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Antibacterial Activity of *Falcataria moluccana* (L) Nielsen Leaf Extracts Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract: *Infectious diseases remain the most prevalent conditions globally, evolving constantly despite medical advancements. Research indicates that sengon (Falcataria moluccana) leaves contain phytochemical compounds with potential antibacterial activity. This study aimed to evaluate the antibacterial activity of F. moluccana leaf extracts against Staphylococcus aureus and Escherichia coli using the gel diffusion method. Samples were obtained using four solvents: distilled water, 70% ethanol, 96% ethanol, and ethyl acetate, with concentrations ranging from 50 to 300 mg/mL. Results indicated that aqueous extracts exhibited no inhibitory effect, whereas ethyl acetate and ethanol extracts demonstrated measurable antibacterial activity. The highest activity was observed in the ethyl acetate extract at 300 mg/mL, yielding inhibition zones of 6.90 mm and 4.97 mm for S. aureus and E. coli, respectively, which are classified within the medium category. Consequently, F. moluccana leaf extracts, particularly ethyl acetate fractions, possess moderate antibacterial properties against Gram-positive and Gram-negative bacteria. The significant influence of solvent type and concentration suggests that non-polar or semi-polar compounds are primarily responsible for this bioactivity.*

Keywords: antibacterial; *Escherichia coli*; *Falcataria moluccana (L) Nielsen*, *Staphylococcus aureus*

Abstrak: Penyakit infeksius menjadi kondisi yang paling umum terjadi secara global dan terus berkembang dari waktu ke waktu. Penelitian sebelumnya menunjukkan bahwa daun sengon (*Falcataria moluccana)* mengandung senyawa fitokimia yang berpotensi sebagai agen antibakteri. Penelitian ini bertujuan untuk mengevaluasi aktivitas antibakteri ekstrak daun *F. moluccana* terhadap *Staphylococcus aureus* dan *Escherichia coli* menggunakan metode difusi gel. Sampel diperoleh menggunakan empat pelarut: akuades, etanol 70%, etanol 96%, dan etil asetat, dengan rentang konsentrasi 50 hingga 300 mg/mL. Hasil penelitian menunjukkan bahwa ekstrak akuades tidak menunjukkan efek penghambatan, sedangkan ekstrak etil asetat dan etanol menunjukkan aktivitas antibakteri yang terukur. Aktivitas tertinggi diamati pada ekstrak etil asetat konsentrasi 300 mg/mL, yang menghasilkan zona hambat sebesar 6,90 mm untuk *S. aureus* dan 4,97 mm untuk *E. coli*, yang tergolong dalam kategori sedang. Oleh karena itu, ekstrak daun *F. moluccana*, khususnya fraksi etil asetat, memiliki sifat antibakteri moderat terhadap bakteri Gram-positif dan Gram-negatif. Pengaruh signifikan dari jenis pelarut dan konsentrasi menunjukkan bahwa senyawa non-polar atau semi-polar bertanggung jawab utama terhadap bioaktivitas ini.

Kata kunci: antibakteri; *Escherichia coli*; *Falcataria moluccana* (L) Nielsen, *Staphylococcus aureus*

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1. Introduction

The management and treatment of diseases remain a primary focus of human civilization, evolving continuously alongside medical and technological advancements. Bacterial infections represent a significant challenge, as specific pathogenic microbes pose persistent threats to human health and other living organisms. Historically, the primary strategy against these pathogens has involved the application of antibacterial compounds, most notably antibiotics. While these substances, derived from microbial secondary metabolites or synthetic derivatives, are designed to maximize therapeutic efficacy, their extensive use has led to critical issues such as bacterial resistance (Absor, 2006). Consequently, there is an urgent need to explore natural antibacterial sources to mitigate the negative impacts associated with conventional antibiotic reliance.

Falcataria moluccana (L) Nielsen, commonly known as the sengon tree, is a member of the Leguminosae family and serves as a major commodity in Indonesia's industrial forest programs (Purwanto, 2007). To date, the utilization of this species has been predominantly limited to its timber, which is highly valued for manufacturing containers, matchsticks, and furniture. Despite the high commercial demand for its wood, the potential biological benefits of other plant parts, particularly the leaves, remain largely unexplored in the medical field. Investigating the antibacterial properties of *F. moluccana* leaves offers a strategic opportunity to diversify the utility of this botanical resource.

Preliminary phytochemical screenings indicate that *F. moluccana* leaves contain various secondary metabolites, including alkaloids, saponins, tannins, phenolics, flavonoids, and triterpenoids (Eleanore, 2013; Harahap, 2006). These compounds are recognized for their distinct antimicrobial mechanisms. For instance, flavonoids are known to disrupt the permeability of bacterial cell walls, microsomes, and lysosomes through interactions with bacterial DNA (Sabir, 2005). Furthermore, the lipophilic nature of flavonoids facilitates the degradation of cell membranes (Ngemenya *et al.*, 2006). Similarly, tannins are hypothesized to inactivate microbial adhesins and transport proteins, while terpenoids exhibit broad activity against bacteria, fungi, and viruses by damaging structural membranes (Sugiharti, 2007).

Despite these promising chemical profiles, comprehensive data regarding the specific antibacterial efficacy of *F. moluccana* leaf extracts are currently lacking. This study aims to evaluate the antibacterial activity of these extracts against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) using the *in vitro* agar well diffusion method. By establishing the inhibitory potential of these extracts, this research seeks to provide scientific evidence that enhances the functional value of *F. moluccana* and contributes new insights into the development of natural antimicrobial agents.

2. Materials and Methods

2.1 Materials

The materials used in this study were sengon (*F. moluccana*) leaves (3–4 years old) collected from Dramaga, Bogor. Biological materials included isolates of *Staphylococcus aureus* and *Escherichia coli*. Chemical reagents comprised ethanol (70% and 96%), ethyl acetate, dimethyl sulfoxide (DMSO), phytochemical reagents, chloramphenicol, Nutrient Broth (NB), and Nutrient Agar (NA).

2.2 Sample Preparation and Extraction (Eleanore 2013)

The *F. moluccana* leaf powder was extracted using four solvents at a 1:10 (w/v) ratio. Aqueous extraction was performed via decoction at 100°C for 2 hours, followed by filtration and concentration using a rotary evaporator at 60°C. Organic solvent extractions (70% ethanol, 96% ethanol, and ethyl acetate) were conducted via maceration for 24 hours with initial 6-hour orbital shaking. The process was repeated in triplicate, and the combined macerates were concentrated at 40°C.

2.3 Water Content and Corrected Yield (AOAC 2005)

Moisture content was determined by drying the samples at 105°C until a constant weight was achieved. The corrected extract yield (Yc) was calculated to account for the moisture content of the initial leaf powder using the following relationship:

$$Y_c = \frac{a}{w-(w\times b)} \times 100\% \quad (1)$$

Where a is the extract weight, w is the initial sample weight, and b is the moisture content fraction (the ratio of evaporated water weight to the initial sample weight.).

2.4 Phytochemical Screening (Harborne 2006)

Qualitative analyses were performed on the ethyl acetate extract to identify secondary metabolites. Alkaloids were tested using Bouchardat and Dragendorff reagents following HCl digestion. Saponins were identified by the persistence of foam after vigorous shaking of the aqueous filtrate. Tannins were detected via the $FeCl_3$ (1%) test. Steroids and triterpenoids

were identified using the Lieberman-Burchard reaction (acetic anhydride and H_2SO_4). Phenolics and flavonoids were confirmed using NaOH (10%) and concentrated H_2SO_4 tests, respectively.

2.5 Antibacterial Activity Assay (Inayati 2007)

S. aureus and *E. coli* were regenerated in NB medium at 37°C for 24 hours and standardized via Optical Density (OD) measurements. The antibacterial activity was evaluated using the agar well diffusion method. 50 µL of bacterial suspension was inoculated into NA media. Wells (5 mm diameter) were loaded with 50 µL of extracts at concentrations ranging from 50 to 300 mg/mL. Chloramphenicol (100 µg\mL) and DMSO served as positive and negative controls, respectively.

Effect of extract concentration on antibacterial activity was further determined using lower extract concentrations (5–40 mg/mL). Inhibition zones were measured in quadruplicate using a digital calliper, and the net zone was calculated by subtracting the well diameter.

2.6. Statistical Analysis (Mattjik and Sumertajaya 2006)

Data were analyzed using a two-factor Split-Plot Design within a Completely Randomized Design (CRD). Statistical significance was determined via ANOVA ($\alpha = 0.05$) using SPSS v.16, followed by Duncan's Multiple Range Test (DMRT) for post-hoc comparisons.

3. Results

3.1 Moisture Content and Yield

The moisture content of the *F. moluccana* leaf powder was determined to be 6.17%. As presented in Table 1, the extraction yield varied significantly depending on the solvent polarity. The highest yield was achieved using 96% ethanol (7.34%), followed by 70% ethanol (6.22%) and distilled water (3.77%). The lowest yield was recorded for the ethyl acetate extract at 3.21%.

Table 1. Moisture content and yield of *F. moluccana* leaf extracts.

Sample	Moisture content (%)	Solvent	Yield (%)
Sengon (*F. moluccana*) leaf	6.17	Water	3.77
		Ethanol 70%	6.22
		Ethanol 96%	7.34
		Ethyl acetate	3.21

3.2 Phytochemical Analysis

Qualitative phytochemical screening of the ethyl acetate extract confirmed the presence of alkaloids, saponins, flavonoids, tannins, phenols, steroids, and triterpenoids (Table 2). Comparative analysis with previous data for aqueous and ethanolic extracts indicates a broad distribution of secondary metabolites across different solvent systems, with the exception of the aqueous extract, which lacked alkaloids.

Table 2. Qualitative phytochemical screening of various extracts from *F. moluccana* leaf.

Phytochemical	Water	Ethanol 70%	Ethanol 96%	Ethyl Acetate
Alkaloids	-	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Tannins and Phenolics	+	+	+	+
Steroids and Triterpenoid	+	+	+	+

* Results indicate the presence (+) or absence (-) of secondary metabolites across different solvent systems.

3.3 Antibacterial Activity

The antibacterial efficacy of the leaf extracts against *S. aureus* and *E. coli* was influenced by both solvent type and concentration (50-300 mg/mL). Statistical analysis revealed that solvent selection and concentration significantly affected the inhibition zone diameters ($P < 0.05$).

For *S. aureus*, the ethyl acetate extract at 300 mg/mL exhibited the highest antibacterial activity (6.90 mm), while the aqueous extract showed minimal inhibition (0.06 mm) at 200 mg/mL (Figure 1). Statistical analysis indicated that the 300 mg/mL concentration produced significantly distinct inhibition compared to lower concentrations.

In the case of *E. coli*, the ethyl acetate extract at 300 mg/mL again demonstrated maximum potency (4.97 mm), whereas the lowest activity was observed in the aqueous extract at 250 mg/mL (0.11 mm) (Figure 2). Statistical analysis results confirmed that both solvent type and concentration exerted significant, independent effects on the growth inhibition of *E. coli*. The positive control (chloramphenicol 100 µg/mL) yielded inhibition zones of 16 mm and 9 mm for *S. aureus* and *E. coli*, respectively, while the negative control (DMSO) showed no activity.

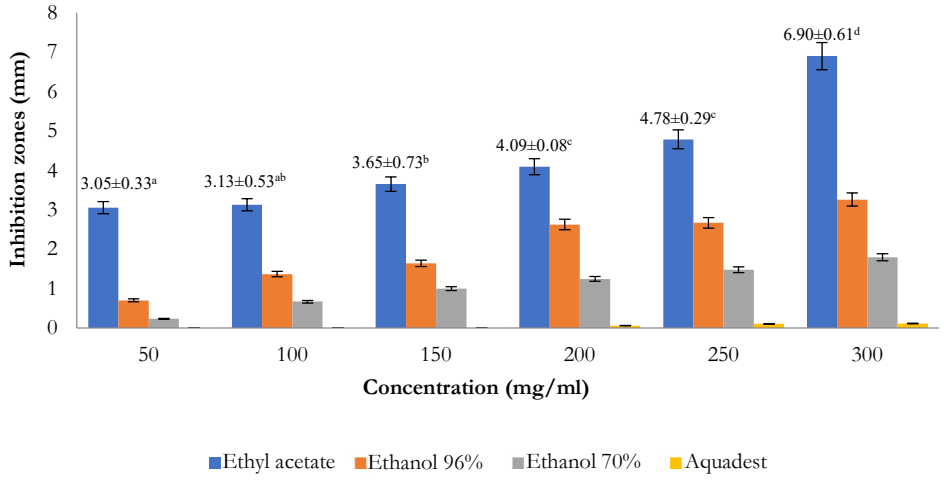

Figure 1. Antibacterial activity of various extracts against S. *aureus*. Different superscript letters (a, b, c, d) indicate significant differences ($p < 0.05$) between concentrations within the same solvent group.

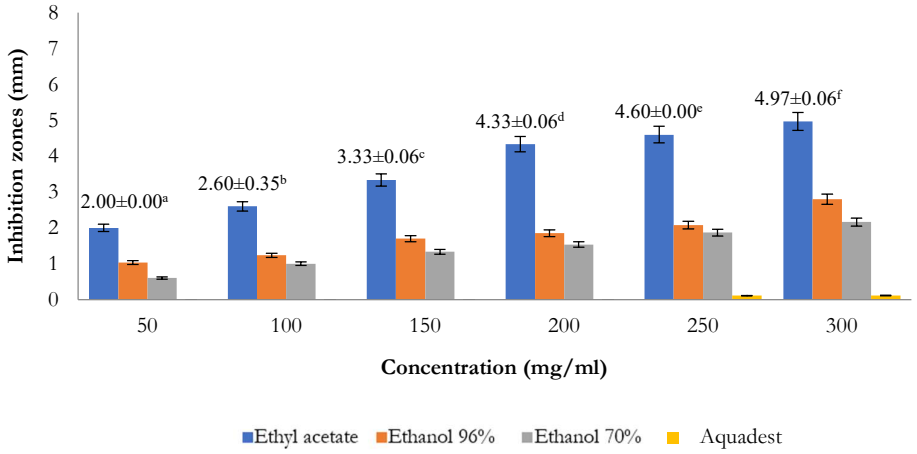

Figure 2. Antibacterial activity of various extracts against *E. coli*. Different superscript letters (a, b, c, d) indicate significant differences ($p < 0.05$) between concentrations within the same solvent group.

3.4 Effect of Extract Concentration on Antibacterial Activity

The concentration on antibacterial activity was evaluated for ethanolic and ethyl acetate extracts at lower concentrations (5-40 mg/mL). The aqueous extract was excluded from this phase due to its lack of efficacy at higher concentrations. As illustrated in Figures 3, the ethyl acetate extract consistently maintained measurable inhibitory effects at lower thresholds compared to the ethanolic counterparts for both Gram-positive and Gram-negative test bacteria.

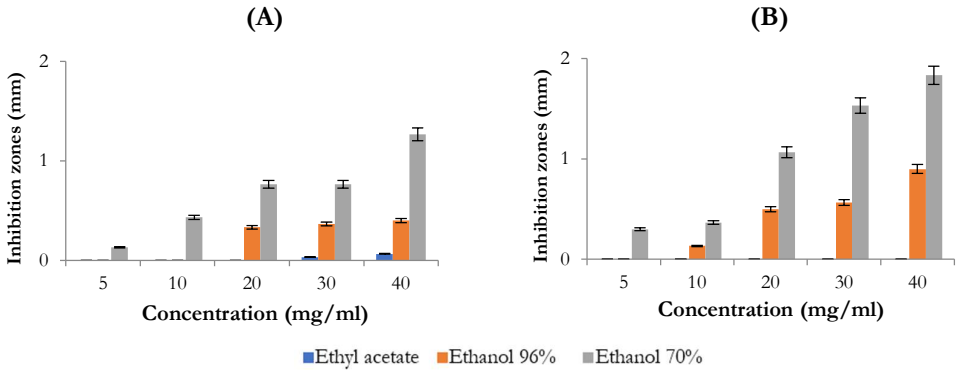

Figure 3. Antibacterial activity of *F. moluccana* leaf extracts at concentrations of 5–40 mg/mL against *S. aureus* (A) and *E. coli* (B)

4. Discussion

4.1 Extraction Yield and Moisture Content

The moisture content of *F. moluccana* leaf powder was recorded at 6.17%, significantly below the 10% threshold required to inhibit microbial degradation and enzymatic activity during storage. This low moisture level ensures long-term stability by preventing the proliferation of prokaryotic and eukaryotic microorganisms that thrive in high-water-activity environments (Harahap, 2006).

Regarding extraction efficiency, the 96% ethanol solvent yielded the highest extract recovery at 7.34%. While this confirms the efficacy of polar organic solvents in mobilizing bioactive constituents, the value is slightly lower than the 9.05% reported by Eleanore (2013). This discrepancy likely stems from variations in thermal regulation during rotary evaporation; excessive heat or prolonged distillation can lead to over-drying and loss of volatile components. The extraction process is governed by the "like-dissolves-like" principle, where mass transfer occurs via diffusion across the plant matrix until a concentration equilibrium is reached (Ayoola *et al.*, 2008).

4.2 Phytochemical Profile and Antibacterial Mechanisms

Phytochemical screening revealed a robust profile of alkaloids, saponins, tannins, phenolics, flavonoids, and triterpenoids within the *F. moluccana* leaf extracts. A critical observation was the absence of alkaloids in the aqueous extract, which directly correlated with its lack of antibacterial activity. This observation aligns with the findings of Koirewoa *et al.* (2012), who noted that free-base alkaloids are generally insoluble in water but highly soluble in organic solvents such as ethyl acetate and ethanol.

The identified secondary metabolites appear to function through distinct yet synergistic antimicrobial mechanisms. Saponins act as surface-active agents that reduce the surface tension of bacterial cell walls, leading to increased permeability and eventual cell lysis (Pratiwi, 2008). Simultaneously, phenolic compounds and flavonoids disrupt the cytoplasmic membrane and inhibit protein synthesis; specifically, flavonoids interfere with peptidoglycan cross-linking, thereby compromising the structural integrity of the cell wall (Ajizah *et al.*, 2007). Furthermore, tannins inhibit microbial growth by coagulating bacterial protoplasm and binding to proline-rich proteins, which hinders essential cell wall biosynthesis (Karlina *et al.*,

2013). In the context of the *F. moluccana* extract, it is hypothesized that saponins and flavonoids first destabilize the bacterial cell wall, facilitating the entry of tannins into the intracellular space to precipitate vital proteins, effectively inhibiting pathogens such as *S. aureus* and *E. coli*.

4.3 Effect of Extract Concentration on Bacterial Growth Inhibition

The antibacterial screening of *F. moluccana* leaf extracts revealed a distinct correlation between solvent polarity and inhibitory efficacy. The observed broad-spectrum activity, indicated by the inhibition of both Gram-positive and Gram-negative bacteria, aligns with the performance of chloramphenicol, the positive control used in this study (Sumardjo, 2009). However, a higher sensitivity was consistently observed in *S. aureus* compared to *E. coli*. This differential susceptibility is attributed to fundamental variations in cell wall architecture. The complex tri-layered cell envelope of *E. coli* (Gram-negative), which includes an outer membrane rich in lipopolysaccharides and a periplasmic space, acts as a selective permeability barrier against hydrophobic and bulky bioactive molecules (Ibrahim, 2007). In contrast, the simpler, porous peptidoglycan layer of *S. aureus* (Gram-positive) facilitates easier penetration of the phytochemicals, resulting in larger inhibition zones (Lathifah, 2008).

Statistical analysis (ANOVA, $p < 0.05$) followed by Duncan's Multiple Range Test confirmed that both solvent type and concentration significantly influenced antibacterial potency. Higher extract concentrations consistently yielded larger inhibition zones, reflecting a dose-dependent response common in botanical antimicrobials. The determination of the MIC further highlighted the potency of the ethyl acetate extract, which maintained inhibitory activity at concentrations as low as 5 mg/mL for both test organisms. In comparison, the 96% ethanol extract required 10–20 mg/mL to initiate inhibition, while the 70% ethanol extract was ineffective against *E. coli* within the tested MIC range.

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