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Antibacterial activity of flavonoid isolated from jamblang leaves (Syzigium Cumini (L.) Skeels) against Staphylococcus aureus

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ABSTRACT
Jamblang leaves (Syzigium cumini (L.) Skeels) is one type of plant used as traditional medicine. Jamblang leaves contain several phytochemical compounds such as alkaloids, flavonoids, resins, tannins, and essential oils. Flavonoid compounds contained in jamblang leaves have an antibacterial ability. The identification of flavonoid compounds in jamblang leaves and the antibacterial activity of flavonoid compounds against Staphylococcus aureus have been carried out. This study was conducted from jamblang leaves (Syzigium cumini (L.) Skeels) at concentrations of 2%, 4% and 6% against the growth of Staphylococcus aureus bacteria and to determine the type and structure of flavonoid compounds that have antibacterial activity. The method used for the extraction is remaceration, the separation of compounds using preparative thin layer chromatography while the antibacterial activity test using the pitting method and identification of flavonoid compounds using UV-Vis spectrophotometry. Extraction of 500 g of jamblang leaf powder using 96% ethanol resulted in a yield of 22.08%. The results of the study tested the antibacterial activity of flavonoid compounds of jamblang leaves obtained an average of clear zones produced at concentrations of 2%, 4% and 6% consecutively amounting to 0.9540 cm; 1.0540 cm; 1.1583 cm and ciprofloxacin 0.005 % by 1.4077%. Based on statistical analysis of the SPSS program with anava test 1 way obtained the results of significant activity differences between concentrations. Identification of the structure in UV-Vis spectrophotometry shows that flavonoid compounds lead to 3,7,4' trihydroxy flavonol.

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1. Introduction

One of the medicinal plants that can be used as herbal medicine is the jamblang plant (Syzigium cumini (L.) Skeels). Jamblang leaves contain many chemical compounds including alkaloids, flavonoids, terpenoids, steroids, phenolics and saponins [1]. Flavonoids are one of the secondary metabolites contained in this plant that serves to treat many diseases such as anti-microbial, wound infection drugs, antifungal, anti-viral, anti-cancer, and anti-tumor. In addition, flavonoids can also be used as anti-bacterial, anti-allergic, cytotoxic, and antihypertensive [2].

Staphylococcus aureus is one of the most common infection-causing bacteria in the world. Staphylococcus aureus bacteria are normal flora in the respiratory tract. Serious infection from Staphylococcus aureus can occur when the immune system is weakened [3]. Infections caused by Staphylococcus aureus bacteria variants from mild infections to severe infections, one of which is pneumonia [4]. Pneumonia is one of the acute respiratory tract infections, namely inflammation or irritation of one or both lungs [5].

In general, infectious diseases can be cured using antibiotics, but the use of antibiotics has a negative impact such as can cause microorganism immunity and increased drug side effects [6]. Due to the negative impact of this antibiotic, it is necessary to have other alternative treatments from safer ingredients such as natural compounds.

A compound of natural materials is usually still mixed with other compounds from the source. Isolation is carried out to obtain one pure compound. The compounds to be isolated are flavonoid compounds. Flavonoids are secondary metabolite compounds found in green plants that have antibacterial activity [7]. Based on the background, research was conducted on the antibacterial activity test of jamblang leaf flavonoid isolate (Syzigium cumini (L.) Skeels) against the growth of Staphylococcus aureus bacteria.

2. Methods

The materials used in this study were jamblang leaves, 96% ethanol, Staphylococcus aureus bacteria, MSA media, NA media, NB media, ciprofloxacin, dimethyl sulfoxide, 1/2 Mc Farland solution, oxalate powder, potassium hexacyanoferate (III) and 1% iron (III) chloride solution, magnesium powder, HCl 2N, HCl p, amyl alcohol, ethanol 95%, boric acid powder, silica gel GF 254 nm, ethyl acetate, n-hexane, ammonia vapor.

The tools used in this study were sieves, kola cloth, baking pans, maceration vessels, stirring rods, water baths, test tubes, rotary evaporators, glass funnels, UV lamps 254 nm and 366 nm, silica gel GF 254 nm plates, glass chambers and caps, drip plates, capillary pipes, ovens, spray bottles, petri dishes, round ose, Laminar Air Flow (LAF), cylinder cups, autoclave, micropipette, tweezers, bunsen, jangkasorong, yellow tip, paper umbrella, mattress string, glassware set and visible spectrophotometer.

Making jamblang leaf extract (Syzigium cumini (L.) Skeels) was carried out by remaceration method for 3 days by soaking 500 grams of simplisia powder in a solvent of 2 liters of 96% ethanol then changing the solvent every 24 hours while stirring occasionally [8]. The liquid extract obtained is concentrated with a rotary evaporator vacuum, then evaporated on a waterbath until a thick extract is obtained. The extracts that have been obtained are then carried out qualitative precursor tests of jamblang leaf flavonoid

compounds including flavonoid tests, glycosides-3-flavonols, taubeck tests, shinoda tests and polyphenol tests [9]. Eluent orientation uses 5 different eluents in the extract using thin layer chromatography to continue the process, namely preparative thin layer chromatography [10]. Each tape on the preparative thin layer chromatography is then scraped off and antibacterial activity is tested. The tape containing the clear zone is then continued with purity tests and antibacterial activity tests using 3 concentrations. The results of flavonoid compound isolates were identified using UV-Vis Spectrophotometry to determine the structure and type of flavonoids based on shifting absorption spectra with various shear reagents [11].

3. Results and Discussion

Extraction of jamblang leaves (Syzigium cumini (L.) Skeels) using the remaceration method obtained a yield of 22.80%. Qualitative preliminary tests on jamblang leaf extract showed positive results in flavonoid, glycoside-3-flavonol tests, taubeck tests, shinoda tests and polyphenol tests [12]. The results of the flavonoid compound affirmation test using thin layer chromatography showed that jamblang leaf extract was positive for flavonoid compounds. The process is carried out by orienting the thin layer chromatography eluent and then proceeding to the preparative thin layer chromatography process. The result of the eluent orientation using 5 different eluents was then selected ethanol eluent: ethyl acetate: n-hexane (2: 2: 6) because the resulting stain is clearly separated, making it easier for the next process, namely preparative thin layer chromatography. From the results of preparative thin layer chromatography, 4 bands were obtained, then each band was separated and antibacterial activity was tested to find out which band could be used as antibacterial Staphylococcus aureus and continued with purity tests.

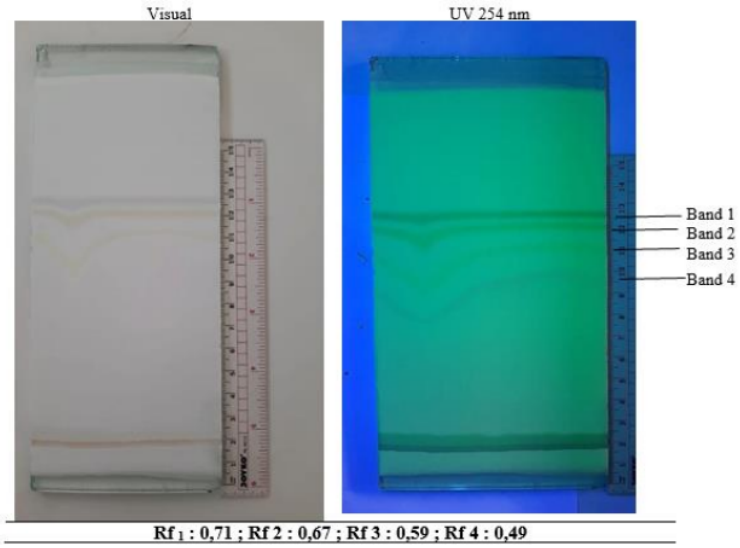

Figure 2. Band from preparative thin layer chromatography

The method carried out is to use three eluent systems with different levels of polarity and 2-dimensional thin layer chromatography. The mobile phase used in thin layer chromatography with different polarities is the ethyl acetate mobile phase: n-hexan (6:4) with a polarity value of 3.36 obtained an Rf value of 0.81. The second mobile phase uses ethyl acetate: chloropone (6: 4) with a polarity value of 4.5264 obtained an Rf value of 0.78. The third mobile phase uses methanol: chloropone (9: 1) with a polarity value of 30.738 obtained an Rf value of 0.73. The 2-dimensional thin layer chromatography test was carried out using Butanol eluent: chloroform (1:9) with a polarity value of 6.1054 and Butanol Eluent: chloroform (1:4) with a polarity indigo of 7.4048 [12]. In the purity test using 2-dimensional thin layer chromatography produced one yellowish-green stain after being given the appearance of ammonia vapor spots suspected of flavonoid compounds. The flavonoid compounds obtained from the preparative thin layer chromatography process were then oriented to the concentration, from the results of the orientation of flavonoid compounds of jamblang leaves (Syzigium cumini (L.) Skeels).

The concentrations of jamblang leaf flavonoid isolate (Syzigium cumini (L.) Skeels) used in this study were 2%, 4%, and 6%. This concentration is used because at a concentration of 2% it can inhibit the growth of bacteria with a clear zone diameter of 1.105 cm. The positive control used was 0.05% ciprofloxacin and the negative control used was DMSO. The amount of suspension grown into the media and the active flavonoid compounds planted is 50 µl. Data on the diameter of the clear zone of jamblang leaf flavonoid compounds (Syzigium cumini (L.) Skeels) against Staphylococcus aureus bacteria can be seen in Table 1.

Table 1. Activity test results of jamblang leaf flavonoid compounds *(syzigium cumini* (l.) skeels)

Replication	**Clear Zone Diameter (cm)**				
	2%	**4%**	**6%**	**K+**	**K-**
1	0,9560	1,0585	1,1575	1,4075	0,000
2	0,9545	1,0570	1,1620	1,4050	0,000
3	0,9520	1,0565	1,1525	1,4105	0,000
4	0,9515	1,0520	1,1525	1,4060	0,000
5	0,9560	1,0460	1,1670	1,4095	0,000
Average	0,9540± 0,0021	1,0540±0,0 051	1,1583±0,0 062	1,4077±0,0 023	0,00 0±0

The average results of the clear zone tested the antibacterial activity of flavonoid compounds of jamblang leaves (Syzigium cumini (L.) Skeels) concentration of 2% which is 0.9540 cm, concentration of 4% is 1.0540 cm, and concentration of 6% is 1.1583 cm. Based on the results of clear zone data, it can be said that an increased concentration of flavonoid compounds causes an increase in the diameter of the bacterial clear zone. The greater the concentration of flavonoid compounds of jamblang leaves (Syzigium cumini (L.) Skeels), the greater the diameter of the clear zone produced.

The results of the clear zone diameter data obtained were then carried out statistical tests using Statistical Program for Social Science. Statistical tests show the results that the data are normally distributed and homogeneous so continued with one way ANOVA and

continued post-anava Least Significant Difference tests to find out which concentrations have significant differences. Post-anava test results can be seen in Table 2.

Table 2. Post anava test results anava compound jamblang compound *(syzigium cumini* (l.) skeels)

Concentration	Significant	Conclusion
2% Vs 4% Concentration	0,000	Significantly different
2% Vs 6% Concentration	0,000	Significantly different
2% Concentration Vs K+	0,000	Significantly different
4% Concentration Vs 6%	0,000	Significantly different
4% Concentration Vs K+	0,000	Significantly different
6% Concentration Vs K+	0,000	Significantly different

The results of Table 2 can be known that the antibacterial activity of flavonoid compounds of jamblang leaves at concentrations of 2%, 4% and 6% and positive control of ciprofloxacin 0.05% differs significantly because the significant value is < 0.05. Ciprofloxacin is an antibiotic to treat various diseases caused by bacterial infections. Based on this, flavonoid structure identification was then carried out using UV-Vis spectrophotometry. UV-Vis spectrophotometry is used to determine the type of flavonoid, flavonoid group and analyze flavonoid structure in terms of OH position in the flavonoid nucleus based on the absorption shift of the spectrum with the addition of various shear reagents [11]. The results of the interpretation of shear reagents can be seen in Table 3.

Table 3. Interpretation results of shear reagents

Treatment	λ max (nm)		Shift λ (nm)		Interpretation
	Ribbon I	**Ribbon II**	**Ribbon I**	**Ribbon II**	
Isolate + methanol	374,00	257,20	-	-	**Flavonols (3 OH)**
Isolate + methanol + NaOH	423,70	279,60	+49,70	+22,40	-
Isolate + methanol + NaOH (5min)	335,10	293,40	-38,90	+36,20	**4' OH** (Absorbance spectrum data does not experience strength reduction after a certain time)
Isolate + methanol + NaOAc	378,40	258,20	+4,40	+1,00	**7-OH** (new band at wavelength 330.60)
Isolate + methanol + NaOAc + H3BO3	387,70	296,50	+13,70	+39,30	**o-diOH on ring B** (addition of spectrur +12 to 36 nm on band I)
Isolate + methanol +AlCl3	445,40	272,50	-2,10	-1,10	-
Isolate + methanol + AlCl3 + HCl	443,30	271,40	+69,30	+14,20	-

The results of the identification of flavonoid compounds are by spectral analysis with UV-Vis spectrophotometry, the structure of flavonoid compounds is obtained which leads to the structure of 3,7,4' trihydroxy flavones [11]. The structure of the 3,7, 4' trihydroxy flavones can be seen in Figure 2.

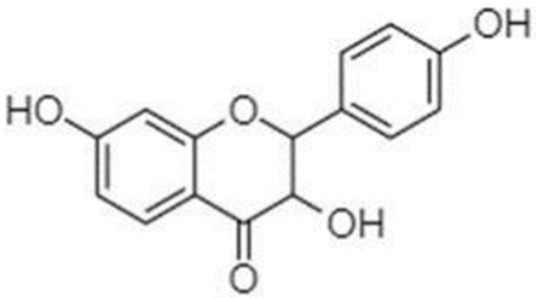

Figure 2. Interpretation of 3, 7, 4' trihydroxy flavone spectra

4. Conclusion

From the results of the research that has been carried out, the following conclusions are obtained, Jamblang leaf flavonoid compounds (Syzigium cumini (L.) Skeels) have antibacterial activity seen from the presence of clear zones against the growth of Staphylococcus aureus bacteria. There are significant differences from the antibacterial activity of flavonoid compounds of jamblang leaves (Syzigium cumini (L.) Skeels) at concentrations of 2%, 4% and 6% against the growth of Staphylococcus aureus bacteria. Analysis of the results of UV-Vis spectrophotometry of flavonoid compounds from jamblang leaves (Syzigium cumini (L.) Skeels) contains flavonoid compounds that lead to the structure of 3,7,4' trihydroxy flavones.

It is necessary to further identify isolates that have been produced to ensure a more complete structure of the compound by using other instruments such as IR, NMR, NMR spectrophotometers to ascertain the name of the compound and it is necessary to develop for pharmaceutical preparations.

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