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ARTICLE

# KESAMBI (*Schleichera oleosa* (Lour) Oken) TREE BARK EXTRACT AND ITS FRACTIONS: PHYTOCHEMICAL SCREENING, DETERMINATION OF TOTAL PHENOLIC CONTENT, AND ANTIMICROBIAL ACTIVITY AGAINST *Staphylococcus aureus, Escherichia coli, AND Candida albicans*

[*Ekstrak dan Fraksi Kulit Batang Kesambi (Schleichera oleosa (Lour). Oken): Skrinning Fitokimia, Penetapan kadar fenolik total dan Aktivitas Antimikroba terhadap Staphylococcus aureus, Escherichia coli, dan Candida albicans*]

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**ABSTRACT**

Microorganisms are becoming increasingly resistant to antibiotics, creating a pressing need for new therapeutic agents to address this issue. One plant that shows promise as an antimicrobial is kesambi (*Schleichera oleosa* (Lour) OKEN). This plant is commonly used in traditional medicine to treat skin infections. This research set out to identify the secondary metabolites in ethanol extract and fractions of kesambi stem bark and their antimicrobial activity against *S. aureus, E. coli,* and *Candida albicans*. Bioactive compounds were extracted through maceration with 96% ethanol, resulting in a concentrated extract yielding 22.34 ± 1.44%. The concentrated extract was gradually separated using n-hexane and ethyl acetate, yielding n-hexane fraction, ethyl acetate fraction, and aqueous fraction of 1.84 ± 0.82%, 16.46 ± 4.90 %, and 40.74 ± 6.27%, respectively. Phytochemical screening of each fraction and extract was carried out using color reagents and ATR-FTIR spectrometry. According to the profiling, the extracts and fractions contain phenolic compounds, flavonoids, alkaloids, steroids, saponins, triterpenoids, glycosides, and tannins. The FTIR analysis provided valuable insights into the presence of different functional groups, including -OH, C=O, $CH_3$, C=C, and C-O esters. Antimicrobial activity was tested using the well diffusion method. The extract displays a more significant inhibition zone against *E. coli* and *Candida albicans* when compared to both the ethyl acetate fraction and the aqueous fraction. However, the ethyl acetate fraction demonstrates a larger inhibition zone against *S. aureus* than both the extract and the aqueous fraction. This suggests a promising potential for these samples to combat these pathogens.

**Keywords**: Antimicrobial; extract; fraction; Kesambi; phytochemical; *Scheichera oleosa;* Total Phenolic Content

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***ABSTRAK***

*Mikroorganisme semakin resisten terhadap antibiotik, sehingga diperlukan agen terapeutik baru untuk mengatasi masalah ini. Salah satu tanaman yang menjanjikan sebagai antimikroba adalah kesambi (Schleichera oleosa (Lour) OKEN). Tanaman ini umumnya digunakan dalam pengobatan tradisional untuk mengobati infeksi kulit. Penelitian ini bertujuan untuk mengidentifikasi metabolit sekunder dalam ekstrak etanol dan fraksi kulit batang kesambi, menetapkan kadar senyawa fenolik dan aktivitas antimikroba mereka terhadap S. aureus, E. coli, dan Candida albicans. Senyawa bioaktif diekstraksi melalui perendaman dengan etanol 96%, menghasilkan ekstrak terkonsentrasi dengan hasil 22,34 ± 1,44%. Ekstrak terkonsentrasi dipisahkan secara bertahap menggunakan n-heksana dan etil asetat, menghasilkan fraksi n-heksana, fraksi etil asetat, dan fraksi air masing-masing sebesar 1,84 ± 0,82 %, 16,46 ± 4,90%, dan 40,74 ± 6,27%. Skrining fitokimia dari setiap fraksi dan ekstrak dilakukan menggunakan reagen warna, dan spektrofotometri ATR-FTIR. Hasil uji dengan pereaksi warna menunjukkan bahwa ekstrak dan fraksi mengandung senyawa fenolik, flavonoid, alkaloid, steroid, saponin, triterpenoid, glikosida, dan tanin. Analisis FTIR memberikan wawasan berharga tentang keberadaan berbagai gugus fungsional, termasuk -OH, C=O, $CH_3$, C=C, dan ester C-O. Aktivitas antimikroba diuji terhadap S. aureus, E. coli, dan Candida albicans menggunakan metode difusi sumuran. Ekstrak menunjukkan zona hambatan yang lebih signifikan terhadap E. coli dan Candida albicans dibandingkan dengan fraksi etil asetat dan fraksi akuatik. Namun, fraksi etil asetat menunjukkan zona hambatan yang lebih besar terhadap S. aureus dibandingkan dengan ekstrak dan fraksi air. Hal ini menunjukkan potensi yang menjanjikan dari ekstrak dan fraksi kulit batang kesambi untuk melawan patogen-patogen tersebut.*

***Kata kunci**: antimikroba; ekstrak; fraksi; Kesambi; Scheichera oleosa*

## INTRODUCTION

Antimicrobial resistance has become a global concern. This has become a significant issue worldwide and a priority of the WHO (Murray *et al*., 2022). Antimicrobial resistance occurs due to genetic changes in bacteria that make the drugs used less effective in treating infections (Walsh *et al*., 2023). Bacteria have an interesting ability to share and develop resistance to the medications used to treat infections. This can be a concern, especially since many hospital patients have weakened immune systems. Plus, with the emergence of new strains resistant to multiple treatments, it's a challenge we need to address together. Microbial resistance continues to spread, and the future of antimicrobial drug use is uncertain. In light of the concerns surrounding this issue, it would be prudent to consider implementing measures such as moderating the use of antibiotics, investing in research to deepen our understanding of the genetic mechanisms behind resistance, and persistently advancing the development of new therapeutic options, both synthetic and natural (Levy & Bonnie, 2004).

It's heartening that traditional medicines resonate with about 60% of the global population. These remedies are vital, especially in rural areas and developing countries, where they often serve as the primary healthcare means. Even in places where modern medicine is prevalent, many still turn to traditional practices, reflecting a deep connection to their cultural heritage and how they care for their communities. Herbal medicines are made solely from medicinal plants, as opposed to traditional drugs, which are made from minerals and organic materials (Bhatia *et al*., 2013). Plants and other natural sources are home to many intricate and unique compounds. Lately, there's been a lot of excitement around studying things like plant and microbial extracts, essential oils, pure secondary metabolites, and even new molecules we've synthesized to see if they can serve as effective antimicrobial agents. It's a fascinating area of research, and there's so much potential to discover (Balouiri *et al*., 2016).

*Schleichera oleosa* (Lour.) Oken is a valuable nutraceutical plant that grows in Indonesia, India, Nepal, Sri Lanka, Thailand, Myanmar, and Malaysia. Kesambi plants are widespread on the Indonesian island of Java, specifically in the provinces of Banten, East Java, and Bali. Kesambi has various chemical contents such as phenols, flavonoids, coumarin, phytosterols (Khandekar *et al*., 2015), triterpenoids (Ghosh *et al*., 2011), tannins, saponins, and steroids (Karthikeyan *et al*., 2023). *Schleichera oleosa*, a member of the Sapindaceae family, has been shown to have antimicrobial (Khandekar *et al*., 2015), antioxidant (Khandekar *et al*., 2015), anti-inflammatory (Karthikeyan *et al*., 2023) anticancer and antidiabetic properties (Goswami & Singh, 2017) and can be used in

biodiesel production (Sarkar *et al*., 2022). This medicinal plant can be used instead of synthetic compounds to prevent and treat various diseases. The ethanol extract of kesambi stem bark has anti-analgesic and anti-inflammatory properties (Khan *et al*., 2016).

The ethyl acetate extract of Kesambi leaves has antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, *Escherichia coli*, and *Klebsiella pneumoniae* (Khandekar *et al*., 2015). Kesambi leaf ethanol extract has potential as an anthelmintic and diabetes treatment due to its ability to inhibit alpha-amylase. Phytochemical screening of various kesambi leaf extracts reveals that ethanol extracts have the highest secondary metabolite content when compared to extracts from other solvents (Goswami & Singh, 2018). Methanol extracts of kesambi bark are effective against *E. coli* bacteria (Susilawati *et al*., 2016) and *S. aureus* (Situmeang *et al*., 2022).

Previous studies have been limited to extracts, whereas the potential fractions from the bark of this plant have not been extensively explored. This study intends to systematically investigate the chemical profile of kesambi stem bark extracts and their respective fractions, in addition to evaluating their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The findings are anticipated to contribute to a better understanding of the therapeutic potential of these parts.

## MATERIALS AND METHODS

The chemicals applied in this research are 96% ethanol (technical) for extraction, n-hexane p.a. (Smart-Lab), ethyl acetate p.a. (Smart-Lab), ethanol p.a. (Smart-Lab), gallic acid (Sigma), HCl, Wagner's reagent, NaOH, $FeCl_3$, acetic anhydride, concentrated sulfuric acid, Mg, chloroform, Folin-Ciocalteu, $Na_2CO_3$, $NaNO_2$, $AlCl_3$, and NaOH. This study used various laboratory glassware, including beaker glass, test tubes, volumetric flasks, volumetric pipettes, micropipettes, and dropper pipettes. UV-Visible spectrophotometry 1800A by Shimadzu, and ATR-FTIR Carry 630 from Agilent.

### Sourcing and preparation of the sample

Kesambi bark (Figure 1) was collected from Wonodadi village in the Kutorejo district of Mojokerto regency, East Java, Indonesia. The cleaned bark was dried and ground into kesambi bark powder. This powder is sieved through a 30/40 mesh sieve (hereafter called the sample).

**Figure 1.** Tree and bark of *Schleichera Oleosa* (Lour.) Oken (*Pohon dan kulit batang Schleichera Oleosa (Lour.) Oken*).

### Extraction and Fractionation

Kesambi bark powder was weighed at 300 grams, placed in a maceration container, and moistened with 96% ethanol before being gradually added to a volume of 3000 mL until the sample was submerged, closed, and left for 3 x 24 hours at ambient temperature with intermittent stirring. The maceration process was repeated five times. The extract was filtered, and the liquid evaporated with a rotary evaporator until a concentrated extract was eventually produced, which was then weighed to determine the yield.

A total of 20 g of extract was dissolved in 25 mL of 96% ethanol and 25 mL of distillate water. The first fractionation with 50 mL n-hexane was stirred for 15 minutes with a magnetic stirrer before being allowed to stand until two layers formed. Refraction was performed twice as frequently. The n-hexane soluble fraction was then collected and evaporated, yielding the n-hexane fraction. Equal quantities of ethyl acetate (1:1) were used to fractionate the n-hexane insoluble fraction. Next, two equal portions of the ethyl acetate insoluble fraction were separated. The ethyl acetate fraction was then obtained by collecting and evaporating the ethyl acetate-soluble fraction. The aqueous fraction was then obtained by collecting and evaporating the ethyl acetate-insoluble fraction (Syukur *et al*., 2023).

**Phytochemical screening and characterization**

The qualitative identification of secondary metabolite compounds in ethanol extracts and fractions of kesambi bark is carried out using standard qualitative methods. Upcoming tests are set to cover a range of components, including alkaloids, glycosides, phytosterols, phenolics, flavonoids, steroids, saponins, triterpenoids, and tannins. The flavonoid test conducted utilized a Shinoda reaction, which involved the application of an extract and filtrate along with 2 N HCl, magnesium powder, and amyl alcohol. A positive result for the flavonoid sample is indicated by the development of a red coloration in the amyl alcohol layer (Purwaningsih *et al*., 2023).

The alkaloid test was carried out using the Wagner test. The tannin test was performed using a $FeCl_3$ solution, which produced a positive blackish-green color. The saponin test was thoughtfully performed by initially adding distilled water to the sample and gently mixing it. This step was followed by incorporating 2 N HCl and shaking the mixture to assess the presence of stable foam, which would suggest a positive indication for saponin. In the case of the terpenoid test, several samples were carefully dissolved in ether, after which the ether was evaporated. Anhydride acetic acid was then added; a resulting red or green color would indicate the presence of terpenoids (Syukur *et al*., 2023).

Glycoside tests are performed by hydrolyzing extracts and fractions with HCl, neutralizing with a 10% NaOH solution, and adding a few Fehling A and Fehling B drops. The color red indicates positive glycosides. Sterol presence was determined by adding acetic anhydride and 2 mL of concentrated $H_2SO_4$ to the sample. A shift in color from purple to blue indicates the presence of sterols (Tuseef *et al*., 2021). The sample's functional groups were identified with an ATR-FTIR spectrometer (Revathi *et al*., 2019).

**The Total Phenolic Content**

*Preparation of Gallic Acid Standard Curve Solution*

The standard solution of gallic acid was prepared by weighing 10.0 mg, dissolving it in 0.5 ml of ethanol p.a, and diluting it to 10 ml with distilled water, then further diluting it to 100 ppm. The 100 ppm intermediate standard was diluted to concentrations of 2, 4, 6, 8, 10, 12, and 14 ppm, adding 0.2 ml of 10% Folin-Ciocalteu reagent and 0.2 ml of 10% sodium carbonate ($Na_2CO_3$), then made up to 5 ml with distilled water. The solution was allowed to stand for an operating time of 48 minutes and measured at a maximum wavelength of 694.7 nm using a UV-Vis spectrophotometer.

*Determination of Operating Time and Maximum Lambda*

Determination of Operating Time and Maximum Lambda The middle stock solution is 8 ppm, pipetted with 0.4 ml, added with 0.2 ml of 10% Folin-Ciocalteu reagent and 0.2 ml of 10% $Na_2CO_3$, topped up with 5 ml of distilled water. The solution is measured every minute to determine the operating time.

The determination of the maximum wavelength was carried out by preparing a standard solution of 8 ppm, by pipetting 0.4 ml and adding 0.2 ml of 10% Folin-Ciocalteu reagent and 0.2 ml of 10% $Na_2CO_3$, then making up to 5 ml with distilled water. The solution was allowed to stand for an operating time of 48 minutes and measured at a wavelength of 400-800 nm.

*Determination of Total Phenolic Content in the Sample*

The determination of the phenolic content of the sample was carried out by weighing 10.0 mg of the n-hexane, ethyl acetate, and aqueous fractions of the kesambi bark, dissolved in ethanol. The solution was added with 0.2 ml of 10% Folin-Ciocalteu reagent and 0.2 ml of 10% $Na_2CO_3$, made up to 10 ml with aquadest, allowed to stand for an operating time of 48 minutes, and the absorbance was measured at a maximum wavelength of 694.7 nm. Total phenolic content is calculated based on equation 1.

$$\mathrm{TPC} = \frac{\mathrm{C.V}}{\mathrm{W}}$$

TPC = Total Phenolic Content
C = concentration of gallic acid (mg/L)
V = volume (L)
w = weight of sample (mg)

**Antibacterial Activity**

Twenty milliliters of each of the following media were transferred into sterilized Petri dishes and allowed to solidify: Mannitol Salt Agar for *Staphylococcus aureus*, Eosin Methylene Blue Agar for *Escherichia coli*, and Potato Dextrose Agar for *Candida albicans*. Using the agar well diffusion technique, six sterile cylinder cups were arranged within the Petri dishes, forming wells in each medium. Five microliters of each medium, initially in liquid form and heated to a temperature of 40-50 °C, were mixed with cultures of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* at a concentration of $1.0x10^8$ CFU/mL. The insides of the cylinder cups were left empty as the microbial-containing medium was poured into the Petri dish over the initial layer (Indriyanti *et al*., 2022, 2023).

Utilizing sterile tweezers, gently take out the cylinder cup to form a well after the bacterial suspension (second layer) media has set. Introduce the positive and negative control solutions into the wells along with 50 µl of each concentration from the test solutions. Incubate at 37°C for a period of 24 hours. Following the incubation period, measure and assess the clear zone that appears at each concentration using a caliper (Wulandari *et al*., 2022). The efficacy of kesambi bark extracts and their fractions against microorganisms was evaluated at concentrations of 5%, 10%, and 15%, with the exception of the n-hexane fraction due to limited sample availability. Ciprofloxacin 20 ppm is the reference standard for testing against *Staphylococcus aureus* and *Eschercia coli.*

## RESULTS

### Yield of Extract and Fractions

**Table 1.** Rendemen extract of Kesambi tree bark *(Rendemen ekstrak kulit batang kesambi*).

| **Extraction** (*Ekstraksi ke-*) | **Tree bark of Kesambi Powder** *(Serbuk batang kesamb)* **(g)** | **Extract** (*Ekstrak*) **(g)** | **Rendemen** (*rendemen*) (%) |
|---|---|---|---|
| 1 | 300.02 | 65.5910 | 23.19 |
| 2 | 300.07 | 65.0102 | 21.66 |
| 3 | 300.02 | 71.1100 | 23.70 |
| 4 | 300.02 | 67.4700 | 22.48 |
| 5 | 300.06 | 69.6500 | 23.21 |
| | average±SD | | **22.85±0.71** |

The ethanol extract from kesambi bark is reddish-brown and has a pleasant aroma. Five extractions were performed, yielding an average of 22.85 ± 0.71% (Table 1). The n-hexane fraction is green, while the ethyl acetate and the aqueous fraction are reddish-brown, with the yields being 2.06±0.79 %, 16.90±3.57 %, and 39.18±5.70 %, respectively (Table 2).

**Table 2.** Rendemen fraction of Kesambi tree bark (*Rendemen fraksi kulit batang kesambi*).

| **Fractionation** (*Fraksinasi ke-)* | **n-hexane fraction** (*Fraksi n-heksana*) **(%)** | **Ethyl acetate fraction** (*Fraksi etil asetat)* **(%)** | **Aqueous fraction** (*Fraksi air*) **(%)** |
|---|---|---|---|
| 1 | 2.89 | 21.56 | 32.93 |
| 2 | 1.47 | 21.56 | 32.93 |
| 3 | 2.00 | 19.29 | 41.54 |
| 4 | 3.01 | 16.25 | 40.55 |
| 5 | 0.95 | 10.93 | 47.96 |
| average±SD | **2.06±0.79** | **16.90±3.57** | **39.18±5.70** |

## Phytochemical Screening

Table 3 shows the results of the phytochemical screening with the color reagent. The ethanol extract of kesambi stem bark contains secondary metabolite compounds like alkaloids, glycosides, phenolics, steroids, saponins, triterpenoids, flavonoids, and tannins. The n-hexane and ethyl acetate fractions contain secondary metabolites similar to the extract. The aqueous fraction doesn't contain steroids.

**Table 3.** Phytochemical screening of Kesambi bark tree (*Skrining fitokimia kulit batang kesambi*).

| **No.** | **Secondary metabolite** (*Metabolite sekunder*) | **Sample** | | | |
|---|---|---|---|---|---|
| | | **Extract** (*Ekstrak*) | **n-hexane fraction** (*Fraksi n-heksana*) | **Ethyl acetate fraction** (*Fraksi etil asetat*) | **Aqueous fraction** (*Fraksi air*) |
| 1 | Alkaloids | + | + | + | + |
| 2 | Glycosides | + | + | + | + |
| 3 | Phytosterols | + | + | + | + |
| 4 | Phenolics | + | + | + | + |
| 5 | Steroids | + | + | + | - |
| 6 | Saponins | + | + | + | + |
| 7 | Triterpenoids | + | + | + | + |
| 8 | Flavonoids | + | + | + | + |
| 9 | Tannin | + | + | + | + |

## Identification of Functional groups of kesabi bark

**Table 4.** Functional groups in the infrared spectrum of the sample (*Gugus fungsi sample dari spektrum inframrah*).

| **No** | **Wave number** (*Bilangan gelombang*) **($cm^{-1}$)** | **Functional groups** *(Gugus fungsi)* | **Extract** (*Ekstrak*) | **n-hexane fraction** (*Fraksi n-heksana*) | **Ethyl acetate fraction** (*Fraksi etil asetat*) | **Aqueous fraction** (*Fraksi air*) |
|---|---|---|---|---|---|---|
| 1 | 3600-3000 | -OH strect | 3320 (s) | 3300 (w) | 3300 (w) | 3200 (s) |
| 2 | 2800-3000 | -CH aliphatic | - | 2922 (sh) | - | - |
| 3 | 1600 | C=O strect | 1591 (s) | 1733 (w) | 1602 (w) | 1600 (s) |
| 4 | 1500-1600 | C=C strect | 1517 (s) | 1517 (w) | 1517 (w) | 1517 (s) |
| 5 | 1400-1500 | OH bend | 1436 (s) | 1436 (w) | 1436 (w) | 1436 (s) |
| 6 | 1276 | C-O ester | 1276 (w) | 1276 (w) | 1276 (w) | 1276 (w) |
| 7 | 1056 | -C-O-H alcohol | 1056 (broad) | 1056 (w) | 1056 (w) | 1056 (broad) |

The functional groups identified in the extract and fractions of keambi include -OH, C=O, C=C, C-OH, C-O ester, and aliphatic -CH, as detailed in Table 4 and illustrated in Figure 2.

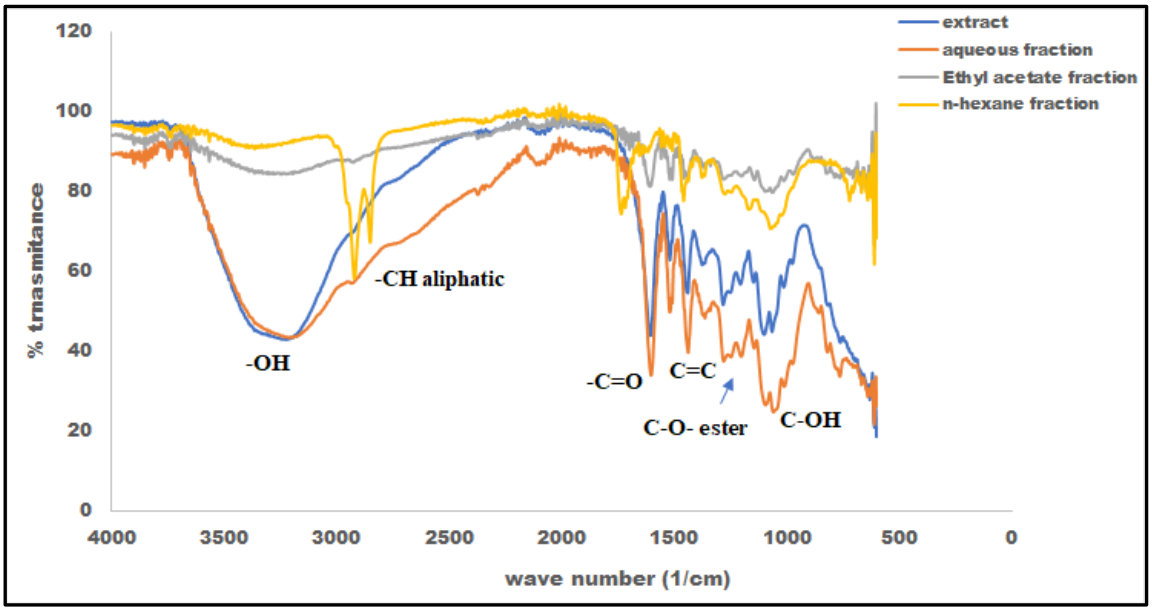


**Figure 2.** Spectrum infrared of sample using ATR-FTIR spectrophotometer *(spektrum inframerah dari sampel menggunakan spectrofotometer ATR-FTIR*).

## Total Phenolic Content of Sample

The colorimetric method for determining phenolic content with Folin-Ciocalteu reagent and gallic acid standard yielded the equation y=0.0688x+0.3273, r=0.9994, with gallic acid concentrations ranging from 2 to 10 ppm (Figure 3). The proportion of phenolic compounds of the extract, ethyl acetate fraction, and aqueous fraction was 7.0795, 8.1227, and 6.7403 milligrams of GAE/g sample, respectively (Figure 4).

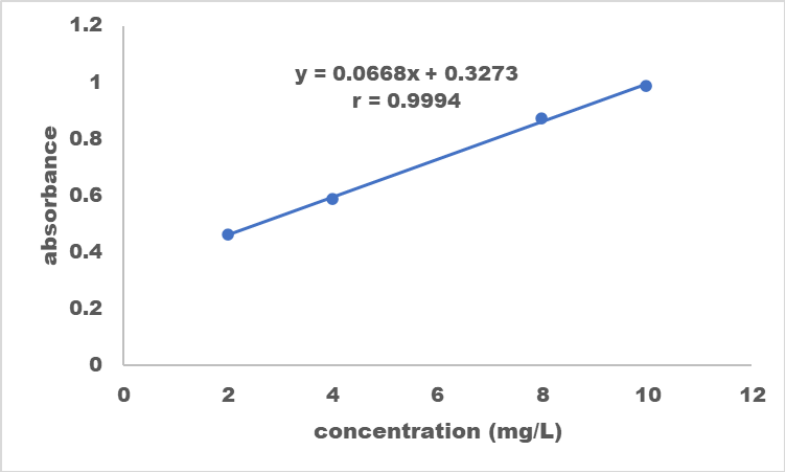


**Figure 3**. Standard curve of gallic acid (*kurva tandard asam galat*).

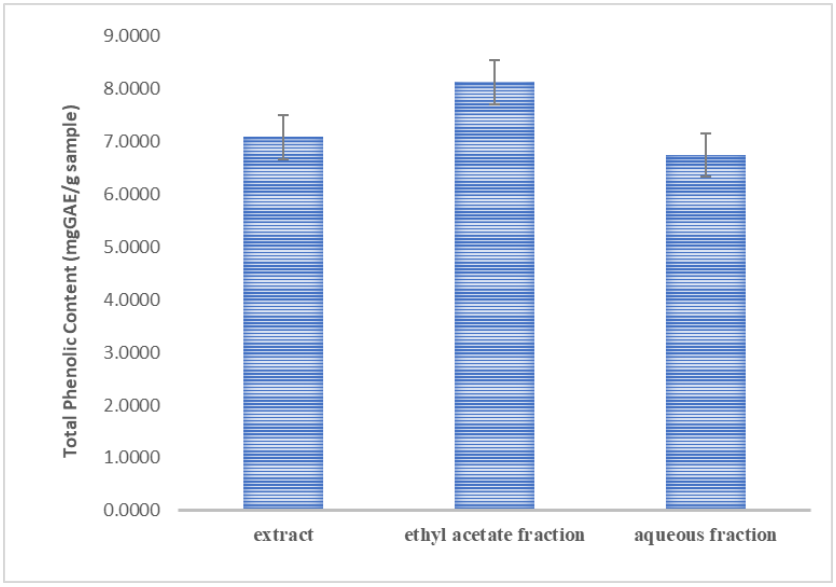


**Figure 4.** The phenolic content of the sample (*Kandungan fenolik sampel*).

**Antimicrobial Activity**

The results showed that the ethyl acetate fraction inhibited the *Staphylococcus aureus* bacteria the most. The positive control, ciprofloxacin at 20 ppm, inhibits more than the ethyl acetate fraction at 5%. The positive control produced a clear zone of 1.456 cm, whereas the ethyl acetate fraction produced a clear zone of 1.424 cm at a 5% concentration. When the extract and ethyl acetate fraction were concentrated to 10%, the clear zone was already more prominent than the positive controls (Figures 5 and 6).

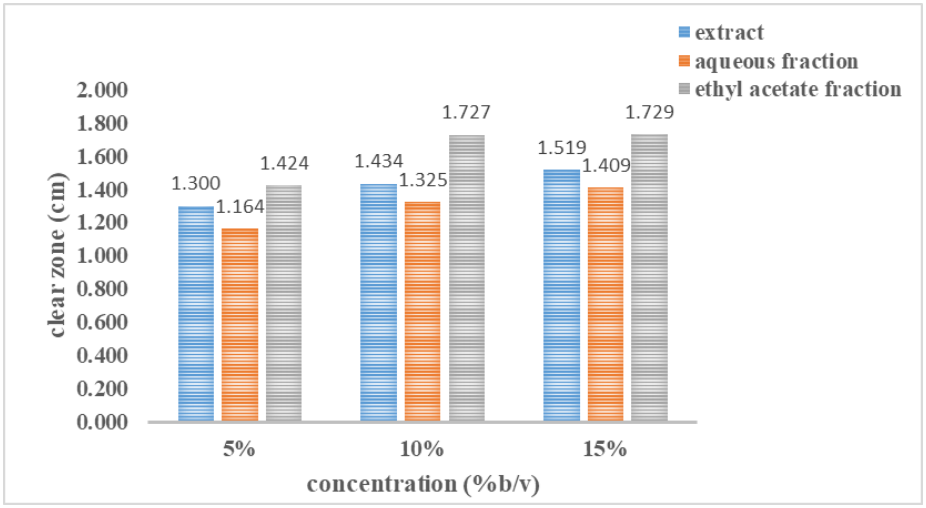


**Figure 5.** Antibacterial activity of kesambi bark against *Staphylococcus aureus* (*Aktivitas antibakteri kulit batang kesambi terhadap Staphylococcus aureus*).

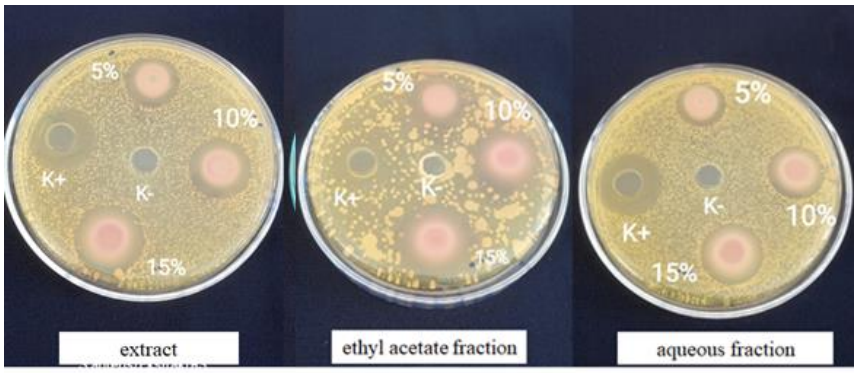


**Figure 6.** Antibacterial activity of Kesambi bark against *Staphylococcus aureus* (*Aktivitas antibakteri kulit batang kesambi terhadap Staphylococcus aureus*).

The activity of kesambi bark extracts and fractions against *Escherichia coli* showed that at all concentrations, the extract provided the best inhibition zone when compared to the fractions of ethyl acetate and aqueous fraction (Figures 7 and 8). The aqueous fraction and the ethyl acetate fraction exhibit nearly equivalent inhibitory effects on this bacterium.

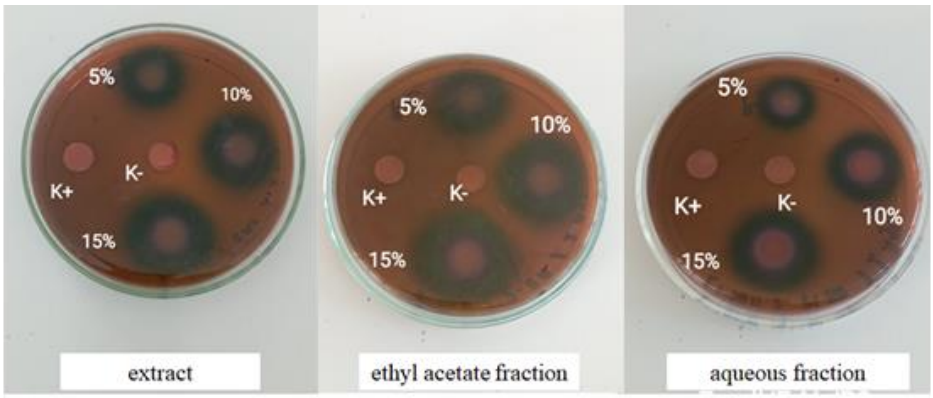


**Figure 7.** Antibacterial activity of Kesambi bark against *E. coli* (*Aktivitas antibakteri kulit batang kesambi terhadap E. coli*).

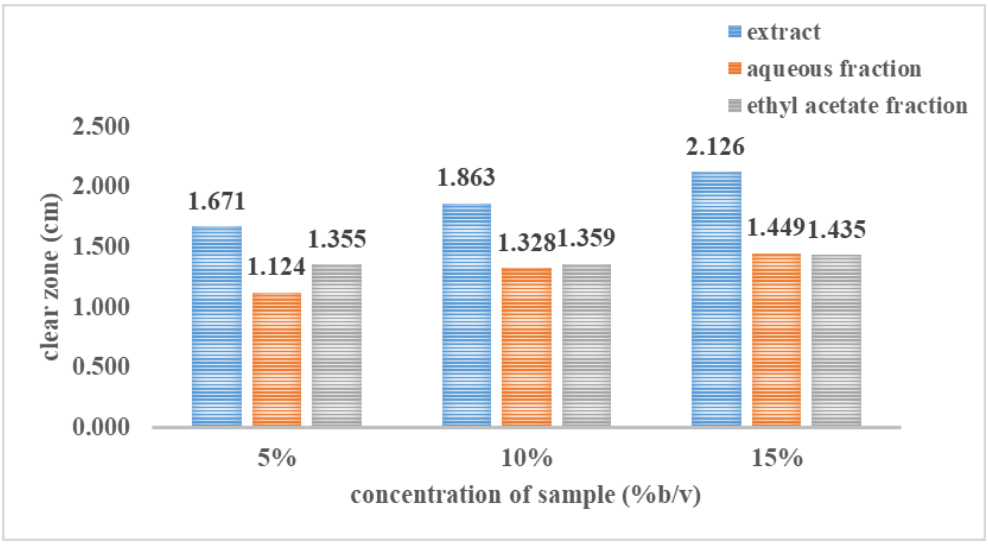


**Figure 8.** Antibacterial activity of kesambi bark against *Escherichia coli* (*Aktivitas antibakteri kulit batang kesambi terhadap Escherichia coli*).

Compared to the fractions at concentrations of 5%, 10%, and 15%, the test results against *Candida albicans* demonstrated that the kesambi bark extract produced the best results (Figures 9 and 10). The inhibition increases with the concentration.

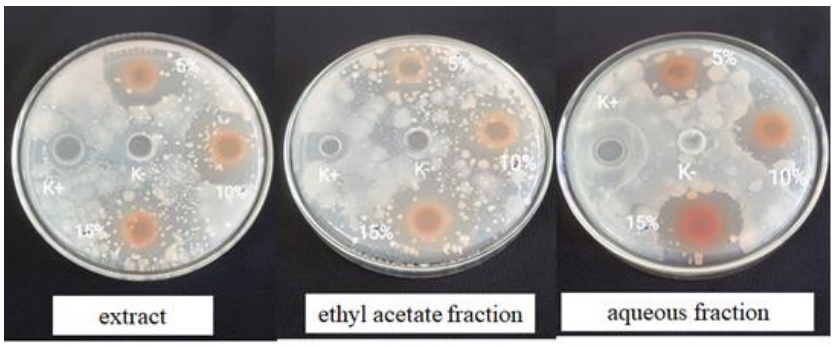


**Figure 9.** The activity of Kesambi Bark against *Candida albicans* (*Aktivitas kulit batang kesambi terhadap Candida albicans*),

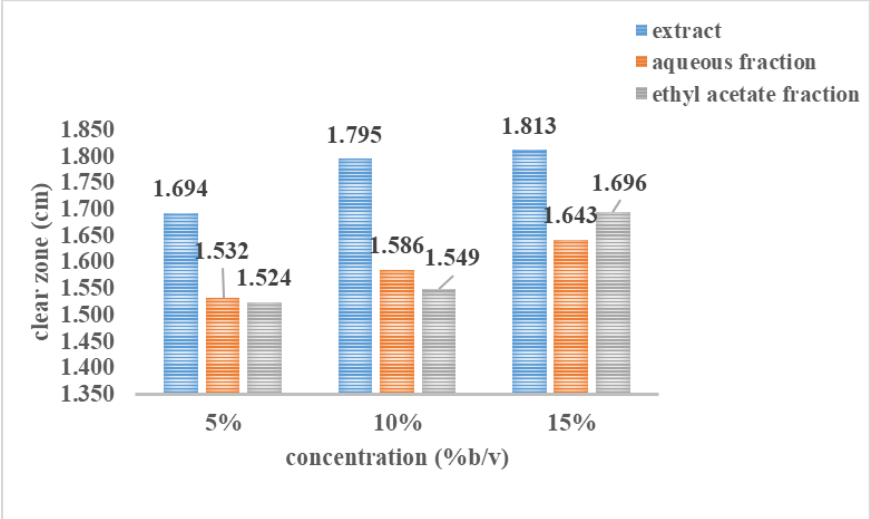


**Figure 10**. The activity of Kesambi Bark against *Candida albicans* (*Aktivitas kulit batang kesambi terhadap Candida albicans*).

## DISCUSSION

### Yield of Extract and Fractions of Kesambi Bark

The yield of the ethanol extract from kesambi bark in this study is 4.97 times higher than that of Alim *et al*. (2022), which employed 70% ethanol. This disparity may be attributed to the fact that the compounds present in kesambi bark are more effectively extracted by 96% ethanol, as opposed to 96% methanol and 70% ethanol. In addition, the extraction results in this study are better compared to extraction using nonpolar solvents like n-hexane and ethyl acetate (Situmeang *et al*., 2022). Ethanol, known as a polar solvent, is capable of efficiently extracting a wide range of both polar and nonpolar compounds. It is especially adept at extracting bioactive substances like phenolic compounds, lipids and fatty acids, and terpenoids, and it has the benefit of being safe for use in food and pharmaceuticals (Lee *et al*., 2024). An additional factor contributing to the observed differences in yield may be attributed to the varying growing environments of the samples, which could result in distinct yield outcomes (Karimnejad & Ghavam, 2024).

Fractionation in this study used liquid-liquid extraction, a separation technique used to isolate various compounds from a mixture based on their polarity (Patel *et al*., 2019). This method employs different solvents to achieve the desired separation: non-polar compounds are extracted using n-hexane, semi-polar compounds are targeted with ethyl acetate, and polar compounds remain as a residue (Zamzani & Tradisti, 2019). This systematic approach allows for the effective partitioning of

complex mixtures into distinct fractions, facilitating further analysis and characterization of the components. The n-hexane fraction yields less than the ethyl acetate fraction, while the aqueous fraction shows the highest yield. It suggests that the compounds in the kesambi stem bark are predominantly polar.

**Screening of Phytochemicals**

The secondary metabolites found in kesambi bark in this study are consistent with those identified in various other studies. This result is also consistent with Sarkar *et al*. (2022) which found that kesambi bark contains dye, tannins, resin, sterols, and triterpenoids. Karthikeyan *et al*. (2023) showed that the bark of kesambi in India contains taraxerone, tricadenic acid, tannin, resin, Sterols, and Triterpenoids. Hence, this experiment's phytoconstituent of kesambi bark is the same as in the other research. The n-hexane and ethyl acetate fractions contain secondary metabolites similar to the extract. The aqueous fraction doesn't contain steroids. Steroids are hydrophobic and do not dissolve in polar solvents like water (Schmidt & Steinhart, 2002).

**Identification of functional groups of the sample using FTIR**

The functional groups in the extract of kesambi bark were identified using FTIR. Figure 2 shows the functional groups present in each sample. The FTIR spectra of ethanol extract, n-hexane fraction, ethyl acetate fraction, and aqueous fraction all show nearly identical patterns. Unlike the n-hexane fraction, which contains an aliphatic -CH group, the other fractions lack that functional group. The extract and fractions share the following functional groups: -OH (3600-3000 $cm^{-1}$), carbonyl C=O (1600 $cm^{-1}$), C=C at 1517 $cm^{-1}$, C-OH at 1056 $cm^{-1}$, and ester C-O at 1276 $cm^{-1}$. The -OH group appears in the 3300 $cm^{-1}$, with a strong signal in the extract and aqueous fractions but a weak signal in the ethyl acetate and n-hexane fractions. A broad absorption band in the 3600-2500 $cm^{-1}$ region indicates the presence of the OH group from carboxylic acids in both the extract and aqueous fractions. The aliphatic C-H group was detected in the n-hexane fraction with a strong signal at 2922 $cm^{-1}$. In contrast, the extract, ethyl acetate fraction, and aqueous fraction did not absorb the group. The n-hexane fraction also showed a weak signal in the 1600 $cm^{-1}$ region, indicating that there aren't many compounds with C=O carbonyl groups in this fraction or the ethyl acetate fraction, in contrast to the extract and aqueous fraction, which displayed a strong signal in this region.

The infrared spectrum of the kesambi bark has a different pattern compared to the spectra of the kesambi leaves and seeds conducted by previous researchers (Khandekar *et al*., 2015). Analysis of the seeds and leaves of the kesambi plant reveals the presence of several functional groups, including N-H (amine), C-N, NCs, and SH. Notably, these functional groups are absent in the spectral analysis of the kesambi bark. This observation may indicate a variation in chemical composition between the kesambi bark and its leaves and seeds. Furthermore, it is also plausible that overlapping absorption bands in the bark's spectra could contribute to the unobservability of these functional groups. The variations in IR spectra across different parts of a plant are attributed to differences in chemical composition and molecular structure specific to each part (Khandekar *et al*., 2015). Various sections of the plant, including leaves, stems, roots, and fruits, contain differing proportions of organic compounds (Sarkar *et al*., 2022). The interaction between IR light and these compounds yields distinct absorption and transmission patterns, resulting in unique IR spectra for each plant part. Furthermore, diverse growing environments yield distinct secondary metabolites, which in turn lead to variations in their infrared (IR) spectra (Chakraborty, 2016).

**Determination of Phenolic Content**

The total phenolic content of the kesambi ethanol extract is lower than that of its ethyl acetate fraction, but higher than that of the aqueous fraction. The high total phenolic content in the ethyl acetate fraction may result from the semi-polar nature of the phenolic compounds present in the kesambi bark. This result is lower than that reported in Alim *et al*. (2022), which found a total phenolic content of 9.20576 mg GAE/g using 70% ethanol for extraction. In contrast, the methanol extract in the same study had a phenolic content of 7.68313 mg GAE/g sample. The observed

variation in phenolic content can be attributed to differing cultivation conditions. Additionally, discrepancies in phenolic content may result from the application of various extraction techniques and the differing polarities of solvents used. Notably, the previous study employed the re-maceration method, whereas the current study utilized the maceration method.

Gallic acid is used as a reference standard since it is part of the group of simple phenolic compounds and serves as a reliable benchmark (Nayeem *et al*., 2016). Gallic acid reacts with the Folin-Ciocalteu reagent, resulting in a yellow color that indicates the presence of phenolic compounds. The addition of $Na_2CO_3$ creates a basic environment, allowing the phenolic compounds in the sample to react with the Folin-Ciocalteu reagent. This reaction results in a notable color change to blue, which is characterized by light absorption at a wavelength of 694.70 nm. The intensity of this light absorption is directly proportional to the concentration of phenolic compounds present. This phenomenon occurs due to the formation of a complex involving phosphomolybdate and phosphotungstate within the reagent. The presence of phenolic compounds reduces this complex to molybdenum, facilitating the measurable change in color and corresponding light absorption (Haminiuk *et al*., 2012).

**Antimicrobial activity**

The results indicated that higher concentrations resulted in larger inhibition zones, demonstrating a stronger activity against *S. aureus* bacteria. Increasing the concentration of the ethyl acetate fraction from 10% to 15% did not result in a significant inhibition zone. Several factors could contribute to this issue, including the presence of additional compounds in the fractions that inhibit the efficacy of the primary active component (Hasan, 2024), limitations of the solvent used (Altemimi *et al*., 2017), or target cell saturation in the bacteria. If the solvent employed to create the fractions cannot fully dissolve the active chemicals at high concentrations, their availability to interact with the bacteria may be compromised, preventing a stronger inhibitory effect. Furthermore, the active ingredients in the fractions may specifically target certain sites on the bacterial cells. Once these sites are occupied or bound by the active compounds, further increases in the fraction concentration may not significantly influence bacterial growth. The ethyl acetate fraction exhibited the highest antibacterial activity, followed by the extract and the aqueous fraction. This greater effectiveness may be attributed to the higher concentration of phenolic compounds in the ethyl acetate fraction compared to the other two fractions (Azad *et al*., 2024).

Susilawati *et al*. (2016) indicated that the extract of the kesambi tree bark, at a concentration of 5%, demonstrated an inhibition zone of 21.67 mm against *E. coli*. These test results differ from those of previous studies, which may be attributed to the use of different extraction solvents. Unlike earlier research that employed methanol as the solvent, this study utilized 96% ethanol. The variation in solvents resulted in the extraction of different chemicals, leading to a diverse range of inhibitory effects on *E. coli* (Omara *et al*., 2021, Nouioura *et al*., 2024).

Compared to the fractions at concentrations of 5%, 10%, and 15%, the test results against *Candida albicans* demonstrated that the kesambi bark extract produced the best results. (Chaidir *et al*., 2020) (Figures 9 and 10). The extract exhibits a stronger antibacterial activity against *Candida albicans* than both the ethyl acetate fraction and the aqueous fraction, due to the higher concentration of chemical compounds present in the extract. A greater variety of chemical compounds with antifungal properties leads to more significant inhibition, thereby enhancing their potential as antifungal agents (Bidaud *et al*., 2021).

According to the description above, kesambi bark has excellent potential as an antimicrobial against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans*. This is due to the secondary metabolite compounds in the extracts and fractions of kesambi bark. Both in extracts and fractions, kesambi bark has several secondary metabolites that can act as antimicrobials, such as phenols, flavonoids (Takó *et al*., 2020), tannin (Jyske *et al*., 2023), triterpenoids (Ghosh *et al*., 2011), and alkaloids.

As phytoconstituents, tannins reduce the availability of vital metal ions by forming metal complexes, inhibit several extracellular bacterial enzymes, and impact microbial metabolism by

preventing oxidative phosphorylation (Farha *et al*., 2020). Alkaloids and flavonoids are also significant plant components with antibacterial and antifungal properties. One flavonoid that inhibits DNA gyrase is quercetin (Chen *et al*., 2023, Yuan *et al*., 2021). By stopping cell division, triterpenoids that were separated from the methanolic extract of *S. oleosa's* outer bark inhibit DNA synthesis in microorganisms (Karthikeyan *et al*., 2023). The antimicrobial qualities of various flavonoids are correlated with their liposome interaction activities. The ability of antibacterial agents to reach their target is determined by their lipophilicity characteristics and how they interact with the cell membrane(Farhadi *et al*., 2019).

## CONCLUSION

The extract showed a greater inhibitory effect against *E. coli* and *Candida albicans* in comparison to both the ethyl acetate fraction and the aqueous fraction. Conversely, the ethyl acetate fraction exhibits a stronger inhibition against *S. aureus* than either the extract or the aqueous fraction. It is essential to evaluate the toxicity of both the extract and the fraction derived from the bark of the kesambi tree in order to develop an effective antibacterial product. Researchers recommend the separation of the secondary metabolite compounds found in the bark of the Kesambi tree to accurately identify those that demonstrate antimicrobial activity.

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## AUTHOR CONTRIBUTIONS

AFM: collecting research data, drafting the article, final revision of the manuscript; YP: Creating research concept, final revision of the manuscript; KPS and MNS: collecting data

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