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Activity Test of Leaf Ethanol Extract Bilajang Bulu *Merremia Vitifolia* Against *Staphylococcus Aureus* Bacteria

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Abstract: This study aims to study the activity of the ethanol extract of the leaves of the Bilajang Bulu *Merremia vitifolia* against *Staphylococcus aureus* bacteria. Bilajang Bulu is one of the plants used as herbal medicine and its efficacy is believed to be an alternative that can be used in the event of injury to diabetics and can reduce blood sugar levels by the community in Luwu Regency, South Sulawesi Province. *M. vitifolia* contains compounds that contain active compounds that function as antibactericides, antivirals and insecticides. The flavonid content in Bilajang Bulu leaves has potential as an antibacterial as it can inhibit the growth of *S. aureus* bacteria and other types of bacteria. The method in this study was through the preparation of Bilajang Bulu leaf samples which were dried with aerated, extraction of samples by maceration method using ethanol 96%, then using its activity using *S. aureus* bacteria. The results obtained the concentration that gives the most optimal activity, namely at a concentration of 20% with an average of 9.5 mm clear zone.

Key word*: ethanol, extraction, metabolites sekunder, merremia vitifolia, staphylococcus aureus.*

INTRODUCTION

Indonesia is a country rich in plants both used as a source of food, and medicine. Some even serve as a cure for certain diseases or media to maintain health. One of them is the *M. vitifolia* plant which is widely used as a medicinal ingredient by the Luwu community. Water from the leaves of Bilajang leaves is believed to reduce blood sugar levels and the leaves are used as medicine to speed healing in the event of injury to diabetics. Meanwhile, the people of Mamuju (West Sulawesi) believe that *M. vitifoilia* can cure malaria (Sukarti, 2016).

One of the active compounds in *M. vitifolia* which plays an important role as a medicinal ingredient is flavonoids. According to Hasanah et al (2019), *M. vitifolia* plants contain flavonoid levels of 163.4 mg / L. Flavonoid is a secondary metabolite compound and is a natural compound from the phenolic group (Mukhriani et al, 2015). Flavonoids are found in all green plants so that they can be found in every plant extract (Andersen and Markham, 2006). Research on the pharmacology of flavonoid compounds shows that some flavonoid compounds show activities such as antifungal, diuretic, antihistamine, antihypertensive, insecticidal, antiviral, inhibiting the work of enzymes and bactericides (Subandono, 2006). Pakekong et al's research (2016) research stated that onion extract containing active compounds flavonoids, saponins and allicin can inhibit S. aureus bacteria with an average resistance of zo diameter reaching 24.32.

S. aureus is a normal bactericide or microflora that causes abscesses (Pakekong et al, 2016). An abscess is a collection of pus locally in a cavity that occurs due to tissue

destruction, usually caused by a piogenic bacterial infection. The pattern of spread of an abscess is influenced by 3 conditions, namely bacterial virulence, tissue endurance, and muscle attachment. High bacterial virulence is able to cause bacteria to move freely in all directions, the resilience of surrounding tissue that is not good causes the tissue to become fragile and easily damaged while the attachment of muscles affects the direction of motion of the pus (Syahrurachman et al, 2010). According to the patient's medical record data in the surgica department of BLU / RSUP Prof. dr. R. D. Kandou from 2009-2013 mentions cases of abscesses caused by dental infections, the most cases being 70-85% (Warbung, 2013).

This preliminary research in the development of natural medicinal drugs aims to find out the effectiveness of *M. vitifolia* leaves in inhibiting the activity of *S. aureus* bacteria using parameters of the flavonoid compound content of polar extract of *M. vitifolia* leaves.

RESEARCH METHODS

Materials and Tools

The ingredients used in this research is *M. vitifolia* leaf, pure culture of *S. aureus* bacteria, NA media, paper level, alumunium foil, ethanol 97%, alcohol 70%, and destilled water.

The tools used in research these are commonly used glassware laboratory, analytical balance, rotary evaporator vacuum , engkas, incubator, paper disk, lamp Bunsen, spoit, caliper, wire ose, autoclave.

Instrumentation

Procedures

Sample preparation

M. vitifolia leaf that was collected, cleaned then dried by aerating without being eksposed to sun light. Next *M. vitifolia* leaf mashed to powder (simplicial).

Sample extraction

Simplisia of *M. vitifolia* leaves are weighed of 500 g, then put in maceration jar, then the solves is added ethanol 96% 2050 mL. Maceration is done three times by changing solvents each 24 hours. The obtained filtrate is combined and concentrated use rotary evaporator with temperature of 40’C until a thick extract is obtained 13,05 g.

Sterilization of tools

At this stage, the tools are sterilized with using autoclave at 121° C with 1 atm pressure with a time of 15-20 minutes. Before sterilized using an autoclave first a sterilizer using 70% alcohol and wrapped in paper and plastic resistant hot. Then the test solution or medium in the pipette of 9 mL was inserted into the tube reaction and clogged using cotton dimensil. Then all the tools are ready put in an autoclave to be sterilized (Biring, 2012).

Making Medium for Growth

Nutrient Agar (NA) is made as a medium for bacterial growth in petri dishes. *Nutrient Agar* (NA) added pepton as much as 3 g and homogenized by heating while stirring. Media which has been homogenized is then plugged with cotton and sterilized again using an autoclave at 121ºC for 15 minutes at 1 atm pressure (Ratnasari, 2017).

Inoculation of S. aureus bacteria

Inoculation of *S. aureus* bacteria with temperature. At this stage bacteria are obtained in the form of pure culture, the inoculation technique is carried out using the pour method on NA media in on 10^{-1} and 10^{-2} dilutions with 2 replications. Where is the pure culture of *Staphylococcus* bacteria then put into the test solution on dilution first 10^{-1} then homogenized, pipetted as much 1 mL at 10 dilutions and inserted into the next 102 dilution homogeneous (Hasrianti et al, 2018).

Antibacterial Activity Testing Based on Area of Inhibition Zones with Diffusion Method

Test the activity of flavonoid compounds against *S. aureus* bacterial growth using the diffusion method that is using sterile encoder paper. Where This test was done 3 treatments plus 1 control group with 3 repetitions in each treatment Aquades is used as control, then NA media were prepared in a petri dish poured as much bacteria 1 ml and allowed to stand for 20 minutes. Then laid the previous sterile disc paper has been immersed in *M. vitifolia* ethanol extract with concentrations between 5% 10% and 15% for 15 minutes aseptically. After that, media that has been ready to be silenced again for 15 minutes and dunked at 37 ° C for 2 × 24 hours. The results were observed at 24 hours and 48 hours and seen at once measured in diameter inhibition zone that forms a region clear paper around the disc with use calipers then the results recorded and documented (Hasrianti et al, 2018).

RESULTS AND DISCUSSION

Based on research conducted regarding the ethanol extract activity of Bilajang Bulu leaves *M. vitifolia* against bacteria *S. aureus* results are obtained:

Table 1. Descriptive statistical analysis

		Statistic	Std. Error
Clear zone diameter	Mean	8.717	1.0537
	95% Confidence Interval for Mean	6.008	
		11.425	
	5% Trimmed Mean	8.591	
	Median	7.850	
	Variance	6.662	
	Std. Deviation	2.5810	
	Minimum	6.2	
	Maximum	13.5	
	Range	7.3	
	Interquartile Range	3.4	
	Skewness	1.566	.845
	Kurtosis	2.729	1.741

S. aureus various concentrations of ethanol extract of *Mvitifolia* leaves can be seen in Table 2 as follows:

Table 2. Average growth inhibition zones of *S. aureus*

Concentration	Diameter Zone Clear (Mm)
3%	7,4
5%	7,5
10%	8,2
20%	9,5
Control (-)	6,2
Control (+)	13,5

This test was carried out to determine the ability of the ethanol extract of Bilajang Bulu leaves in inhibiting bacteria which is characterized by the formation of a clear zone around the well that contains the test extract. Clear zone indicates that the extract has antibacterial activity. Results measurements on the tests of the extract calculated the average diameter of the clear zone are presented in Table 1 above.

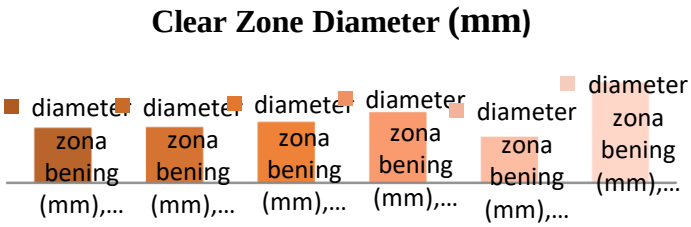

Figure 1. The average zone of *S. aureus* growth retardation

Test the activity of ethanol extract of *M. vitifolia* against *S. aureus* was carried out by the diffusion method. Where the results obtained from observations and measurements of the average diameter of the clear zone. At a concentration of 3% a clear zone of 7.4 mm was seen, at a concentration of 5% the average area of a clear zone was 7.5 mm, at a concentration of 10% a clear zone of 8.2 cm was obtained and at an average concentration of 20% clear zone 9.5 mm. In addition, control (-) obtained a 6.2 mm clear zone increased and in control (+) a 13.5 mm clear zone was obtained.Observation and measurement of the average clear zone diameter.

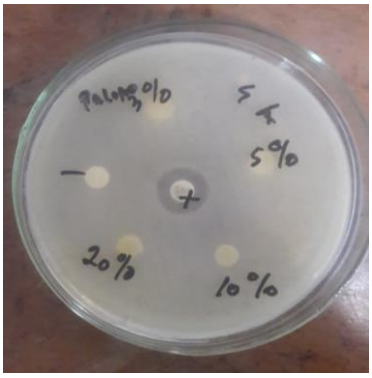

Figure 2. Clear zone

According to Davis and Stout, (1971) for inhibits the growth of a bacterium, if the greater the concentration used then the greater the ability of the extract to inhibit bacterial growth. Based on the strength of the antimicrobial power with Inhibitory zone diameters can be grouped into 4 parts, namely: a) weak, inhibitory zone 5 mm or less; b) medium, 5-10 inhibitory zone mm; c) strong inhibition zone of 10-20 mm; and d) very strong, inhibition zone of 20 mm or more. In the extract *M. vitifolia* with a concentration of 20% including the medium inhibit zone and the controls (+) is a strong category inhibitory zone, so the results obtained are significant results.

Basically, *S. aureus* bacteria are gram-positive bacteria that have a thick cell layer with 90% of the peptidoglycan layer (Lestari, 2013). Where S. aureus bacterial cells can be damaged with compounds contained in the extracts of *M. vitifolia* leaves, especially large flavonoid compounds contained therein. Flavonoid compounds are secondary metabolites which have the ability to inhibit bacterial growth. The activity caused by these compounds is damaging the cell membrane and inhibiting the synthesis of bacterial cell macromolecules (Mawan et al, 2018).

Flavonoids have a mechanism of action on antibacterial can be divided into 3 parts including inhibiting the synthesis of nucleic acids in which ring A and ring B which play a role in the hydrogen bonding process are able to accumulate nucleic acid bases that inhibit the formation of DNA and RNA. At the location of the hydroxyl group in position 2 ', 4' or 2'6 'will be hydroxied in ring B while 5.7 will be hydroxylized by ring A. So that the flavonoid compounds will cause damage to the permeability of bacterial cell walls, lysosomes and microsomes between these interactions (Cushnie et al, 2005). In the working system of flavonoids in inhibiting the function of cell membranes there are 2 namely first forming complex compounds with dissolved extracellular proteins followed by the release of intacellular compounds and secondly disrupting cell membrane permeability and inhibiting the binding of enzymes such as ATPase and phospholipase. Flavonoid compounds in inhibiting energy metabolism by inhibiting oxygen and inhibiting cytochrome C reductase so that the energy metabolism needed by bacteria for macromolecular biosynthesis will be disrupted (Rijayanti et al, 2014).

CONCLUSIONS

Based on the results of research on the test ethanol extract activity of *M. vitifolia* leaves against *S. aureus* bacteria, can concluded that the concentration gave power the most optimal inhibitor of ethanol extract M. vitifolia leaves against *S. aureus* bacteria is at a concentration of 20% namely with an average of 9.5 mm clear zone which is medium category.

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