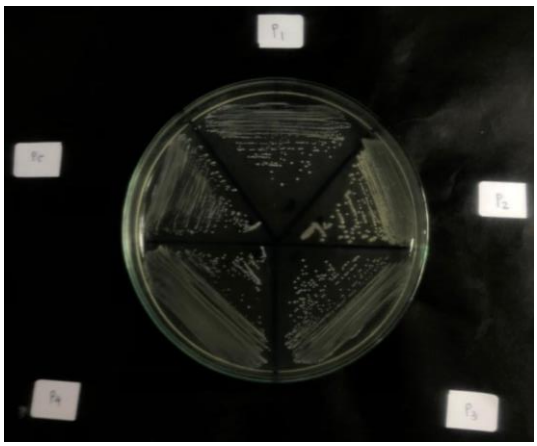

Figure 2. Streaking Agar Results of Treatment Group P1-P5

According to the inhibitory percentage graph (**figure 1**), concentration 62,5 µg/mL has a higher inhibitory percentage than the other concentrations. Concentration 500 µg/mL has a higher inhibitory percentage compared to 125 µg/mL and 1.000 µg/mL. concentration 250 µg/mL has the lowest inhibitory percentage of the other concentration. In conclusion, the MBC value of red ginger ethanol extract is not in the concentration range of 62,5 µg/mL-1.000 µg/mL.

DISCUSSION

The spectrophotometer result shows that red ginger (*Zingiber officinale var. rubrum*) ethanol extract does not meet the MIC value criteria. The highest inhibitory percentage was 75% at 62,5 µg/mL, while the other concentrations showed an inhibitory percentage lower than 75%.

The streaking test result on MHA (**figure 2**) did not meet the MBC criteria. The P1-P5 treatment group shows *Staphylococcus epidermidis* bacteria growth in which P1 showed a sparser density than P2-P5, which means P1 has a better ability to inhibit bacteria growth than the treatment group P2-P5.

Several factors are suspected to be the cause of MIC and MBC not being fulfilled. The first factor is the resistance of *Staphylococcus epidermidis*. *Staphylococcus epidermidis* can form a

biofilm layer that reduces bacteria sensitivity to antibacterial components and the host's immune system. This statement is supported in a review titled *"Staphylococcus epidermidis device-related infections: pathogenesis and clinical management"* written by Maureen McCann (2008)[8] in *Journal of Pharmacy and Pharmacology*. The review states that *Staphylococcus epidermidis* has a very resistant biofilm layer that creates bacteria protection against the host's immune system and lowers bacteria sensitivity towards harmful molecules such as antibiotics, cytokines, and antibacterial peptides, decreasing inflammation reaction on site of infection. *Staphylococcus epidermidis* also has *multidrug-resistant* (MDR) characteristics, making said bacteria more resistant than other bacteria[8].

The second factor is the concentration of the extract used in this research. Firnanda's research (2018)[9] using red ginger (*Zingiber officinale var. rubrum*) extract using tube dilution and disc diffusion method to *Staphylococcus aureus* bacteria show a MIC value at a concentration of 1.000 µg/mL. The concentration used in this research follows the concentration from previous research done by Firnanda (2018)[9]. It changes the solvent to ethanol 96% hoping that the red ginger's secondary metabolite component can be better extracted since the higher the solvent's concentration, the greater the component can be extracted[10]. This statement is supported in Luginda's (2018) research using *Pluchea Indica* leaves extracted using ethanol 60%, 70%, 80%, and 96%. The average yield of *Pluchea Indica* leaves shows that ethanol 96% has the best ability to extract compounds[10]. The different methods and bacteria used in the research may also influence the MIC and MBC values even though the concentration of the extracts are the same.

The third factor is the methods used in making the extract. Kartika did the research (2013)[6] using fresh red ginger extract against *S. aureus* dan *E. coli* resulted in the inhibitory zone of 15,83 mm and 15,33 mm. In this research, the extract was made using the maceration method, where the red ginger was made into powder and packed before it was extracted. The process of making the red ginger into powder and the packing process was presumed to harm or oxidize the compounds contained in the red ginger before the maceration process.

CONCLUSIONS

Red ginger (*Zingiber officinale var. rubrum*) ethanol extract in the concentration range of 62,5-1.000 µg/mL has no MIC and MBC value against *Staphylococcus epidermidis.*

REFERENCES

1. Wang W, Arshad MI, Khurshid M, Rasool MH, Nisar MA, Aslam MA, et al. Antibiotic resistance : a rundown of a global crisis. Infect Drug Resist. 2018;1645–58.
2. Sweeney MT, Lubbers B V., Schwarz S, Watts JL. Applying definitions for multidrug resistance, extensive drug resistance and pan drug resistance to clinically significant livestock and companion animal bacterial pathogens. J Antimicrob Chemother. 2018;73(6):1460–3.
3. Scarborough M. Color Atlas of Medical Microbiology, 2nd ed. Vol. 65, Journal of Hospital Infection. 2007. 91 p.
4. Cabrera-Contreras R. Antibiotic Resistance and Biofilm Production in Staphylococcus epidermidis Strains, Isolated from a Tertiary Care Hospital in Mexico City. ISRN Microbiol. 2013;97(29):1503–7.
5. Zakaria AA, Sofiana L. Correlation between nurse knowledge and attitude with hand hygiene compliance. J Kedokt dan Kesehat Indones. 2018;9(2):74–81.
6. Sari, Kartika Indah Permata, Periadnadi, Nasir N. Uji Antimikroba Ekstrak Segar Jahi-

Jahan (Zingiberaceae) Terhadap Staphylococcus aureus , Escherichia coli dan Candida albicans Antimicrobial test of ginger fresh extract (Zingiberaceae) against Staphylococcus aureus , Escherichia coli and Candida al. J Biol Univ Andalas. 2013;2(1):20–4.

7. Putri DA. Pengaruh Metode Ekstraksi dan Konsentrasi terhadap Aktivitas Jahe Merah sebagai Antibakteri. J Penelit Bengkulu. 2014;1–46.
8. McCann MT, Gilmore BF, Gorman SP. <I>Staphylococcus epidermidis</I> device-related infections: pathogenesis and clinical management. J Pharm Pharmacol. 2008;60(12):1551–71.
9. Dewi FI, Wahyunitisari MR. AKTIVITAS DAYA HAMBAT EKSTRAK JAHE MERAH (ZINGIBER OFFICINALE VAR RUBRUM) TERHADAP PERTUMBUHAN KUMAN STAPHYLOCOCCUS AUREUS. J Vocat Heal Stud [Internet]. 2018;01(01):113–6. Available from: www.e-journal.unair.ac.id/index.php/JVHS
10. Luginda RA, Lohita B, Indriani L. PENGARUH VARIASI KONSENTRASI PELARUT ETANOL TERHADAP KADAR FLAVONOID TOTAL DAUN BELUNTAS (Pluchea indica (L.)Less) DENGAN METODE Microwave – Assisted Extraction (MAE). 2018;