**Antioxidant and Antibacterial Activities of Ethanol Extract of Kesambi Leaves (*Schleichera oleosa*) from Alor District, Indonesia**

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**Abstract.** Human health is increasingly threatened by oxidative stress and microbial infections, emphasizing the need for effective antioxidant and antibacterial agents. *Schleichera oleosa* (Kesambi), a tropical plant native to South and Southeast Asia, is rich in bioactive secondary metabolites such as alkaloids, phenolics, tannins, and flavonoids. This study investigated the antioxidant and antibacterial activities of ethanol extracts from Kesambi leaves collected in highland and lowland areas of Alor Regency, Indonesia. Antioxidant activity was assessed using the DPPH assay, while antibacterial activity was evaluated against *Pseudomonas aeruginosa* and *Bacillus subtilis* through the well diffusion method. The highland extract displayed stronger antibacterial activity, particularly against *P. aeruginosa*, whereas the lowland extract showed higher antioxidant potency. Both extracts produced larger inhibition zones against *P. aeruginosa* than *B. subtilis*, indicating species-specific susceptibility. In the antioxidant assay, the lowland extract exhibited an IC₅₀ value of 4.81 ± 1.00 ppm, surpassing the positive control ascorbic acid (IC₅₀ 6.07 ± 1.03 ppm), while the highland extract showed an IC₅₀ of 7.16 ± 0.61 ppm. These findings suggest that Kesambi leaves hold significant potential as natural therapeutic agents, with lowland samples as strong antioxidants and highland samples as promising antibacterial candidates, warranting further investigation for pharmaceutical and biomedical applications.

# INTRODUCTION

Human health is currently confronted with significant challenges, particularly those arising from oxidative stress and microbial infections. Oxidative stress occurs when there is an imbalance between the excessive production of free radicals and the body’s defense mechanisms to neutralize them. [1]. Free radicals are chemical species containing one or more unpaired electrons, making them highly reactive with other molecules to achieve stability. Among these, reactive oxygen species (ROS) represent oxygen-derived free radicals, which may exist in the form of ions, atoms, or molecules. Although ROS are normally generated in the body, their excessive accumulation poses serious risks if not effectively eliminated, as they can induce biomolecular oxidation in both in vivo and in vitro systems. Such oxidative processes may result in cellular damage and apoptosis and ultimately contribute to the onset of various diseases, including Parkinson’s, Alzheimer’s, cardiovascular disorders, and certain cancers [2]. Antioxidants play a crucial role in mitigating these effects, as they are compounds capable of preventing the oxidation of other molecules. Specifically, antioxidants counteract the harmful impact of free radicals by donating electrons, thereby stabilizing reactive species and protecting cells from damage or death [3].

In addition to oxidative stress, pathogenic microorganisms such as fungi, bacteria, and algae also play a significant role in causing diseases in humans, animals, and plants [4]. Infections, particularly those caused by fungi and bacteria, are among the leading causes of mortality in higher organisms. Therefore, the discovery and development of new antioxidant and antibacterial agents are urgently needed. Antibacterial agents function to inhibit the growth of harmful bacteria, thereby preventing infection, controlling disease transmission, and protecting biological as well as non-biological materials from microbial damage [5]. Considering these challenges, natural resources such as medicinal plants have gained increasing attention as potential sources of safe and effective antioxidant and antibacterial compounds, including Kesambi leaves.

*Schleichera oleosa* (Kesambi) is a tropical tree of the Sapindaceae family, widely distributed across South and Southeast Asia, including Cambodia, India, Indonesia, Myanmar, Sri Lanka, Thailand, and Vietnam, and known by various local names such as Kesambi, Sambi, Kusambi, and Usapi [6]. Kesambi leaves contain secondary metabolites in the form of alkaloids, phenolics, tannins, and flavonoids. Several studies have shown that Kesambi leaf extract has high antioxidant and antibacterial activity, with varying effectiveness depending on the type of solvent used. According to [7], the strongest antioxidant activity was obtained from extracts prepared with acetone (IC₅₀ = 6.33 µg/mL) and methanol (IC₅₀ = 13.46 µg/mL). Conversely, [6] reported methanol extract as the most active (IC₅₀ = 16.12 µg/mL), followed by ethanol (20.43 µg/mL), with water extract showing the weakest effect (IC₅₀ = 904.28 µg/mL). Furthermore, [8] noted that ethyl acetate extract of Kesambi leaves exhibited the highest antioxidant potential among different plant parts (IC₅₀ = 5.50 µg/mL), while bark extracts also showed strong activity, particularly with ethyl acetate (IC₅₀ = 7.72 ppm). In terms of antibacterial effects, Kesambi leaf extract was found to be more effective against *Staphylococcus aureus* than *Escherichia coli*. Similarly, [9] reported that 96% ethanol extract of Kesambi leaves inhibited the growth of *P. aeruginosa* and *S. aureus*, producing inhibition zones of 17.3 mm and 25 mm, respectively, at 15% concentration. Beyond these antioxidant and antibacterial effects, *S. oleosa* has been associated with several other pharmacological properties. The methanol fraction demonstrated strong antimalarial potential against *Plasmodium falciparum* (IC₅₀ = 0.780 µg/mL) [(10)]. Fruit extracts also exhibited high antioxidant effects across multiple assays, while seed extracts demonstrated anticancer activity against breast cancer (MCF-7) cells by inhibiting proliferation, migration, and colony formation, as well as modulating apoptosis and cell cycle-related pathways [11]. A novel compound, 3,5-dihydroxy-4-methoxybenzoic acid, isolated from the leaves, demonstrated superior α-glucosidase inhibition compared to acarbose, indicating antidiabetic potential [12].

Kesambi plants grow naturally in various regions of Alor District, both in the highlands and lowlands, with different environmental conditions. In the highlands, Kesambi grows in cooler temperatures, high rainfall, and soil conditions that tend to be rocky. Meanwhile, in the lowlands, this plant thrives in hotter temperatures, high humidity, and more fertile soil. These environmental differences can affect the physiological and morphological characteristics of Kesambi plants, such as leaf size, stem thickness, and growth patterns. Therefore, it is important to conduct antioxidant and antibacterial activity tests on Kesambi leaf extracts from the two regions. This test aims to determine whether differences in growing places also affect the biological potential of plants, so that the results can be used in the development of more effective natural materials according to their geographical origin.

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# MATERIALS AND METHODS

**Materials**

The study utilized Kesambi leaves sourced Fanating Village (lowland) and Otvai Village (highland), Alor District, Indonesia; 70% ethanol, *P. aeruginosa* and *B. subtilis bacteria* cultures were supplied by the Microorganism Chemistry Laboratory, Department of Chemistry, FSAD, ITS; DPPH *(Merck*);DMSO (*Merck)*;ascorbic acid (*Merck*; gallic acid (*Merck*); nutrient agar (*Sigma Aldrich*); nutrient broth (*Himedia*); and methanol.

**Sample Preparation and Extraction**

Kesambi leaves from the highlands and lowlands were washed clean, chopped, air-dried, and ground. Kesambi leaf powder was weighed as much as 50 g and macerated using 70% ethanol solvent as much as 1 L for 2x24 hours, and the filtrate was taken. The filtrate was evaporated with a rotary evaporator. The end of this process obtained pure extract, which will be used for antioxidant activity testing and antibacterial activity testing.

**Antibacterial Activity Test**

*B. subtilis* and *P. aeruginosa* were initially inoculated into 20 mL of sterile nutrient broth (NB) and pre-incubated at 37ºC for 24 hours with agitation at 120 rpm. The optical density (OD) of the resulting cultures was determined at 600 nm (OD600) using a UV-Vis spectrophotometer, and the suspensions were subsequently diluted to achieve an absorbance of 1. Thereafter, 20 mL of the standardized culture was transferred into 180 mL of sterile NB and incubated until reaching an OD of 0.5.

Ethanol extracts of Kesambi leaves from both highland and lowland origins were each weighed at 1 mg and dissolved in 1 mL of DMSO to achieve a final concentration of 1000 ppm. The antibacterial activity was evaluated using the well diffusion method. A mixture of 5 mL Nutrient Agar (NA) medium and 1 mL of bacterial suspension was poured into sterile Petri dishes to ensure even distribution. After solidification, wells were created in the NA medium, and each was loaded with 5 µL of the test solution (1000 ppm extract), negative control (DMSO), and positive control (chloramphenicol). The plates were then incubated at 37ºC for 41 hours. Following incubation, the inhibition zones were examined, and the antibacterial activity was determined by measuring the clear area surrounding the wells. The diameter of the inhibition zone was subsequently calculated using Equation 1 [13]:

(1)

Dv = Vertical diameter

Dh = Horizontal diameter

Ds = Well diameter

**Antioxidant Activity Test**

The DPPH assay was employed to assess antioxidant activity. To prepare the DPPH solution, 2.4 mg of DPPH was dissolved in 100 mL of methanol. A 1000 ppm stock solution of Kesambi leaf extract was prepared by dissolving 10 mg of extract in 10 mL of DMSO, followed by serial dilution to 100, 10, 1, and 0.1 ppm. For each concentration, 33 μL of the sample was mixed with 1 mL of DPPH solution and incubated at 37 ºC for 30 minutes. The reduction of DPPH by antioxidants was indicated by a color change from purple to pale yellow. Absorbance values were measured at 515 nm using a UV spectrophotometer. The blank consisted of 33 μL of DMSO with 1 mL of DPPH in methanol. Ascorbic acid and gallic acid were used as reference standards. The free radical scavenging activity was determined using Equation 2:

(2)

# RESULT AND DISCUSSION

**Sample Preparation and Extraction**

The preparation and extraction process of Kesambi leaves in this study were meticulously designed to maximize the yield and quality of bioactive compounds. The selection of the 4th leaf from the shoot is a critical step, as these leaves are physiologically mature and contain higher concentrations of phytochemical constituents like tannins, alkaloids, and flavonoids [14]. This aligns with findings from other studies, which emphasize that mature leaves often exhibit greater bioactive potential due to their fully developed metabolic pathways [15]. Air-drying the leaves at room temperature was chosen to preserve the integrity of heat-sensitive compounds. This method avoids the degradation of thermolabile constituents, such as phenolics and flavonoids, which can occur with high-temperature drying techniques [16]. Additionally, air-drying minimizes enzymatic reactions and fungal growth, ensuring the chemical composition remains stable during storage [14]. The subsequent grinding of the dried leaves increases the surface area, facilitating more efficient solvent penetration during extraction [17].

The maceration process using 70% ethanol as the solvent is particularly noteworthy. Ethanol is a versatile solvent capable of extracting a wide range of polar and nonpolar compounds, including alkaloids, flavonoids, saponins, and tannins [18]. Its effectiveness is attributed to its ability to disrupt cell membranes and solubilize intracellular materials. The choice of 70% ethanol is further justified by its optimal balance between polarity and extraction efficiency, which enhances the recovery of bioactive compounds compared to pure ethanol or water [7]. The maceration duration of 2x24 hours ensures thorough extraction by allowing sufficient time for solvent penetration and compound dissolution. This extended period is critical for achieving high yields, as it accounts for the gradual release of compounds from the plant matrix [16]. The use of a rotary evaporator for filtrate concentration is a standard practice to remove solvents while preserving the bioactive properties of the extract. This step is essential for obtaining a pure, concentrated extract suitable for subsequent biological assays [18].

In summary, the sample preparation and extraction protocol in this study is well-optimized to maximize the recovery of bioactive compounds from Kesambi leaves. The methods employed are supported by extensive literature, ensuring the reliability and reproducibility of the results. Future studies could explore the impact of alternative extraction techniques, such as ultrasound-assisted extraction, to further enhance efficiency and yield.

# Antibacterial Activity

The antibacterial test was performed using the well diffusion technique, which was selected for its simplicity in measuring inhibition zone diameters. The inhibition zone was identified as a clear region surrounding the well, indicating suppression of bacterial growth by the test sample (DMSO). A wider zone reflects stronger antibacterial activity of the tested compound. The concentrated Kesambi leaf extract was diluted with DMSO, a solvent capable of dissolving both polar and nonpolar compounds. Chloramphenicol served as the positive control because of its broad-spectrum activity against Gram-positive and Gram-negative bacteria [19]. Its antibacterial effect is exerted through the inhibition of bacterial protein synthesis. After applying the test and control solutions, the inoculated media were incubated at 37°C for 41 hours, as the inhibition zone was found to reach its maximum diameter at this incubation period, ensuring optimal visualization of antibacterial activity.

The highland Kesambi leaf extract exhibited a wider inhibition zone diameter than the lowland extract. Both extracts inhibited the growth of *P. aeruginosa* and *B. subtilis*, with the inhibition being more pronounced against *P. aeruginosa* (Gram-negative). This trend is consistent with previous studies showing that Gram-negative bacteria, despite the presence of an outer membrane, can be more susceptible to certain plant-derived compounds due to differences in membrane permeability [20]. By contrast, Gram-positive bacteria such as *B. subtilis* possess a thick peptidoglycan layer that may hinder the penetration of antibacterial agents [21]. The notable effect against *P. aeruginosa* suggests that the bioactive compounds in Kesambi leaves may interfere with the lipopolysaccharide (LPS) barrier or efflux pump systems, leading to cell damage [22]. The antibacterial activity results of the ethanol extract are presented in Table 1.

**TABLE 1.** Antibacterial test results of Kesambi Leaf Ethanol Extract

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Bacteria** | **Sample** | **Inhibition Zone (mm)** | | **Inhibition Zone (mm)** |
| **Horizontal** | **Vertical** |
| *P. aeruginosa* | DT | 19.5 | 20 | 12.75 |
| DR | 13 | 15 | 7 |
| Chloramphenicol | 15 | 16 | 8.5 |
| DMSO | 0 | 0 | 0 |
| *B. subtilis* | DT | 13 | 14 | 6.5 |
| DR | 10 | 12 | 4 |
| Chloramphenicol | 26 | 29 | 20.5 |
| DMSO | 0 | 0 | 0 |

Note: Values represent single measurements; no replicates were performed.

DT = Kesambi Highlands,

DR = Kesambi Lowlands

Table 1 shows that the highland extract is more effective than the lowland extract. Table 1 shows that the highland extract is more effective than the lowland extract. This is reflected in the larger inhibition zones observed for the highland extract. The *S. oleosa* leaf extract demonstrated stronger activity against *P. aeruginosa* compared to the lowland extract, with inhibition zones in some cases similar to or slightly exceeding those of chloramphenicol. In contrast, chloramphenicol showed a markedly higher inhibitory effect against *B. subtilis* than either of the extracts. According to [23], the inhibition zones observed for the lowland and highland *S. oleosa* leaf extract treatments fall within the resistant category for *P. aeruginosa* and *B. subtilis*, as the measured zones were ≤13 mm.

Bioactive compounds from *S. oleaosa* leaf exert their biological effects via multiple mechanisms. Flavonoids disrupt the synthesis of nucleic acids by targeting key bacterial enzymes like DNA gyrase and topoisomerase, which are essential for replication [20]. Tannins compromise bacterial cell integrity by binding with proteins and polysaccharides, thereby weakening the cell wall [19]. Saponins, with their surfactant-like behavior, increase the permeability of bacterial membranes, leading to the leakage of intracellular components [21]. Meanwhile, alkaloids work by inhibiting bacterial efflux systems and interfering with quorum sensing, thereby diminishing the bacteria's virulence and ability to form biofilms [18].

The superior antibacterial activity of highland Kesambi extracts compared to lowland extracts may be linked to environmental stress factors, such as cooler temperatures and higher UV exposure, which can enhance the production of defensive secondary metabolites [6]. This phenomenon is consistent with studies showing that plants grown in harsher conditions often accumulate higher levels of antimicrobial compounds as a survival strategy [7]. While the inhibition zones of Kesambi extracts were smaller than those of chloramphenicol (a broad-spectrum antibiotic), their activity against *P. aeruginosa* (12.75 mm for highland extract) is noteworthy, given the increasing resistance of this pathogen to conventional antibiotics [19]. This suggests that Kesambi extracts could serve as complementary or alternative treatments, particularly in cases of antibiotic-resistant infections.

# Antioxidant Activities

The antioxidant activity of Kesambi leaf extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. For this assay, incubation was carried out for 30 minutes to homogenize the sample with DPPH, allowing complete interaction between the antioxidant compounds and DPPH radicals through hydrogen atom donation. The reduction of DPPH is indicated by a visible color change from deep purple to yellow, which results from the proton donation of natural antioxidants to DPPH [24]. This color transition serves as the basis for measurement using a visible light spectrophotometer. Following absorbance determination with a UV-Vis spectrophotometer, the percentage of inhibition was calculated. The inhibition values obtained for both highland and lowland Kesambi leaf extracts are presented in Table 2.

**TABLE 2.** Percentage of inhibition of Kesambi leaf ethanol extract

|  |  |  |
| --- | --- | --- |
| **Concentration (ppm)** | **% Inhibition of Highland Kesambi ± SD** | **% Inhibition of Lowland Kesambi ± SD** |
| 1000 | 90.98 ± 1.12 | 90.82 ± 1.62 |
| 100 | 75.93 ± 5.14 | 89.83 ± 0.90 |
| 10 | 61.53 ± 2.03 | 64.56 ± 6.85 |
| 1 | 31.64 ± 1.19 | 30.71 ± 2.74 |
| 0.1 | 6.22 ± 4.86 | 10.59 ± 2.14 |

\*Data points represent means and standard deviations (n=3).

After obtaining the percentage of inhibition, a calibration curve was constructed by plotting % inhibition against the sample concentration, from which a linear regression equation (y = ax + b) was generated to determine the IC₅₀ value. The antioxidant activity of Kesambi leaf extract was expressed using the IC₅₀ parameter, which reflects the concentration of extract required to inhibit 50% of free radicals in the DPPH assay. A lower IC₅₀ value indicates stronger antioxidant activity [25].

**FIGURE 1.** Antioxidant activity of *S. oleosa* leaf extracts from lowland and highland regions, compared to standard antioxidants (ascorbic acid and gallic acid), measured as IC₅₀ (ppm) using the DPPH assay. Samples were incubated for 30 minutes at room temperature.

From Figure 1, ascorbic acid as a positive control has the lowest IC₅₀ value (1.74±0.13 ppm), meaning it has the strongest inhibitory activity compared to other samples. Lowland Kesambi extract (4.81±1.00 ppm) is more effective than highland Kesambi extract (7.16±0.61 ppm) and gallic acid as a positive control (6.07±1.03 ppm). The differences observed between highland and lowland samples may be attributed to varying abiotic stress conditions. Lowland plants are typically exposed to higher temperatures and oxidative stress, which can stimulate the production of antioxidant compounds such as flavonoids as a defense mechanism [6]. In contrast, highland plants, which experience cooler environments, may allocate more metabolic resources toward cold-adaptive compounds rather than antioxidants, resulting in slightly reduced antioxidant activity [3]. This pattern is consistent with findings in other species, where environmental factors like UV exposure and drought in lowland regions have been shown to enhance antioxidant capacity [20].

# The antioxidant activity increases with higher flavonoid content. Flavonoids and phenolic compounds are recognized as potential antioxidants. As polyphenolic compounds, flavonoids are capable of donating hydrogen atoms to free radicals, thereby contributing to antioxidant activity through neutralization of free radicals or by terminating ongoing chain reactions. The ability of flavonoids to complex with metal ions such as iron allows it to increase its antioxidant effect [26]. Gallic acid and ascorbic acid are natural antioxidants that function to protect the body from oxidative damage. Gallic acid is derived from phenolic compounds with strong antioxidant activity and additional benefits in the treatment of diseases, while ascorbic acid is vitamin C, which is very important for metabolism and the immune system, with antioxidant abilities that are also very effective [27].

# CONCLUSION

# The leaf extract of *S. oleosa* (Kesambi) from Alor Regency demonstrated notable bioactivity. Antioxidant analysis revealed that lowland samples possessed stronger activity (IC₅₀ 4.81 ppm) than highland samples (IC₅₀ 7.16 ppm), highlighting their potential as superior natural antioxidants. In contrast, antibacterial assays showed that highland leaf extracts were more effective against *P. aeruginosa* (12.75 mm inhibition zone), while activity against *B. subtilis* was lower than that of chloramphenicol. Overall, these findings indicate that lowland Kesambi leaves are promising sources of antioxidants, whereas highland leaves exhibit stronger antibacterial properties, particularly against Gram-negative bacteria. Future studies should include MIC and MBC assays, as well as the isolation and characterization of bioactive compounds, in order to provide a more comprehensive evaluation and validation of the therapeutic potential of *S. oleosa* (Kesambi) leaves.

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