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Cytotoxic and Antibacterial Activity of Hexana Plants Extract *Scurrula ferruginea* Jack Danser (*Coffee Parasite*)

Rosnani Nasution[1], Nisa Aldila[1], Muhammad Bahi[1], Marianne Bastian [2]
[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
[2]Department of Pharmacology Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia
*Corresponding Author: rosnani@usk.ac.id

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ABSTRACT
Cytotoxic compounds are substances that can inhibit the growth of cancer cells, whereas antibacterial compounds inhibit microbial growth. This study was conducted to evaluate the cytotoxic and antibacterial effects of *Scurrula ferruginea* Jack Danser. The cytotoxic activity was assessed against *Artemia salina* larvae using the Brine Shrimp Lethality Test (BSLT), while antibacterial activity was evaluated against *P. acnes* using the agar diffusion method. The results showed that *S. ferruginea* exhibited cytotoxic activity against *A. salina* larvae. The tested concentrations were 50, 100, 250, 500, and 1000 ppm. The LC_{50} value of *S. ferruginea* against *A. salina* larvae was 19.49 ppm. The antibacterial activity of the n-hexane extract of *S. ferruginea*, tested at concentrations of 0.5%, 2%, 5%, 7.5%, and 12%, showed no inhibitory effect against *P. acnes* (positive control inhibition zone: 20.66 mm). GC-MS characterization revealed that the n-hexane extract of *S. ferruginea* contained 14 compounds, with lupeyl acetate (44.99%) identified as the dominant compound.

Keywords: *Scurrula ferruginea Jack Danser, cytotoxic, antibacterial*

ABSTRAK
Senyawa sitotoksik merupakan senyawa atau zat yang dapat menghambat pertumbuhan sel kanker, sedangkan senyawa antibakteri merupakan senyawa yang dapat menghambat pertumbuhan mikroba. Penelitian ini dilakukan untuk mengetahui efek sitotoksik dan antibakteri *Scurrula ferruginea* Jack Danser terhadap larva *Artemia salina* menggunakan metode *Brine Shrimp Lethality Test* (BSLT) dan antibakteri terhadap bakteri *P. acnes* menggunakan metode difusi agar. Hasil penelitian ini menunjukkan bahwa tanaman *S. ferruginea* memiliki efek sitotoksik terhadap larva *A. salina*. Konsentrasi uji sitotoksik adalah 50 ppm; 100 ppm; 250 ppm; 500 ppm dan 1000 ppm, diperoleh LC_{50} larva *S. ferruginea* terhadap *A. salina* adalah 19,49 ppm. Hasil antibakteri ekstrak *n*-heksan tanaman *S. ferruginea* menggunakan konsentrasi 0,5%; 2%; 5%; 7,5% dan 12% tidak dapat menghambat bakteri *P. acnes* (zona hambat kontrol positif, 20,66 mm). Hasil karakterisasi dengan GC-MS, ekstrak n-heksana tanaman *S. ferruginea* mengandung 14 senyawa, dengan senyawa yang paling dominan adalah lupeyl acetate (44,99%).
Keywords: *Scurrula ferruginea* Jack Danser, sitotoksisitas, antibakteri

1. Introduction

The *Scurrula ferruginea* Jack Danser plant (coffee parasite) is a parasitic plant that comes from the Loranthaceae family and is commonly found in the tropical regions of the Southeast Asian continent, such as Indonesia, Malaysia, Singapore, Vietnam, Thailand, Myanmar, the Philippines, Cambodia, China, and Laos [1].

These parasitic plants usually attach to tree branches. They are also known to be very harmful because they can damage their hosts [2]. Still, they are plants that are well-known to the public and have

been used as traditional medicines, which can function as inhibitors of abnormal cell growth, cancer drugs, diuretics, pain relievers, and care after childbirth [2], antioxidants, antimicrobials, anticancer [3], anti-allergy, anti-tumor, cold medicine, and other degenerative diseases [4]. The *S. ferruginea* plant is also used as a medicine for herpes zoster, malaria, high blood pressure, wound healing, and snake bites to relieve painful urination and hypertension [1], antioxidant, antimicrobial, and anticancer [3].

Scurrula ferruginea plants have secondary metabolites such as alkaloids, flavonoids, saponins, phenols, and steroids [5]. [6] reported that the *S.ferruginea* plant contains secondary metabolites, namely the flavonol glycoside compound 4-O-acetylquestitrile. The Indonesian Food and Drug Supervisory Agency [7] also reported that *S. ferruginea* leaves contain compounds belonging to the flavonoid class, namely quercetin, quercitrin, and rutin. The ethyl acetate fraction of the *S. ferruginea* plant contains flavonoid compounds such as quercetin, quercitrin, and 4"-O-acetyl quercitrin [8].

Based on this, this research was conducted to obtain new information and references regarding the cytotoxic activity of the *n*-hexane extract of *S. ferruginea* against *Artemia salina* larvae. and antibacterial activity against *P. acnes* bacteria.

The cytotoxic test used the Brine Shrimp Lethality Test (BSLT) method as a first step for compounds suspected of having anticancer properties because this test has a positive correlation with their potential as anticancer and certain physiologically active properties [9]. Antimicrobial testing of *P. acnes* was carried out using the agar diffusion method. *P. acnes* bacteria are one of the causes of acne on human skin.

2. Material and Method

2.1. Plant material and bioindicator

The samples used in this study were all parts of the *S. ferruginea* plant from the Bener Meriah District, Aceh Province. Sampling was carried out in early January 2021 and continued with identification at the Herbarium Laboratory, Department of Biology, FMIPA USK. The bioindicator used in this study was the bacterium *P. acnes,* which is one of the bacteria that causes acne. The *P. acnes strain* (ATCC 27853) was purchased from the Biology Laboratory, University of North Sumatra.

2.2. Plant Extraction of *S. ferruginea*

Scurrula ferruginea Jack Danser plants as much as 1.725 kg, which has been air-dried and finely ground, macerated using *n*-hexane solvent for 3 x 24 hours. Maceration was repeated until a clear filtrate was obtained. The filtrate was filtered and evaporated with a rotary evaporator, and 16.972 g (0.2725%) of hexane extract was obtained. The residue is air-dried and re-macerated using ethanol solvent for 3 x 24 hours and filtered. The filtrate with ethanol solvent was evaporated using a vacuum rotary evaporator, and the ethanol extract was obtained as much as 50 g. (2.105%).

The hexane extract obtained was tested for phytochemicals, tested for their antimicrobial activity against *P. acnes* bacteria, and their toxicity, and the extract was characterized by using GC-MS.

2.3. Brine Shrimp Lethality Test (BSLT) Method Cytotoxic Test

The cytotoxic test of plant extract used the BSLT method according to the procedure carried out by Tianandari and Rasidah [10] and [11].

2.3.1. Preparation of *A. salina* larvae

Preparation of *A. salina* Leach larvae is carried out by preparing a container for hatching shrimp eggs. In one of the chambers in the container, filtered seawater is placed, and a lamp is placed above it to warm the hatching temperature. As much as 50-100 mg of *A. salina* larvae were put into seawater and put into the aerator as a source of oxygen for 48 hours to incubate the larvae. Ten shrimp larvae to be tested were taken with a pipette and put into a tube filled with seawater.

2.3.2. Preparation of test solution

The plant extract of *S. ferruginea* was weighed as much as 50 mg and dissolved in 25 mL of seawater to obtain a mother liquor concentration of 2000 ppm. The extract was first dissolved with 5% DMSO and carried out by the method of sonication so that it was completely dissolved. The mother liquor was then diluted to a concentration of 1000 ppm, 500 ppm, 250 ppm, 100 ppm, and 50 ppm. While the negative control was prepared by adding 5 mL of seawater with 3 drops of 5% DMSO.

2.3.3. Cytotoxic test procedure with the Brine Shrimp Lethality Test (BSLT) method

The *n*-hexane extract of the *S. ferruginea* plant, which was tested for its cytotoxic activity, was varied in solution concentrations of 1000 ppm, 500 ppm, 250 ppm, 100 ppm, and 50 ppm from a 2000 ppm mother liquor. To dissolve the *n*-hexane extract of the *S. ferruginea* plant, 3 drops (5% DMSO) are needed and left overnight. Dimethyl Sulfoxide is an aprotic solvent, which is a solvent that can dissolve polar and nonpolar compounds and dissolves in various organic solvents and water [12]. Then it is sonicated with

filtered seawater solvent because the extract does not completely dissolve, only with the addition of DMSO. In addition, a negative control was also made in the form of seawater and 5% DMSO, with as many as 3 drops without the addition of extract. Then, 18 test tubes were prepared as BSLT test containers, and each concentration of the test solution was repeated three times. Pipette each test tube with the test solution and seawater, and then pipette 10 *A. salina* shrimp. For the control test, 1 ml of seawater and 10 *A. salina* Leach pipettes were pipetted. Evaluation of the death of *A. salina* was carried out after 24 hours of treatment, and the number of experimental animals that died was counted to calculate the LC_{50} value.

2.3.4. Calculation of Lethal Concentration (LC_{50})

The LC_{50} value is determined using the bill test table, where the LC_{50} value is the concentration of the test substance that can kill shrimp larvae by 50%.

2.4. Antibacterial Testing Process

Testing the antibacterial activity of the *n*-hexane extract of the *S.ferruginea* plant against *P. acnes* was carried out by the diffusion method using discs. A total of 1-2 test ose bacteria were inoculated into Lactose Broth (LB), then incubated in an incubator at 37°C for 24 hours. The test bacterial suspension was equalized to the turbidity of a 0.5 McFarland solution. The medium used for the antibacterial activity test was Muller-Hinton Agar (MHA) media. Muller-Hinton Agar is used because it has good nutritional content for most bacterial cultures. In addition, Muller-Hinton Agar (MHA) is also neutral, so it does not affect the antibacterial test procedure. The thickness of Muller-Hinton Agar (MHA) media in Petri dishes is also uniformized by equalizing the volume used, which is 25 mL in one Petri dish. A total of 20µL of inoculum in Lactose Broth (LB) medium was spread aseptically on Muller-Hinton Agar (MHA) medium. *S. ferruginea n*-hexane extract (concentration 0.5%; 2%; 5%; 7.5% and 12%), solvent as the negative control, and chloramphenicol antibiotic solution with a concentration of 30µg/mL as the positive control, respectively dripped onto different paper discs and incubated for 18 hours at 37°C. After the incubation process, the diameter of the inhibition zone was measured in the form of a clear zone around the disc, which showed inhibition of microbial growth or antibacterial activity [13].

3. Results And Discussion

3.1. Phytochemical Test of n-Hexane Extract of *S. ferruginea*

The phytochemical test is a qualitative test to identify the content of secondary metabolites found in *S. ferruginea*. The results of the phytochemical test of the hexane extract of the *S. ferruginea* plant can be seen in Table 1.

Table 1. Phytochemical test results of the n-hexane extract of *S. ferruginea*

Secondary metabolite	Test method	Results
Alkaloids	Mayer	-
	Wagner	-
	Dragendorff	-
Steroids	Lieberman-Burchad	+
terpenoids	Lieberman-Burchad	-
Saponins	stired	-
Flavonoids	0.5 Mg and HCl	-
Phenolic	$FeCl_3$	+

The phytochemical test results of the *n*-hexane extract of the *S. ferruginea* plant (Table 1) were positive for the presence of steroid secondary metabolites, indicated by the formation of a green color after being reacted with the Lieberman-Burchard reagent. The presence of phenolic compounds was indicated by the formation of a blue precipitate after being reacted with $FeCl_3$.

3.2. Cytotoxic Activity Test of Hexane Extract of *S. ferruginea* Plant

Cytotoxic activity test using the BSLT method to determine the most active concentration. The BSLT testing method is based on active ingredients from plants that are toxic and capable of killing *A. salina* larvae are used as test animals. The test animal used in this test was *A. salina,* which was 48 hours old because it has a complete digestive tract, so it is sensitive to a substance added [14]. The larvae of *A. salina* shrimp have sensitive characteristics and will die if the foreign substances or compounds are toxic [15]. The

results of observing the cytotoxic effect of various concentrations of the n-hexane extract of the *S. ferruginea* plant on *A. salina* larvae can be found in Table 2 below. 6

Table 2. The effect of various concentrations of the *n*-hexane extract of the *S. ferruginea* plant on *A. salina* larvae

Concentration	Concentration Log	Number of Dead o (%)	Total Life	The number of dead larvae in the control	Accumulated Death Count	Accumulated Life Count	Ratio	Mortality (%)
1000	3	9.33	0,67	0	42.65	0.67	0.99	99
500	2.69	9	1		33.32	1.67	0.96	96
250	2.39	9	1		24.32	2.67	0.9	90
100	2	7.66	2.34		16.66	5.01	0.77	77
50	1.69	7.66	2.34		9	7.35	0.56	56

Based on Table 2, it can be seen that the highest mortality percentage of A. salina larvae was at a concentration of 1000 ppm and the lowest at a concentration of 50 ppm. The percentage of mortality increased with an increase in the concentration of the test solution, whereas in the control, there were no dead *A. salina* larvae, so it can be concluded that the death of *A. salina* larvae was caused by the *n*-hexane extract of the *S. ferruginea* plant. The correlation curve of % mortality with log concentration can be seen in Figure 1.

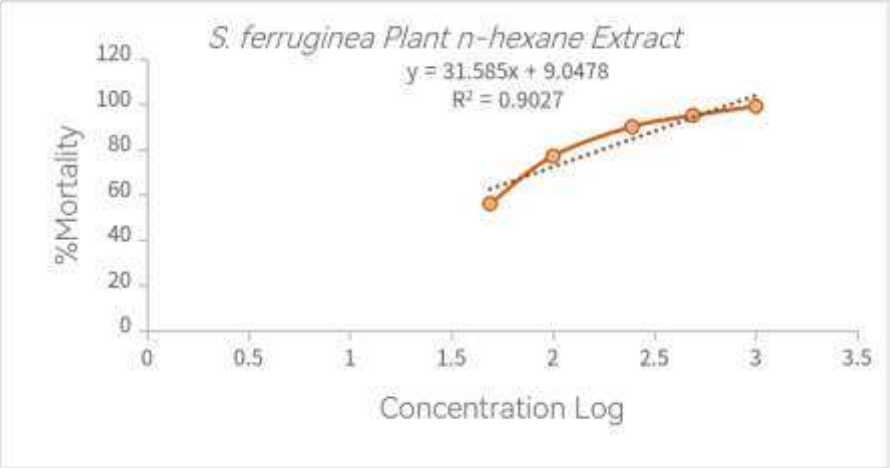

Figure 1. Correlation curve of % mortality with the log of concentration

Based on Figure 1, data is obtained for calculating the linear regression equation for the relationship between Y (% mortality value) and X (log concentration). The LC_{50} value can be calculated from the resulting regression equation by replacing the Y value with 50. The value of 50 is obtained from the 50% death rate of the test animal, so it will produce an X value. The LC_{50} value is obtained by converting the X value into the antilog of the X value. [16]. The LC_{50} value parameter, which indicates the level of toxicity of a sample, is grouped into three groups, namely very toxic (<30 ppm), toxic (<1000 ppm), and not toxic (>1000 ppm) [17]. From Figure 1, the value of Y = 31.585x + 9.0478 is obtained, so the LC_{50} value is 19.49 ppm. The LC_{50} results obtained show that the *n*-hexane extract of the *S. ferruginea* plant has very toxic activity because the LC_{50} produced is <30 ppm.

3.3. Antibacterial Activity Test of n-hexane Extract of *S. ferruginea*

Testing the antibacterial activity of *S. ferruginea* plant extract was carried out to determine the activity of the extract in inhibiting the growth of *P. acnes* bacteria. The choice of this test bioindicator is because the *P. acnes* bacteria are one of the causes of acne on human skin [13]. Variations in the concentration of *S. Ferruginea* plants used in this study were 0.5%; 2%; 5%; 7.5% and 12% (w/v). The results of measuring the diameter of the inhibition zone for each extract can be seen in Figure 2.

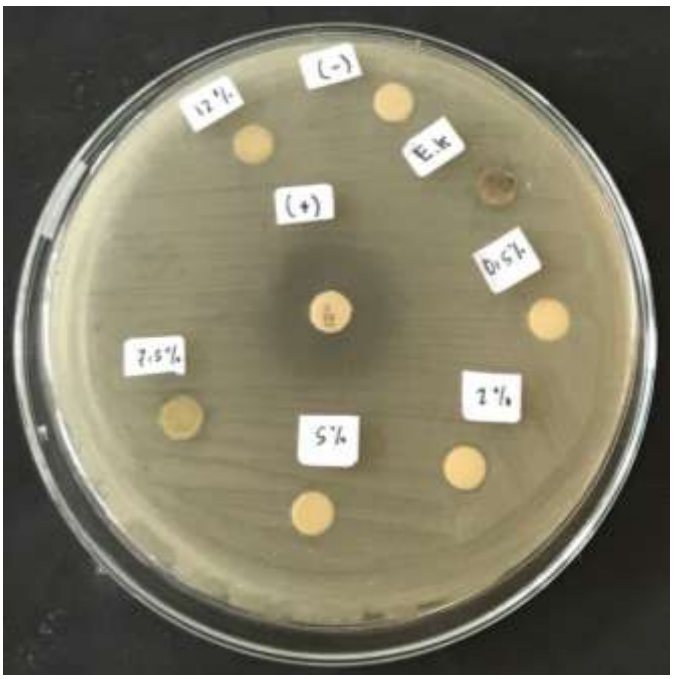

Figure 2. Inhibition zone for the antibacterial activity of the hexane extract of *S. ferruginea*

The antibacterial activity test used chloramphenicol as a positive control and *n*-hexane as a negative control. Chloramphenicol is widely used because, apart from being an antibacterial, chloramphenicol has a broad spectrum, which means it can inhibit the growth of gram-negative and gram-positive bacteria [18]. Based on the antibacterial test that was carried out in this study it showed that the positive control, namely chloramphenicol produced an inhibition zone of 20.66 mm while the concentration of the *n*-hexane extract of the *S. ferruginea* plant was 0.5%; 2%; 5%; 7.5% and 12% showed no antibacterial activity as indicated by the absence of clear zones around the discs that were unable to kill the test bacteria (Figure 2). Factors that can inhibit this activity are the presence of active substances trapped in the extract, so that it will have difficulty passively diffusing out of the extract towards the target location, namely the bacterial growth medium [19]. Because these compounds are not soluble in hexane solvent, so they cannot interact with the bacteria in the agar medium.

3.4. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of *n*-Hexane Extract of *S. ferruginea* Plant

The characterization results of the *S. ferruginea* hexane extract using gas chromatography (GC) identified 14 chemical compounds, which were further characterized by mass spectrometry (MS). Based on the MS library data, the identified compounds along with their potential benefits are presented in Table 3. One dominant compound, accounting for 44.99% of the total composition, was detected at a retention time of 27.218 minutes with a similarity index of 83%; this compound was identified as lupeyl acetate.

Table 3. GC-MS results of the hexane extract of *S. ferruginea*

No	Name of compound	Retention time (minutes	Area (%)	Similarity (%)	Activity	Reference
1.	1-Hexadecene	10.735	1.71	97	-	-
2.	9-Octadecene	13.058	2.83	97	Antimicrobial	[20]
3.	Heptadecane	13.133	1.23	95	-	-
4.	Pentadecanoic acid, 14-methyl-, methyl ester	14.527	1.44	94	Anticancer	[21]
5.	9-Eicosene	15.225	5.49	97	Antioxidant	[22]
6.	Pentadecane	15.225	0.89	93	-	-
7.	1-Nonadecene	18.865	2.57	95	-	-
8.	1,2-Benzenedicarboxylic acid	20.294	1.55	95	Antifungal	[23]
9.	Nonacosanol	20.493	1.95	94	-	-

No	Name of compound	Retention time (minutes	Area (%)	Similarity (%)	Activity	Reference
10.	Eicosane	21.283	0.85	93	-	-
11.	1-Heptacosanol	22.005	1.32	91	-	-
12.	Tetratetracontane	22.732	31.9	98	Anti-bacterial and Anti-inflammatory	[21]
13.	Tritetracontane	23.409	1.28	89	-	-
14.	Lupeyl acetate	27.218	44.99	83	Anticancer, Antioxidant, Anti-inflammatory, and Antimicrobial	[24]

4. Conclusion

The *n*-hexane extract of the *S. ferruginea* plant had a cytotoxic effect on *A. salina* larvae; the LC_{50} value of the hexane extract of the *S. ferruginea* plant on *A. salina* larvae was 19.49 ppm, but it could not inhibit the growth of *P. acnes* bacteria (positive control inhibition zone, i.e., 20.66mm).

Based on the results of characterization using GC-MS, the *n*-hexane extract of *S. ferruginea* contained 14 compounds, the most dominant compound being lupeyl acetate (44.99%).

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6. Conflict of Interest

Authors declare no conflicts of interest.

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