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**ORIGINAL RESEARCH**

# Activity Antibacterial Assay of Coffee Mistletoe Leaves (*Loranthus Ferrugineus* Roxb) Ethanol Extract is Against Bacteria *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

Coffee mistletoe (*Loranthus ferrugineus* Roxb) is a plant that has been researched globally and contains metabolite compounds such as flavonoids, tannins, saponins, and alkaloids, which have been extensively researched to have natural antimicrobial and antioxidant effects. For centuries, mistletoe has been widely used in traditional medicine as an anti-cancer agent for diarrhea and other infectious diseases. The study's goals are to find out what kind of metabolite compounds are in the ethanol extract of mistletoe coffee leaves and how well that extract kills *Staphylococcus aureus* and *Escherichia coli* bacteria. This research method is experimental, which includes collecting plant material, identifying plants, processing samples of coffee mistletoe into an ethanol extract of coffee mistletoe with a 96% ethanol solvent, and then testing phytochemical screening against coffee mistletoe simplisa. Antibacterial activity of an ethanol extract of coffee mistletoe against *Staphylococcus aureus* and *Escherichia coli* bacteria using the well diffusion test method. The ethanol extract of coffee mistletoe provides phytochemical screening results, including phenyl, terpenoids, and alkaloids. From the test results, it can be seen that the ethanol extract of coffee mistletoe can kill bacteria with the inhibitory power value of the extract on *Staphylococcus aureus* and *Escherichia coli* bacteria, with a minimum inhibitory power of 7.5 mm at a concentration of 3.125 mg/ml against *Staphylococcus aureus* and 8.9 mm at a concentration of 3.125 mg/ml against *Escherichia coli* bacteria. Judging from the inhibitory zone value, it is in the weak category for the lowest concentration in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria.

**Keywords**: Coffee parasite, antibacterial, *Staphylococcus aureus Escherichia coli*

## Introduction

Mistletoe is a parasitic plant that resembles a shrub and grows by attaching itself to the stems or branches of other plants. It obtains nutrients from its host plant, and if left unchecked, the parasite can proliferate and lead to the gradual decline and eventual death of the host plant (Oloruntola & Ayodele, 2022). Mistletoe mostly contains flavonoids, tannins, amino acids, carbohydrates, alkaloids, and saponins. Flavonoids, tannins, and saponins have been found to possess antibacterial properties, as indicated by multiple conducted research (Pebiansyah et al., 2023).

*Escherichia coli* and *Staphylococcus aureus* are pathogenic bacteria responsible for causing illnesses. The use of antibiotics for treating illnesses has led to the emergence of bacteria that exhibit resistance to many medicines. Therefore, it is imperative to explore other sources of antibacterial agents. Common infectious disorders that frequently affect individuals include dermatological infections and gastrointestinal disturbances such as diarrhea. *Escherichia coli* and *Staphylococcus aureus* are potential causes of diarrhea. (Gurning et al., 2019; Nasri, Kaban, Syahputra, et al., 2022). The use of antibacterial medications for the treatment of bacterial-induced infectious disorders has been prevalent. However, the present challenge lies in the manifestation of adverse effects experienced by users, including diarrhea,

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allergies, and other toxic hazards, in addition to the elevated expenses associated with treatment. The prevalence of bacterial illnesses, the occurrence of adverse effects associated with antibacterial medications, and the exorbitant expenses associated with treatment underscore the imperative for conducting research aimed at developing novel antibacterial agents, particularly derived from natural sources (Kaban et al., 2023; Lubis et al., n.d.).

*Escherichia coli* is a gram-negative bacterium that ranks second in terms of infection causation, following *Streptococcus*. *Escherichia coli*-induced meningitis results in a mortality rate of 20-40% among affected infants. *Staphylococcus aureus* is a Gram-positive bacterium that is ubiquitously present, including within the human body. If the bacteria in the human body are present in normal quantities, they do not possess the capacity to induce illness. Epidemiological studies indicate a global rise in *Staphylococcus aureus* infections during the past two decades. Available data from the United States and Europe indicates that *Staphylococcus aureus* is the predominant pathogenic bacterium responsible for infections, with a prevalence ranging from 18% to 30%. In Asia, the frequency of *Staphylococcus aureus* and *Pseudomonas aeruginosa* is about identical.

Previous studies used coffee mistletoe leaf extract (Loranthus ferrugineus Roxb.). Yulian and Safrijal (2018) have reported that coffee mistletoe leaf extract contains alkaloids, flavonoids, saponins, and tannins. This composition suggests its potential application as an anti-inflammatory agent for burns. Given this situation, researchers are excited about the prospect of examining the effectiveness of coffee mistletoe leaves in promoting the growth of *Escherichia coli* and *Staphylococcus aureus* germs.

# MATERIALS AND METHOD

## Materials

The research utilized a range of equipment including a rotary evaporator, oven, incubator, refrigerator, autoclave, caliper, petri dish, tube needle, analytical balance, blender, Erlenmeyer flask, test tube, glass beaker, test tube rack, volume pipette, separating funnel, vials, hot plates, micro pipettes, paper discs, stirring rods, Bunsen burners, and spatulas.

The materials utilized in this study include coffee mistletoe, a 5% solution of FeCl3, a 1% solution of CeSO in 10% H2SO4, Bouchardat's reagent, Dragendorf's reagent, Meyer's reagent, Chloramphenicol, Mueller Hinton Agar (MHA), Nutrient Agar (NA), Nutrient Broth (NB), Dimethylsulfoxide (DMSO), and cultures of *Staphylococcus aureus* and *Escherichia coli*.

## Methods

### Botanical Classification

The determination of plants involves comparing the morphological properties of the plants to be examined with information found in the literature. This process ensures that there are no mistakes in picking plants for research purposes. The process of identifying the plant species was conducted at the Basic Biology Laboratory, which is part of the Faculty of Mathematics and Natural Sciences at the University of North Sumatra.

### Preparation of the Sample

The samples included in this investigation consisted of coffee mistletoe leaves (Loranthus ferrugineus Roxb) and were selected using a purposive sampling strategy. Specifically, the samples were not compared with those obtained from identical plants in different regions. The samples were collected from the Simpang Balik area in the Wih Pesam District of Bener Meriah Regency, located in Nanggroe Aceh Darussalam.

### Management of the Sample

After the coffee parasite is removed from the twigs, it undergoes a cleaning process, followed by thorough washing and drying in a drying closet. Pulverize the coffee mistletoe simplicia by blending it to

get a powder. Macerate the powder in a 96% ethanol solvent for 5 days, ensuring it is shielded from sunlight. Finally, filter the mixture. The filtrate and residue are placed in separate containers. The leftover coffee mistletoe residue undergoes a remaceration process lasting 2 days. The filtrate is concentrated using a rotary evaporator to generate a dense extract of coffee mistletoe (Arora & Itankar, 2018; Harahap et al., 2020). The finely pulverized simplicia powder underwent a phytochemical screening test to ascertain the presence of secondary metabolites in coffee mistletoe.

## Assessment of the Antibacterial Efficacy of Ethanol Extracts Derived from Mistletoe Coffee Leaves

**Tool sterilization**

The glassware employed is resistant to thermal damage, and may be sterilized in the oven at a temperature of 171℃ for a duration of 1 hour. Glassware that is susceptible to high heat damage, on the other hand, is sanitized in an autoclave at a temperature of 121ºC for a duration of 15 minutes. The osé needle is incinerated using a spirit lamp. Any tools used in the antibacterial activity test, regardless of whether they are reused or not, must be promptly sterilized by boiling them in a steamer with water and disinfectant liquid for a duration of 30 minutes. (Gurning et al., 2021; Nasri, Kaban, Gurning, et al., 2022).

**Producing Bacterial Inoculum**

Preparation of bacterial inoculum for *Staphylococcus aureus* and *Escherichia coli*. The culture sample was collected using a sterile needle and transferred into a test tube containing 10 ml of nutrient broth media. The sample was then mixed thoroughly using a vortex and incubated at a temperature of 37°C for 18-24 hours. Subjected to incubation at a temperature of 37°C (Amalyuri et al., 2022).

**Preparing Different Dilutions of Ethanol Coffee Mistletoe Leaf Extract**

The ethanol extract was prepared at concentrations of 100 mg/ml, 50 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml. DMSO served as the negative control, while the antibiotic chloramphenicol at a concentration of 10 mg/ml was employed as the positive control.

**Assessment of the Antibacterial Efficacy of Ethanol Extract Derived from Mistletoe Coffee Leaves**

10 mL of Mueller Hinton Agar media was added to a sterile petri dish and allowed to harden at room temperature. First, introduce 0.1 mL of bacterial inoculum with a concentration of 900 x 106 Colony Forming Units (CFU)/mL. Next, add 25 mL of liquid Mueller Hinton Agar (MHA) media. Homogenize the mixture in the shape of a figure eight. Then, place a metal backer and adjust the distance accordingly. Finally, label the concentration under the petri dish where it aligns with the metal backer. Once the Mueeler Hinton Agar (MHA) has hardened, raise the backer to create a depression. Then, add 0.1 mL of the ethanol extract test solution of coffee mistletoe leaves to each depression, with concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml. DMSO served as the negative control, while a 10 mg/ml concentration of chloramphenicol antibiotic was employed as the positive control. Subsequently, it was placed in an incubator set at a temperature of around 35ºC for a duration of 24 hours. The well area was assessed and quantified using a caliper with millimeter (mm) units of measurement, based on data obtained from the outcomes of three treatments (Rani et al., 2022).

## Data Analisys

Analyzed using SPSS, the study examined the impact of inhibitory zone diameter on the suppression of bacterial growth in samples of coffee mistletoe ethanol extract. The data is organized in a tabular format and analyzed based on the acquired results.

## Result and Discussion

### Results of Plant Identification

The findings of a study conducted on the ethanol extract of Loranthus ferugineus Roxb, often known as coffee mistletoe leaves, have been acquired. The plant identification conducted at the Medanese Herbarium (MEDA) at the University of North Sumatra confirms that the plant under study is coffee mistletoe leaves.

### Results of Phytochemical Screening of Ethanol Extract of Coffee Mistletoe Leaves

A phytochemical screening test was performed on the leaves of the coffee mistletoe (*Loranthus ferrugineus* Roxb.) to determine the presence of phenolic chemicals, terpenoids, alkaloids, and saponins. The test results are displayed in Table 1.

**Table 1**. Phytochemical screening test findings for the ethanol extract of coffee mistletoe leaves.

| No | Compounds of Secondary Metabolites | Extract of ethanol from coffee mistletoe | Simplicia Benalu Coffee |
|---|---|---|---|
| 1. | Alkaloids | Positive | Positive |
| 2. | Phenolic | Positive | Positive |
| 3. | Saponin | Positive | Positive |
| 4. | Terpenoids | Positive | Positive |

Phytochemical screening is the initial stage of research that seeks to give a comprehensive analysis of the types of chemicals present in the plant under investigation, namely the ethanol extract of coffee mistletoe leaves. The findings from the phytochemical analysis of the ethanol extract of coffee leaves in this study revealed the presence of alkaloid, saponin, phenolic, and terpenoid components in mistletoe coffee leaves.

Testing the ethanol extract of coffee mistletoe leaves revealed the presence of alkaloids, as shown by the production of a brown precipitate when treated with the Wagner reagent, and a change in the color of the extract to brown when treated with the Dragendorff reagent (Yulianto, 2020). Saponin is a polar compound because it is in the glycoside form of sapogenin. When agitated in water, it has the ability to generate foam (Kristansti,dkk., 2008). The foam observed in the saponin test indicates the existence of glycosides, which possess the capacity to generate foam when mixed with water and then degraded into glucose and other chemicals. Terpenoid testing relies on the capacity of the component to produce color when combined with a solution containing 1% CeSO4 in 10% concentrated H2SO4 in the ethanol extract of coffee mistletoe leaves. Positive results are shown by the creation of a brownish red color, which confirms the presence of terpenoids (Cahyaningsih et al., 2019).

### Results of the antibacterial activity test show that the ethanol extract of coffee mistletoe leaves (*Loranthus ferrugineus* Roxb) has an inhibitory effect on the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria.

The inhibitory zone seen in the growth of *Escherichia coli* and *Staphylococcus aereus* bacteria during the antibacterial activity test demonstrated the antibacterial qualities of the coffee mistletoe leaves (*Loranthus ferrugineus* Roxb.) ethanol extract. Multiple concentrations were used in the experiment: 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml. The positive control in the trial was the antibiotic chloramphenicol, which was used at a dosage of 10 mg/ml. Gram-positive and gram-negative bacteria can both be inhibited and killed by the broad-spectrum antibiotic chloramphenicol. To dissolve the extract in this study, ethanol and DMSO solvents are used as negative blanks. In order to ensure that the results obtained are unaffected by the extract's antibacterial capabilities, testing employing negative controls is done to show that the solvent does not provide positive results throughout the testing process. The results of the antibacterial activity tests are shown in Table 2 below.

**Table 2.**. Phytochemical screening test results for the ethanol extract of coffee mistletoe leaves

| No | Concentration (mg/ml) | Ethanol Extract | |
|---|---|---|---|
| | | ***Escherichia coli*** **(mm)** | ***Staphylococcus aureus*** **(mm)** |
| 1. | 100 | 18.2 | 16.85 |
| 2. | 50 | 17.2 | 15.8 |
| 3. | 25 | 16.4 | 14.15 |
| 4. | 12.5 | 15.1 | 11.6 |
| 5. | 6.25 | 10.6 | 9.2 |
| 6. | 3.125 | 8.9 | 7.4 |
| 7. | Chloramphenicol | 19.7 | 19.6 |
| 8. | DMSO | 0 | 0 |

Table 2 demonstrates that the ethanol extract of mistletoe coffee leaves significantly inhibits the antibacterial activity of *Escherichia coli* and *Staphylococcus aureus*. The diameter of the inhibitory zone seen at all concentrations clearly shows the substance. As the concentration of the drug under test rises, so does the diameter of the inhibition zone for the development of *Escherichia coli* bacteria. The diameter of the inhibition zone is 18.2 mm at a dosage of 100 mg/ml. The diameter measures 17.2 mm when the concentration is 50 mg/ml. The diameter measures 16.4 mm when the concentration is 25 mg/ml. The diameter measures 15.1 mm at a concentration of 12.5 mg/ml. The diameter measures 10.6 mm when the concentration is 6.25 mg/ml. At 3.125 mg/ml of concentration, the diameter.

The ethanol extract of coffee mistletoe leaves demonstrates inhibitory effects on the antibacterial activity of *Staphylococcus aureus*. The inhibitory zone diameter decreases as the concentration of the extract decreases: at a concentration of 100 mg/ml, the diameter is 16.85 mm; at 50 mg/ml, it is 15.8 mm; at 25 mg/ml, it is 14.15 mm; at 12.5 mg/ml, it is 11.6 mm; at 6.25 mg/ml, it is 9.2 mm; and at 3.125 mg/ml, it is 7.4 mm. In comparison, the positive blank (chloramphenicol) produces an inhibition zone of 19.6 mm.

Based on the aforementioned studies, it is evident that the ability to limit the growth of *Escherichia coli* bacteria is superior to that of *Staphylococcus aureus*. This discrepancy may arise due to the distinct composition of cell wall components in each bacterium. Gram-positive bacteria possess a substantial and intricate cell wall construction, ranging from 20 to 80 mm in thickness, with peptidoglycan constituting more than 50-80% of the total weight. This massive and complex cell wall hinders the penetration of antibiotic chemicals into the cell. Gram-negative bacteria possess a narrower cell wall structure (5-10 nm) and have a lower amount of peptidoglycan compared to gram-positive bacteria. This allows antibacterial agents to penetrate the cells more readily (Brooks et al., 2007).

The differences in the inhibition zone for each concentration can be seen in Figure 1 below.

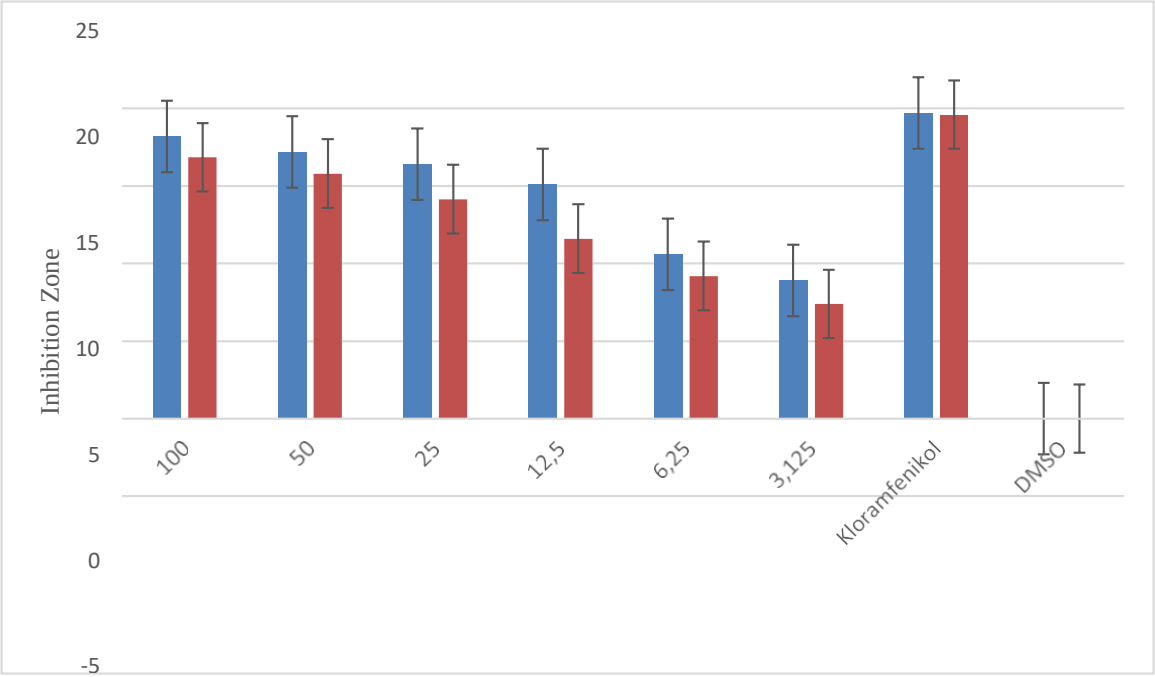


**Figure 1.** The diameter of the antimicrobial inhibition zone of the ethanol extract of coffee mistletoe leaves (Loranthus ferrugineus Roxb) against the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria

The antibacterial efficacy against *Escherichia coli* and *Staphylococcus aureus* germs was assessed using one-way ANOVA testing using SPSS 24 software to determine the statistical significance of the obtained data. The analysis of *Escherichia coli* and *Staphylococcus aureus* bacteria indicated that the data had a normal distribution across different treatments, as evidenced by the p-values (>0.05) obtained from each treatment test. Analysis of the research test data reveals that the data exhibits homogeneity, as shown by a significance level of 0.002 ($p<0.05$). According to the One-way ANOVA analysis test using the Tukey method, the treatment with a concentration of 100 mg/mL demonstrated the highest level of test effectiveness.

## Conclusion

At a minimum inhibitory concentration (MIC) of 3.125 mg/ml, the ethanol extract of coffee mistletoe leaves (Loranthus ferrugineus Roxb.) effectively suppresses the development of *Escherichia coli* bacteria, resulting in an inhibitory zone diameter of 8.9 mm. Likewise, at the same dose, it also inhibits the growth of *Staphylococcus aureus* germs, with a 7.4 mm inhibition zone diameter. Consequently, the growth of *Escherichia coli* bacteria is inhibited more effectively by coffee mistletoe leaf extract than it is by *Staphylococcus aureus* bacteria.

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