

EFFECTIVENESS OF WRESAH (AMOMUM DEALBATUM) EXTRACT IN INHIBITING THE GROWTH OF STAPHYLOCOCCUS AUREUS

Ajeng Dian Pertiwi¹[™] [□], Musparlin Halid² [□]

¹Department of Pharmacy, Politkenik Medica Farma Husada Mataram ²Department of Medica Record and Health Information, Politeknik Medica Farma Husada Mataram

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ABSTRACT

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Corresponding Author:

Wresah plant (Amomum dealbatum) is one of the typical fruit species from Lombok Island which has a distinctive fragrance and is a member of the ginger-ginger tribe. This study aims to test the ethanol extract of Wresah skin (Amomum dealbatum) as an antibacterial (Staphylococcus aureus) with concentrations of 15%, 30% and 50%. The research method conducted was laboratory experimental. The antibacterial test of Staphyloccocus aureus was carried out with five treatment groups and five repetitions, namely positive control (ciprofloxacin), negative control (distilled water), concentrations of 15%, 30% and 50% using well method and then incubated for 24 hours at a temperature of 370 C. The results of the antibacterial test of ethanol extract of Wresah fruit peel showed inhibition of Staphylococcus aureus bacteria and at concentrations of 15%, 30%, and 50% which produced the largest diameter of the inhibition zone with an average diameter of 2.0 mm. The Kruskal Wallis test showed a statistically significant difference between the three concentrations tested (p < 0.05), which means that Wresah fruit peel extract is able to inhibit the growth of Staphylococcus aureus bacteria. In conclusion, the extract of Wresah fruit peel has demonstrated antibacterial properties against Staphylococcus aureus and can be used as a natural alternative to conventional antibiotics.

ABSTRAK

Tanaman wresah (Amomum dealbatum) adalah salah satu jenis buah khas dari Pulau Lombok yang memiliki wangi yang khas dan termasuk dalam suku jahe-jahean. Penelitian ini bertujuan untuk menguji ekstrak etanol kulit buah wresah (Amomum dealbatum) sebagai antibakteri (Staphylococcus aureus) dengan konsentrasi 15%, 30% dan 50%. Metode penelitian yang dilakukan adalah eksperimental laboratorium. Uji antibakteri Staphyloccocus aureus dilakukan dengan lima kelompok perlakuan dan lima kali pengulangan, yaitu kontrol positif (siprofloksasin), kontrol negatif (akuades), konsentrasi 15%, 30% dan 50% dengan metode sumuran kemudian diinkubasi selama 24 jam pada suhu 370 C. Hasil uji antibakteri ekstrak etanol kulit buah wresah menunjukkan adanya daya hambat terhadap bakteri Staphylococcus aureus dan pada konsentrasi 15%, 30%, dan 50% yang menghasilkan diameter zona hambat paling besar dengan diameter rata-rata 2,0 mm. Hasil uji Kruskal Wallis, menunjukkan nilai signifikansi Asymp signifikansi sebesar 0,000 atau p < 0,05 yang berarti ekstrak kulit buah wresah mampu menghambat pertumbuhan bakteri Staphylococcus aureus. Dengan demikian, ekstrak kulit buah Wresah dapat digunakan sebagai antibakteri.

Ajeng Dian Pertiwi Department of Pharmacy, Politeknik Medica Farma Husada Mataram Telp. 081237920249 Email: addian90@gmail.com

INTRODUTION

An infectious disease is caused by a biological agent, such as a virus, bacterium, or parasite. It can be transmitted from one person to another, either directly or through an intermediary. Broadly speaking, infectious diseases can be transmitted through direct media, i.e. from person to person, for example through the skin surface. The air media is referred to as an airborne disease, an example of a disease that can be transmitted and spread directly or indirectly through the respiratory air. Through the water, the medium is referred to as waterborne disease or water-related disease, an example of a disease transmitted through water. Through the medium of vectors, often referred to as vector-borne

diseases are diseases that are often endemic or epidemic and often pose a danger of death. Generally, this type of infectious disease is also called environment-based disease. This is because the onset of the disease is caused by human interaction with the surroundings that have the potential for disease. Infectious diseases are a major contributing factor to the high morbidity and mortality rates in the world. Infectious disease is a disease factor that is widely suffered in Indonesia and in the world. In addition to viruses, bacteria are also one of the causes of infection (Tun, 2018).

Our bodies are exposed to viruses, parasitic fungi and bacteria all the time. Serious physiological abnormalities or even death are caused by infectious agents that attack the body to the internal organs (I. A. Dewi & Adhi, 2016). In addition to being exposed to pathogenic infections, we are often exposed to infections by excessive levels of the normal flora that can cause disease, such as *Staphylococcus sp*, *Escherichia coli*, *Streptococcus sp*, and others. *Staphylococcus sp* bacteria can cause ulcers, pneumonia, meningitis, urinary tract infections and others (Rizky, 2018).

Epidemiological studies show that infections caused by *Staphylococcus aureus* in the world have increased in the last two decades. Data in the United States and Europe show that *Staphylococcus aureus* is the most common pathogenic bacteria causing infections with a prevalence of 18-30% (Toy et al., 2015). Meanwhile, in Asia and Indonesia, *Staphylococcus aureus* has almost the same incidence of infections (Sundari & Nuryanto, 2016).

Staphylococcus aureus is also a bacterium that causes many nosocomial infections in Indonesia. In Jakarta in the other period of 2019-2021 there was an almost fourfold increase in the incidence of *Staphylococcus aureus* infection from 2.5% to 9.4% (Perwira, 2014).

According to research conducted by Nuryah et al. (2019) revealed that there were 23 cases of postoperative wound infection caused by *Staphylococcus* aureus (Nuryah et al., 2019). Not only in Indonesia, in developed countries, such as the United States, there are 20,000 deaths every year due to nosocomial infections (Tandanu, 2020). Worldwide, 10% of hospital inpatients experience new infections during their stay, totaling 1.4 million infections each year (Salim & Soleha, 2017). According to WHO in 55 hospitals in 14 countries around the world, 8.7% of hospital patients suffer from infections during hospitalization (Roni et al., 2019). Whereas in developing countries there are more than 40% of patients with nosocomial infections. The most common bacteria found in cases of infection is *Staphylococcus* aureus (Lutpiatina, 2017).

With the advancement of modern science and technology, traditional medicine in Indonesia has a very large role in public health services in Indonesia, so traditional medicine has the potential to be developed (Dewi *et al.*, 2019). Indonesia is rich in medicinal plants, which are still not optimally utilized for health. Indonesia is known to have the second largest biodiversity in the world after Brazil (Dewa et al., 2019).

Indonesia's tropical forests contain 30,000 species of plants, approximately 9,600 of which are known as medicinal plants. There are so many medicinal plants in Indonesia both in traditional and modern medicine. One of the medicinal plants that has been quite widely used is the Wresah plant (Adiyasa & Meiyanti, 2021). Wresah is one type of flora that also has the ability to treat various diseases. Wresah is a kind of spice substitute for cardamom which has a slightly sour taste and has a Latin or scientific name as *Amomum dealbatum* (Nurcahyati & Ardiyansyah, 2018). Wresah is still classified in the rhizome or ginger-ginger tribe, the benefits of Wresah include being able to eliminate and overcome red eyes, whether it is due to illness or irritation due to pollution. Another advantage or benefit of Wresah is that it can treat dizziness and nearsightedness after childbirth (Lianah et al., 2020).

In addition, infectious diseases have their problems with various risks such as immunocompromised local and systemic status in patients, microbial resistance to antibiotics, and microbial types that sometimes require specific antibiotics that are expensive and prolonged. The main basis for antibiotic selection in the management of a disease is based on the results of secretion culture and cell sensitivity. However, a very important problem in antibiotic therapy is the formation of resistance mechanisms to antibiotics, so it is very necessary to think about utilizing natural materials in their use for antibiotic therapy. Based on several studies, the dominant bacterial colonies found in skin diseases are Staphylococcus aureus colonies.

27

Until now there has been no research report on the potential of this plant in inhibiting bacterial growth, so researchers are interested in conducting research aimed at determining the inhibition of Wresah fruit peel extract against the growth of *Staphylococcus aureus bacteria*. The purpose of the study was to determine the effectiveness of Wresah fruit peel extract (*Amomum dealbatum*) in inhibiting *Staphylococcus aureus* bacteria.

MATERIALS AND METHOD

This research is an experimental study used to see the effect of Wresah fruit peel extract (*Amomum dealbatum*) in inhibiting the growth of *Staphylococus saureus* bacteria. This research was conducted at the Biology Laboratory, Politeknik Medica Farma Husada Mataram from March to November 2022. The sample used in this study was the ripe fruit peel of Wresah (*Amomum dealbatum*), as much as 250 grams which had been dried. The total sample size used in the study was 25 Petri dishes containing *Staphylococcus aureus* bacteria. The 25 petri dishes were divided into 5 test groups, each test group consisting of 5 petri dishes. The instruments in this study are:

- a. Tools. The tools used in this research are autoclave, blender, rotary evaporator, incubator, digital scale, micropipette, ruler, triangle, bunsen lamp, funnel, filter paper, test tube, gloves, mask and tissue.
- b. Material. The materials used in this study were Wresah fruit peel (*Amomum dealbatum*), *Staphylococcuss aureus*, NA media, MHA (Muller Hinton Agar), 96% ethanol solution, distilled water and aluminum foil.

Work Procedure

Sampling

Wresah fruit peel samples were collected in East Lombok Regency. Wresah fruit peels taken were 3 months old. The skins were then collected and wet sorted. Wresah fruit skins are washed with running water so that the dirt attached to the Wresah fruit skin is lifted until clean and then drained. After drying for several days in an open place that is not exposed to direct sunlight, it is then sorted dry and mashed using a blender. Wresah fruit peel powder is stored in a closed container.

Extract Preparation

The extract of Wresah fruit peel (*Amonum dealbatum*) was made by maceration method which was carried out by weighing 250 grams of Wresah fruit peel simplisia powder then put in a container and given 96% ethanol. Maceration was carried out for 3 days in a room protected from direct sunlight while stirring repeatedly, then the extract obtained was filtered and stored in a closed container.

Antibacterial Test of Wresah Fruit Peel Extract

- a. Sterilization of tools and materials
 - 1) Wash tools and materials to be used
 - 2) Wrap and sterilize equipment such as glassware, petri dishes, test tubes, Erlenmeyer, volume pipettes by using an oven at 175° C for 2 hours.
- b. Prepare test microorganisms

The test microorganism that will be carried out in this study is *staphylococcus aureus*. To multiply the population of organisms, pure cultures of *staphylococcus aureus* bacteria are taken aseptically using an ose needle and then scratched zik-zak and then incubated for 24 hours.

c. Preparation of *Staphylococcus aureus* 0.5 Mc Farlan suspension

In one ose tip, *staphylococcus aureus* colonies from clinical culture were suspended in sterile 0.95 NaCl (5 ml) and compared with 0.5 Mc farlan turbidity standard until the same.

d. Preparation of MHA (Mueller Hinton Agar) media

Weighed 20 grams of MHA media using an analytical balance. Put into an erlenmeyer flask then dissolved with 150 ml of distilled water until homogeneous. Stirred and heated on a *hot* plate while stirring until the media dissolved well. Covered the media with cotton, then sterilized with an *autoclave* at a temperature of 121° C for 15 minutes. Poured media after

sterilization into sterile petri dishes with a thickness of 4 mm, waited until the media solidified before use.

e. Inhibition test

To determine the effect of ethanol extract of Wresah fruit peel on the growth of *staphylococcus aureus* bacteria using the well method, namely: prepare a clinical suspension of *staphylococcus aureus* with a turbidity of 0.5 Mc farlan. Prepare MHA with a thickness of 4 mm, take a sterile cotton swab, then enter 100 μ ml of test bacteria into each petri dish, 10 petri dishes are then leveled using a triangle evenly, allowed to dry for 5 minutes. Wells were made using a sterile *blue tip* placed on the surface of the MHA media, each cup made 4 wells. After that, the ethanol extract of Wresah fruit peel as much as 4 μ ml was included in each well with a concentration of 15%, 30%, and 50%. Given a wide enough distance until the clear zone does not overlap. Incubated at 37° C for 24 hours not upside down so that the ethanol extract of Wresah fruit peel plant does not spill. Observed the inhibition zone around the wells, the inhibition zone formed was measured with a ruler and expressed in millimeters.

Data collection techniques were carried out by conducting tests that have been observed from a physical perspective including organoleptic tests, homogeneity tests and antibacterial inhibition tests. This research data uses qualitative data analysis and quantitative data. Qualitative and quantitative data analysis was carried out by testing antibacterial inhibition. The research data that has been obtained was first tested for normality using the *kalmogorov-smirnov test*. Followed by a parametric test using *oneway anova* with a confidence level of 95% ($\alpha = 0.05$) this anova test is used to determine the mean difference from the source of variation, namely the treatment group and the control group.

RESEARCH RESULTS

Positive

water)

(ciprofloxacin)

Negative control (distilled 0

control

3,7

5,3

0

The results in Table 1 show that at a concentration of 15% there is no inhibition zone because the concentration is too small, the smallest inhibition zone at a concentration of 30% with an average inhibition zone of 1 mm. While the largest inhibition zone was at a concentration of 50% with an average inhibition zone of 1.48 mm (Table 2). This means that the higher the concentration of ethanol extract of Wresah fruit peel, the greater the inhibition zone formed.

Table 1. Extraction of Wresah Fruit Peel			
Simplisia	Weight of simplisia	Extract (gram)	Yield %
Wresah fruit peel	250 grams	4 grams	1,6%

	Inhibition Zone DiameterAverageatment(mm)TotalTest				Average	Category		
Treatment					Total	Test	of	
	Ι	II	III	IV	V	-	Results	Barriers
15% Concentration	0	0	0	0	0	0	0	Weak
30% concentration	1	1	1	1	1	1	1	Weak
50% Concentration	11	13	1.0	2.0	2.0	74	1 48	Weak

5,3

0

3,7

0

71,5

0

4,34

0

Weak

Weak

3,7

0

Research Data Analysis

Based on the measurement results of the inhibition zone formed, the *Shapiro Wilk* normality test was carried out to determine whether the data was normally distributed or not.

Table 3.Normality Tes	st			
Tests of Normality				
	Shapiro-Wilk			
	Sig.			
Extent of Zone of Inhibition	.002			
Concentration	.009			

Table 3 shows that the results of the *Shapiro Wilk* normality test of the data obtained a significance value (Sig.) of 0.002 and 0.009 < 0.05 respectively, which means that the data is proven not to be normally distributed, from these results the test is continued to the *Kruskal Wallis* test.

Table 4. Kruskal Wallis Difference Test			
Test Statistics			
Extent of Zone of Inhibition			
Asymp. Sig.	.000		

Table 4 shows that the results of the Kruskal Wallis test in determining the inhibition zone and from the data obtained a significance value of 0.000 < p=0.05, which means that the data has a significant value.

Multiple Comparisons				
Dependent Var	iable: Extent of Zone o	f Inhibition		
(I) Concentration	(J) Concentration	Sig.		
500/	15%	.001		
30%	30%	.452		

Table 5 shows that the results of the *Post Hoc* test data obtained a significance value (Sig.) between 50% and 15% concentrations of 0.001 < 0.05 which means that the data has a significant difference. While, the significance value (Sig.) between 50% and 30% concentrations of 0.452 > 0.05 which means that the data is not significant.

Table 6. Post Hoc Test Results				
Multiple Comparisons				
Dependent Variable: Extent of Zone of Inhibition				
(I) Concentration	(J) Concentration	Sig.		
Positive Control	15% 30%	.000		
	50%	.000		

Table 6 shows that the results of the Post Hoc test data obtained a significance value (Sig.) between the Positive concentration and the Concentration of 15%, 30%, and 50%, which is 0.000

<0.05, which means that the ethanol extract of Wresah fruit peel with a concentration of 15%, 30%, and 50% is able to inhibit the growth of *Staphylococcus aureus* bacteria

DISCUSSION

The aim of this study was to test the activity of ethanol extract of Wresah fruit peel (Amonum dealbatum) against Staphylococcus aureus bacteria. This study was conducted at the Biology Laboratory, Politeknik Medica Farma Husada Mataram with three different extract concentrations (15%, 30%, 50%), and ciprofloxacin as the positive control. To ensure the accuracy of the results and minimize errors, the experiment was conducted with five replications. Each treatment group produced a zone of inhibition indicating the presence of inhibition on *Staphylococcus aureus* bacteria, but at a concentration of 15% there was no zone of inhibition because the concentration dose was small. The zone of inhibition is a clear area around the wells that can indicate that there is inhibited bacterial activity. Based on the results of the research that has been done, the ethanol extract test of Wresah fruit peel (Amonum dealbatum) is able to inhibit Staphylococcus aureus bacteria at concentrations of 13%, 30%, and 50% with a weak inhibition category. The data was then analyzed statistically, the first step was the Kalmogorov-smirnov normality test. Based on this test, the asymp sig value is 0.002 and 0.009 > 0.005, which means that the data distribution is not normal. Thus, the test was continued with the Kruskal wallis test because the data tested did not meet the requirements for the Oneway Anova test where to use this test the data must be from a population or sample of interval or ratio type, the population tested must be normally distributed, the variation or population must be the same and the data groups must have the same sample size. The Kruskal wallis test is a non-parametric statistic in an independent group procedure and is used to compare two variables measured from unequal samples. The significance value obtained from the Kruskal wallis test (attached) at 0.000 <0.005 means there is a difference between concentrations.

Antibacterial sensitivity test is a method to determine the level of susceptibility of bacteria to antibacterial substances and to determine pure compounds that have antibacterial activity. The bacterial sensitivity test method is a method of how to find out and get natural products that have the potential as antibacterial ingredients and have the ability to inhibit bacterial growth at each concentration. The principle of this method is the inhibition of the growth of microorganisms, namely the zone of inhibition will be seen as a clear area around the well. Furthermore, it is said that the wider the diameter of the inhibition zone formed, the more sensitive the bacteria are.

Based on the results of a study conducted by Muliasari et al. (2019) which states that the active compounds that play a role in inhibiting *Staphylococcus aureus* bacteria are alkaloids (Muliasari et al., 2019). Alkaloids can interfere with bacteria by poisoning protoplasm, damaging and penetrating cell walls and precipitating proteins (Wulandari et al., 2021). Alkaloid components can also denature enzymes responsible for spore proliferation. Alkaloid compounds are able to break peptidoglycan bonds when breaking through the cell wall (Hamrat & Rita, 2021).

This peptidoglycan bond mechanically gives strength to the bacterial cell. The type of bacteria used are gram-negative bacteria with cell walls that have thin or very little peptidoglycan and are between the outer membrane and the inner membrane of the cell wall (Alam & Singh, 2021). The cell wall of gram-negative bacteria contains phospolipids, lipopolysaccharides, and lipoproteins. After breaking through the cell wall, the compound will cause leakage of cell contents by breaking hydrophobic bonds which results in increased membrane permeability (Garg et al., 2016). The occurrence of damage to the cell membrane results in pressure inhibiting the activity and biosynthesis of specific enzymes needed in metabolic reactions (Huong et al., 2015).

CONCLUSION

Ethanol extract of Wresah fruit peel (*Amonum dealbatum*) has the potential to inhibit the growth of Staphylococcus aureus bacteria. Wresah fruit peel extract is able to inhibit the growth of *Staphylococcus aureus* bacteria at concentrations of 15%, 30%, and 50%.

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