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Research Article

Phytochemical screening, antibacterial, antioxidant, and anticancer activity of Coffee parasite acetone extract (*Loranthus ferrugineus* Roxb)

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Abstract

This study aimed to perform a phytochemical screening and test the antibacterial, antioxidant, and anticancer activities of acetone extracts of the Coffee parasite (*Loranthus ferrugineus* Roxb). A phytochemical screen was performed using specific reagents. Antimicrobial testing was performed using the paper disc diffusion method. The antioxidant activity test used the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Anticancer activity test against HeLa and A549 cells based on the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay method. Acetone extract *L. ferrugineus* Roxb contains alkaloids, flavonoids, triterpenoids, and tannins compounds. The acetone extract of *L. ferrugineus* Roxb showed activity against all bacteria tested, with the inhibition zone diameter ranging from 6.2 mm - 11.1 mm. Acetone extract of *L. Ferrugineus* Roxb had a very strong antioxidant activity with a value of IC_{50} = 48.7122 µg/mL. The anticancer activity test showed cytotoxic activity against HeLa cells with a value of IC_{50} = 47.62 µg/mL and for A549 cells with a value of IC_{50} = 192.83 µg/mL.

Keywords

antibacterial, antioxidant, anticancer, *Loranthus ferrugineus* Roxb, phytochemical screening

Introduction

North Sumatra is the main coffee-producing province in Indonesia. Many parasitic plants grow on coffee plants. The parasite is a parasitic organism that lives on or in its host plant branches and twigs and absorbs nutrients, minerals, and water from its host. The parasite was originally considered a harmful plant because it damaged its host plant. However, several countries such as Thailand, Korea, Japan, Indonesia, Malaysia, China, Saudi Arabia, and Nigeria have used several species of parasites in ethnomedicine to treat various diseases (Kwanda et al. 2012; Moghadamtousi et al. 2013).

One of the most widely used parasite plants is from the Loranthaceae family. Calvin and Wilson (2006) reported that *L. micranthus* had been widely used to treat schizophrenia, diabetes, and hypertension and as a booster for the immune system. *Scurrula ferruginea* Danser is used for postpartum treatment, beriberi, malaria, snake bites, wounds, and fever (Lohézic et al. 2002). *L. ferrugineus* is used to treat high blood pressure and gastrointestinal complaints (Ameer et al. 2015). It is also used to treat stomach and back pain and can treat cancer (Kwanda et al. 2012). *L. parasiticus* is used for the treatment of brain diseases. The genus *Loranthus* is used to treat diabetes, inflammation, and cancer (Noman et al. 2019).

The use of parasites in traditional medicine is popular. Therefore it attracted researchers to research it. Several studies published regarding parasite plant activity of the genus *Loranthus*, including *L. micranthus*, have found antihypertensive, antimicrobial, hypolipidemic immunomodulatory activity, antidiabetic antioxidant, and antidiarrheal (Onunogbo et al. 2012; Moghadamtousi et al. 2013). On the other hand, *L. acaciae* Zucc has antidiabetic, antiinflammatory and antioxidant activity (Noman et al. 2019).

Meanwhile, *L. regularis* Steud ex Sprague has antiinflammatory and antioxidant activity (Mothana et al. 2012). The extracts of *L. europaeus* twigs and stems showed antioxidant activity (Katsarou et al. 2012), and *L. ferrugineus* Roxb, which has antioxidant activity (Yulian & Safrijal, 2019). Several researchers reported related phytochemical screening and isolation of secondary metabolites from several *Loranthus* species. From these reports, it is known that the *Loranthus* Genus contains secondary metabolites of flavonoids, alkaloids, steroids, triterpenoid esters, and phenolic glycides (Lohézic et al. 2002; Moghadamtousi et al. 2013; Noman et al. 2019).

Materials and methods

Plants extract preparation

The research was carried out from April - November 2020; antibacterial testing and phytochemical screening were carried out at the Universitas Negeri Medan laboratory, while antioxidant and anticancer testing was carried out at the Central Lab of Universitas Padjadjaran Bandung. A 200 g of *L. ferrugineus* Roxb sample was macerated using acetone as a solvent to obtain a crude extract. This process is carried out for 3 × 24 hours at room temperature. The extract was filtered with Whatman filter paper, then evaporated at low pressure using a rotary evaporator at a temperature of 50 °C (Juwitaningsih et al. 2020a).

Phytochemical screening

Phytochemical screening was carried out, referring to the method that was used by Harborne (1987) using specific reagents. These tests include the alkaloid flavonoids, triterpenoids, steroids, saponins, and tannins test.

Antibacterial activity test

Antibacterial activity test was carried out by using the paper disc diffusion method (Juwitaningsih et al. 2020a) against *Bacillus cereus* ATCC 1178, *Salmonella enterica* ATCC 14028, *Propionibacterium acnes* ATCC 27853 and *Streptococcus mutans* ATCVC 35668 bacteria. The test solution was prepared at a concentration of 1% (10.000 µg/mL) in 10% Dimethyl sulfoxide (DMSO).

Antioxidant activity test

The antioxidant test used the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Molyneux 2004; Soltanzadeh et al. 2018). The concentrations of each of the test solutions were 100, 80, 60, 40, and 20 ppm. The test was replicated twice. Antioxidant activity was determined by the inhibition concentration (IC_{50})value that was calculated using the equation obtained from the linear regression curve y = a+bx, where x is the sample concentration, a is the regression coefficient, b is the intercept, and y is the % inhibition value. The IC_{50} value is determined by the formula:

$$IC50 = \frac{50 - a}{b}$$

Anticancer activity test

The anticancer activity test was carried out as in previous studies, namely the MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Assay (Juwitaningsih et al. 2020b). The anticancer activity test was carried out on HeLa and A2549 cells. The absorbance measurement used a Multimode Reader at a wavelength of 595 nm. Based on the absorbance value obtained, the percentage of cell viability is determined using the formula:

$$\%\ Viability = \frac{(\text{absorbance of the treatment} - \text{absorbance of media controls})}{\text{absorbance of control cells} - \text{absorbance of media controls}} \times 100\%$$

The IC_{50} value obtained from the linear regression equation y = a + bx. The graph was made with the concentration of the test sample (ppm) as abscissa (x-axis) against the percent Viability as the ordinate (y-axis) - data processed with Excel version 2016.

Results and discussion

Phytochemical screening

Based on the phytochemical screening result against *L. ferrugineus* Roxb, the results are summarized in Table 1. It shows that the results of the phytochemical test of *L. ferrugineus* Roxb acetone extract attached to the coffee tree contain secondary metabolites of the alkaloid, triterpenoid, flavonoid, and tannin.

The results obtained were similar to *L. ferrugineus* Roxb from Bener Meriah Regency Aceh, which was extracted with ethanol (Yulian and Safrijal 2019). There is a similarity in results even though using different extracting solvents. This is because the polarity of acetone and methanol is almost the same, so the compounds that are extracted are the same.

Table 1. Phytochemical test results of *L. ferrugineus* Roxb acetone extract.

Secondary Metabolites	Test Method	Test Results
Flavonoids	$FeCl_3$ 5%	+
	NaOH 10%	+
Alkaloids	Dragendorff's reagent	+
	Wagner's reagent	+
	Mayer's reagent	+
Saponins	Aquades	+
Triterpenoids	Liebermann-Burchard's reagent	+
Steroids	Liebermann-Burchard's reagent	-
	$FeCl_3$ 1%	+

Note: (+) Detected, (-) Not detected.

Previous research has shown that the chemical compound content includes flavonoids, alkaloids, lectins, polypeptides, arginine, histamine, tannins, terpenoids, acid compound steroids, glycosides, gallic acid (Ameer et al. 2015). The host plant may influence the composition of the chemical compounds of parasites (Narayanasamy and Sampathkumar 1981; Moghadamtousi et al. 2013). Methanol extract from *L. micranthus* leaves attached to *Periplaneta americana* contains steroids, terpenoids, resins, oils, proteins, tannins, flavonoids, saponins, reducing sugars, alkaloids, glycosides, with alkaloids as the main component. Secondary metabolites of the flavonoid group isolated from *L. acaciae* Zucc grown in Saudi Arabia, namely quercetin 3-O-β-D-glucopyranoside, quercetin 3-O-β- (6-O-galloyl) -glucopyranoside, (-) catechins, and catechins 7-O-gallate (Noman et al. 2019). Whereas, Falvanoid is isolated from *Loranthus regularis* Steud. ex Sprague namely quercetin 3-O-β-l-galactopyranoside, quercetin 3-O-β-l-araβinopyranoside, and quercetin 3-O-α-l-rhamnopyranoside. Apart from flavonoid compounds, it has βeen isolated from *L. micratus* compounds. Lupinine alkaloids, steroid compounds of Loranthoid, 3β-hydroxystigmast-23-ene (stigmast-23-en-3β-ol, stigmast-7,20 (21)-diene-3β-hydroxy-6-one, triterpenoid compounds Lupeol, Triterpenoid esters namely 7β, 15α-dihydroxy- lup-20 (29)-en-3β-O-palmitate, 7β, 15α-dihydroxy- lup-20 (29) -en-3β-O-stearate and Phenolic glycoside, namely linarin gallate, walsuraside B (Moghadamtousi et al. 2013). The diversity of secondary metaβolites indicates that the parasite plant may βe influenced by the species of the host tree (Moghadamtousi et al. 2013) and the environment in which the host grows (Kutchan et al. 2001).

Antibacterial activity

In this study, the acetone extract of *L. Ferrugineus* Rox was tested against three gram-positive bacteria, namely *P. acnes* ATCC 27853, *S. mutans* ATCV 35668, *B. cereus* ATCC 1178, and one gram-negative bacterium, *S. enterica* ATCC 14028, so chloramphenicol was used as control positive which is able to inhibit the growth of bacteria with a broad spectrum. The acetone extract of *L. ferrugineus* Roxb has antibacterial activity against all tested bacteria, with a clear zone diameter between 6.2–11.1 mm. The results of the observations are summarized in Table 2.

Table 2. Antibacterial activity.

	Mean Value of Inhibition Zone (mm)			
	P. acnes ATCC27853	*S. mutans* ATCC35668	*S. enterica* ATCC14028	*B. cereus* ATCC1178
Negative control (DMSO 10%)	0	0	0	0
Chloramphenicol positive control	22.2	26	30.7	25.9
Acetone extract of *L. ferrugineus* Roxb	7.6	11.1	6.2	7.2

P: *Propionibacterium*, S: *Streptococcus*; S: *Salmonella*, B: *Bacillus*.

Based on the results of observations on the in vitro antibacterial activity test, the acetone extract solution of *L. ferrugineus* Roxb showed strong activity against *Streptococcus mutans*, and other bacteria showed moderate activity. This is based on the following criteria: an extract has very strong antibacterial activity if the inhibition zone diameter is ≥ 20 mm, strong if the inhibition zone diameter is 10–20 mm, and moderate if the inhibition zone is 5–10 mm (Davis et al. 971).

Previous studies of the genus *Loranthus* showed antibacterial activity, such as *L. micranthus*, which was extracted with methanol, ethanol, chloroform, and petroleum ether solvent, which showed antibacterial activity against the test bacteria of *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumonia* (Osadebe and Akabogu 2006). Likewise, *L. micranthus*, grown to *Periplaneta americana*, provided stronger antibacterial activity against *Pseudomonas aeruginosa* than amoxicillin, while *L. micranthus* extracts that are attached to *Azadirachta indica*, *Hydrangea macrophylla*, and *Irvingia gabonensis* showed antibacterial activity against *S. typhi* and *B. subtilis* (Osadebe and Ukwueze 2004).

Based on these observations, the antibacterial activity is related to the content of secondary metabolites of *L. ferrugineus* Roxb, namely alkaloids, terpenoids, flavonoids, and tannins. These secondary metabolites have an antibacterial effect with different mechanisms of action. The alkaloid action mechanism is by disrupting the peptidoglycan component of bacterial (Darsana et al. 2012). The mechanism of flavonoids' action as antimicrobials can be through 3 ways, inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism (Cushnie et al. 2005). Mechanism of terpenoids' action by disrupting the function of cell membranes (Saleem et al. 2010). Moreover, the mechanism of tannins' action as antibacterials by causing cells to become lysed. This happens because tannins have a target on the polypeptide wall of the cell wall, so the cell wall formation becomes less than perfect. This causes bacterial cells to become lysed due to osmotic and physical pressure so that the bacterial cells will die (Zega et al. 2021; Silaban et al. 2022).

Antioxidant activity

The test parameter for antioxidant activity is Inhibition Concentration (IC_{50}), which is the concentration of an antioxidant substance that causes 50% DPPH to lose its radical character or its concentration antioxidant substance, which gives an inhibitory percentage of 50% (Nursid et al. 2013). The amount of antioxidant activity is inversely proportional to the IC_{50} value, meaning that the greater the antioxidant activity, the smaller the IC_{50} value is obtained. The acetone extract of *L. ferrugineus* Roxb has antioxidant activity with an IC_{50} value of 48.7122 µg/ml), which was the mean value of two repetitions. The results of IC_{50} calculations can be seen in Table 3. The IC_{50} value was determined from the linear regression graph in Fig. 1.

Table 3. Antioxidant activity data (DPPH).

Sample	IC_{50} (µg/ml)		
	1st repetition	2nd repetition	Mean
Acetone extract of *L. ferrugineus* Roxb	49.0650	48.3593	48.7122

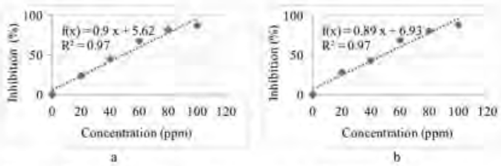

Figure 1. Diagram of antioxidant activity (DPPH) of *L. ferrugineus* Roxb acetone extract. **a**: 1st repetitions; **b**: 2nd repetitions.

If the IC_{50} value is < 50 µg/ml, the antioxidant activity is categorized as very strong. If the value is 50–100 µg/ml is classified as strong, and if the IC_{50} value is 101–250 µg/ml, it is categorized as moderate (Jun et al. 2003). Based on these criteria, the acetone extract of *L. ferrugineus* Roxb has strong antioxidant activity. Antioxidant activity may be related to secondary metabolites of phenolics (for example, flavonoids, tannins, phenolic acids, etc.) which can donate hydrogen atoms or electrons to free radicals (Sharififar et al. 2009). The results of previous studies of the genus *Loranthus* showed antioxidant activity, such as the *L. acaciae* Zucc chloroform fraction showed high inhibitory activity against DPPH radicals of 88.3% (Noman et al. 2019). Eleven compounds consisting of terpenoids and flavonoids isolated from the methanol extract of *L. micranthus* leaf twigs showed antioxidant activity in the range of IC_{50} = 23.8–50.1 µM (Moghadamtousi et al. 2013).

Anticancer activity

In Indonesia, through the Global Burden of Cancer Study (Globocan) report from the World Health Organization (WHO), the number of cancer patients reached 396,914 cases of cancer in 2020, and 54% of cases occurred in women. Cervical cancer ranks second with 36,633 cases, and lung cancer with 34,783 cases out of total cases. The anticancer activity test was carried out in vitro using the MTT assay method. This test aims to determine the toxicity of a compound. The IC_{50} value of *L. ferrugineus* Roxb acetone extract against Hela cells was 47.62 (µg/mL), and the A549 cell was 192.83 (µg/mL). These values were obtained based on the linear regression equation (Figs 2, 3).

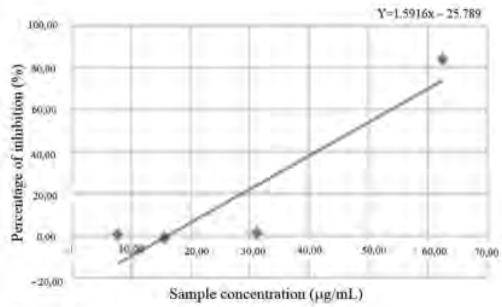

Figure 2. Curve results of *L. ferrugineus* Roxb acetone extract test against Hela Cells.

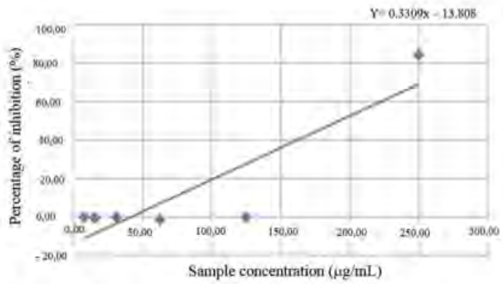

Figure 3. Test results curve of *L. ferrugineus* Roxb acetone extract against A549 cells.

NCI (National Cancer Institute) has established criteria for anticancer activity, namely an extract is declared active to have anticancer activity if it has a value of IC_{50} < 30 µg/mL, moderate active if it has a value of $IC_{50} \geq 30$ µg/mL and IC_{50} < 100 µg/mL, it is said to be inactive if the value of IC_{50} > 100 µg/mL (Suffness et al. 1990). Based on these provisions, the acetone extract of *L. ferrugineus* Roxb has strong anticancer activity against cervical cancer cells with an IC_{50} value of 47.62 µg/mL. It is the result of the observations of HeLa cells and A549 cell morphology. *L. ferrugineus* Roxb. Acetone extract at the concentration of 62 µg/mL could kill all HeLa cells and against A549 cells at a concentration of 500 g/mL (Figs 4, 5).

In the cell morphology (Figs 4, 5), there is a difference between the positive control (media + cells) and the acetone extract sample treatment with various concentrations and cisplatin as a negative control. Changes in cell morphology that may result from exposure to certain active compounds are a reflection of biochemical conditions, namely the activation of various endonucleases and proteases so that DNA is broken into fragments of different lengths that can lead to cell death either by necrosis or apoptosis. Apoptosis is an active process that requires specific gene transcription and translation and also requires the use of intracellular energy sources, whereas necrosis is cell death with a pathological condition. The difference in the fundamental mechanism of the two types of cell death is reflected in the morphology of cells that

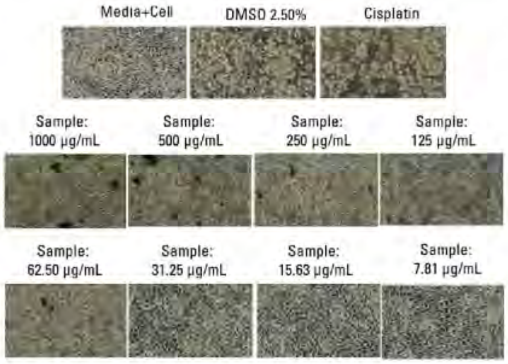

Figure 4. Hela cell morphology results of *L. ferrugineus* Roxb acetone extract test.

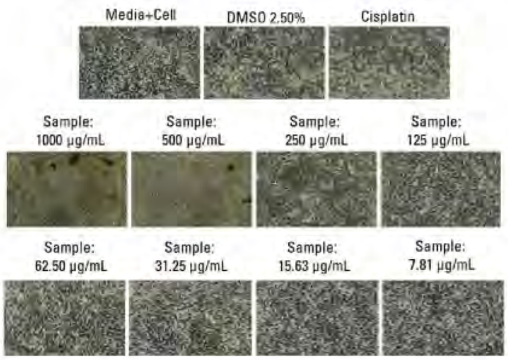

Figure 5. A549 cell morphology results of *L. ferrugineus* Roxb acetone extract test.

experience death (Nursid et al. 2013). In cells that undergo apoptosis, their cytoplasm volume decreases, the cell nucleus shrinks, and the membrane and organelles remain united. On the contrary, in cells that undergo necrosis, the cell looks bulging or has swelled, the cell nucleus undergoes lysis, the plasma membrane and nuclear membrane are damaged, and cell organelles undergo disintegration (Nursid et al. 2013).

In Fig. 4, normal living HeLa cells are polygonal with a clearly visible nucleus. At a sample concentration of 62 μg/mL, there was a change in cell morphology. The cells became rounder, and the cell density was lower than the control and could kill all HeLa cells. The cells experienced shrinkage and lost contact with surrounding cells (Hutomo et al. 2016). At a sample concentration of 62 μg/mL, all HeLa cells died by apoptosis. Although *L. ferrugineus* Roxb acetone extract did not show activity against A549 lung cells, *L. ferrugineus* Roxb acetone extract has potential as a lung anticancer. This is based on figure 5 of cell morphology; at a concentration of 250 μg/mL, some of the A549 cells have died, the cell density was already reduced, and the cell morphology was similar to the positive control, namely cisplatin. At a concentration of 500 μg/mL, all A549 cells died by apoptosis.

In many studies carried out, phenolic, terpenoids, and alkaloids group compounds are known to have activities that can inhibit growth and kill cancer cells. Flavonoids such as Epicatechin, quercetin, and catechin are also isolated from *L. micranthus* (Moghadamtousi et al. 2013). Quercetin is a flavonoid derivative that has antioxidant, antiinflammatory, and anticancer activity (Alexander et al. 2014). The mechanism of flavonoid's action as an anticancer is by inhibiting the activity of DNA topoisomerase I/II, decreasing the expression of Bcl-2 and Bcl-xl genes, as well as activating endonuclease (Ren et al. 2003). The mechanism of the alkaloid action is by inhibiting cell growth in the G_1 phase through the exposure of p53 (Murti et al. 2007). The terpenoids by blocking the cell cycle in the M phase (mitosis), namely when cancer cells interact with terpenoid compounds, it will cause the mitotic stage to be inhibited, which follows there will be inhibition of cell proliferation and triggers apoptosis.

Conclusions

The acetone extract of parasitic *L. ferrugineus* Roxb in coffee trees contains flavonoids, alkaloids, triterpenoids, and tannins, and exhibits antibacterial, antioxidant, and anticancer activity. Acetone extract of *L. ferrugineus* Roxb is the potential as a source of antibiotic, antioxidant, and cervical anticancer compounds.

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