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Anti-Bacterial Activity of Ethanol Extract of the Stem of Coffee Parasite (*Scurulla Ferruginea* (Robx. Ex Jack) *Danser*) and its Secondary Metabolite Isolation

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Abstract. The aim of this study is to determine the antibacterial activity of the ethanol extract of the stem of the coffee parasite (Scurulla ferruginea (Robx. Ex Jack) Danser) using disc diffusion and microdilution methods. In this study, secondary metabolites were isolated from coffee parasite stems using Vacuum Liquid Chromatography and Column Chromatography continued by characterized the compounds using GC-MS (Gas chromatography-mass spectroscopy). Based on the identification using GC-MS, it was known that the stem of the coffee parasite contains diisooctyl phthalate. The results of the antibacterial activity test of the disc diffusion method with an extract concentration of 1% against *Staphylococcus aureus*, *Salmonella typhi*, *Streptcoccus viridans*, *Escherichia coli*, *Streptococcus mutans*, and *Bacillus aureus* bacteria were 6.35 mm, 6.7 mm, 8.95 mm, 7 mm, 8.75 mm and 8.2 mm respectively. This result shows that the ability to inhibit bacterial growth of 1% extract is in the medium category. The MIC values for *Escherichia coli*, *Streptococcus mutans*, and *Bacillus aureus* bacteria were 2500 g/mL, while the MIC values for *Staphylococcus aureus*, *Staphylococcus typhi*, *Streptcoccus viridans* were 5000 g/mL. The MBC value of *Streptcoccus viridans*, *Streptococcus mutans*, and *Bacillus aureus* bacteria was greater than 5000 g/mL, only *Escherichia coli* bacteria can be killed at a concentration of 5000 g/mL and *Salmonella typhi* at a concentration of 2500 g/mL. The test results showed that the stem extract of *Scurulla ferruginea* was only as an inhibitor.

INTRODUCTION

The parasite plant is one of the plants that has been widely used as traditional medicine. The chemical components contained in this plant are phenolics, tannins, alkaloids and saponins [1]. Component act as a disinfectant is the phenolic content. Based on phytochemical tests, beside the flavonoid and terpenoid groups it is also contained alkaloids and steroids. Previous researches reported secondary metabolites that were isolated and identified generally found is in the nonpolar fraction, flavonoids and terpenoids, there were no report on the types of alkaloids and steroids found in parasites. The best phenolic compounds and bioactivity were found in polar and stem fractions of the coffee parasite, but the specific type of flavonoid has not been reported. [2-4] Phytochemical tests on samples of the ethanolic extract of the parasite coffee showed the presence of alkaloids, flavonoids, steroids, and saponins. In addition, the methanol extract of the leaves of the cacao parasite proved the presence of groups of flavonoid, phenolic, terpenoid and saponin compounds [5]. Saponins have molecules that are hydrophilic (attracts water) and lipophilic (dissolve fat) so that they can reduce cell surface tension which in turn can cause the destruction of bacteria [6].

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The general composition of loranthus family parasites is 82.28% crude fiber, 9.21% water, 2.44% ash, 2.70% crude protein, 0.77% crude fat and 2.60% of other components [7]. Secondary metabolites in coffee parasites that have been identified include fatty acids: oleic acid, linoleic acid, linolenic acid, octadeca-8-10-dinoic acid, (Z)-octade-12-ene-8-10-dioic acid. and octadeca-8-10-12-trinoic acid; quercithrin, quercetin, rutin, icarisid B2, aviculin, (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-O-gallate and (-) epigalocatechin-3-O- gallate. Sesquiterpenes are the type of compounds that are isolated from ethanol extract of the leaves of the coffee parasite [8]. Three natural flavonol compounds have been isolated from the ethyl acetate fraction of Scurrula ferruginea Danser (Loranthaceae) namely quercetin and quercitrin, flavonol glycoside 4-O-acetylquercitrin [9].

MATERIALS AND METHODS

This study was conducted at the Research Laboratory and Mirobiology Laboratory, Department of Chemistry, Medan State University, Williem Iskandar Pasar V Street, Medan Estate, Deli Serdang, North Sumatera.

Materials

The materials used in this study is a stem coffee parasite (Scurulla ferruginea (Robx. Ex Jack) Danser) TLC plate, silica gel merck 60 GF254, silica gel merck (230-400 mesh), ethanol, n-hexane, ethyl acetate, and chloroform. Antibacterial test materials were used Mueller-Hinton Agar (MHA), Mueller-Hinton Broth (MHB), dimethyl sulfoxide (DMSO), chloroamphe-nicol, 0.9% NaCl, bacterial cultures of *Staphylococcus aureus*, *Salmonella typhi*, *Streptcoccus viridans*, *Escherichia coli*, *Streptococcus mutans*, and *Bacillus aureus.*
The tools used in this research is analytical balance, a set of glasses, TLC chamber, UV lamp (UVP UVGL-55), erlenmeyer flask, vacuum pump, Buchner funnel, column for chromatography, column for vacuum liquid chromatography (VLC), rotary Heidolph Base Hei-VAP HL evaporator, incubator, autoclave TOMY ES-315, oven, petridish, microplate and GCMC.

Methods

Extraction is done by maceration method. A total of 1.3 kg *Scurulla ferruginea* stems that have been mashed and dried, macerated with ethanol 3 x 24 hours. The macerated ethanol extract is combined and filtered. The obtained filtrate was evaporated using a rotary evaporator at low pressure, resulting in ethanol extract [10]

Bacterial Suspension

The bacteria are cultured before being used for testing. From bacteria that have been sterile taken bacteria and smeared on MHA media, then put into the incubator to be incubated at 37°C for 24 hours. After that the bacteria are suspended into 0.9% NaCl to the McFarland 0.5 standard (0.5 mL $BaCl_2$ 0.048 mol/L in 99.5 mL H_2SO_4 0.18 mol/L). The suspension of bacteria into 0.9% NaCl is the same as bacterial dilution of 10-6 CFU/mL (McFarland Standard 0.5). [10-11]

Paper Disc Diffusion Method Test

In this test the concentration of test extracts used were 100, 50, 25 and 12.5 g/µL with DMSO solvents [12]. The 100 L/µL test extract was prepared by weighing 0.01 g extract and dissolved in 1 mL DMSO. Concentrations of 50, 25 and 12.5 g/µL were made by performing serial dilution extracts with DMSO. Bacterial suspension was taken with a cotton bud and smeared on MHA media which has been prepared in advance. Each petri dish made with a diagram of 6 parts and each section placed in a disc paper that has been dripped with 20 g of test extract.

The positive control used was 30 g of chloramphenicol antibiotic disc while the DMSO solvent was used for negative control [12]. Positive control disc paper, negative control and discs that had been dripped with extract solution, placed on each part and then incubated at 37°C for 24 hours. The clear area around the disc showed no bacterial growth which was then measured using a caliper run. The inhibition zone measurement results are classified based on Table 1.

TABLE 1. Classification of Antibacterial Activities [13]

Diameter of Inhibition Zone (mm)	Antibacterial Activity
≤ 10	No Activity
10 – 15	Weak
15 – 20	Medium
≥ 20	Strong

Microdilution Method Test

Before the antibacterial test, a test solution (sample) was prepared using a DMSO solvent with a concentration of 1000 g/mL. MHB liquid media that had been suspended with 100 L of bacteria was inserted into each microplate hole. In the first hole, 100 L of test solution was added. The solution concentration series was carried out by transferring 100 L of solution from the first hole to the second hole, from the second hole 100 L was taken and put into the third hole, the same was done to the tenth hole.

The next two holes were used for two control solutions. For the first control the hole was filled with 100 L of liquid medium and 100 L of bacterial suspension (growth control), while for the control of the two holes only filled with 200 L of liquid medium (sterility control). Microplate was then incubated at 37°C for 24 hours. Antibiotic control used in this study was chloramphenicol. The determination of MIC was carried out based on observations on the concentration that began to appear bacterial growth and MBC was determined by placing 5 L of samples that were not overgrown with bacteria in the agar medium, then incubated again for 24 hours. The determination of MBC was based on bacteria that do not grow in the media. [10-11]

Isolation

A. Separation with VLC and TLC

The ethanol extract was fractionated using liquid vacuum chromatography with a column diameter of 10 cm and adsorbent height 5 cm. Fractionation was carried out using 60 GF254 silica gel merck as a stationary phase and mobile phase in the form of eluent n-hexane, n-hexane:ethyl acetate, ethyl acetate (variation based on polarity level) with various eluent comparisons. The resulting fraction was then monitored by TLC with the n-hexane mobile phase : ethylacetate for eluent test. The same Rf values then be grouped into several combined fractions, which are then tested for phytochemicals again [10].

B. Separation by Gravity Column Chromatography

Combined fraction with the same pattern of Rf results from VLC, fractionated further by GCC with a silica gel merck 60 and motion fraction n-hexane:ethyl acetate. The fractions obtained from the eluent were monitored by TLC with the n-hexane: ethyl acetate mobile phase with a comparison obtained from the previous TLC. The same Rf value was combined and then one of the fractions was selected based on the incorporation of the Rf value in the TLC with the three-eluent test namely n-hexane: ethyl acetate (1:1), n-hexane: chloroform (1:1) and ethyl acetate: chloroform (1:1) until it indicates a single spot. [10].

RESULTS AND DISCUSSION

The sample used in this study is the stem of the plant *Scurulla ferruginea* (Roxb. Ex Jack) *Danser* which was obtained from Sidikalang district, North Sumatra. The results of plant identification, that have been carried out at the Medanese botanical research center, show that the plants used in this study are plants from the *Loranthaceae* family.

Antibacterial Activity Test Results with Disc Diffusion Method

Data of the antibacterial activity test using disc diffusion method is summarized and can be seen in Table 2.

TABLE 2. Results of Measurement of Inhibitory Zone Diameter in Antibacterial Test Using Paper Disc Diffusion Method

Test Bacteria	Test Concentration	Inhibition Zone Diameter(mm)		
		d1	d2	$\bar{d}$
E. coli	1% coffee parasite stem extract	6.8	7.2	7
	K+	20.8	24.1	22.45
	K-	0	0	0
S. mutans	1% coffee parasite stem extract	8.6	8.9	8.75
	K+	17.2	15.4	16.3
	K-	0	0	0
B. cereus	1% coffee parasite stem extract	8.4	8.0	8.2
	K+	18.1	14.9	16.5
	K-	0	0	0
S. aureus	1% coffee parasite stem extract	6.7	6.0	6.35
	K+	8.2	17.8	13
	K-	0	0	0
S. typhi	1% coffee parasite stem extract	7.2	6.2	6.7
	K+	16.0	15.0	15.5
	K-	0	0	0
S. viridans	1% coffee parasite stem extract	9.0	8.9	8.95
	K+	18.5	21.5	20,0
	K-	0	0	0

Information:
d1 : diameter of inhibition zone 1 d2 : diameter of inhibition zone 2
K+ : Paper disc chloramphenicol (30 g) K- : DMSO

Results of MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bacterial Concentration)

The results of the study to obtain the MIC value in determining the concentration of inhibitory bacteria can be seen in Table 3.

TABLE 3. Value of MIC and KBM With Microdilution Method

Test Bacteria	Test Concentration	Test Value	
		MIC (µg/ml)	MBC (µg/ml)
S. aureus	Extract 5000 ppm	5000	>5000
	Chloramphenicol 500 ppm	500	500
S. typhi	Extract 5000 ppm	5000	2500
	Chloramphenicol 500 ppm	3.90	1250
S. viridans	Extract 5000 ppm	5000	>5000
	Chloramphenicol 500 ppm	62.5	>500
E. coli	Extract 5000 ppm	2500	5000
	Chloramphenicol 500 ppm	500	5000
S. mutans	Extract 5000 ppm	2500	>5000
	Chloramphenicol 500 ppm	500	5000
B. cereus	Extract 5000 ppm	2500	>5000
	Chloramphenicol 500 ppm	500	5000

Isolation with Vacuum Liquid Chromatography

A total of 20 grams of ethanolic extract of the stem of *Scurrula ferruginea* (Roxb. Ex Jack) *Danser* was fractionated by liquid vacuum chromatography (LVC) using silica gel merck 60 G F254 as the stationary phase and n-hexane, n-hexane:ethyl acetate, ethyl acetate: ethanol, and ethanol as the mobile phase which polarity was continuously increased by gradient by adjusting the eluent ratio so that 16 fractions were produced. The results of

the combined fraction (F14 – F15) that had been removed from the solvent were then monitored again by thin layer chromatography (TLC) using n-hexane:ethyl acetate ((8:2), (4:6), (2:8), and (1:9)). It aims to find the appropriate eluent ratio in the separation of a good compound. TLC results showed good separation in the ratio of n-hexane:ethyl acetate eluent (1:9).

Based on the results of the GC-MS analysis, it shows that there are 10 compounds contained in fraction 4 of the ethanolic extract of the parasite coffee stem, where the chromatogram displays the main peak and includes the highest peak of the analysis spectrum with the highest abundance. The peaks that appear dominant at retention times are 9.201 and 11.917 is the compound with the highest abundance contained in fraction 4 of the ethanol extract of the coffee parasite stem. The results of the analysis showed the characteristics of the diisooctyl phthalate compound with a base peak at 149.1 and a molecular formula of $C_{24}H_{38}O_4$ for fragmentation. The fragmentation peaks of the compound can be seen in Figure 1.

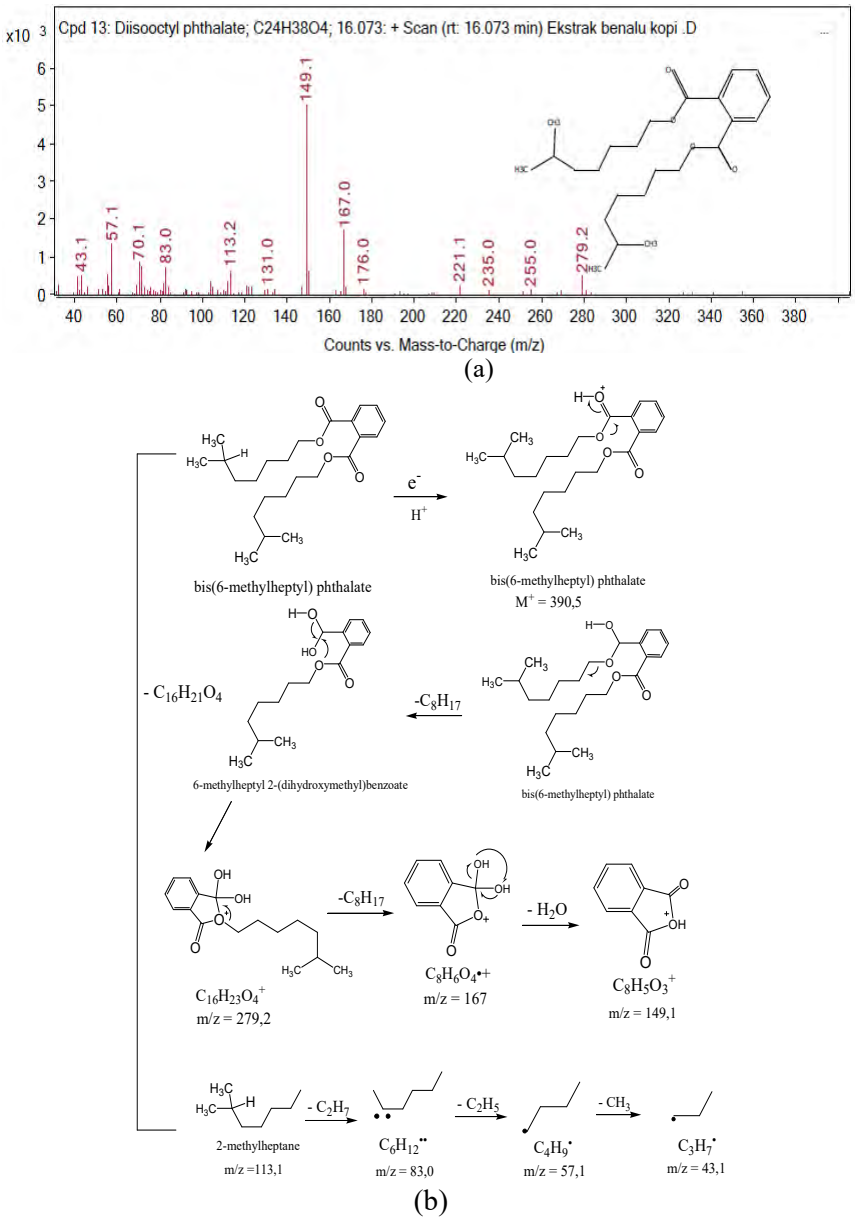

FIGURE 1. a. Peak Fragmentation of Diisooctyl Phtalate Compounds b. Diisooctyl Phtalate Compound Fragmentation

Spectrum analysis of GC-MS fraction 4 is known to have a molecular weight of 390.5 with a basic peak at 149.1 m/z. The peak at m/z 279.2 comes from $C_{16}H_{23}O_4^+$ caused by the release of $C_8H_{17}^\bullet$ from the molecular ion followed by the release of C_8H_{17} to form $C_8H_6O_4^{\bullet+}$ which appears at m/z 167. This ion then releases H_2O forming a base peak at m/z 149.1. This fragmentation also occurs in the CO ester bond, where the molecular ion releases $C_6H_{21}O_4^\bullet$ to form $C_8H_{17}^+$ which appears at m/z 113.2 then releases C_2H_7 to form $C_6H_{12}^{\bullet\bullet}$ which appears at m/z 83. Peak at m/z 57.1 derived from $C_4H_9^\bullet$ caused by the release of C_2H_5 from $C_6H_{12}^{\bullet\bullet}$ which was followed by the release of CH_2 to form $C_3H_7^\bullet$ as seen at m/z 43.1.

Disooctyl phthalate is an ester compound of phthalic acid. Disooctyl phthalate compound has bioactivity as an antimicrobial against *Bacillus subtilis*, *Candida albicans*, *Cryptococcus neoformans* and *Escherichia coli* [14]. In addition, the compound Disooctyl phthalate was also reported to have a cytotoxic effect on more aggressive PC3 cells. This suggests the compound has the potential to inhibit the development of prostate tumors.

CONCLUSION

The results of the antibacterial activity test of the disc diffusion method with an extract concentration of 1% against *Staphylococcus aureus*, *Salmonella typhi*, *Streptcoccus viridans*, *Escherichia coli*, *Streptococcus mutans*, and *Bacillus aureus* bacteria were 6.35 mm, 6.7mm, 8.95 mm, 7 mm, 8.75 mm and 8.2 mm respectively. This result showed that the ability to inhibit bacterial growth of 1% extract was in the medium category. The MIC value for bacteria was 5000 g/mL. The MBC values of *S. aureus*, *S. viridans*, *S. mutans*, and *B.aures*, *E.coli*, *S. mutans*, and *B.aureus* bacteria were 2500 g/mL, while *S. aureus*, *S. typhi*, *S. Viridans* were greater than 5000 g/mL, only *E. coli* can be killed at a concentration of 5000 g/mL and *S. typhi* at a concentration of 2500 g/mL. The test results showed that the stem extract of *S. ferruginea* was only as an inhibitor. The results of the isolation of secondary metabolites from the ethanol extract of the coffee bean parasite which were analyzed using GC-MS showed that fraction 4 contained 10 components with the greatest similarity of 83.45 which was a diisooctyl phthalate compound.

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