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# Phytochemical Screening and Antibacterial Activity of Ethyl Acetate, n-Hexane, and Aqueous Fractions of White Teak (*Tectona grandis* Linn. f.) Leaves against *Escherichia coli*

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**ABSTRACT**

This research was designed to perform phytochemical screening and assess the antibacterial potential of white teak (*Tectona grandis* Linn. f.) leaf fractions, namely ethyl acetate, n-hexane, and aqueous extracts, against *Escherichia coli*. The phytochemical analysis aimed to detect bioactive constituents with antibacterial relevance, including flavonoids, tannins, saponins, alkaloids, and terpenoids. Antibacterial testing employed the disk diffusion and broth dilution techniques to determine inhibition zone diameters, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC). The diffusion assay revealed that the ethanolic extract (11.3 ± 1.02 mm) and ethyl acetate fraction (18.1 ± 2.46 mm) demonstrated notable inhibitory activity, whereas the n-hexane and aqueous fractions exhibited no inhibition zones. A significant difference among the treatments was revealed by statistical examination using ANOVA ($F = 163.29$; $p < 0.05$). The ethyl acetate fraction showed a 12.5% MIC and a 25% MBC in the broth dilution results. These findings point to the presence of secondary metabolites including tannins, alkaloids, and flavonoids in the ethyl acetate fraction of T. grandis leaves as the source of its significant antibacterial activity.

**Keywords:** antibacterial, *Escherichia coli*, leaf extract, ethyl acetate fraction.

## Introduction

Tectona grandis leaves have long been used in traditional medicine for treating various ailments, including skin infections and digestive disorders. Despite several studies highlighting the antibacterial potential of these leaves, research specifically examining their effectiveness against *Escherichia coli*, a major pathogen responsible for gastrointestinal and urinary tract infections, remains limited (Kumar et al., 2019). This study aims to address this gap by evaluating the antibacterial activity of different solvent fractions ethyl acetate, n-hexane, and water from *T.*

*grandis* leaves. The selection of these solvents is essential, as solvent polarity significantly affects the extraction of bioactive compounds, which may possess notable antibacterial activity (Sasidharan et al., 2011; Sarker & Nahar, 2012)."

The Gram-negative bacteria Escherichia coli is a leading cause of UTIs and gastrointestinal illnesses. The increasing prevalence of E. coli strains that are resistant to many drugs emphasizes how critical it is to find new antibacterial medicines that are derived from natural sources as soon as possible. (Akinjogunla et al., 2021). Phytochemical screening, as a preliminary stage of pharmacognostic evaluation, is essential for identifying bioactive constituents in plant materials that may contribute to antibacterial activity (Harborne, 1998).

The polarity of solvents significantly affects the types of compounds extracted from plant matrices. Ethyl acetate is a semi-polar solvent capable of isolating phenolics and flavonoids, while n-hexane extracts non-polar compounds such as steroids and essential oils. Conversely, water, being highly polar, is efficient for extracting alkaloids and saponins (Sasidharan et al., 2011). Although teak leaf extracts have been reported to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, investigations against *E. coli* remain limited (Singh et al., 2018). Standard antibacterial evaluation methods, such as disk diffusion and dilution assays, provide quantitative and qualitative assessments of inhibitory activity by determining inhibition zones and Minimum Inhibitory Concentrations (MIC) (Balouiri et al., 2016). The antibacterial mechanisms of flavonoids and tannins involve disruption of bacterial cell membranes, enzyme inhibition, and interference with DNA replication (Cushnie & Lamb, 2011).

Testing the antibacterial efficacy of teak leaf fractions against *E. coli* is particularly relevant because the bacterium is a major causative agent of diarrheal diseases, particularly in children, often leading to dehydration and mortality (Gu et al., 2020). The escalating resistance of *E. coli* to antibiotics underscores the necessity for natural antibacterial alternatives that are both effective and safe (Ventola, 2015). A comparative analysis of the antibacterial activity of different solvent fractions—ethyl acetate, n-hexane, and water—can provide valuable insights into which solvent most effectively extracts bioactive compounds with significant antibacterial potential (Sarker & Nahar, 2012). Therefore, this study aimed to perform phytochemical screening and antibacterial testing of *T. grandis* leaf fractions against *E. coli*. Phytochemical identification was carried out using qualitative color reaction methods, including Mayer's and Wagner's tests for alkaloids, $FeCl_3$ for tannins, alkaline reagents for flavonoids, and Salkowski's test for terpenoids (Rauf *et al*., 2022).

The antibacterial activity was further evaluated using disk diffusion, MIC, and MBC assays following the Clinical and Laboratory Standards Institute (CLSI, 2018) guidelines. This research is expected to contribute to the pharmacological understanding of *T. grandis* and to support its potential use as a natural antibacterial agent effective against resistant strains of *E. coli*.

## Methodology

### Materials and Equipment

The experimental work utilized a variety of standard laboratory apparatus, including Erlenmeyer flasks, pipettes, Petri dishes, incubator, autoclave, micropipettes, laminar air flow cabinet, inoculating loops, and a Bunsen burner. The main materials consisted of white teak (*Tectona grandis*) leaves, magnesium powder, 2N hydrochloric acid (HCl), ferric chloride ($FeCl_3$), ethanol 96%, Nutrient Agar (NA), Brain Heart Infusion (BHI), tetracycline, *E. coli* isolate, ethyl acetate, n-hexane, and distilled water.

**Preparation of Teak Leaf Extract**

A total of 300 g of powdered teak leaves was subjected to maceration using 96% ethanol in a ratio of 1:10 (w/v). The process was carried out in a covered glass container at room temperature for 24 hours with intermittent stirring. The filtrate was separated through Whatman filter paper and remacerated with half of the initial solvent volume to ensure maximum extraction. The combined filtrates were concentrated under vacuum using a rotary evaporator at 70°C to obtain a viscous crude extract. The yield percentage was determined according to the following equation:

$$\% \text{ Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Sample Powder}}$$

**Fractionation of Teak Leaf Extract**

Liquid–liquid partitioning of the ethanolic extract was performed sequentially using n-hexane, ethyl acetate, and water as solvents. Ten grams of the crude extract was dissolved in hot water, placed into a separatory funnel, and partitioned three times with 50 mL of n-hexane. The n-hexane phase was collected and evaporated under reduced pressure at 50°C. The remaining aqueous layer was subsequently partitioned three times with 50 mL of ethyl acetate. The ethyl acetate fraction was concentrated under reduced pressure at 50°C, and the residual aqueous fraction was thickened using a water bath until a concentrated form was obtained.

**Phytochemical Screening**

**Alkaloids**

1 mg of each extract or fraction was dissolved in 5 mL of 2N HCl, divided into three test tubes, and reacted separately with Dragendorff's and Mayer's reagents. The formation of orange and white/yellowish precipitates indicated the presence of alkaloids.

**Flavonoids**

After boiling for 5 minutes, 2 milligrams of extract or fraction was added to 10 milliliters of hot water. After shaking, a 5-milliliter portion of the filtrate was treated with magnesium powder, one milliliter of strong hydrochloric acid, and one milliliter of amyl alcohol. If the amyl alcohol layer became red, yellow, or orange, it meant that the flavonoids were present.

**Tannins**

The tannins were extracted by boiling 0.1 g of the extract or fraction in 5 mL of distilled water for 5 minutes, filtering the mixture, and then treating it with a 1% $FeCl_3$ solution. The presence of tannins was proven when a dark green or blue-black

coloring developed.

**Saponins**

A shaker was used to dilute a 0.1 g sample with 5 mL of distilled water. Saponins were thought to be present when a stable foam layer about 1 cm tall persisted for 15 minutes.

**Steroids**

2 mL of the test solution was evaporated until it was dry, and the remaining solid was dissolved in half a milliliter of chloroform. Then, along the wall of the test tube, 2 mL of concentrated $H_k\ SO_4$ and 0.5 mL of acetic anhydride were added. Steroids were detected when a bluish-green ring formed.

**Antibacterial Activity Assay**

**Media Preparation**

20 grams of *Nutrient Agar* (NA) powder were dissolved in 1 L of distilled water and heated until the solution became clear*. Brain Heart Infusion* (BHI) broth was prepared according to manufacturer instructions and distributed into sterile test tubes. Both media were sterilized by autoclaving at 121°C for 30 minutes.

**Bacterial Rejuvenation**

A pure culture of *Escherichia coli* was streaked onto sterile NA slants using a sterilized inoculating loop and incubated at 37°C for 24 hours.

**Preparation of Bacterial Suspension**

A loopful of the 24-hour culture was inoculated into BHI broth and incubated at 37°C for another 24 hours. The turbidity was adjusted to the 0.5 McFarland standard, corresponding to approximately $1.5 \times 10^8$ CFU/mL.

**Antibacterial Testing**

**Disk Diffusion Method**

Sterile Petri dishes and glass bottles were prepared. Each bottle contained 15 mL of NA medium and 0.2 mL of bacterial suspension, which were mixed and poured into Petri dishes to solidify. Sterile 6 mm paper disks were loaded with the extract and its n-hexane, ethyl acetate, and aqueous fractions. Chloramphenicol and 5% DMSO served as positive and negative controls, respectively. Tests were performed in triplicate, and plates were incubated at 37°C for 24 hours.

**Dilution Method**

The MIC and MBC of the most active fraction were determined using the broth dilution method. Serial dilutions were prepared in 5% DMSO to obtain concentrations ranging from 75% 50%, 25%, 12.5%,
6.25%, 3.125%, 1.56%, and 0.78%. Each tube contained 0.5 mL of BHI broth and 0.5 mL of the bacterial suspension. Tubes were incubated at 37°C for 24 hours, and bacterial growth was assessed by turbidity. For MBC determination, clear tubes were streaked on NA plates and incubated for 24–48 hours. The absence of visible colonies indicated bactericidal activity.

**Data analysis**

Using the Shapiro-Wilk test, we checked whether the data followed a normal

distribution; a p-value greater than 0.05 was considered significant. To find significant differences between groups, normally distributed data were first examined using One-Way ANOVA ($p < 0.05$), and then Tukey's post hoc test was used. When the data did not conform to the assumptions of normality or homogeneity, non-parametric tests were used. An analysis was performed using SPSS version 24 for statistical purposes.

## Result and Discussion

Sequential maceration of 500 g of *Tectona grandis* leaf powder yielded three fractions: 8.4 g of a yellowish-green, oily-scented n-hexane extract after 3 × 24 h extraction; 12.7 g of a reddish-brown, mildly pungent ethyl acetate fraction; and 15.2 g of a dark brown, viscous aqueous fraction obtained at approximately 60°C with a characteristic herbal aroma. These results demonstrate the influence of solvent polarity on extraction yield and compound solubility.

Previous studies have reported the presence of secondary metabolites such as flavonoids, tannins, alkaloids, and saponins in *Tectona grandis* leaf extracts (Nurdianti et al., 2017; Ramesh et al., 2020). However, most of these studies were limited to general phytochemical screening and did not correlate the phytochemical composition of specific solvent fractions with their antibacterial activity. Furthermore, data describing the relationship between phytochemical constituents of different fractions and their antibacterial effectiveness against *Escherichia coli* are still scarce, despite the clinical relevance of this bacterium as a major cause of gastrointestinal and urinary tract infections (Gu et al., 2020; Akinjogunla et al., 2021). This study addresses this research gap by integrating phytochemical screening with antibacterial assays of individual solvent fractions of *T. grandis* leaves, thereby providing a more comprehensive evaluation of the fraction with the highest potential as a natural antibacterial agent.

## Phytochemical Screening

Phytochemical testing of the crude extract and its solvent fractions revealed qualitative differences in secondary metabolite content, as shown in Table 1.

**Table 1. Phytochemical screening of teak leaves**

| Test Sample | Alkaloid | Flavonoid | Tannin | Saponin | Steroid |
|---|---|---|---|---|---|
| Extract | + | + | + | + | + |
| *n-Hexane* fraction | - | - | - | + | - |
| Ethyl acetate fraction | + | + | + | + | + |
| Aqueous fraction | - | + | + | + | + |

The crude ethanolic extract and the ethyl acetate and aqueous fractions contained alkaloids, flavonoids, tannins, saponins, and steroids, consistent with the solvent's moderate-to-high polarity that facilitates extraction of polar metabolites. In contrast, the n-hexane fraction revealed only saponins, reflecting the non-polar nature of n-hexane, which favors lipophilic compounds while excluding polar constituents.

These findings align with previous reports. Nurdianti *et al* (2017) identified flavonoids, polyphenols, and tannins in 96% ethanolic extracts of teak leaves, while Syahadat *et al* (2020) androgynus ethanol extracts included triterpenoids, tannins, alkaloids, flavonoids, and saponins. Supporting the findings of Supomo et al. (2019), this investigation validated the detection of alkaloids by seeing the production of white precipitates with Mayer's reagents and orange precipitates with Dragendorff's reagents.

The positive flavonoid reaction was indicated by a reddish-brown color upon the addition of magnesium and 2N HCl, resulting from the reduction of the benzopyran ring to flavilium salts (Syahadat *et al*., 2020). The foam test verified the presence of saponins through the formation of a stable froth, while tannins produced a blue-black coloration when reacted with $FeCl_3$ due to their phenolic nature and ability to form complexes with proteins (Azzahra, 2022).

**Antibacterial Activity Test Results**

**Diffusion Test Results of the Extract and Fractions of *Tectona grandis* Leaves against *Escherichia coli***

The antibacterial efficacy of the extract and fractions against *Escherichia coli* was assessed using the disk diffusion method, as summarized in Table 2.

**Table 2. Inhibition zones against *E. coli***

| Sampel | Concentration | Replication | | | Mean ± SD |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| Extract | 50 mg/mL | 11,5 mm | 12,5 mm | 10 mm | 11,3±1,02 |
| n-Hexane fraction | 50 mg/mL | 0 | 0 | 00 | |
| Ethyl acetate fraction | 50 mg/mL | 18,5 mm | 21 mm | 15 mm | 18,1±2,46 |
| Aqueous fraction | 50 mg/mL | 0 | 0 | 00 | |
| Positive control | 20 µg/mL | 38,5 mm | 37,5 mm | 32 mm | 36±2,85 |
| Kontrol negatif | 0 | 0 | 0 | 00 | |

The assay results confirmed antibacterial activity against *E. coli*, with inhibition zones of 11.3 mm for the extract and 18.1 mm for the ethyl acetate fraction, indicating strong efficacy. The n-hexane and aqueous fractions, however, showed minimal or undetectable inhibitory zones.

The differences observed are influenced by solvent polarity. N-hexane extracts non-polar, lipophilic compounds, whereas the active antibacterial agents in *T. grandis* are polar and insoluble in this solvent. The aqueous fraction, rich in sugars and hydrophilic metabolites, generally exhibits low antibacterial activity. In contrast, organic solvents like ethanol and methanol effectively extract polar bioactive compounds with greater antibacterial potential. ANOVA revealed significant differences in antibacterial activity among the tested groups ($F = 163.29$, $p < 0.05$). The ethyl acetate fraction (18.1 ± 2.46 mm) and crude extract (11.3 ± 1.02 mm) exhibited measurable inhibition, whereas the n-hexane fraction, aqueous fraction, and negative control showed none. The positive control produced the

largest inhibition zone (36 ± 2.85 mm), consistent with its known antibacterial efficacy. These results demonstrate the superior antibacterial strength of the ethyl acetate fraction over the crude extract. A Tukey HSD post hoc test is recommended to specify intergroup differences.

**Dilution Test Results of the Extract and Fractions against *E. coli***

The MIC of the ethyl acetate fraction was determined using the broth dilution method at varying concentrations 75%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78%. The results are presented in Table 3.

**Table 3. MIC results for the ethyl acetate fraction of *T. grandis* leaves**

| Concentration (%) | Replicate I | Replicate II | Replicate III |
|---|---|---|---|
| 75 | - | - | - |
| 50 | - | - | - |
| 25 | - | - | - |
| 12,5 | + | + | + |
| 6,25 | + | + | + |
| 3,12 | + | + | + |
| 1,56 | + | + | + |
| 0,78 | + | + | + |
| **Tetracycline** | - | - | - |
| **BHI (control)** | + | + | + |

*Note:*
(-)=Nobacterialgrowth
(+) = Bacterial growth present

Bacterial growth was inhibited at concentrations of 25% and above, while visible turbidity appeared at 12.5% and lower concentrations. Accordingly, the MIC value of the ethyl acetate fraction was determined to be 12.5%. The antibacterial activity of the ethyl acetate fraction of *Tectona grandis* leaves against *Escherichia coli* is likely related to the presence of flavonoids, tannins, and alkaloids. Flavonoids can disrupt bacterial cell membranes and inhibit nucleic acid synthesis, tannins inactivate bacterial enzymes through protein precipitation, and alkaloids interfere with peptidoglycan synthesis, leading to cell wall damage (Cowan, 1999; Cushnie & Lamb, 2011). These results are consistent with previous studies showing that ethyl acetate fractions are more effective in extracting phenolic compounds with antibacterial activity compared to non-polar and aqueous fractions (Sasidharan et al., 2011; Sarker & Nahar, 2012).

**Table 4. *Minimum Bactericidal Concentration* (MBC) results for the ethyl acetate fraction of *T. grandis* leaves**.

| Concentation(%) | Ethyl Acetate Fraction | | | Tetracycline | BHI control |
|---|---|---|---|---|---|
| | R1 | R2 | R3 | | |
| 75 | - | - | - | - | + |
| 50 | - | - | - | - | + |

| | | | | | |
|---|---|---|---|---|---|
| 25 | - | - | - | - | + |

The MBC of the ethyl acetate fraction was observed at a concentration of 25%, confirming its bactericidal activity against *E. coli*. Tetracycline, serving as the positive control, acted by binding to the 30S ribosomal subunit and inhibiting aminoacyl-tRNA binding during protein synthesis (Tariq et al., 2018). The antibacterial effects of the ethyl acetate fraction can be attributed to the synergistic presence of tannins, flavonoids, and alkaloids. Tannins inhibit bacterial growth by binding to cell wall proteins and metal ions, disrupting membrane integrity and causing lysis (Suryani, 2014). Flavonoids compromise bacterial structural stability by forming hydrogen, hydrophobic, and covalent bonds with essential proteins and enzymes, thereby inhibiting nucleic acid synthesis (Cowan, 1999). Alkaloids, on the other hand, disrupt peptidoglycan synthesis, weaken the cell wall, and interfere with protein and energy metabolism (Balsundram *et al*., 2006; De Oliveira et al., 2011). This study has several limitations. The antibacterial activity was evaluated only through *in vitro* assays, which may not fully represent *in vivo* conditions. In addition, the number of experimental replications was limited. Furthermore, the identification of bioactive compounds was based solely on qualitative phytochemical screening and has not been conclusively confirmed using advanced analytical techniques such as HPLC or LC–MS.

## Conclusion

The ethyl acetate fraction of *Tectona grandis* leaves exhibited the strongest antibacterial activity against *Escherichia coli*, with an MIC of 12.5% and an MBC of 25%. The presence of flavonoids, tannins, and alkaloids likely contributes to this activity. These results support the potential development of *T. grandis* leaf fractions as natural antibacterial agents.

## Declaration of Competing Interest

The authors declare that there are no competing interests, either financial or non-financial, that could have influenced the work reported in this article.

## Reference

Ahmad, I., Imran, M., & Khan, M. S. (2020). Antimicrobial properties of medicinal plants. *Biomedicine & Pharmacotherapy, 130*, 110599.

Akinjogunla, O. J., Enabulele, O. I., & Ajayi, A. O. (2021). Antibacterial resistance of *Escherichia coli*. *Journal of Microbiology, 59*(4), 389–397.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis, 6*(2), 71–79.

Clinical and Laboratory Standards Institute (CLSI). (2018). *Performance standards for antimicrobial susceptibility testing* (28th ed.). CLSI supplement M100.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*(4), 564–582.

Cushnie, T. P. T., & Lamb, A. J. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents, 38*(2), 99–107.

Dey, P., Saha, M. R., & Chaudhuri, T. K. (2020). Phytochemical screening methods and their applications. *Journal of Plant Research, 133*(4), 455–470.

Eze, E. A., Oruche, N. E., & Iroha, I. R. (2013). Phytochemical and antimicrobial properties of some Nigerian medicinal plants. *Journal of Medicinal Plants Research, 7*(20), 1418–1424.

Gu, B., Cao, Y., Pan, S., Zhuang, L., Yu, R., & Peng, Z. (2020). *Escherichia coli* infections and resistance mechanisms. *Journal of Medical Microbiology, 69*(5), 522–534.

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman & Hall.

Kumar, P., Sharma, R., & Singh, V. (2019). Medicinal properties of *Tectona grandis*: A review. *International Journal of Herbal Medicine, 7*(4), 34–38.

Prescott, L. M., Harley, J. P., & Klein, D. A. (2017). *Microbiology* (10th ed.).

McGraw Hill Education.

Ramesh, H. P., Nagaraja, M. P., & Joshi, C. G. (2020). Bioactive compounds of *Tectona grandis* and their biological activities. *Phytotherapy Research, 34*(8), 1866–1880

Rauf, A., Uddin, G., Siddiqui, B. S., Arfan, M., Khan, H., Mubarak, M. S., & Patel, S. (2022). Phytochemical, antimicrobial, antioxidant and cytotoxic activities of *Caralluma tuberculata* N.E.Br. *Saudi Journal of Biological Sciences, 29*(7), 103391.

Kumar, P., Sharma, R., & Singh, V. (2019). Medicinal properties of *Tectona grandis*: A review. *International Journal of Herbal Medicine*, 7(4), 34–38.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Yoga Latha, L. (2011). Extraction, isolation, and characterization of bioactive compounds from plant extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(1), 1–10.

Sarker, S. D., & Nahar, L. (2012). An introduction to phytochemical analysis. *Phytochemical Analysis*, 23(1), 3–9.