

Antibacterial Activity of *Scurrula ferruginea* (Roxb. Ex Jack) Leaves Extract Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract. The mistletoe (*Scurrula ferruginea*) is a parasitic plant obtaining nutrients and water from host plants at Nglingga tea plantation, Kulon Progo, Yogyakarta. Despite the numerous benefits of *Scurrula ferruginea* leaf, substantiation regarding its pharmacological activity is still lacking. Therefore, this research aimed to identify *Scurrula ferruginea* species and obtain secondary metabolites with antibacterial activity. The experiment was carried out by extracting *Scurrula ferruginea* leaf using maceration methods with 96% ethanol, followed by qualitatively and quantitatively phytochemical screening, inhibitory zone test, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) test. Several compounds, such as flavonoids, tannins, phenols, saponins, alkaloids, terpenoids, and steroids, were identified in the mistletoe extract. The results showed total flavonoid, phenolic, and tannin levels were 7.41 ± 0.38 mg QE/mg, 1672 ± 50.99 mg GAE/g, and 1.43 ± 0.15 mg TAE/g, respectively. *Scurrula ferruginea* leaf ethanolic extract showed the potential to form an inhibition zone against *S. aureus* in the moderate category at 525 mg/mL, but no significant activity was observed against *E. coli*. The MIC results of *Scurrula ferruginea* leaf ethanolic extract against *S. aureus* were 0.6%, and the MBC was at 2.4%. Furthermore, the MIC value against *E. coli* was 14%, and the MBC value was 28%. Based on these results, it could be concluded that *Scurrula ferruginea* leaf ethanolic extract showed high effectiveness in inhibiting and killing *S. aureus* bacteria compared to *E. coli*, with MIC and MBC values of 0.6% and 2.4%, respectively

Keywords: antibacterial activity, Mistletoe, Phytochemical screening, *Scurrula ferruginea*

Citation

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INTRODUCTION

The mistletoe (*Scurrula ferruginea*) is a parasitic plant that survives depending on the host's nutrients and water. This parasitic plant attacks twigs on the roots of trees, thriving on various types of hosts. Moreover, *Scurrula ferruginea* is divided into 2 types: holoparasites lacking chlorophyll and hemiparasites capable of photosynthesis. In Yogyakarta region, *Scurrula ferruginea* tea plants are often found in the Kulon Progo area, specifically in the Nglinggo tea plantations. This area has local tea plantations infected with *Scurrula ferruginea*, threatening agriculture. Due to insufficient information, people around tea plantations have used *Scurrula ferruginea* as herbal medicine without adequate knowledge of the type and compounds. Therefore, Aditiyarini et al. (2022) conducted an investigation and identified strong antioxidant activity of tea *Scurrula atropurpurea* in Nglinggo, Kulon Progo, with IC₅₀ 0.33 ppm. The results showed that *Scurrula atropurpurea* contains flavonoids, saponins, steroids, tannins, and terpenoids, with total levels of 96.06 ± 1.87 mg TAE/g and 36.70 ± 1.16 mg QE/g, respectively. These compounds are secondary metabolites that have health benefits, with flavonoids functioning as antibacterial agents by inhibiting the performance of bacterial cell membranes. Based on morphological observation in tea plantation area, the mistletoe species is presumed to be *Scurrula ferruginea*. Compared to *Scurrula atropurpurea*, which has identified phytochemical compounds, *Scurrula ferruginea* has not been extensively investigated.

Justine et al. (2018) reported that the methanolic extract of *Scurrula ferruginea* provided superior antibacterial activity against *Staphylococcus aureus* bacteria compared to *Escherichia coli* with a diameter of 9.63 ± 0.13 mm and 8.20 ± 0.02 mm, respectively. These pathogenic bacteria pose a significant risk to human health by initiating infectious diseases. Specifically, *S. aureus* belongs to a group of Gram-positive bacteria causing wound infections, food poisoning, and skin diseases such

as pustules and ulcers (Dewi, 2013), while *E. coli* is recognized as normal floral bacteria. These bacteria are commonly found in the digestive tract of livestock and humans, with *E. coli* causing diarrheal, meningitis, and hemolytic uremic syndrome (Bria et al., 2022). However, the phytochemical composition of the methanolic extract of *Scurrula ferruginea*, which contributes to moderate antibacterial, remains unknown. Since methanol is a polar solvent that tends to solve a polar compound, this research uses ethanol solvent during extraction. This is because ethanol is less polar and can dissolve various compounds with a broader polarity spectrum. Therefore, this research aimed to identify the phytochemical composition of *Scurrula ferruginea* using quantitative and quantitative methods along with the antibacterial activity against *S. aureus* and *E. coli*. The samples of *Scurrula ferruginea* leaf were obtained from Nglinggo, Kulon Progo Regency, Yogyakarta, Indonesia

MATERIALS AND METHODS

Research Methods

This research was conducted through experimental methods at the Laboratory of Biotechnology, Universitas Kristen Duta Wacana. Samples were collected from Nglinggo, Pagerejo, Samigaluh, Kulon Progo, Yogyakarta. Soil phosphate (P), potassium (K), and nitrogen (N) content assays were conducted at the Laboratory of the Faculty of Agriculture, STIPER Yogyakarta. The plant determination test was conducted at the Laboratory of Biology, Universitas Gadjah Mada, Yogyakarta. The minimum inhibitory concentration (MIC) assay was conducted at the Faculty of Medicine, Universitas Kristen Duta Wacana, Yogyakarta.

Identification of the mistletoe species

Mistletoe species were determined in the Laboratory of Plant Systematics in the Faculty of Biology, Universitas Gadjah Mada, Yogyakarta. The samples consist of stems, leaves, and flowers of tea mistletoe from Nglinggo, Kulon Progo, Yogyakarta.

Samples Preparation

Scurrula ferruginea leaf samples were sorted, rinsed using running water, drained, and dried with wind-dry method for 24 hours. Subsequently, the drying process was continued in the oven for 12 hours at 40°C and weighed to obtain dry weight. The samples were pulverized using a blender and stored in the refrigerator.

Sample Extract

The extraction method was carried out through maceration using 96% ethanol with a ratio of 1:10 (sample: solvent), with approximately 4 repetitions. Subsequently, the extract was concentrated using a rotary evaporator (IKA HB 10) at 40°C.

Phytochemical screening

Phytochemical screening was conducted to identify alkaloids, flavonoids, terpenoids, saponins, tannins, steroids, and phenol compounds, following the procedure of Aditiyarini et al. (2022). Alkaloid was identified by dissolving 0.5 g of extract with 9 mL of distilled water and HCl 2N, which was heated and filtered to obtain the pellet and supernatant. Subsequently, the supernatant was placed into 4 test tubes and added with reagents. Tubes A, B, C, and D were used for Mayer, Wagner, Bouchradat, and Dragendorf reagents. Tannin was identified by dissolving 0.05 g of extract with 2 mL of 96% ethanol and 3 drops FeCl₃ 10%. Phenol was identified by dissolving 0.05 g extract with 2 mL ethanol 70% and 3 drops FeCl₃ 10%. The terpenoids and steroids were identified by dissolving 0.05 g of extract with 0.05 mL of anhydrous acetate, followed by adding 2 mL of concentrated H₂SO₄ through the tube wall. Flavonoids were identified by dissolving 0.05 g of extract with 2 mL of 96% ethanol and inserting 1 cm Mg and 1 mL concentrated HCl. Saponins were identified by dissolving 0.05 g of extract with 10 mL of distilled water, followed by homogenization until foam was formed.

Total Flavonoids Content

Total flavonoid content was initiated by preparing a standard solution of 100 ppm quercetin. Initially, approximately 10 mg of quercetin was dissolved in 10 mL of 80% ethanol to obtain 1000 ppm quercetin stock solution, which was diluted to obtain concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 ppm. Each concentration was taken 0.5 mL and added with 1.5 mL of 80% ethanol, 0.1 mL of 1M potassium acetate, 0.1 mL of 10% AlCl₃, and 2.8 mL of distilled water, which stood for 30 minutes. The absorbance value was measured using a spectrophotometer with a wavelength of 415 nm. The results were used to make a standard curve of quercetin to obtain a regression equation $y = ax \pm b$ by plotting the sample concentration (x) against the absorbance value (y). The extract of 0.5 g was dissolved with 10 mL of 80% ethanol to calculate total flavonoids. Subsequently, 0.5 mL was taken from the extract solution and added with 1.5 mL of 80% ethanol, 0.1 mL of 10% AlCl₃, 0.1 mL potassium acetate 1 M, and 2.8 mL of distilled water. The absorbance value was observed using a spectrophotometer with a wavelength of 415 nm, which was conducted with three replicates.

Total Phenolic Content

Total phenolic content was initiated by making a standard solution of gallic acid of 10 mg dissolved with 10 mL of distilled water. This was carried out to obtain a stock solution of gallic acid 1000 ppm that was diluted to achieve concentration series of 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 ppm. The folin ciocalteu reagent 0.4 mL was added at each concentration and allowed to stand for 4-8 minutes. The procedure followed by adding 4 mL of 20% Na₂CO₃ and aqua until a volume of 10 mL. The sample was incubated for 2 hours at room temperature, and the absorbance value was calculated using a UV-Vis spectrophotometer with a wavelength of 662.85 nm. The absorbance value was used to obtain the linear regression

value. Total phenol content was measured by taking 10 mg of extract dissolved with 10 mL of 96% ethanol. Subsequently, 0.4 mL of folin ciocalteu reagent was added to 1 mL extract and allowed to stand for 4-8 minutes. This was followed by adding 4 mL Na₂CO₃ 20% and 10 mL aqua bidestilata to the solution. The samples were incubated for 2 hours at room temperature, and the absorbance value was measured using a UV-Vis spectrophotometer with a wavelength of 662.85 nm.

Determining total phenol content commenced by making a standard solution of 100 ppm quercetin, which was dissolved in 10 mL of 80% ethanol to obtain 1000 ppm quercetin stock solution. Subsequently, 1000 ppm quercetin stock solution was diluted to obtain concentrations of 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 ppm. Approximately 0.5 mL of each concentration was added with 1.5 mL of 80% ethanol, 0.1 mL of 1M potassium acetate, 0.1 mL of 10% AlCl₃, and 2.8 mL of distilled water. The solution was allowed to stand for 30 minutes, and the absorbance value was calculated using a spectrophotometer with a wavelength of 415 nm. The results were used to make a standard curve of quercetin to obtain a regression equation $y = ax \pm b$ by plotting the sample concentration (x) against the absorbance value (y). The extract of 0.5 g was dissolved with 10 mL of 80% ethanol to calculate the total phenol content. A total of 0.5 mL was taken from the extract solution and added with 1.5 mL of 80% ethanol, 0.1 mL of 10% AlCl₃, 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water. The absorbance value was measured using a spectrophotometer with a wavelength of 415 nm, which was conducted with three replicates.

Total Tannin Content

A 10 mg Tannin acid was dissolved with 10 mL folin ciocalteu reagent and homogenized using a magnetic stirrer. The solution was allowed to stand for 5 minutes and added with 20% sodium carbonate until the volume became 100 mL. Di-

lutions were made to each concentration of 0.125, 0.25, 0.5, 1, 2, 4, 8, and 16 ppm, followed by incubation for 30 minutes at room temperature. The absorbance value was measured using a UV-Vis spectrophotometer at a wavelength of 720 nm. The 100 mg extract was extracted with diethyl-ether 10 mL and allowed to stand for 20 hours. The results were added to distilled water until the volume became 10 mL. Subsequently, 0.1 mL of Folin ciocalteu reagent was added, vortexed, and allowed to stand for 5 minutes, followed by 2 mL of 20% Na₂CO₃. The solution was added with distilled water until the total volume was 10 mL and incubated for 30 minutes. The absorbance value was measured using a UV-Vis spectrophotometer at a wavelength of 760 nm. Moreover, this assay was carried out with three replicates to ensure accuracy.

Inhibition Zone Diameter Assay

The Kirby Bauer method on Muller Hinton Agar (MHA) determined the inhibition zone diameter. The medium was inoculated with a suspension of *S. aureus* and *E. coli* bacteria that was adjusted to McFarland 0.5 using sterile cotton sticks. Paper discs with a diameter of 6 mm that was soaked in extract solutions with each concentration of 25, 150, 275, 400, 525 mg/mL on *S. aureus* bacteria and a concentration series of 25, 150, 275, 400, 525, 750, 1000, 1250, 1500, 1750 mg/mL on *E. coli*, was placed on MHA media, and incubated for 24 hours at 37°C. After 24 hours, the resulting inhibition zone was measured using a Vernier caliper. The concentrations that inhibited bacteria were used to determine the minimum inhibitory concentration (MIC) assay.

Minimum Inhibitory Concentration Assay

The minimum inhibitory concentration (MIC) test was carried out using the microdilution method, where the extract was dissolved with 5% DMSO to obtain 100% extract stock. In antibacterial assay against *S. aureus*, twofold dilution was carried out using Brain-heart Infusion Broth (BHIB) media as a diluent to obtain an extract

concentration series of 38.4%, 19.2%, 9.6%, 4.8%, 2.4%, 1.2%, 0.6%, 0.3%, 0.15%. For antibacterial assay against *E. coli*, the extract was prepared using BHIB media to obtain concentrations of 56%, 28%, 14%, 7%, 3.5%, and 1.75%. The negative control was 5% DMSO solution with BHIB, while ampicillin 5 ppm served as negative control. After preparation, microplate 96 wells containing bacteria in media and extract were incubated at 37°C for 24 hours. The optical density (OD) value on a microplate reader was measured with a wavelength of 570 nm. The result was inserted into the formula to determine the OD of bacteria using the formula:

$$\text{OD of bacteria} = \text{OD extract} - \text{OD blank}$$

The concentration that could inhibit bacteria was used to determine the minimum bactericidal concentration (MBC) assay.

Minimum Bactericidal Concentration Assay

Minimum Bactericidal Concentration (MBC) assay of *Scurrula ferruginea* leaf ethanolic extract against *S. aureus* bacteria was carried out through inoculation with concentrations of 0.6%, 1.2%, 2.4%, and 4.8% in 96 well microplates on MHA media with the streak plate method then incubates at 37°C for 24 hours. In *E. coli* bacteria using a concentration series of 56%, 28%, 14%, and 7%, MBC values were determined by looking at the growth of bacterial colonies on MHA media. The lowest concentration with no bacterial growth was the MBC value.

RESULTS AND DISCUSSION

Phytochemical of Ethanolic Extract of Mistletoe Tea Leaves

Using colorimetry, phytochemical screening is applied to identify chemical compounds contained in plant extracts. In phytochemical screening, there will be a change in color or shape, showing the content of compounds in a plant extract (Putri et al., 2014). This research shows phytochemical screening in Table 1, where alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, and steroids are detected in the *Scurrula ferruginea* leaf ethanolic extract. The results showed that the *Scurrula ferruginea* leaf ethanolic extract has high secondary metabolite diversity with pharmacological potential. The selection of solvent type affected the diversity of secondary metabolite compounds in the extract. Therefore, careful selection is required, as the proper solvent can attract active compounds perfectly. The principle of solubility of substances is a similar polarity, namely 'like dissolve like', where the compounds with similar polarity dissolve each other (Suhendra et al., 2019; Vifta & Advistasari, 2018).

Table 1. Phytochemical screening of *Scurrula ferruginea* leaf ethanol extract

Test component	Result	Description
Alkaloids	+	Orange, Brown, White precipitation, and Brown precipitation
Flavonoids	+	Deep orange
Tannins	+	Dark green
Phenolic	+	Dark blue
Terpenoids	+	Purple rings
Steroids	+	Brown rings
Saponins	+	Stable foam

Description: (+) : Positive or detected; (-) : Negative or not detected

Total Content of Flavonoids, Phenolic, and Tannins of *Scurrula ferruginea* leaf Ethanolic Extract

As shown in Table 2, total flavonoid, phenolic, and tannin content of *Scurrula ferruginea* leaf ethanolic extract are 7.41 ± 0.38 mg QE/g extract, 1672 ± 50.99 mg GAE/g extract, and 1.43 ± 0.15 mg TAE/g extract, respectively. These results showed that the content of phenolic compounds in the *Scurrula ferruginea* leaf ethanol extract was higher than flavonoids and tannins.

Table 2. Total flavonoids, phenolic, and tannins content of *Scurrula ferruginea* leaf ethanolic extract

No.	Compounds	Amount
1	Flavonoids	7.41 ± 0.38 mg QE/g extract
2	Phenolic	1672 ± 50.99 mg GAE/g extract
3	Tannins	1.43 ± 0.15 mg TAE/g extract

Description :

QE : Quercetin Equivalent ; GAE : Gallic Acid Equivalent ; TAE : Tannic Acid Equivalent

Production of secondary metabolites increased in the presence of biotic or abiotic stress. Specifically, secondary metabolites play significant roles in plants as a defense mechanism against pathogens and abiotic stress, attractants, and others (Perangin-angin et al., 2019). Plants that encounter stress will cause disturbances in cellular redox homeostasis to facilitate the production of reactive oxygen species (ROS). Phenolic compounds can scavenge free radicals, forming complex reactions with metals. Furthermore, phenolic compounds will catalyze oxygen reactions and become inhibitors of oxidizing enzymes (Król et al., 2014).

Inhibition activity of *Scurrula ferruginea* leaf ethanolic extract

The antibacterial activity of *Scurrula ferruginea* leaf ethanolic extract is represented in Figure 1 and Table 3, indicating the formation and measurement of clear zone diameter,

respectively. *Scurrula ferruginea* leaf ethanolic extract results against *S. aureus* and *E. coli* bacteria showed that the concentration of 25 mg/mL did not form an inhibition zone. A clear zone for medium activity was observed at extract concentrations of 150, 275, 400, and 525 mg/mL. The extract could not form an inhibition zone on *E. coli* bacteria. In previous investigations, David et al. (2017) reported that the distilled water of *Scurrula ferruginea* ethanolic extract was unable to form an inhibition zone of *E. coli* bacteria but inhibited the growth of *S. aureus* at a concentration of 500 mg/ml with an inhibition zone diameter of 10.67 ± 0.58 mm. Justine et al. (2018) also reported that 100% methanol extract inhibited the growth of *S. aureus* bacteria with an inhibition zone diameter of 9.63 mm, while *E. coli* bacteria had 8.20 mm. However, in this research, *Scurrula ferruginea* leaf ethanolic extract in Nglinggo, Kulon Progo, has antibacterial activity with moderate strength against *S. aureus* bacteria. At the same time, there was no activity observed for *E. coli* bacteria.

The variations in results were caused by several factors, such as the type of solvent, organism sensitivity, diffusion speed of the extract, concentration, microorganisms, and secondary metabolites contained (Putri et al., 2014). Previous research has established that selecting the suitable solvent is based on the ability to dissolve chemical compounds possessing bioactivity as antibacterial solvents (Vifta & Advistasari, 2018). The results of inhibition zone diameter in *S. aureus* bacteria are more significant than in *E. coli* bacteria. This occurs because *S. aureus* is classified as Gram-positive while *E. coli* is Gram-negative, which is determined by the structure of the cell wall. Specifically, Gram-negative bacteria consist of outer, middle, and inner layers, inhibiting the potential of antibacterial compounds to damage cells (Lestari et al., 2016). Gram-positive bacteria consist of

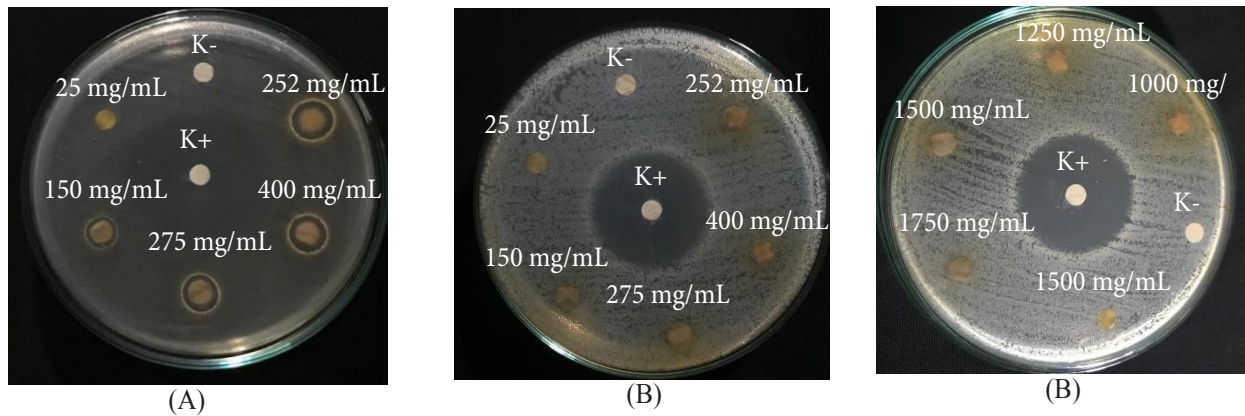


Figure 1. Bacterial growth at various concentrations of *Scurrula ferruginea* ethanolic extract by agar Muller-Hinton Kirby Bauer method (A) *S. aureus* FNCC 0047 (B) *E. coli* ATCC 25922

many peptidoglycans and few cell walls. This structural difference indicates that gram-negative bacteria are relatively more complex than gram-positive bacteria (Novita, 2016). Gram-positive bacteria tend to be more sen-

sitive to antibacterial due to straightforward cells, which facilitates easy access to antibacterial compounds (Runtuwene et al., 2017).

Table 3. Zone Diameter of Inhibition against *S. aureus* and *E. coli*

Bacteria	Concentration	Diameter (mm ± SD)	Category
<i>S. aureus</i>	Ampicillin 0.25 ppm (K+)	35.90 ± 0.55	Strong
	Negative control (K-)	0 ± 0	Na
	25 mg/mL	0 ± 0	Na
	150 mg/mL	4.78 ± 0.49	weak
	275 mg/mL	5.66 ± 0.69	Moderate
	400 mg/mL	7.55 ± 0.19	Moderate
	525 mg/mL	8.18 ± 0.02	Moderate
<i>E. coli</i>	Ampicillin 0.25 ppm (K+)	26.88 ± 1.18	Strong
	Negative control (K-)	0 ± 0	Na
	25 mg/mL	0 ± 0	Na
	150 mg/mL	0 ± 0	Na
	275 mg/mL	0 ± 0	Na
	400 mg/mL	0 ± 0	Na
	525 mg/mL	0 ± 0	Na
	1000 mg/mL	0 ± 0	Na
	1250 mg/mL	0 ± 0	Na
	1500 mg/mL	0 ± 0	Na
1750 mg/mL	0 ± 0	Na	

Description: Na = Not available

Minimum Inhibitory Concentration of *Scurrula ferruginea* leaf ethanolic extract

A minimum inhibitory concentration (MIC) test was conducted to determine the smallest concentration capable of inhibiting bacterial growth. Due to the small amount

of material required, this test was carried out using the microdilution method. The microdilution principle calculates OD value of bacteria using a microplate reader instrument.

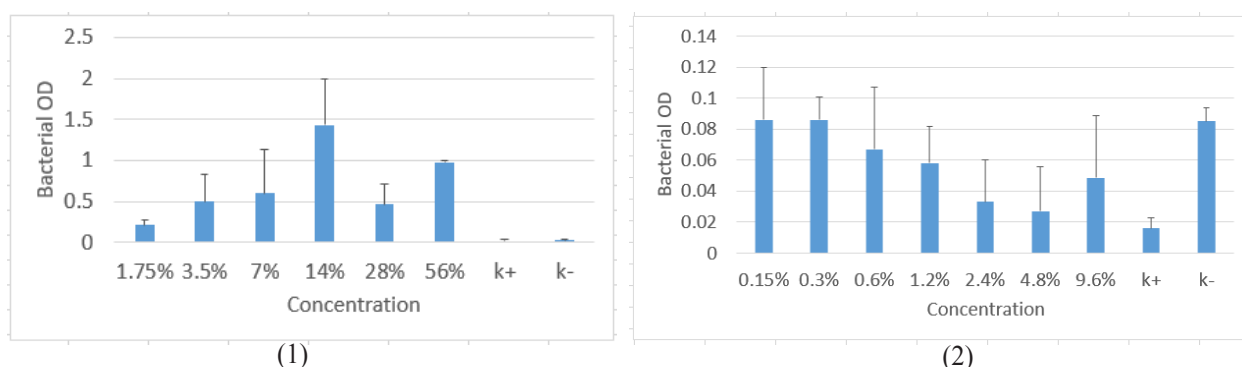


Figure 2. Bacterial growth at various concentrations of *Scurrula ferruginea* ethanolic extract by broth microdilution method at 570 nm wavelength (1) *S. aureus* FNCC 0047 (2) *E. coli* ATCC 25922

The results of OD value for *E. coli* bacteria from the microplate reader are represented in Figure 1. The statistical test showed significance results < 0.05 , indicating differences between treatments, where 56% and 28% concentrations met standard requirements. Furthermore, OD bacterial at 7% extract was lower than the negative control but close to the OD value of the positive concentration. The smallest concentration of *Scurrula ferruginea* leaf ethanolic extract leaf that can inhibit bacteria was 7% or equivalent to 7000 mg/mL. This result is smaller when compared to Justine et al. (2018), where the MIC value of 100% methanol extract of *Scurrula ferruginea* leaf against *E. coli* bacteria was at a concentration of 900 mg/mL. In this research, OD value of *S. aureus* from the microplate reader is represented in Figure 2. The statistical test showed a significance result > 0.05 , indicating that the data cannot be used. Based on the comparison, the bacterial growth at 0.6% extract was lower than OD of the negative control but higher compared to

positive control. The smallest concentration of *Scurrula ferruginea* leaf ethanolic extract that inhibited bacteria was 0.6% or equivalent to 600 mg/mL. This result is smaller when compared to Justine et al. (2018), which showed the MIC value of 100% methanol extract of *Scurrula ferruginea* against *S. aureus* at a concentration of 450 mg/mL. The variation indicated that the antibacterial activity of *Scurrula ferruginea* leaf ethanolic extract was higher than the 100% methanol extract due to the different extraction solvents. Consequently, solvents for extraction must be capable of dissolving chemical compounds with the potential to be antibacterial. Ethanol solvent can distill almost simplistic, non-polar, semi-polar, and polar content (Runtuwene et al., 2017; Vifta & Advistasari, 2018)..

Minimum Bactericidal Concentration of *Scurrula ferruginea* leaf ethanolic extract

Minimum Bactericidal Concentration (MBC) value is the smallest concentration that can kill bacterial growth. In this research,

MBC value was determined by the concentration of extract that has no bacterial growth. As shown in Figure 3, MBC value of *Scurrula ferruginea* leaf ethanolic extract against *Staphylococcus aureus* bacteria was 2.4%, while for *E.coli* bacteria was 28% (Figure 4). This value was higher compared to Justine et al. (2018), where $750 \pm 259.81 \mu\text{g/mL}$ and $900 \pm$

$0.00 \mu\text{g/mL}$ were obtained using 100% methanol extract against *S. aureus* and *E. coli* bacteria, respectively. The results suggested that *Scurrula ferruginea* leaf ethanolic extract did not inhibit the growth of *S. aureus* or *E. coli*.

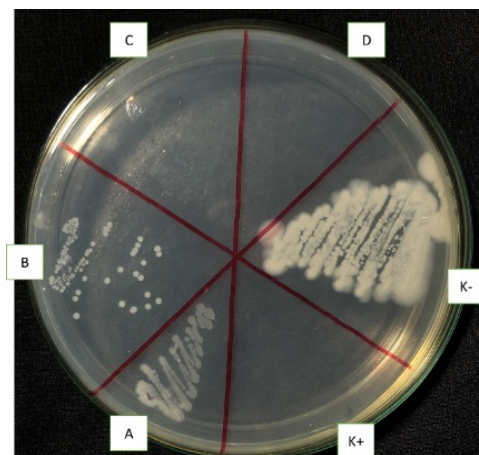


Figure 3. Bacterial growth in the Minimum Bactericidal Concentration test on *S. aureus* bacteria. Positive control (K+), negative control (K-), 0.6% concentration (A), 1.2% concentration (B), 2.4% concentration (C), 4.8% concentration (D)

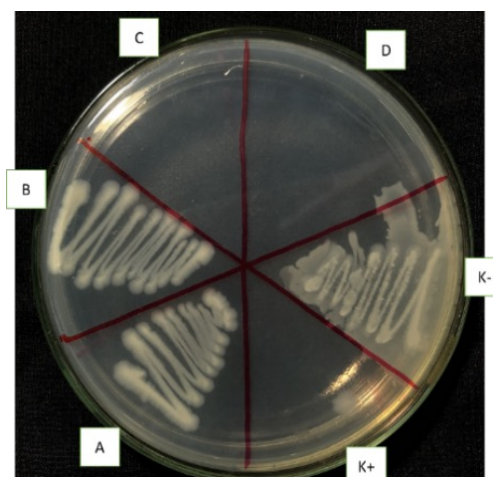


Figure 4. Bacterial growth in the Minimum Bactericidal Concentration test on *E. coli* bacteria. Positive control (K+), negative control (K-), 7% concentration (A), 14% concentration (B), 28% concentration (C), 56% concentration (D)

The antibacterial activity of *Scurrula ferruginea* leaf ethanolic extract is attributed to secondary metabolite compounds that have bioactivity as antibacterial. Moreover, antibacterial activity shows the potential of compounds to inhibit and kill bacteria, affecting both physiological and psychological aspects in humans (Akbar et al., 2016). *Scurrula ferruginea* leaf contains secondary metabolites such as alkaloids, flavonoids, tannins, steroids, terpenoids, and saponins. In the total content assay, phenolic compounds were the highest compared to tannin and flavonoid compounds, playing an essential role as antibacterial. This is due to hydroxyl groups in chemical structures of phenolic compounds functioning as inhibitors. At low concentrations, phenolic compounds damage the cytoplasmic membrane to facilitate the leakage of bacterial cell nuclei, while high concentrations enhance coagulation with cellular proteins. This activity is significantly influential when bacteria perform cell division, where the cell's phospholipid layer is very thin, causing high susceptibility to damage (Novita, 2016). Another mechanism occurs by inhibiting cellular enzymes influenced by the activity of phenolic compounds with cell membranes for permeability changes to occur in cytoplasmic membranes.

Flavonoids in *Scurrula ferruginea* leaf extract have bioactivity as antibacterial by inhibiting the function of bacterial cell membranes. When inhibition occurs, flavonoids form complex compounds with extra-cellular proteins to damage bacterial cell membranes. Flavonoids also inhibit the use of oxygen by bacteria to ensure an inhibition of energy metabolism. Since bacteria require energy to biosynthesize macromolecules, the lack of energy will inhibit the formation of complex molecules (Sapara et al., 2016). Tannic acid also damages the peptidoglycan wall of bacteria, followed by cell walls due to spas-

molytic properties that cause wrinkles and disrupt cell permeability, thereby inhibiting growth (Rahmawati, 2021). Terpenoids bind to transmembrane proteins on the outer part of the bacterial cell wall and form polymer bonds. This phenomenon causes damage to cell membrane proteins, affecting cell wall permeability reduction, resulting in a lack of nutrients for bacteria (Rollando et al., 2019).

CONCLUSION

In conclusion, this research showed that *Scurrula ferruginea* leaf ethanolic extract contained tannins, flavonoids, saponins, steroids, terpenoids, and alkaloids. The total phenolic content was higher than flavonoids or total tannin content, with 1672 ± 50.99 mg GAE/g. Regarding secondary metabolites, *Scurrula ferruginea* leaf ethanolic extract showed a higher antibacterial activity for *S. aureus* than *E. coli*, with MIC 0.6% and MBC 2.4%.

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