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Antibacterial Activity Test of Suji Leaf Fraction (Dracena Angustifolis (Medik.) Roxb) Agains the Growth of Staphylococcus Epidermidis An Acna-Causing Bacteria

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Abstract: **Background**: The suji plants is a plant that has benefits as an antibacterial, one of which is the leaves. Suji leaves contain chemical compounds such as flavonoids, alkaloids, saponins, steroids, terpenoids, and tannins. The compounds can function as antibacterials. The study aims to determine the value of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of suji leaf fractions against Streptococcus epidermidis bacteria. The antibacterial test method used is liquid dilution and solid dilution. The results obtained were the most active fraction that was able to inhibit Staphylococcus epidermidis was the n-hexane fraction with a KHM value of 6.25% and KBM 12.5%. The results obtained show that the N-Hexane fraction of suji leaves which is the most active fraction in inhibiting bacterial growth is not as good as clindamycin and ethanol extract in inhibiting Staphylococcus epidermidis bacteria, where ethanol extract has a KBM value of 6.25%. Meanwhile, clindamycin which is a positive control has been able to inhibit and kill the growth of Staphylococcus epidermidis bacteria at a concentration of 0.1%. The results of KBM values obtained from the most active samples are ethanol extract > n-hexane fraction > ethyl acetate fraction > ethyl acetate insoluble fraction. Bioautography KLT results show that the compounds responsible for antibacterial activity are thought to be flavonoids, steroids, and triterpenoids. Acne is one of the skin infectious diseases caused by microorganisms such as fungi, viruses, and bacteria. Bacterial species that dominate the skin and cause acne are Staphylococcus epidermidis, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus, Enterobacter coli and Propionibacterium acnes. Acne treatment using irrational antibiotics often causes antibiotic resistance, therefore other alternatives are needed by using natural antibiotics from natural materials such as suji leaves. The suji plant is a plant that contains chemical compounds in the form of flavonoids, saponins and polyphenols which are proven to have activity as antibacterial agents, so it has the potential for acne treatment. **Aim**: The study aims to determine the value of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of suji leaf fractions against Streptococcus epidermidis bacteria and determine the most active fraction that is able to inhibit bacteria as well as the compound responsible for antibacterial activity. **Material and Methods**: Suji leaf extract was prepared by maceration using 96% ethanol solvent in a ratio of (1:10). The extract was then fractionated using solvents of different polarity, namely n-hexane and ethyl acetate so that the n-hexane fraction, ethyl acetate fraction and ethyl acetate insoluble fraction were obtained. The samples were then screened to determine the metabolite compounds contained and quantitative tests of total flavonoids were carried out. Samples of ethanol extract, n-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction were carried out antibacterial tests with a concentration series made of 3.125%; 6.25%; 12.5%; and 25%. The negative control used was 10% DMSO and the positive control used was clindamycin. Tests were carried out by liquid dilution method to determine the Minimum Inhibitory Concentration (MIC) value and solid dilution to determine the Minimum Bactericidal Concentration (MBC). The most active fraction in inhibiting bacteria was then subjected to further tests in the form of KLT Bioautography to determine the compounds responsible for antibacterial activity. **Results**: The results of this test are the most active fraction in antibacterial activity is the n-hexane fraction with an MIC value of 6.25% and MBC 12.5%. This antibacterial activity is influenced by the presence of secondary metabolite compounds contained, namely flavonoids, steroids, triterpenoids and tannins. Flavonoid levels in the most active n-hexane sample were 3.40 ± 0.08 mgQE/g. Bioautography KLT test results showed that the compounds responsible for antibacterial activity were found in spots with rf values of 0.25; 0.51; and 0.63 which were thought to be flavonoids, steroids, and tannins obtained after qualitative identification of compounds.

Keywords: antibacterial, suji leaves, fraction, Staphylococcus epidermidis

INTRODUCTION

Acne is a skin disease that is often experienced and is felt to be quite disruptive to the appearance of some people, especially in adolescents because it can lead to loss of self-confidence. Acne is caused by follicular hypercreatinization, bacterial colonization, increased sebum and inflammation. Acne is characterized by the chronic development of blackheads, erythematous papules on the face, neck and body (Leung et al., 2020).

Acne is one of the skin infectious diseases caused by microorganisms such as fungi, viruses, and bacteria. Bacterial species that dominate the skin and cause acne are Staphylococcus epidermidis, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus, Enterobacter coli and Propionibacterium acnes (Kumar et al., 2016). Staphylococcus epidermidis is a normal bacterial flora that resides on the skin and is a bacterium that causes acne

that develops in the sebaceous glands so that there is a blockage that causes irritation or inflammation in the skin tissue. Acne can be treated by reducing sebum production, reducing inflammation in the skin and reducing or inhibiting the growth of acne-causing bacteria such as Staphylococcus epidermidis (Afifi et al., 2018). In general, acne is treated using antibiotics to help inhibit or kill the growth of bacteria that cause acne. However, irrational use of antibiotics will cause bacterial resistance (Baroroh et al., 2018). Therefore, there is a need for other alternatives in overcoming problems due to bacterial infection with Staphylococcus epidermidis. One of them is by developing research on natural antibacterials derived from natural materials such as plants that are easily available in Indonesia, which are expected not to cause problems, such as those caused when using antibiotics (Riswana et al., 2022). One of the plants that is believed to be used as medicine is the suji plant. The suji plant is also a plant that contains chemical compounds in the form of flavonoids, saponins and polyphenols which are proven to have activity as antibacterial agents, so it has the potential for acne treatment (Sukmawati et al., 2017).

Yuniarni (2022) has conducted research on the antibacterial activity of suji leaves. The results obtained were that the ethanol extract of suji leaves extracted using the maceration method could inhibit the growth of Staphylococcus epidermidis bacteria (Yuniarni et al., 2022). Other research states that suji leaf ethanol extract contains chemical compounds in the form of flavonoids, saponins, tannins and steroids/triterpenoids, where these compounds have the potential to inhibit bacterial growth (Putriyana & Ridwanto, 2023). Thus, further research is needed on the antibacterial potential of suji leaves against the growth of Staphylococcus epidermidis bacteria that cause acne.

Research on the antibacterial activity of suji leaves against Staphylococcus epidermidis bacteria is still limited to extracts, but research on the antibacterial activity of fractions has never been done. Therefore, researchers want to conduct research related to the antibacterial activity of the fraction of suji leaf extract against Staphylococcus epidermidis bacteria. Fractionation is a method used to separate the components of active compounds from the resulting extract based on their polarity. Thus, it is expected to obtain the most active fraction in inhibiting the growth of Staphylococcus epidermidis bacteria that cause acne by looking at the MIC and MBC values produced. Testing the antibacterial activity of the suji leaf ethanol extract fraction was carried out using the dilution method to see the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

MATERIAL and METHOD

Tools

The tools used in this study are blender, a set of glass mat, autoclave, petri dish, oven, sterile cutton bud, tweezers, micropipette, centrifuge, vortex, evaporator, analytical balance, spirit lamp, sterile cotton, hot plate, refrigerator, incubator, ose needle, UV spectrophotometer.

Materials

The materials used in this study were suji leaves, Staphylococcus epidermidis test bacteria, ethanol, n-hexanes, ethyl acetate, aquadestilata, DMSO, toluene, acetone, formic acid, NaCl 0.9%, $AlCl_3$ reagent, Mayer reagent, Libermann-Burchard reagent, $FeCl_3$ 10% reagent, nutrient broth, nutrient agar, clindamycin positive control.

Determination

Plant determination is carried out with the aim of knowing and ensuring the correctness of the identity of plants that will be used in research. Suji leaf samples to be used were identified at the Biology Laboratory of Universitas Negeri Semarang.

Preparation of Suji Leaf Ethanol Extract

The preparation of suji leaf ethanol extract was carried out by weighing 500 g of suji leaf simplisia. The ratio between the simplisia and the solvent used in the maceration extraction process is 1: 10 (Rohadi & Ahidin., 2021). The suji leaf simplisia that has been weighed is carried out the extraction process using the maceration method by immersing it in a macerator containing 5 liters of 96% ethanol liquid for 24 hours and occasionally stirring. The filtered filtrate was then concentrated using a rotary evaporator, then evaporated on a waterbath until a thick extract was obtained. The thick extract obtained was then calculated the yield.

Fractionation

The ethanol extract of suji leaves was weighed as much as 1 gram then dissolved with 10 ml of N-Hexan solution and put into a centrifuge tube. Then the solution was vortexed for 5 minutes or until dissolved. Then continue the same

process using ethyl acetate solvent as much as 10 ml. The ethyl acetate soluble extract was taken as ethyl acetate fraction while the insoluble extract was taken as ethyl acetate insoluble fraction. All collected fractions were then evaporated on a waterbath to obtain a thick fraction.

Phytochemical Screening of Extracts and Fractions using KLT

Samples of suji leaf extracts and fractions were taken as much as 10 mg and then dissolved using 1 ml of 96% ethanol, then bottled on a silica gel F254 KLT plate. Elution using N-Hexan: Ethyl Acetate in a ratio of 3:1.

Table 1. Reagents to Phytochemical Screening

Test	Reagent	Positive result
Flavonoids	$AlCl_3$	Yellow color spot
Alkaloids	Mayer	Greenish-yellow spots
Steroids/ triterpenoids	Liebermann Burchard	Blue or blue-green spots
Tannins	$FeCl_3$	Black colored spots

Antibacterial Activity Test

Antibacterial activity tests were conducted on samples of ethanol extract, N-Hexan fraction, ethyl acetate fraction and insoluble fraction of suji leaves. Material samples were made with concentration series of 10%, 5%, and 2.5%. Each concentration was taken as much as 1 mL and put into a test tube that contained 1 mL of Nutrient Broth (NB). Furthermore, each test tube was added with 1 mL of test bacterial suspension. The negative control used was 1 mL of Nutrient Broth (NB) which was put into a test tube. Furthermore, for the Positive Control, 0.9 mL of Nutrient Broth (NB) was inserted into the test tube and added with 0.1 mL of test bacterial suspension. Then each tube was vortexed until homohen and incubated using an incubator for 18-24 hours at 37oC. The tubes were then observed for turbidity by comparing them with the control. The Minimum Inhibitory Concentration (MIC) is seen from the minimum concentration of the sample that is able to inhibit bacterial growth as seen by the presence of clarity.

Minimum Bactericidal Concentration can be determined by taking one ose of test media from the tube and inserted into Mannitol Salt Agar (MSA) media. Furthermore, Mannitol Salt Agar media is incubated for 18-24 hours at 37oC. The Minimum Kill Concentration of the ethanol extract fraction of suji leaves can be seen by looking at the presence or absence of bacterial colonies that grow on Mannitol Salt Agar media. The KBM value is determined from the absence of bacterial colonies that grow on Mannitol Salt Agar media. The most active fraction that can inhibit and kill Staphylococcus epidermidis bacteria is the ethyl acetate fraction.

Testing the most active fraction with KLT Bioautography Method

The fraction that has the greatest antibacterial activity, namely the N-Hexan fraction, is then continued to test antibacterial activity with KLT Bioautography. Bioautography test is done by putting 20µL of test bacteria and 25 mL of NA medium into a petri dish. The diluted chromatogram plate was then placed on the surface of the NA medium that had solidified. NA medium containing silica plates was then incubated for 18-24 hours. The presence of a clear zone around the plate indicates the presence of antibacterial activity in the compound group.

Total Flavonoid Content

Determination of Maximum Wavelength

A total of 100µl of 100 ppm quercetin solution was added with 10µl of 10% AlCl3. The solution was then measured using UV-Vis Spectrophotometry with a wavelength of 350-450 nm.

Determination of Quercetin Standard Curve

Quercetin standard solution was used to make a series solution with levels of 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm. each series solution was then taken as much as 100µl and put into a test tube. Furthermore, it was reacted using 10µl AlCl3. The solution was then allowed to stand for operating time and absorbance readings were taken using a spectrophotometer at the maximum wavelength.

Determination of total flavonoids

Fractions and ethanol extract of suji leaves were made into a concentration of 100 ppm. then the fraction was taken as much as 100µl and added with 10% AlCl3 as much as 10µl. The solution was allowed to stand for operating time. Then the absorbance was read with a spectrophotometer at the maximum wavelength.

RESULT AND DISCUSSION

Sample Preparation of Suji Leaf Extract

Suji plants obtained in the Magelang area were determined to ensure that the plants used were really the suji plants in question. The results obtained were that the plants used were really suji plants or Dracaena angustifolia (medik) Roxb. The maceration method is used in the extraction process because the process is relatively simple and does not require heating so that it can prevent damage or loss of active substances that are not resistant to heating. In the extraction process, 96% ethanol solvent is used, where ethanol is a universal solvent that has the ability to attract metabolite compounds contained in simplisia both polar and nonpolar. The mechanism of maceration with ethanol solvent is that there is a breakdown in the cell wall and cell membrane of the simplisia due to different pressures between inside and outside the cell which results in the secondary metabolites contained will be dissolved by ethanol9. Rendemen ekstrak yang didapatkan dari 500 gram simplisia adalah 8,8%. The results obtained have not met the requirements of a good yield value according to the Indonesian Herbal Pharmacopoeia 2017, which is a yield of not less than 10%10. Results that do not meet the requirements can be caused by the reduced volume of solvent in the maceration process, leaf age, plant environmental conditions, particle size of the sample and also how to stir the sample (Herdiansyah et al., 2023).

Fractinations

Suji leaf extract as much as 20 grams was fractionated using solvents with different polarity. The purpose of fractionation is to separate compounds based on the level of polarity. Fractionation was carried out using n-hexane and ethyl acetate solvents. The fractionation results can be seen in the following table:

Table 2. Yield of Suji Leaf Ethanol Extract Fractions

Extract weight	Fractions	Fraction weight (gram)	Yield (%)
20 gram	N-Hexan	2,62	13,1
	Ethyl acetate	1,06	5,3
	Insoluble ethyl acetate	2,19	10,95

The table above shows that the fraction that produces the largest yield value is the N-Hexan fraction, which is 13.1% with a fraction weight of 2.62 grams. The resulting yield value gives a relatively large value, this is because most of the secondary metabolite compounds contained in the ethanol extract of suji leaves are non-polar so that the results obtained in fractionation using non-polar solvents, namely N-Hexan, are relatively large. N-hexan solvent is used as a non-polar solvent so that non-polar compounds contained in suji leaf ethanol extracts such as steroids and triterpenoids will be extracted. While semi-polar compounds, namely phenolic compounds such as flavonoids, will be extracted in ethyl acetate solvents.

Phytochemical Screening

Phytochemical screening was carried out with the aim of knowing the presence of secondary metabolite compounds contained in ethanol extract samples, N-Hexan fractions, ethyl acetate fractions and ethyl acetate insoluble fractions of suji leaves. Samples that have antibacterial activity against Staphylococcus epidermidis are caused by the presence of secondary metabolite compounds where these compounds have a mechanism to inhibit or kill bacteria. Based on the results of phytochemical screening carried out on extract samples and fractions of suji leaves, they contain secondary metabolite compounds, including:

Table 3. Phytochemical Screening Results

Test	Sample			
	E Etanol	F N-H	F EA	F TLEA
Alkaloids	+	-	-	-
Flavonoids	+	+	+	+
Steroids	+	+	+	+
Triterpenoids	+	+	-	+
Tannins	+	+	+	-

Based on the results of table 4.2 shows that the ethanol extract positively contains metabolite compounds in the form of alkaloids, flavonoids, steroids, triterpenoids and also tannins. The n-hexane fraction which is a non-polar

solvent contains flavonoids, steroids, triterpenoids, van tannins that can dissolve in polar solvents. The ethyl acetate fraction contains flavonoids, steroids, and tannins, this is because ethyl acetate is a semi-polar solvent so that it can extract polar and non-polar compounds.

Secondary metabolite compounds such as alkaloids, flavonoids, steroids, triterpenoids, and tannins contained in the extract samples and fractions of suji leaves have antibacterial activity with different inhibitory mechanisms. Alkaloids have antibacterial activity by disrupting the preparation of peptidoglycan in bacterial cells so as to cause the formation of bacterial cell walls intact and by disrupting the process of bacterial peptidoglycan synthesis. Furthermore, flavonoid compounds have the ability to inhibit bacterial growth by disrupting the synthesis process of bacterial cell walls, bacterial polysaccharides and bacterial enzymes, causing bacteria to lysis. Steroids have a mechanism in inhibiting bacteria by connecting with bacterial lipid membranes so that bacterial lysosomes leak. While triterpenoids have a mechanism in inhibiting bacterial growth by forming polymer bonds on the outer membrane of the bacterial cell wall, causing a lack of permeability of the bacterial cell wall. And tannins are included in the phenol compound group with the mechanism of action in inhibiting bacterial growth is by inactivating microbial adhesins, enzymes, and protein transport in the cell layer (Harefa et al., 2022).

Total Flavonoid Content (TFC) calculation results

Flavonoids are one of the main compounds that have antibacterial activity. Quantitative analysis of flavonoids is carried out using UV-Vis spectrophotometry because flavonoids are conjugated aromatic compounds that will produce strong absorption bands in the ultraviolet and visible light spectra. Testing the total flavonoid content aims to determine the amount of flavonoid content in the sample. This test was carried out by colorimetric method with AlCl3 reagent with the standard used is quercetin. This method is done because flavonoids will react to form a color complex with AlCl3. The quercetin standard is used in the determination of total flavonoid content because it belongs to the flavonol group and has a hydroxyl group that neighbors flavones and flavonols.

The determination of the maximum wavelength aims to determine the maximum absorbance value that is selective to be used to analyze the total flavonoid content of the standard solution and sample. The use of the maximum wavelength in sample readings is used because it will provide the largest change in absorbance at each concentration, so it will minimize errors in re-measurement or replication of each test sample (Suharyanto & Prima, 2020). Wavelength determination is done by screening at a wavelength of 350-450 nm. The maximum wavelength obtained in the test is 430 nm. The wavelength obtained is 430 nm and then used to read the absorbance of the standard solution. In making the standard curve after the concentration series is added with AlCl3, the samples that will be read by uv-vis spectrophotometry are allowed to stand for an operating time of 30 minutes using a wavelength of 430 nm. Based on the standard curve, the resulting standard shows the equation y = 0.0473x + 0.0982 with a linear regression 0.9912 or close to 1. This means that there is a good correlation between the concentration of quercetin standard sample and the resulting absorbance where the increase in concentration is directly proportional to the increase in sample absorbance. The resulting standard curve was then used to perform calculations in determining the concentration of flavonoids in each sample.

Table 3. Calculation of Total Flavonoid Contents

Sampel	KFT
EE	3,70±0,29
FN-H	3,40±0,08
FEA	4,04±0,15
FTLEA	3,04±0,24

Based on the results obtained in table 4.5 above, it was found that among the N-Hexan fraction, Ethyl Acetate fraction and Ethyl Acetate Insoluble fraction, the largest flavonoid content was obtained in the ethyl acetate fraction of suji leaves with the value of the content obtained was 4.04 ± 0.15 mgQE/g. This can be caused because ethyl acetate is a semi-polar solvent so that it can attract flavonoid compounds that are polar and nonpolar. In addition, it can also be caused by the content of some flavonoids that are more soluble in semipolar solvents such as flavones, flavonols and flavones.

Testing the total flavonoid content that has been done gets the result that the lowest flavonoid content is found in the ethyl acetate insoluble fraction < N-Hexan fraction < ethanol extract < ethyl acetate fraction. The largest flavonoid content was obtained by the ethyl acetate fraction of suji leaves, which amounted to 4.04 ± 0.15 mgQE/ g

sample. The test results show that the p-value obtained (>0.05) which means there is no significant difference between samples.

Determination of the most active fraction as antibacterial

Antibacterial activity testing was carried out using the dilution method with several sample concentrations. Determination of antibacterial activity was carried out using the liquid dilution method to see the Minimum Inhibitory Concentration value and solid dilution to determine the Minimum Kill Concentration of the sample. The selection of this dilution method is because it has advantages compared to the diffusion method, where with the dilution method the bacterial suspension is evenly distributed on the media used so as to facilitate interaction with the test sample.

The sample concentrations of ethanol extracts and N-Hexan fractions, ethyl acetate fractions and ethyl acetate insoluble fractions of suji leaves used in the test were 25%; 12.5%; 6.25%; and 3.125%. The preparation of the test solution of the suji leaf fraction was carried out by dissolving the fraction using 10% DMSO solvent. The use of DMSO to be used as a solvent of ethanol extract and the three test fractions is due to its properties that can dissolve all compounds both polar and non-polar. DMSO is also used as a negative control because DMSO does not have the ability to inhibit bacterial growth so that its use will not affect the results of the antibacterial test of the suji leaf fraction against Staphylococcus epidermidis bacteria.

The positive control used is clindamycin 0.01% where clindamycin is an antibiotic that has a broad spectrum and is optimal against gram-positive and anaerobic bacteria such as staphylococcus epidermidis. Clindamycin is an antibiotic that is often used to treat acne problems caused by bacteria such as Staphylococcus epidermidis. The mechanism of clindamycin is by inhibiting protein synthesis. The use of clindamycin as a positive control is to determine at what concentration the ethanol extract and fraction of suji leaves are able to inhibit Staphylococcus epidermidis bacteria similar to the inhibition produced by clindamycin.

Minimum Inhibitory Concentration (MIC) is the lowest concentration value of the sample that is able to inhibit bacterial growth. Determination of the Minimum Inhibitory Concentration (MIC) value on the sample is done by visually observing the presence or absence of turbidity in the test concentration solution. The concentration where there is no bacterial growth characterized by the turbidity of the test concentration solution is determined as the Minimum Inhibitory Concentration (MIC).

Table 4. Results of Minimum Inhibitory Concentration (MIC)

Samples Test	Consentrations				MIC
	3,125%	6,25%	12,5%	25%	
EE	+	-	-	-	6,25%
FN-H	+	-	-	-	6,25%
FEA	+	+	-	-	12,5%
FTLEA	+	+	-	-	12,5%

Description:

(+) = there is bacterial growth (cloudy)

(-) = no bacterial growth

The Minimum Inhibitory Level (KHM) value is characterized by a change in the clarity of the test tube adjusted to the clarity of the tube containing the positive control used, namely clindamycin. Based on the results of the Minimum Inhibitory Level (KHM) test conducted on ethanol extracts, N-Hexan fractions, ethyl acetate fractions, and ethyl acetate insoluble fractions, it shows that ethanol extracts and fractions of suji leaves are able to inhibit Staphylococcus epidermidis bacteria with the greatest strength in inhibiting bacteria contained in ethanol extract samples and N-Hexan fractions > ethyl acetate fractions and ethyl acetate insoluble fractions. KHM results obtained in each sample are characterized by the absence of turbidity in the test tube. One factor that can affect the ability to inhibit bacteria (bacteroistatic) is the level of concentration, where higher concentrations will provide higher antibacterial inhibitory activity.

Minimum Bactericidal Concentration is the lowest concentration required for a sample to kill a bacterium. The Minimum Bactericidal Concentration value is characterized by the absence of bacterial colonies that grow on the agar medium used. (Gumilar & Susanti, 2024).

Table 5. Results of Minimum Bactericidal Concentration (MBC)

Test	Consentations				MBC
	3,125%	6,25%	12,5%	25%	

EE	+	-	-	-	6,25%
FN-H	+	+	-	-	12,5%
FEA	+	+	+	-	25%
FTLEA	+	+	+	-	25%
Klindamisin 0,1%	-				

Description:

(+) = there is growth of bacterial colonies

(-) = no growth of bacterial colonies

Based on the results of antibacterial testing on suji leaf extracts and fractions that have been carried out, the MBC value produced has a greater concentration than the MIC value. Therefore, it can be concluded that to be able to kill the test bacteria, it is necessary to increase the concentration of the test solution which is greater than the Minimum Inhibitory Concentration (MIC) value. The results of the Minimum Bactericidal Concentration (MBC) obtained in this study were that the ethanol extract of suji leaves was able to kill Staphylococcus epidermidis bacteria at a concentration of 6.25%; the n-hexane fraction was able to kill at a concentration of 12.5%; while the ethyl acetate fraction and ethyl acetate insoluble fraction were able to kill at a concentration of 25% which was characterized by the absence of bacterial colonies that appeared on agar media.

The results obtained in this study showed that the fraction with the best antibacterial activity was the N-Hexan fraction. The N-Hexan fraction sample has antibacterial activity with a MIC value of 6.25% and MBC at a concentration of 12.5%. The results obtained show that the N-Hexan fraction of suji leaves which is the most active fraction in inhibiting bacterial growth is not as good as clindamycin and ethanol extract in inhibiting Staphylococcus epidermidis bacteria, where ethanol extract has a MBC value of 6.25%. Meanwhile, clindamycin which is a positive control has been able to inhibit and kill the growth of Staphylococcus epidermidis bacteria at a concentration of 0.1%. The results of MBC values obtained from the most active samples are ethanol extract > n-hexan fraction > ethyl acetate fraction > ethyl acetate insoluble fraction. The most active fraction, namely n-hexan, was then subjected to further tests, namely bioautography KLT test.

Bioautography KLT Test Results

Bioautography KLT testing was carried out on the most active fraction of suji leaves, namely the N-Hexan fraction. This test was carried out to determine the active compounds contained in the N-Hexan fraction which had antibacterial activity against Staphylococcus epidermidis. Bioautography testing was carried out using the contact method where the eluted KLT plate was placed directly on a medium containing a suspension of Staphylococcus epidermidis bacteria. This method was chosen because of its ease of operation and the results obtained were clearer. The result of this test is the appearance of an inhibition zone in the form of a clear zone that appears on the surface of the agar where the silica plate is attached, indicating the location of compounds that have antibacterial activity.

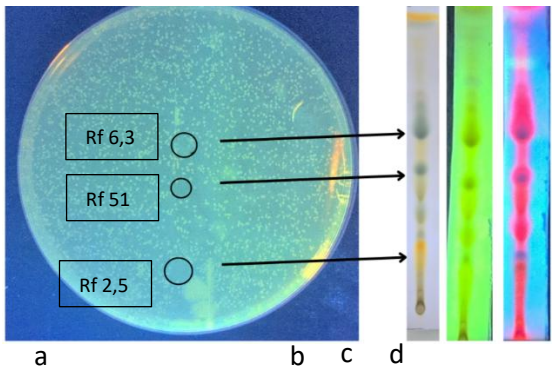

Figure 1. Observation of Bioautography KLT Results of N-Hexan Fraction on (a) Petri dish containing Staphylococcus epidermidis bacteria, (b) KLT observation in visible light, (c) KLT observation in UV 254 nm, (d) KLT observation in UV 366 nm.

The results obtained in Figure 4.3 show that in the n-hexan sample which is the most active fraction, there are 5 spots that appear after elution observed in visible light, UV 254 and UV 366 KLT observations. However, there are only 3

spots that show inhibitory activity against Staphylococcus epidermidis bacteria characterized by the absence of bacterial colonies that grow with an rf value of 0.25; rf 0.51 and rf 0.63.

Table 6. Zone of Inhibition of Bioautography KLT Test

Rf Value	Diameter of Zone of Inhibition (mm)
0,25	6,5 mm
0,51	3 mm
0,63	5 mm

The results obtained in table 4.6 show that the greatest inhibition is owned by the spot with an rf value of 0.25 > 0.63 > 0.51. Furthermore, the characterization of the components contained in the N-Hexane fraction samples that have been eluted using ethyl acetate eluent: N-Hexane (1:3) by using AlCl3, and Liebermann Burchard spotter. AlCl3 is used to identify the presence of Flavonoid compounds while Liebermann Burchard is used to identify steroid compounds and triterpenoids.

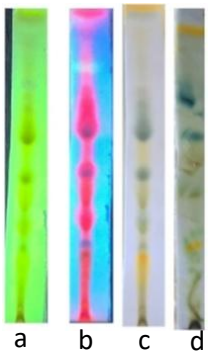

Figure 2. Identification results of chemical compounds on chromatogram (a). KLT at UV light 254 nm, (b) KLT at UV light 366 nm, (c) KLT with AlCl3 spot, (d) KLT with Liebermann Burchard spot.

The results of the identification of chemical compounds in the N-Hexane fraction sample showed that the N-Hexane fraction positively contained flavonoid compounds characterized by the presence of yellow spots with an rf value of 0.25 in the KLT test with AlCl3 spotting. While the KLT test with Liebermann Burchard spotter produced positive results on triterpenoids with an rf value of 0.47 which is characterized by the presence of brownish green spots and steroid compounds at rf 0.58 and rf 0.66 which are characterized by blue spots. The results obtained indicate that the compounds thought to be responsible for the antibacterial activity of the suji leaf fraction are flavonoids, steroids and triterpenoids.

Relationship of Total Flavonoid Level with Antibacterial Activity

Table 8. Relationship of Total Flavonoid Level with Antibacterial Activity

Samples	Total Flavonoids Content (mgQE/g)	MIC	MBC	Phytochemical Test Results
EE	3,70±0,29	6,25%	6,25%	Alkaloids, flavonoids, steroids, triterpenoids, tannins
FN-H	3,40±0,08	6,25%	12,5%	Flavonoids,steroids, triterpenoids, tannins
FEA	4,04±0,15	12,5%	25%	Flavonoids, steroids, tannins
FTLEA	3,04±0,24	12,5%	25%	Flavonoids,steroids, triterpenoids

The results obtained in table 8 also show that the high levels of flavonoids do not affect the decrease in Minimum Inhibitory Concentration (KHM) and Minimum Kill Concentration (KBM) of the sample. In testing flavonoid levels, which is one of the antibacterial compounds, the results show that the highest flavonoid levels are in the ethyl acetate fraction, while based on the results of the antibacterial test, the most active fraction of suji leaf ethanol extract in inhibiting the growth of Staphylococcus epidermidis bacteria is the N-Hexane fraction. However, flavonoid levels in the ethyl acetate fraction with the N-Hexane fraction have an insignificant difference characterized by the p-value obtained 0.051 > 0.05. This can indicate that flavonoids are compounds that play a role in antibacterial activity, but there are other compounds that also have antibacterial activity in the N-Hexane fraction sample of suji leaves. The compounds contained and thought to have antibacterial activity besides flavonoids in the N-Hexane fraction are

steroids, triterpenoids and tannins, where the results of phytochemical screening in table 4.4 of the N-Hexan fraction are positive in testing these compounds.

CONCLUSION

Based on the results of the research that has been conducted and obtained, it can be concluded that:

N-Hexane Fraction, Ethyl Acetate Fraction and Ethyl Acetate Insoluble Fraction of suji leaves have antibacterial activity against Staphylococcus epidermidis with the largest to smallest being the n-hexane fraction > ethyl acetate fraction > ethyl acetate insoluble fraction. The most active fraction that can inhibit the growth of Staphylococcus epidermidis bacteria is the N-Hexane fraction with a KHM value of 6.25% and KBM 12.5%. However, the inhibition is not as good as ethanol extract and clindamycin in its inhibition. Secondary metabolite compounds that are active in antibacterial activity by KLT bioautography are shown in spots with rf values of 0.25; 0.51; and 0.63 which are thought to be flavonoid, steroid and triterpenoid compounds seen from the results of qualitative identification of compounds.

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CONFLICT OF INTEREST

We declare that we don't have any conflict of interest.

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