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ANTIMICROBIAL EFFECT OF ARROWROOT (*Maranta arundinacea* L.) METHANOLIC EXTRACT AGAINST *Staphylococcus aureus* BACTERIAL GROWTH

Muhammad Ghaza Syahputra[1], Arlita Leniseptaria Antari[2], Winarto [2], Endang Sri Lestari [2]
[1] Undergraduate Student, Faculty of Medicine, Diponegoro University
[2] Lecturer, Department of Microbiology, Faculty of Medicine, Diponegoro University

ABSTRACT

Introduction: *Staphylococcus aureus* is a spherical gram-positive bacterium that forms clusters and is frequently resistant to antibiotics. Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus* that is resistant to beta-lactam and cephalosporin groups of antibiotics, it is one of the biggest problems in resistant bacteria. Plants that have flavonoids in their extract can inhibit the growth of bacteria by impairing their cytoplasmic membrane. Arrowroot is an example of plants that have flavonoids in their extracts. **Objectives:** This research aims to determine the antimicrobial effect of arrowroot (*Maranta arundinacea* L.) methanolic extract against the growth of *Staphylococcus aureus* in vitro. **Methods**: This is true experimental research with a post-test control group design with two control groups and eight experimental groups. The sample of this research utilizes the MRSA strain of *S. aureus* received from the Microbiology Laboratory of Diponegoro University Faculty of Medicine that was eligible with the inclusion criteria. The research was conducted by using arrowroot extract concentrations 6.5%, 12.5%, 25%, 50%, and 100% to determine the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and inhibitory zone diameter. **Results:** MIC and MBC of arrowroot methanolic extract against MRSA is 100%. The arrowroot methanolic extract to have yielded an inhibitory zone diameter is 100% with a mean inhibitory zone diameter of 15.5mm. **Conclusion:** Arrowroot methanolic extract can inhibit the bacterial growth of MRSA

Keywords: *Staphylococcus aureus,* growth, *M. arundinacea* L. methanolic extract

INTRODUCTION

Infection caused by bacteria is one of the biggest issues in developing countries because it causes 50.000 deaths each day. (Permenkes, 2011; Nirosha dan Mangalanayaki, 2013) Antibiotics are the most frequently used agent to resolve this problem, however, there are two massive problems in the usage of antibiotics to cure infections caused by bacteria which are rationality of use and resistance. (Sufi, 2015) Resistance, in this case, means that the bacteria can still grow within the chemical that was meant to kill the bacteria or inhibit its growth. (NIAID, 2009) The main cause of bacterial resistance against an antibiotic is the irrational use of the drug. (Permenkes, 2011)

Resistance may occur through two mechanisms which are selection pressure and dissemination. Selection pressure means the growth of resistant bacteria in the human body which increases the number of resistant bacteria. The dissemination mechanism is the spread of resistance through plasmids. (Permenkes, 2011)

Staphylococcus aureus is a spherical gram-positive bacteria that forms clusters.S. aureus acts as a commensal bacteria that live predominantly in the throat and nasal cavity. (Brooks *et al.*, 2013; Kuntaman *et al.*, 2016) *S. aureus* is a species of bacteria that is frequently resistant to antibiotics.

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Methicillin-Resistant *S. aureus* (MRSA) is a strain of S. aureus that is resistant to beta-lactam and cephalosporin groups of antibiotics, it is one of the biggest problems in resistant bacteria, the prevalence of MRSA in the human throat and nasal cavity is 5.4% in the throat and 1.2% in the nasal cavity. (Al-Quorain, 2016; Kuntaman *et al.*, 2016)

Indonesia has a large amount of plant that has antimicrobial properties, particularly plants that have flavonoids in their extract. Flavonoids can inhibit the growth of bacteria by impairing its cytoplasmic membrane (Manik, T dan H, 2014). Arrowroot is an example of plants that have flavonoids in their extracts and it can be found easily throughout Indonesia. Arrowroot has been proven in research done by Amrutha Jayakumar to have bioactive properties such as alkaloids, glycosides, flavonoids, terpenes, saponins, and tannin. (Jayakumar dan Suganthi, 2017)

Many pieces of research investigate the antimicrobial effects of plant material, one of which is the antimicrobial effect of *S. indicus* extract against *Eschericia coli* and *Klebsiella pneumoniae*. The result of said research is *S. indicus* extract has antimicrobial effect against *E. coli* and *K. pneumoniae*. (Irfan, Ahmed dan Sharma, 2014)

Not many information as regards to the antimicrobial effect of arrowroot (*Maranta arundinacea L.*) against *S. aureus*. Based on the reasons stated above, the researcher is interested to investigate the antimicrobial effect of arrowroot methanolic extract against *S. aureus*.

METHODS

This research is true experimental research with a post-test control group design with two control groups and eight experimental groups. The sample of this research utilizes the Methicillin-Resistant *S. aureus* (MRSA) strain of *S. aureus* received from the Microbiology Laboratory of Diponegoro University Faculty of Medicine. The inclusion criteria consist of MRSA that has been cultured in a growth medium and incubated at 37oC for 1 x 24 hours. The exclusion criteria consist of MRSA cultured that has been contaminated by fungi and other species of bacteria. Based on the sample determination formula the experiment is repeated 3 times.

The independent variables in this research consist of arrowroot extract concentrations 6,25%, 12,5%, 25%, 50%, and 100%. The dependent variable in this research is the growth of MRSA.

For the dilution method, the experiment is conducted by preparing 7 tubes of bacterial suspensions which consists of MRSA in Mueller Hinton Broth (MHB) equal to McFarland 0,5 which is then given equal amount of arrowroot methanolic extract concentrations 6.25%, 12.5%, 25%, 50%, 100%, and positive and negative control. It is then incubated at 37oC for 1 x 24 hours. The concentration that yields clear tubes are then cultured in Mueller Hinton Agar (MHA) using the streak plate method and then incubated at 37oC for 1 x 24 hours. This is done to determine the sterility of the clear tubes.

For the diffusion method, the experiment is conducted by streaking the bacterial suspension on MHA that has been given 6 wells, then the arrowroot extracts concentrations 6.25%, 12.5%, 25%, 50%, 100%, and positive control are poured into the wells. It is then incubated at 37oC for 1 x 24 hours. This is done to determine the inhibitory zone diameter of arrowroot methanolic extracts.

ETHICS APPROVAL

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Before data collection, ethical approval was obtained from the Medical Research Ethical Committee of Kariadi. The Ethical Clearance of this research was No. 128/EC/KEPK/FK-UNDIP/X/2019.

DATA ANALYSIS

The obtained data were processed using a computer program and firstly analyzed with a normality test. The hypothesis regarding the differences between MIC and MBC between the intervention group and positive control was tested using the Kruskal Wallis test because data were not normally distributed. The differences of inhibitory zone diameter between the intervention group and positive control were tested using the Kruskal Wallis test because data were not normally distributed.

RESULTS

The dilution method results showed that the concentration of arrowroot methanolic extracts began to show clarity in MRSA was 100%. Arrowroot concentrations 50%, 25%, 12.5%, and 6.25% all showed turbidity. It can be determined that the MIC of arrowroot methanolic extracts is 100%. The diffusion method results showed that the concentration of arrowroot methanolic extracts began to show inhibitory zone in MRSA was 100% with a mean diameter of 15.88 mm, which is still not as good as the inhibitory zone diameter of vancomycin with a mean diameter of 28.75 mm. It is further proven by the results of the Mann Whitney test which shows that there is a significant difference between the inhibitory zone diameter of arrowroot methanolic extract concentration 100% and vancomycin.

Table 1. Arrowroot Methanolic Extract MIC Results

Groups	Repetition 1	Repetition 2	Repetition 3	Repetition 4
Positive Control	Clear	Clear	Clear	Clear
Negative Control	Turbid	Turbid	Turbid	Turbid
100%	Clear	Clear	Clear	Clear
50%	Turbid	Turbid	Turbid	Turbid
25%	Turbid	Turbid	Turbid	Turbid
12,5%	Turbid	Turbid	Turbid	Turbid
6,25%	Turbid	Turbid	Turbid	Turbid

Table 2. Arrowroot Methanolic Extract MBC Results

Groups	Repetition 1	Repetition 2	Repetition 3	Repetition 4
Positive Control	Growth -	Growth -	Growth -	Growth -
Negative Control	Growth +	Growth +	Growth +	Growth +
100%	Growth -	Growth -	Growth -	Growth -

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Table 3. Arrowroot Methanolic Extract Inhibitory Zone Diameter Results

Groups	Repetition 1	Repetition 2	Repetition 3	Repetition 4
Positive Control	29mm	28,5 mm	29 mm	28,5 mm
Negative Control	–	–	–	–
100%	16mm	16mm	16mm	15,5mm
50%	–	–	–	–
25%	–	–	–	–
12,5%	–	–	–	–
6,25%	–	–	–	–

DISCUSSION

The results showed that the MIC and MBC for arrowroot methanolic extract against MRSA was 100%. This shows that arrowroot methanolic extract affects bacterial growth MRSA. Arrowroot contains a variety of biologically active plant chemicals including flavonoids and terpenoids which in some studies have antibacterial effects. (Jayakumar dan Suganthi, 2017)

There are several antibacterial mechanisms of flavonoids which are the inhibition of nucleic acid synthesis, cytoplasm membrane function, and energy metabolism. Other mechanisms include forming complexes with proteins through nonspecific reactions such as hydrogen bonds and hydrophobic effects and by forming covalent bonds. (Cushnie dan Lamb, 2005; Kumar dan Pandey, 2013; Ren *et al.*, 2014) Terpenoids have a toxic effect on the structure and membrane function of a bacteria, terpenoids are also able to inhibit bacterial respiration and ion transport. (Trombetta *et al.*, 2005)

The results also showed that the minimum concentration of arrowroot methanolic extract that forms inhibitory zone diameter is 100% with a mean inhibitory zone diameter of 15.5mm while the mean inhibitory zone diameter for vancomycin against MRSA is 28.5mm. the data analysis shows that there is a significant difference between the inhibitory zone diameter of arrowroot methanolic extract and vancomycin. This is maybe due to the bioactive compounds that have an antibacterial effect in arrowroot methanolic extract is not enough to equal the antimicrobial effect of vancomycin.

This study has a weakness in which the concentrations tested were only 100%, 50%, 25%, 12.5%, and 6.25%, so there is a possibility of inaccuracies in the MIC and MBC test. In the dilution study of arrowroot methanolic extract against MRSA, it was found that only the 100% concentration was clear so the MIC could be between 100% and 50%.

The strength of this research is that this research utilizes two methods in antimicrobial susceptibility testing which are dilution and diffusion tests to determine the MIC, MBC, and inhibitory zone diameter of arrowroot methanolic extract against MRSA where other researches only measure the inhibitory zone diameter of an extract.

CONCLUSION AND SUGGESTIONS

Conclusion

The results of this study conclude that arrowroot methanolic extract can inhibit

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the growth of MRSA with the most effective concentration is 100%.

Suggestions

Further research is needed regarding the antimicrobial effect of arrowroot methanolic extract against the bacterial growth of MRSA with a shorter interval of concentration, the synergistic effect of arrowroot methanolic extract and other antimicrobial substances, and the antimicrobial effect of arrowroot extract using a different solvent other than methanol.

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