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ANTIBACTERIAL ACTIVITY TEST OF WHITE GALANGAL RHIZOME EXTRACT (ALPINIA GALANGA L.) AGAINST THE BACTERIST STAPHYLOCOCCUS AUREUS AND IDENTIFICATION OF ACTIVE MATERIAL

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ABSTRACT
Infectious diseases caused by Staphylococcus aureus bacteria can be treated by using natural ingredients, one of which is white galangal. White galangal is a shrub that contains secondary metabolites that can inhibit bacterial growth. This study aimed to determine the antibacterial activity of white galangal rhizome extract against Staphylococcus aureus bacteria. Sample extraction was carried out using the maceration method, while antibacterial activity testing was conducted by using the diffusion well method. The results of the antibacterial activity test of white galangal rhizomes showed that the ethyl acetate fraction at a concentration of 20% was the most effective in inhibiting the growth of Staphylococcus aureus bacteria compared to the other 3 fractions, and the minimum inhibitory concentration was obtained at a concentration of 1.56% with an inhibition zone of 7,25 mm. Based on the identification results shown by LC-MS/MS it was found that the compounds contained phenol derivative compounds of the phenylpropanoid group, such as cinnamaldehyde compounds containing aldehyde functional groups and alkenes conjugated to benzene rings. Consequently, White galangal rhizomes can inhibit Staphylococcus aureus bacteria.

KEYWORDS: *antibacterial, Staphylococcus aureus, white galangal.*

INTRODUCTION
The activities of modern society have caused many problems, one of which is health problems due to a polluted environment. A polluted environment results in the growth of harmful bacteria and causes various diseases such as infectious diseases. Pathogenic bacteria that often causes infections in humans is Staphylococcus aureus.

Staphylococcus aureus is a bacterium that causes infections of skin tissue in the human body such as acne, and boils (Harian, 2008). Infectious diseases caused by bacteria are usually treated with antibiotics. However, there is currently a problem of bacterial resistance to some commonly used antibiotics. Antibiotic resistance is the growth of bacteria that is not inhibited by the administration of antibiotics and has become a global problem because it causes reduced therapeutic effectiveness and difficulty in managing bacterial infections. One alternative that can be done is the use of natural ingredients. Alternative medicine using natural ingredients is necessary to prevent side effects of excessive antibiotic use and minimize bacterial resistance.

Galangal is one of biopharmaceutical plants, which are plants that are useful for medicine, consumed from parts of the plant that come from the leaves, flowers, fruits, tubers (rhizomes), or roots. In general, there are two types of galangal, namely red galangal (Alpinia purpurata) and white galangal (Alpinia galanga L. Willd.). White galangal is usually used as seasoning and red galangal is utilized as medicine. Pharmacologically. White galangal (Alpinia galanga L.) is an annual shrub plant, belonging to the Zingiberaceae family. The part of the white galangal plant that is often utilized is the rhizome. white galangal has activity as an antioxidant, antibacterial Klebsiella pneumonia and S. aureus, antidiabetic, anticancer, anti-tuberculosis, and anti-tuberculosis, prevent kidney damage.

Azkiyah (2020) detailed that ginger rhizome extricate was able to hinder the concentration of Staphylococcus aureus bacterial development with an restraint zone of 10.37 mm, where the most effective concentration

inhibits bacterial growth is 80%. Inquire about by Fransiska (2017), appeared that white galangal rhizome mixture was able to repress the development of Staphylococcus aureus microbes with an hindrance distance across of 19.40 which was included within the solid category. Until now, the test of antibacterial activity of white galangal rhizome extract (Alpinia galanga L) is still little done.

Based on the above analysis, it is necessary to further investigate to determine the antibacterial ability contained in galangal (Alpinia galanga L.) against the growth of Staphylococcus aureus bacteria.

MATERIALS AND METHODS

Research Materials and Equipment

White galangal rhizome (Alpinia galangal) was obtained from the Badung market, where the samples are fresh samples obtained directly from the white galangal plantation in Bali. Plant determination was carried out at Yayasan Generasi Biologi Indonesia, Gresik.

The chemicals used are, methanol, n-hexane, ethyl acetate, n-butanol, ethanol, distilled water, Tetracylin, Nutrient agar (NA), Nutrient broth (NB), clear agar powder, FeCl3 10%. The bacteria used were Staphylococcus aureus.

The equipment used in this research includes a set of glassware, are Beker, glass funnel, stirring rod, volumetric flask, separatory funnel, micro pipette, petri dish, cotton, gauze, aluminum foil, scissors, knife, blender, ose needle, sieve, analytical balance, rotary vacuum evaporator, bunsen, pot, incubator, autoclave, laminar and stove.

Method

Sample preparation

A total of 8000 g white galangal rhizomes were washed using running water and then dried. The dried simplicia is sorted dry, then ground using a blender until it becomes powder. The results were put into a closed glass container.

Extraction of White Galangal Rhizome ((*Alpinia galangal*)

A total of 800 g of dry powder samples of white galangal rhizomes obtained were macerated using methanol as a solvent. Extraction was carried out in 3x24 hours. The obtained macerate is then evaporated to obtain a thick methanol extract. The thick methanol extract is then dissolved in a mixture of methanol: water (7: 3). The extract mixture is then evaporated back to its methanol to obtain water extract. The aqueous extract was then partitioned with a variety of different levels of polarity.

Antibacterial Activity Test

Antibacterial activity tests were carried out on crude extracts, and fractionated extracts, where extracts that showed the largest diameter of inhibition would be identified for their active compounds. The Staphylococcus aureus antibacterial activity test using the pitting diffusion method includes several stages, namely tool sterilization, making test media, and sample testing.

Tool Sterilization

All tools used in antibacterial testing must be sterilized first. Rubber-based equipment is sterilized using alcohol, test media, micropipette tips and petri dishes are sterilized using an autoclave for 60 minutes, while ose needles and perforators are sterilized by heating directly over a Bunsen flame.

Preparation of Test Media

Media for the antibacterial test was made by Weighed as much as 0,4 g of nutrient agar, 0,3 g of clear agar powder, and 20 ml of distilled water for test media, then the ingredients are heated on a water bath until it boils and becomes clear. The media and blue tip tools were sterilized in an autoclave for 60 minutes. Meanwhile, the tube needle and tweezers equipment is sterilized by heating it over a Bunsen flame. Media that has been sterilized by autoclaving, put into Petri dishes with a uniform well diameter of ± 0.5 cm.

Sample Testing

Staphylococcus aureus bacteria were first inserted into a Petri dish as much as 200 µL, then the test media was inserted into the Petri dish. The media was perforated using a perforator with a hole diameter of 0.5 cm. The sample, positive control, and negative control were inserted as much as 20 µL into each well. The test media containing microbes was at that point brooded for 24 hours at 37℃. After incubation, the clear zone shaped region around the well could be a bacterial hindrance zone.

Phytochemical Screening

The foremost dynamic antibacterial division was at that point tried for phytochemicals to decide the substance of secondary metabolites contained within the division.

Flavonoid Test

The flavonoid test was carried out with Wilstatter reagent by adding Mg powder and 2-3 drops of concentrated HCl to the sample. The formation of orange color indicates the presence of flavonoid compounds.

Phenolic Test

Phenolic test is done by adding 1% FeCl3 solution into the sample extract. The formation of a blue- black color indicates the presence of phenolic compounds.

Alkaloid Test

Alkaloid test is done by the addition of Wagner reagent, where a certain amount of sample is added ammonia, chloroform, and H2SO4. The formation of a brown precipitate indicates the presence of alkaloid compounds.

Saponin Test
The saponin test is carried out by adding distilled water to the sample extract then shaken until foam is formed, then a little HCl is added again. The formation of stable foam after the addition of HCl indicates the presence of saponin compounds.

Triterpenoid and Steroid Test
Triterpenoid and steroid tests were carried out using Limbeman burchard reconstitution Several samples were added with chloroform, anhydrous acetic acid, and concentrated H2SO4. The formation of red-purple color indicates the presence of terpenoid compounds, while the formation of green-blue color indicates the presence of steroids.

Identification Compound by LC-MS/MS
The character of isolated compounds is determined by LC.MS/MS where a mass spectrometer is used to determine the molecular weight or molecular formula and or used to identify compounds from the fragmentation pattern of the compound molecules separated by liquid chromatography (Karmita et al, 2019).

RESULTS AND DISCUSSION

Extraction of White Galangal Rhizome ((*Alpinia galangal*)
The extraction process of white galangal rhizome powder is carried out by maceration method, where the working principle of maceration method is based on the contact between solvent and white galangal rhizome powder. The solvent will enter the cavity of the simplisia and attract the active substances present to move into the solvent. The maceration results obtained were from 800 g of white galangal powder with 5 L of methanol solvent 42 grams of methanol thick extract which is dark brown in colour. The more compounds extracted causes the colour of the extract to be darker and more concentrated. The thick water extract was partitioned with non- polar (n-hexane), semi-polar (ethyl acetate), and polar (n-butanol) solvents according to their polarity. The partition results obtained 2.47 g yellow n-hexane thick extract, 9.07 g blackish brown ethyl acetate solvent, 13.42 g black brown n-butanol solvent and 10.65 g brown water solvent. The use of methanol solvent is very effective in extracting polar to nonpolar compounds contained in the sample. This is because methanol has a hydroxy group (-OH) which is polar and a methyl group (-CH3) which is nonpolar.

Antibacterial Activity Test
The antibacterial movement test of white galangal rhizome extricate was carried out to decide the capacity and potential of the extricate in hindering the development of Staphylococcus aureus microbes. The positive control used was Tetrasilin 0.3% while the negative control was tween-80 with a concentration of 10%. The function of the negative control is to determine whether or not the solvent affects the growth of the two bacteria, while the positive control acts as a comparison to see the activity provided by samples with various concentrations.

Antibacterial activity testing was carried out on all four extracts, namely n-hexane, ethyl acetate, n-butanol, and water thick extracts. The test extracts were made at the same concentration of 20%. The positive control used was Tetrasilin 0.3% while the negative control was tween-80 at 10% concentration.

The results of antibacterial activity testing are presented in Table 1.

Table 1: Antibacterial Activity Test of Partitioned Viscous Extract against Staphylococcus aureus Bacteria at a Concentration of 20%.

No.	Test fraction	Inhibitionpower (mm) ± SD	Category
1.	N-hexaneextract	11.50 ±0.5000	S
2.	Ethyl acetateextract	23,75 ± 0,0866	VS
3.	N-butanolextract	0,00 ± 0,0000	-
4.	Waterextract	9,60 ± 0,57735	M
5.	Negativecontrol	0,00 ± 0,0000	-
6	Positive Control	36,67 ± 0,5204	VS

Based on the results of the antibacterial activity test in Table 1, it is known that ethyl acetate extract has the highest inhibition among other extracts in inhibiting the growth of Staphylococcus aureus. The inhibition diameter of Staphylococcus aureus is 23.75 mm which is included in the very strong category at a concentration of 20%. The results of the negative control antibacterial activity test, and the n- butanol extract did not produce an inhibition zone against the growth of Staphylococcus aureus bacteria.

The semi-polar nature of ethyl acetate causes the fraction to be able to attract more complex secondary metabolite compounds than the polar and nonpolar fractions. This resulted in the ethyl acetate fraction being the most active fraction by forming the greatest inhibition and most active compared to methanol extract, n-hexane fraction, and water fraction (Sreelatha et al., 2013).

The n-butanol fraction is known to be unable to inhibit the growth of staphylococcus aureus bacteria at a concentration of 20% where it is thought that this is because at this concentration, the secondary metabolite compounds contained in the fraction do not damage the bacterial cell membrane enough so that the bacteria can still multiply their cells.

Minimum Inhibitory Concentration

The most active antibacterial ethyl acetate extract was then determined to be the Minimum Inhibitory Concentration (KHM). KHM determination was carried out with various concentrations, namely 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78%. The results of the ethyl acetate fraction antibacterial activity test with various concentrations can be seen in Table 2.

Table 2: Results of Antibacterial Activity Test of Ethyl Acetate Fraction with Various Concentrations.

No	Ethyl acetate extract concentration %(b/v)	Zone of inhibitionof extract against Staphylococcus aureus bacteria (mm) ± SD
1	100	35,41 ± 0.9464
2	50	30,08 ± 0.1443
3	25	25,25 ± 2.4622
4	12,5	18,33 ± 1.2332
5	6,25	13,50 ± 0.9827
6	3,12	9,91± 0.5204
7	1,56	7,25 ± 0.2500
8	0,78	-

Table 2 shows that the minimum concentration on Staphylococcus aureus bacteria is 1.56% with an inhibition zone of 7.25 mm and is categorised as moderate (5-10 mm), and at a concentration of 0.78% it is unable to inhibit the growth of Staphylococcus aureus bacteria. The resulting inhibition zone indicates that the white galangal rhizome extract has antibacterial activity against Staphylococcus aureus bacteria. Table 2 shows that the magnitude of the inhibition zone produced is directly proportional to the sample concentration, where the diameter of the inhibition zone produced decreases as the concentration of the extract decreases. The higher the concentration of the tested material, the more active substances contained in it, so the stronger the inhibitory power (Halimathussadiahh, 2021).

Phytochemical Test

Based on the results of phytochemical tests that have been carried out, it is known that the ethyl acetate extract of white galangal rhizome is known to contain alkaloid, phenolic, steroid, and flavonoid compounds. The results of the phytochemical test of ethyl acetate extract of white galangal rhizome contain steroid, flavonoid, alkaloid and phenolic compounds. This is supported by research conducted by Sangadji (2021), which reports that white galangal contains secondary metabolite compounds of alkaloids, flavonoids, tannins, phenolics, and triterpenoids, where it is known that these secondary metabolite compounds have the ability as antibacterials. The difference in the results of this test is thought to be the diverse planting sites by several factors, such as environmental stress, plant age, growing area and physical factors such as weather, humidity, and temperature (Figueiredo et al. 2008).

As an antibacterial compound, alkaloid compounds work by damaging the components and constituents of peptidoglycan in bacterial cell walls (Kurniawan, 2015), flavonoids as antibacterials work by forming complex compounds with extracellular proteins, phenols as antibacterials work by breaking bonds in bacterial cell proteins which result in the death of bacteria (Marfuah, 2018), and finally steroids as antibacterials work by damaging cell wall membranes in bacteria which can result in cell wall leakage in bacteria (Monalisa, et al., 2011).

Identification of Active Compounds

The most antibacterial active ethyl acetate fraction was identified using LC-MS/MS. In the process of identifying active compounds contained in of ethyl acetate extract of white galangal rhizome with LC-MS/MS, there is 1 chromatogram peak with a retention time of 5.79 minutes that can be identified. The compound identified at the retention time of 5.79 minutes is the sinamaldehyde compound. The identified compound is a compound that belongs to the phenolic group. It is known that sinamaldehyde compounds are semi-polar phenol compounds.

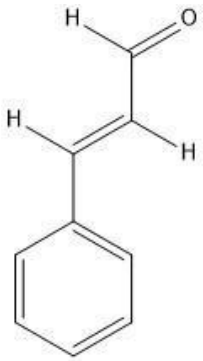

Figure 1: Sinamaldehyde compound.

Sinamaldehyde compounds included in the phenylpropanoid group are derivatives of phenol compounds, where these compounds include semipolar compounds. Phenol derivative compounds can interact Phenolic-derived compounds can interact with bacterial cells through an adsorption process involving hydrogen bonding. At low levels, phenol-protein complexes form with weak bonds and are immediately degraded, after which the phenol enters the cell and causes precipitation (crosslinking of antigen molecules dissolved in body fluids) and denaturation (changes in protein structure due to damage or partial breakage of bonds in proteins) proteins. At high levels phenol causes protein coagulation and cell membrane lysis (Hallimatussadiah, 2021).

Sinamaldehyde compounds are the main compounds that are often found in cinnamon plants, which in these plants are the components responsible for the antibacterial properties of cinnamon. Sinamaldehyde mechanism in inhibiting bacterial growth by damaging the cell wall and plasma membrane of bacteria which then causes leakage

to result in cytoplasm flowing out and causing bacterial death (Larasati, 2022).

Based on research conducted by Tokan, et al (2019), it is known that sinamaldehyde compounds can inhibit antibacterial activity on Staphylococcus aureus bacteria. Where in this study it is known that the inhibition produced is almost the same as the positive control used, namely amoxicillin.

CONCLUSION

From the results of the study, it is known that extracts from white galangal rhizomes have the ability as antibacterial agents against Staphylococcus aureus bacteria, where among the 4 extract fractions, ethyl acetate extract is the most effective antibacterial inhibitor. Ethyl Acetate Extract of White Galangal Rhizome has an inhibition of 7.5 mm at a minimum inhibition concentration of 1.56%. The antibacterial active compound contained in the ethyl acetate fraction is thought to be a sinamaldehyde compound from the phenolic compound group.

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