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ANTIBACTERIAL ACTIVITY TEST OF n-HEXAN, ethyl acetate AND WATER FROM ETHANOL EXTRACT OF KITOLOD LEAF (Isotoma longiflora (L.) C. Presl.) AGAINST Staphylococcus aureus ATCC 25923

Tatiana Siska Wardani*, Triya Choirin Nisa, Kusumaningtyas Siwi Artini
Faculty of Health Sciences, Universitas Duta Bangsa, Jl. K.H Samanhudi No.93, Sondakan, Kec. Laweyan, Kota Surakarta, Central Java 57147
*siskatatiana@gmail.com

ABSTRACT

Kitolod plant (isotoma longiflora (L) C.Presl) contains alkaloids, flavonoids, and saponins. The content of this compound has antibacterial activity. This study aims to determine the antibacterial activity of the n-hexane, ethyl acetate and water fractions from the ethanolic extract of kitolod leaves against Staphylococcus aureus, and to determine the most active fraction in inhibiting bacteria. This research is an experimental descriptive study. The ethanolic extract of kitolod leaves was obtained by maceration method using 96% ethanol solvent, the viscous extract obtained was fractionated with three solvents, and dissolved in 1% DMSO then tested for its antibacterial power by diffusion and dilution method against Staphylococcus aureus at concentrations of 15%, 20%, 25%, and 30% using ofloxacin positive control. The test results obtained were analyzed using the One Way Anova test followed by the Post Hoc Test using the Tukey method using SPSS16. The results of the test using the diffusion method showed that the ethanol extract, n-hexane fraction, ethyl acetate fraction and water fraction had antibacterial activity against Staphylococcus aureus. The most active fraction was the water fraction at a concentration of 30%, with an average inhibition zone diameter of 19.0 mm. The inhibitory power was lower than the positive control ofloxacin (28.3 mm). The dilution method of the water fraction showed a minimum inhibitory concentration and a minimum kill concentration at a concentration of 15%.

Keywords: antibacterial; diffusion; dilution; fraction; kitolod; staphylococcus aureus

INTRODUCTION

Infectious disease is a disorder that often occurs in the community, infection can be caused by fungi, viruses and bacteria. Some bacteria that can cause infection include Escherisia coli, Pseudomonas aeruginosa, Salmonella typhi and Staphylococcus aureus (Jewet, Melnick & Adelberg's, 2001). One of the bacteria that often causes infection is Staphylococcus aureus, this is a normal flora found on human skin. Especially on the skin, nose and throat (Buckle, et al, 2007). Many diseases can be caused by Staphylococcus aureus bacteria, such as pneumonia, meningitis, endocarditis or sepsis with suppuration in each organ (Jewet, Melnick & Adelberg's, 2001). Multilevel extraction or fractionation is an extraction method that uses a solvent based on the polarity of the compound to be searched, ranging from non-polar solvents to polar solvents (Endang, 2015).

The kitolod plant (Isotoma longiflora (L.) C. Presl.) has been empirically proven to treat diseases of the iris or conjunctivitis of the eye caused by bacterial, fungal and viral infections (Ali, 2013). Kitolod plants contain alkaloid compounds (lobelin, lobelamin and isotomin) (Dalimartha, 2008). People use the leaves and flowers of the kitolod plant to treat inflammation or infection. Alkaloids, flavonoids, saponins are antibacterial compounds found in the kitolod plant. This study was conducted to determine the antibacterial activity of the n-hexane, ethyl acetate and water fractions from the ethanolic extract of kitolod leaves and to determine the most active fraction in inhibiting

Staphylococcus aureus bacteria grown on Muller Hilton Agar medium. In previous studies, the antibacterial activity of the ethanolic extract of kitolod leaf against Staphylococcus aureus was carried out, while this study used three fractions with concentrations of 15%, 20%, 25% and 30% which were tested on Staphylococcus aureus bacteria.

METHOD

Tools and Materials

The tools used in this study were Beaker glass, blender, porcelain dish, glass funnel, measuring cup, watch glass, oven, water bath, a set of maceration tools, separating funnel, vacuum rotary evaporator, Moisture Balance, analyte scales, water bath, microscope, preparations glass, autoclave, Bunsen burner, 500 ml beaker glass, petri dish, 5 ml measuring cup, 10 ml measuring cup, gloves, incubator, caliper, needle loop, 10 ml volumetric flask, mask, micropipette, disc, dropper, test tube rack, small test tube, LAF (Laminar Air Flow), yellowtip, chamber, UV 254 lamp, and UV 366. The materials used in this study were Kitolod leaves, 96% ethanol, 3% hydrogen peroxide, the test bacteria were Staphylococcus aureus cultures, gram A, gram B, gram C, cotton, chloroform, methanol, Nutrient Agar (NA) medium, Nutrient Broth medium (NB), Muller Hinton Agar (MHA) medium, NaCL 0.9%,, piper disk (oxxoid), aquadest, DMSO 1%, antibiotic ofloxacin 0.4%, silica gel GF 254, cytoborate reagent, liberman bourchardat reagent, Dragendroff's spray reagent.

Work Procedures

The first stage in this study was the determination of kitolod plants carried out at B2P2TOOT Tawangmangu. Based on the results of the determination, it is known that the plants used in this study are kitolod plants. Second, the characteristics of simplicia powder were tested, namely drying shrinkage test and moisture content test using the Moisture Balance tool. Extraction of kitolod simplicia with 96% ethanol using the maceration method. The kitolod leaf powder was weighed as much as 300 grams, soaked in a brown bottle with 2 liters of 96% ethanol, tightly closed and left for 3 days protected from sunlight, with shaking every 6 hours. The extracted liquid was filtered through filter paper. The pulp is then soaked again with 1 liter of 96% ethanol. The filtrate obtained was then concentrated with a vacuum rotary evaporator at a temperature of 40ºC and a water bath at a temperature of 60ºC to obtain a thick extract of kitolod leaves. The results of the extract were then tested free of ethanol with the aim that the extract did not contain ethanol which has antibacterial activity (Praeparandi 2006).

Phytochemical screening of ethanolic extract of kitolod leaves by color test and thin layer chromatography (TLC) method. The extract was spotted on a silica gel plate and put into a saturated solvent. The results of the separation from the spots are then sprayed with color reagents. Chemical screening tests carried out were alkaloid test, flavonoid test, and saponin test. Fractionation uses non-polar, semi-polar and polar solvents, the solvents used are n-hexane, ethyl acetate and water. The extract was weighed 10 grams, dissolved with 75 ml aquadest, after completely dissolved, 75 ml n-hexane was added and shaken for 10 minutes, occasionally opening a separating funnel to remove gas. The residue of n-hexane was then continued by adding 75 ml of ethyl acetate and shaking for 10 minutes. Before carrying out the antibacterial test, the concentration of the three fractions and the positive control of ofloxacin were made first. All samples were dissolved with 1% DMSO, so that the concentrations of 15%, 20%, 25%, and 30% v/v were obtained.

The bacteria to be used are cultured first to obtain bacterial stocks and are easier to use to make bacterial suspensions. Bacterial suspension was prepared by adding 0.9% NaCl, until the turbidity was equivalent to 0.5 Mc Farland. Then the identification of Staphylococcus aureus ATCC 25923 was carried out with gram staining and biochemical tests. The bacterial suspension was then implanted into Muller Hilton Agar (MHA) and Nutrient Broth (NB) media and then tested by diffusion and dilution. Diffusion test using a piper disk that has been saturated in four different concentrations of 15%, 20%, 25%, 30% in one type of sample. The dilution test was carried out using 7 sterile tubes. The concentration of the stock solution made was 15%, then diluted with 1% DMSO solvent. Aseptically, from the stock solution, a series of concentrations was made below it, namely control (-); 15%; 7.5%; 3.75%; 1.87% and control (+). All petri dishes and test tubes that have been treated are put in an incubator for 18-24 hours at 37°C to optimize bacterial growth. Observations were made on the antibacterial activity of the test and positive control solutions. All inhibition zones were measured using a caliper and then calculated. Analysis of the data used in this study using ANOVA using SPSS16.

RESULTS AND DISCUSSION

Extraction Randement Results

The results of the extraction of simplicia leaves of kitolod (Isotoma longiflora L.) as much as 300 grams with 96% ethanol solvent (3 liters) obtained an extract weight of 38.63 grams. The yield of kitolod extract obtained was 12.87%.

Ethanol Free Test Results

In the ethanol-free test, the extract was added with H2SO4 and CH3COOOH, heated, it did not form a distinctive ester odor from ethanol (Praeprapandi, 2006). In this experiment, using kitolod leaf extract, there was no ester odor.

Drying Shrinkage Test Results

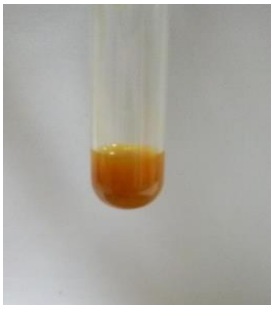
The maximum limit of drying loss according to the Herbal Pharmacopoeia is not more than 10% (Depkes RI, 2008). In this experiment, drying loss was carried out by weighing 2 grams of simplicia powder and then adding it to the Moisture Balance tool. The drying shrinkage obtained from the drying of kitolod leaves was found to be 9.29% meeting the drying shrinkage standard.

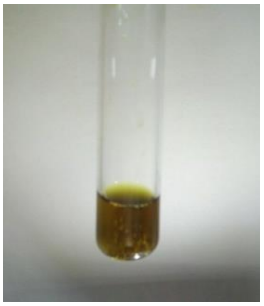
Phytochemical Screening

The results of the phytochemical screening test in the ethanol extract of kitolod leaves contained alkaloids, flavonoids, and saponins. The results can be seen in the table.

Table 1.
Phytochemical Screening

Compound test	Reagent	Teory	Result	Note
Alkaloid	2 mg HCL 2% and Dragendroff 2-3 drops	There is an orange precipitate	There is an orange precipitate	Positive
Flavonoid	Concentrated Mg and HCL powder 5 drops	Orange red	Orange red	Positive
Saponin	Aquades and KOH 10 drops	There is a solid foam as high as 1 cm and it remains stable for 15 minutes	There is foam that doesn't go away 15 minutes	Positive


Alkaloid Test


Flavonoid Test

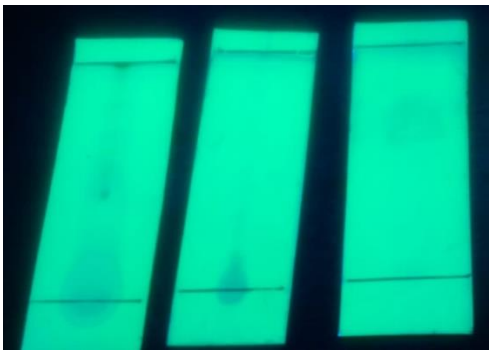

Saponin Test

Figure 1. Phytochemical screening

TLC identification

Table 2.
Identification of TLC

No	Test	Stationary Phase	Motion Phase	Detection	Result	Note
1	Flavonoid	Silica Gel GF 254	kloroform : methanol (1:1)	UV 254 dan UV 366	Greenish yellow spots	+
2	Alvoloid	Silica Gel GF 254	n-heksana : etil asetat (7:3)	UV 254 dan UV 366	Brownish-orange spots	+
3	Saponin	Silica Gel GF 254	kloroform : etil asetat (1:1)	UV 254 dan UV 366	Violet-blue spots	+


UV light 366


UV light 254

Figure 2. TLC test (Flvonoids, Alkaloids, Saponins)

Antibacterial Activity Test Results

Diffusion Test

The n-hexane, ethyl acetate, and water fractions from the ethanolic extract of kitolod leave had antibacterial activity against Staphylococcus aureus. The results of the diameter of the inhibition zone can be seen in table 3. Based on the results in the table, it can be seen that the n-hexane, ethyl acetate, and water fractions of the ethanol extract of kitolod leaves have antibacterial activity. The resulting inhibition zone increases with increasing concentration. The water fraction of the ethanolic extract of kitolod leaves at a concentration of 30% was able to inhibit the most optimum bacterial growth compared to other concentration series.

Table 3.
Results of Inhibitory Zone Diameter

Solvent	Concentration	Inhibition			Average
		I	II	III	
Extract	15%	5	5	6	5,3
	20%	7	7	8	7,3
	25%	10	13	12	11,6
	30%	15	14	14	14,3
N-Heksana	15%	4	3	3	3,3
	20%	6	6	5	5,6
	25%	10	10	9	9,6
	30%	14	13	12	13
Etil Asetat	15%	9	8	7	8
	20%	11	10	11	10,6
	25%	12	12	13	12,3
	30%	20	17	16	17,6
Water	15%	12	11	10	11
	20%	14	15	13	14
	25%	16	18	17	17
	30%	20	19	18	19
Control (+) Ofloxacin 0,4%	15%	19	20	20	19,6
	20%	23	23	24	23,3
	25%	26	27	27	26,6
	30%	28	27	30	28,3
Control (–) DMSO 1 %	15%	0	0	0	0
	20%	0	0	0	0
	25%	0	0	0	0
	30%	0	0	0	0

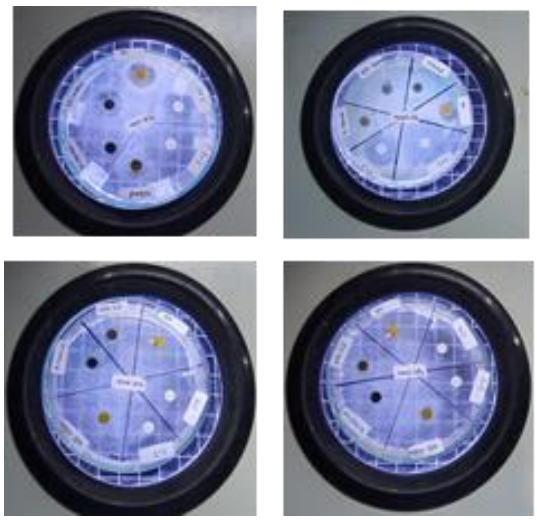


Figure 3. Diffusion Test

Dilution Test

The dilution test was carried out using liquid media, taking into account the turbidity of the tube containing bacteria which were incubated for 1 to 24 hours in the incubator. The dilution test was carried out from the most active fraction, namely the water fraction. The most active fraction in inhibiting with 15% MIC. Characterized by the clarity of the tube that has been filled with ethyl acetate fraction plus bacterial suspension, and liquid media NB. The concentration of the fraction used is 15%; 7.5%; 3.75%; 1.87%.

Table 4.
Dilutional Antibacterial Activity Test

Water Fraction Concentration	Replication		
	I	II	III
Ofloxacin 0,4%	-	-	-
Water Fraction 15%	-	-	-
7,5%	+	+	+
3,75%	+	+	+
1,87%	+	+	+
DMSO (-)	+	+	+
Normal (Media NB)	+	+	+

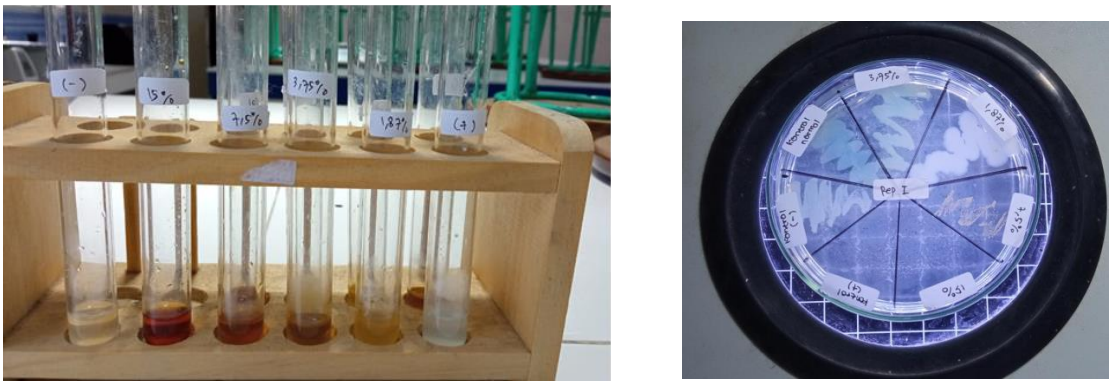


Figure 4. Dilution Test

Data Analysis

From the results of the Kolmogorove-Sminov One-Sample test, sir. 0.994 > 0.05, then H0 is accepted, the data is declared normally distributed. Furthermore, Homogeneity Test The probability value of Levene Statistic is 0.065 > 0.05, then H0 is accepted, which means that the four samples have the same variance. The significance result of the ANOVA test data is 0.000 < 0.05, which means that the four samples have differences in the diameter of the inhibition zone. Tukey's analysis showed that there were differences in the concentration of each treatment in the study that affected the inhibition of bacteria.

CONCLUSION

The experimental results using the diffusion method showed that the ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction had antibacterial activity against Staphylococcus

aureus. The most active fraction was the water fraction at a concentration of 30%, with an average inhibition zone diameter of 19.0 mm. The inhibitory power was lower than the positive control ofloxacin (28.3 mm). The dilution method of the water fraction showed a minimum inhibitory concentration and a minimum kill concentration at a concentration of 15%.

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