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Phytochemical Composition and Antibacterial Potential of *Vitex trifolia* L. Against Pneumonia Causing Bacteria

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Abstract

This study investigated the phytochemical composition and antibacterial activity of *Vitex trifolia L.* (Legundi) in response to the increasing need for alternative therapeutic agents against pneumonia-causing bacteria. The research aimed to evaluate secondary metabolite distribution across roots, stem bark, leaves, and fruits, and to assess their effectiveness against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae*. A laboratory-based experimental design was employed, involving maceration extraction using 70% ethanol, qualitative phytochemical screening, and antibacterial testing through the agar well diffusion method at multiple extract concentrations. Results reveal that all plant parts contain flavonoids, tannins, saponins, and steroids, with terpenoids present in bark and fruits. Leaves and fruits exhibited the strongest antibacterial responses, particularly at 80% and 100% concentrations, reflecting their richer phytochemical profiles. *S. aureus* showed the highest susceptibility, followed by *S. pneumoniae* and *K. pneumoniae*, consistent with differences in bacterial cell-wall structure. These findings demonstrate Legundi's potential as a natural antibacterial agent, provide new insights into plant-part variation and dose-dependent effects, and strengthen the scientific foundation for developing plant-based therapeutic alternatives. Further research on compound isolation, quantitative assays, and in vivo validation is recommended to enhance the clinical relevance of these results.

Keywords: *Vitex trifolia* L.; Legundi; phytochemical screening; antibacterial activity; pneumonia-causing bacteria; medicinal plants.

INTRODUCTION

The use of medicinal plants has long been an integral part of human healthcare systems worldwide, playing an important role as a resource for both traditional and modern medicine. Among these plants, Legundi (*Vitex trifolia* L.), also known as the three-leaf chaste tree, has attracted considerable attention due to its remarkable pharmacological properties. This plant, a member of the Verbenaceae family, is widely distributed in tropical and subtropical regions, including Southeast Asia, Australia, and parts of Africa. Traditional medicine extensively utilizes the leaves, fruits, and roots of Legundi (*Vitex trifolia* L.) to treat conditions such as inflammation, pain, infections, respiratory disorders, and digestive problems (Ghafari et al., 2021; Liou et al., 2018).

In recent years, modern scientific research has increasingly validated the health benefits of Legundi (*Vitex trifolia* L.). Its phytochemical profile reveals a wealth of bioactive compounds, including flavonoids, iridoids, and diterpenes, which significantly contribute to its therapeutic potential. For instance, casticin, a major flavonoid isolated from this plant, has been shown to possess strong anti-inflammatory and antioxidant properties, making it a promising candidate for treating inflammatory disorders (Devi & Singh, 2014; Liou et al., 2014). Moreover, several studies indicate that Legundi (*Vitex trifolia* L.) extracts can modulate immune responses by inhibiting the production of pro-inflammatory cytokines and other mediators in macrophages (Matsui et al., 2012; Wee et al., 2020).

Another noteworthy property of *Vitex trifolia* L. is its hepatoprotective effect. Research shows that its extracts can protect liver cells from toxic agents such as carbon tetrachloride, demonstrating its potential for treating liver disorders (Manjunatha & Vidya, 2008; Mathankumar et al., 2015). In addition, the plant's essential oil exhibits larvicidal activity, further highlighting its benefits in addressing public health challenges (Arpiwi et al., 2020; Trilokesh et al., 2019).

The Indonesian government has identified efforts to reduce pneumonia and diarrhea in children as strategic issues and priority agendas within the national action plan for 2023–2030 (Iswandi, Alfinella Izhar et al., 2023). The Ministry of Health of the Republic of Indonesia, in collaboration with UNICEF and other relevant institutions, has formulated integrated strategies to reduce child mortality caused by these two diseases.

These measures include improving access to healthcare services, strengthening immunization, educating communities about clean and healthy living behaviors, and ensuring proper antibiotic use to prevent antimicrobial resistance. The Deputy for Education, Health, and Human Development has also committed to effectively communicating this policy to the President and Vice President of the Republic of Indonesia to ensure optimal implementation (Ministry of Health, 2023).

Pneumonia, an acute respiratory infection, remains a leading cause of morbidity and mortality worldwide, especially among children under five years of age. In Indonesia, pneumonia accounts for a significant proportion of child deaths, exceeding the combined number of deaths caused by AIDS, malaria, and measles (Ministry of Health, 2023). These alarming statistics underscore the importance of identifying effective strategies to combat pneumonia. Although antibiotics are the primary treatment for bacterial pneumonia, the increasing prevalence of antibiotic resistance poses a serious threat to health. Irrational antibiotic use has been linked to decreased effectiveness, higher treatment costs, and severe side effects, particularly in children whose body systems are still developing (Janna & Yuliana, 2024). Given these challenges, exploring alternative therapeutic options that are both effective and safe has become increasingly urgent. Natural products, especially medicinal plants with proven antibacterial properties, offer a promising path for addressing antibiotic resistance while minimizing side effects. In this context, Legundi (*Vitex trifolia* L.) emerges as an attractive candidate for further research.

The traditional use of Legundi (*Vitex trifolia* L.) aligns closely with pharmacological needs in managing pneumonia. The plant has been documented to exhibit strong antibacterial activity against various pathogens, including both Gram-positive and Gram-negative bacteria (Huong, 2023; Nur Rejai Salmah Abdul Hakeem et al., 2016). Key phytochemicals such as flavonoids and terpenoids play an essential role in its antimicrobial efficacy by disrupting bacterial membranes and inhibiting enzymatic processes (Cushnie & Lamb, 2005; Devi & Singh, 2014). Additionally, research by Supiana (2022) showed that ethanol extracts of Vitex trifolia L. effectively inhibit the growth of Escherichia coli and Staphylococcus epidermidis, common pathogens in respiratory infections. The inhibition zones observed at various extract concentrations (10%–60%) ranged from weak to strong, with higher concentrations demonstrating significant antibacterial activity.

The bioactivity of Legundi in treating respiratory diseases has also been explored. Several studies indicate that this plant has immunomodulatory and anti-inflammatory effects in asthma (Liou et al., 2018). Legundi leaves have also been studied and shown to exhibit antituberculosis activity against Mycobacterium tuberculosis in vitro (Munthe et al., 2024). This strengthens the possibility of antibacterial activity against pneumonia, which shares similarities as a respiratory illness.

Indonesia, home to the second-highest biodiversity in the world, is rich in medicinal plants. The traditional knowledge of ethnic communities across the archipelago has contributed to the use of various plants to treat diseases, including respiratory disorders (Evizal et al., 2013). In Lombok, West Nusa Tenggara, Legundi (Vitex trifolia L.), locally known as daun lego or glundih, thrives in humid environments such as foothills and roadside areas. Despite its abundance, awareness of its medicinal benefits remains limited among local communities. Mount Rinjani National Park, one of Lombok's biodiversity centers, has identified Legundi as one of its medicinal plants (Rianto et al., 2015). However, its potential as a natural remedy for pneumonia and other respiratory infections has not been fully explored.

Preliminary studies reveal the rich phytochemical composition of this plant, including secondary metabolites such as steroids, saponins, alkaloids, flavonoids, terpenoids, and tannins (Zulkifli et al., 2021). These compounds collectively contribute to its antibacterial, anti-inflammatory, and antioxidant activities. Yet, research specifically focusing on the antibacterial properties of Legundi against pneumonia-causing pathogens remains limited. The plant's rich phytochemical composition, combined with traditional uses and scientific findings on its various bioactivities, positions Legundi as an important candidate for further exploration.

MATERIALS AND METHODS

Study Site and Plant Material Collection

Plant material was sourced from two primary locations within the Mount Rinjani National Park region, specifically the Bebidas and Lemor areas, recognized for their high ecological diversity and optimal conditions for Legundi growth. Plants were selected based on morphological characteristics consistent with botanical identification standards for *Vitex trifolia* L. Collection included roots, stem bark, leaves, and fruits, ensuring representation of all major anatomical parts relevant to phytochemical and antimicrobial assessment. Samples were transported in sterile, breathable containers to maintain phytochemical stability prior to processing. Environmental variables such as altitude, humidity, and temperature were documented to support reproducibility and contextual interpretation of phytochemical variability.

Sample Preparation and Drying Procedures

All collected plant materials were cleaned to remove soil and impurities using distilled water, followed by shade-drying at ambient temperature to preserve thermolabile compounds. Drying continued until moisture content fell below 10%, which is the recommended threshold for minimizing microbial contamination and preventing enzymatic degradation of phytochemicals. Dried samples were subsequently milled into fine powder using a mechanical grinder equipped with stainless steel blades to avoid metal contamination. The uniformity of particle size was maintained to ensure consistent solvent penetration during extraction.

Extraction of Bioactive Compounds
Extraction was conducted using the maceration method with 70% ethanol as the solvent, chosen for its effectiveness in extracting both polar and semi-polar secondary metabolites. For each plant part, approximately 500 grams of powdered material were submerged in solvent at a ratio of 1:10 (w/v). The mixtures were stirred periodically and allowed to macerate for 72 hours at room temperature. After maceration, the extract was filtered using Whatman No. 1 filter paper to separate the solvent phase from the plant residue. The filtrate was then evaporated using a rotary evaporator at 40–45°C to remove excess solvent while preserving thermosensitive compounds. Concentrated extracts were stored in amber glass containers at 4°C to minimize oxidative degradation until further analysis.

Phytochemical Screening
Phytochemical screening was performed to identify major classes of secondary metabolites present in the extracts, including flavonoids, saponins, tannins, steroids, terpenoids, and alkaloids. Standard qualitative tests were used for each compound class. The presence of flavonoids was determined using the Shinoda test, which detects color changes indicating flavonoid-metal complex formation. Saponins were identified using the foam test, where persistent froth indicates saponin presence. Tannins were detected using ferric chloride reagent, producing a blue-black or greenish precipitate. Steroid presence was assessed using the Liebermann–Burchard reaction, while terpenoids were detected through the Salkowski test. Alkaloid detection was performed using Mayer's and Dragendorff's reagents. Positive and negative controls were included to validate each qualitative result.

Bacterial Strains and Culture Preparation
Three clinically relevant pneumonia-causing bacteria were selected for antibacterial testing: *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae*. These strains were obtained from accredited microbiology laboratories and maintained on nutrient agar slants at 4°C prior to use. For experimental assays, each bacterial strain was subcultured onto fresh agar plates and incubated for 24 hours at 37°C to ensure optimal viability. Standardized inoculum density was prepared using the McFarland 0.5 turbidity standard, corresponding to approximately 1.5 × 10^8 CFU/mL. This standardization ensured consistency across all antibacterial tests.

Antibacterial Assay Procedures
Antibacterial activity was assessed using the agar well diffusion method. Mueller–Hinton agar was prepared according to manufacturer guidelines and poured into sterile Petri dishes. Once solidified, agar surfaces were inoculated with bacterial suspensions using sterile cotton swabs to achieve uniform lawn growth. Wells with a diameter of 6 mm were created using a sterile cork borer. Extracts were prepared at concentrations of 20%, 40%, 60%, 80%, and 100% by diluting the concentrated extract with 70% ethanol. For each concentration, 50 µL of extract solution was dispensed into designated wells. Amoxicillin served as the positive control due to its established efficacy against the tested pathogens, while sterile aquadest functioned as the negative control. Plates were incubated at 37°C for 24 hours. After incubation, inhibition zones were measured using digital calipers to ensure accuracy. Measurements were taken in millimeters, including the diameter of the well. All tests were performed in triplicate to ensure reliability.

Data Categorization and Measurement Criteria
The antibacterial strength of each extract concentration was categorized based on standardized inhibition zone ranges. Inhibition zones of less than 5 mm indicated weak activity; 5–10 mm moderate activity; 10–20 mm strong activity; and greater than 20 mm very strong activity. These ranges were applied consistently across all assays to facilitate comparative analysis between plant parts, bacterial strains, and extract concentrations. Data were recorded in structured tables to support clear visualization of trends.

RESULTS AND DISCUSSION

This chapter presents the results of the phytochemical analyses and antibacterial assays conducted on the extracts of *Vitex trifolia L.* (Legundi) derived from four distinct plant parts: roots, stem bark, leaves, and fruits. The results are organized to facilitate systematic interpretation of the chemical composition and antibacterial behaviour of each extract across multiple concentration levels. The findings highlight the distribution of major secondary metabolites and the varying inhibitory effects of extracts against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae*. All results retain the integrity of the original observations and citations from the source document.

Phytochemical Composition of Plant Parts
Qualitative phytochemical screening confirmed the presence of several classes of secondary metabolites across all examined plant parts. Flavonoids, saponins,

tannins, and steroids were consistently detected in root, bark, leaf, and fruit extracts. Terpenoids were identified in the stem bark and fruit extracts, whereas alkaloids were absent from all samples. These results demonstrate that Legundi contains a diverse chemical profile known to contribute to antimicrobial activity. The widespread presence of flavonoids and tannins supports the hypothesis that these compounds may play a critical role in the antibacterial effects observed later in the study (Table1). Phytochemical screening analysis was carried out to identify the presence of active compounds in the root, stem bark, fruit, and leaf extracts of *Legundi* (*Vitex trifolia* L.). Various tests were performed, including assays for flavonoids, steroids, terpenoids, tannins, alkaloids, and saponins. These tests aimed to determine the types of secondary metabolites contained in the extracts. The results showed that none of the plant parts contained alkaloids, while terpenoids were not detected in the leaves and roots of *Legundi*.

Table 1. Phytochemical Screening Results of the roots, bark, fruit, and leaves of *Vitex trifolia*.

Phytochemical Test	Reagent(s) Used	Bark	Leaves	Root	Fruit
Flavonoids	Concentrated HCl + Mg metal	+	+	+	+
Steroids	Liebermann–Burchard	+	+	+	+
Terpenoids	Liebermann–Burchard	+	–	–	+
Alkaloids	Dragendorff / Wagner	–	–	–	–
Saponins	HCl	+	+	+	+
Tannins	$FeCl_3$	+	+	+	+

Note: (+) indicates a positive reaction; (–) indicates a negative reaction.

Antibacterial Activity Assay

In the antibacterial activity assay, ethanol extracts of *Legundi* (*Vitex trifolia* L.) leaves and stem bark were tested against three pneumonia-causing bacteria: *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Streptococcus pneumoniae*. The antibacterial test was performed using the well diffusion method. The well diffusion method has the advantage of allowing easier measurement of the inhibition zone area because the active compounds diffuse not only on the surface of the nutrient agar but also into deeper layers.

Antibacterial Activity Against *Staphylococcus aureus*.

All plant extracts exhibited antibacterial activity against *Staphylococcus aureus*, with inhibition zones generally increasing alongside extract concentration. At 20% concentration, the inhibition zones were typically categorized as moderate, ranging from small visible halos to clearly defined but limited inhibition regions. As concentrations were increased to 40% and 60%, the inhibition zones expanded noticeably, indicating stronger antibacterial effects. Extracts at 80% and 100% concentrations demonstrated strong to very strong inhibitory responses, with leaves and fruits consistently producing the largest inhibition zones (Table 2, 3, 4, and 5).

Table 2. Diameter of inhibition zones of *Legundi* (*Vitex trifolia* L.) root extract against the growth of *Staphylococcus aureus*.

Concentration	Replicate I	Replicate II	Replicate III	Mean	Category
K1	9.7	9.2	9.3	9.40	Moderate
K2	11.2	11.7	11.3	11.40	Strong
K3	13.2	13.8	13.8	13.60	Strong
K4	14.7	14.7	14.8	14.73	Strong
K5	16.2	17.2	17.9	17.10	Strong
C+	40.06	40.96	39.92	40.31	Very strong
C–	–	–	–	–	–

Table 3. Diameter of inhibition zones of *Legundi* (*Vitex trifolia* L.) stem bark extract against the growth of *Staphylococcus aureus*.

Concentration	Replicate I	Replicate II	Replicate III	Mean	Category
K1	9.7	8.7	8.3	8.90	Moderate
K2	11.2	11.3	11.9	11.45	Strong
K3	13.7	14.0	14.0	13.90	Strong
K4	15.2	16.7	17.1	16.33	Strong
K5	18.6	19.0	18.7	18.77	Strong
C+	40.03	40.63	40.12	40.26	Very strong
C–	–	–	–	–	–

Table 4. Diameter of inhibition zones of *Legundi* (*Vitex trifolia* L.) fruit extract against the growth of *Staphylococcus aureus.*

Concentration	Replicate I	Replicate II	Replicate III	Mean	Category
K1	13.2	13.7	13.7	13.53	Strong
K2	15.2	15.8	14.9	15.30	Strong
K3	17.2	17.3	17.2	17.23	Strong
K4	18.7	18.6	18.9	18.73	Strong
K5	20.5	20.7	21.0	20.73	Strong
C+	39.45	40.13	39.12	39.56	Very strong
C–	–	–	–	–	–

Table 5. Diameter of inhibition zones of *Legundi* (*Vitex trifolia* L.) leaf extract against the growth of *Staphylococcus aureus*.

Concentration	Replicate I	Replicate II	Replicate III	Mean	Category
K1	14.2	14.7	13.9	14.26	Strong
K2	16.2	16.8	16.7	16.57	Strong
K3	18.7	18.7	18.7	18.70	Strong
K4	20.0	20.7	20.8	20.50	Strong
K5	21.6	21.9	22.0	21.83	Very strong
C+	40.02	39.96	40.09	40.02	Very strong
C–	–	–	–	–	–

Antibacterial Activity Against *Streptococcus pneumoniae*.

Antibacterial inhibition data for *Streptococcus pneumoniae* using root, stem bark, fruit, and leaf extracts of *Legundi* are presented in the following tables (6,7,8, and 9)

Table 6. Diameter of inhibition zones of *Legundi* (*Vitex trifolia* L.) root extract against the growth of *Streptococcus pneumoniae.*

Concentration	Replicate I	Replicate II	Replicate III	Mean	Category
K1	10.2	10.2	10.0	10.13	Moderate
K2	12.1	12.7	12.2	12.33	Strong
K3	13.6	14.2	14.7	14.16	Strong
K4	15.2	15.9	15.7	15.60	Strong
K5	17.9	17.7	17.9	17.83	Strong
C+	24.8	24.7	24.3	24.60	Very strong
C–	–	–	–	–	–

Table 7. Diameter of inhibition zones of *Legundi* (*Vitex trifolia* L.) stem bark extract against the growth of *Streptococcus pneumoniae*.

Concentration	Replicate I	Replicate II	Replicate III	Mean	Category
K1	6.2	6.2	6.2	6.20	Moderate
K2	10.7	10.8	10.7	10.73	Moderate
K3	12.2	12.2	12.9	12.43	Strong
K4	14.2	14.2	14.7	14.36	Strong
K5	16.6	16.7	16.2	16.50	Strong
C+	23.6	23.9	23.2	23.57	Very strong
C–	–	–	–	–	–

Table 8. Diameter of inhibition zones of *Legundi* (*Vitex trifolia* L.) fruit extract against the growth of *Streptococcus pneumoniae.*

Concentration	Replicate I	Replicate II	Replicate III	Mean	Category
K1	6.2	5.2	5.2	12.70	Moderate
K2	10.2	11.0	10.2	14.53	Strong
K3	12.8	12.2	12.9	16.13	Strong
K4	14.7	14.5	14.7	18.36	Strong
K5	16.6	16.7	16.2	20.23	Strong
C+	24.6	24.9	24.9	24.80	Very strong
C–	–	–	–	–	–

Table 9. Diameter of inhibition zones of *Legundi* (*Vitex trifolia* L.) leaf extract against the growth of *Streptococcus pneumoniae.*

Concentration	Replicate I	Replicate II	Replicate III	Mean	Category
K1	8.2	8.2	8.2	8.20	Moderate
K2	12.7	12.6	12.7	12.67	Strong
K3	14.7	14.3	14.9	14.63	Strong
K4	16.2	16.7	16.7	16.53	Strong
K5	18.0	18.9	18.2	18.36	Strong
C+	26.6	26.2	26.1	26.30	Very strong
C–	–	–	–	–	–

Antibacterial Activity Against *Klebsiella pneumoniae*

Antibacterial inhibition data for *Klebsiella pneumoniae* using root, stem bark, fruit, and leaf extracts of *Legundi* are presented in the following of tables 10,11, 12, and 13

Table 10. Diameter of inhibition zones of *Legundi* (*Vitex trifolia* L.) root extract against the growth of *Klebsiella pneumoniae.*

Concentration	Replicate I	Replicate II	Replicate III	Mean	Category
K1	10.2	10.2	10.2	10.20	Moderate
K2	15.7	15.2	15.2	15.36	Strong
K3	17.2	17.3	17.3	17.27	Strong
K4	19.2	19.2	19.3	19.23	Strong
K5	21.6	21.6	21.6	21.60	Very strong
C+	35.2	35.2	35.7	35.36	Very strong
C–	–	–	–	–	–

Table 11. Diameter of inhibition zones of *Legundi* (*Vitex trifolia* L.) stem bark extract against the growth of *Klebsiella pneumoniae.*

Concentration	Replicate I	Replicate II	Replicate III	Mean	Category
K1	14.2	14.2	14.1	14.16	Strong
K2	16.7	16.7	16.2	16.53	Strong
K3	18.2	18.3	18.2	18.23	Strong
K4	20.2	20.8	20.7	20.56	Strong
K5	23.1	23.9	23.9	23.63	Very strong
C+	35.4	35.0	35.7	35.36	Very strong
C–	–	–	–	–	–

Table 12. Diameter of inhibition zones of *Legundi* (*Vitex trifolia* L.) fruit extract against the growth of *Klebsiella pneumoniae.*

Concentration	Replicate I	Replicate II	Replicate III	Mean	Category
K1	12.7	12.2	13.2	12.70	Strong
K2	14.2	14.7	14.7	14.53	Strong
K3	16.2	16.0	16.2	16.13	Strong
K4	18.7	18.2	18.2	18.36	Strong
K5	20.5	20.2	20.0	20.23	Strong
C+	35.2	35.2	35.7	35.36	Very strong
C–	–	–	–	–	–

Table 13. Diameter of inhibition zones of *Legundi* (*Vitex trifolia* L.) leaf extract against the growth of *Klebsiella pneumoniae.*

Concentration	Replicate I	Replicate II	Replicate III	Mean	Category
K1	14.2	14.1	14.2	14.10	Strong
K2	16.2	16.2	16.7	16.36	Strong
K3	18.1	18.1	18.2	18.13	Strong
K4	20.1	20.9	20.0	20.33	Strong
K5	22.9	22.7	22.7	22.77	Very strong
C+	35.7	35.7	35.7	35.70	Very strong
C–	–	–	–	–	–

Discussion

The phytochemical screening results indicated that all parts of the *Legundi* plant (roots, stem bark, leaves, and fruits) contained flavonoids, saponins, tannins, and steroids, whereas alkaloids were not detected in any of the four parts. Terpenoids were only detected in the stem bark and fruit. This pattern is consistent with the literature, which states that the vegetative and generative parts of *Vitex trifolia* L. are rich in secondary metabolites such as flavonoids, saponins, tannins, terpenoids, and steroids, all of which contribute substantially to antibacterial activity (Manaf & Daud, 2016; Parkhe & Bharti, 2019; Zulkifli et al., 2021).

Flavonoids are among the key components due to their ability to disrupt bacterial cell membranes, interfere with enzymatic function, and inhibit DNA and protein synthesis (Maulidah et al., 2020; Sepriani et al., 2019). Tannins enhance this activity by precipitating proteins and compromising the integrity of bacterial cell walls (Noipha et al., 2023; Toma et al., 2019; Xie et al., 2017). Saponins, which were found in all plant parts in this study, act by disrupting bacterial cell membrane permeability through their surfactant properties and their ability to interact with membrane sterols (Gupta et al., 2025; S. Z. Khan et al., 2024; Zulkifli et al., 2021). Steroids and terpenoids, particularly those detected in the stem bark and fruit, may further exacerbate membrane damage and alter bacterial membrane fluidity (Ajayi et al., 2019; Bhattarai et al., 2023; Sudhan et al., 2021).

The screening results showed an absence of alkaloids in all plant parts, which contrasts with several previous reports that have documented the presence of alkaloids in *Vitex trifolia* L. (for example, general phytochemical studies of *Legundi* from other regions). These discrepancies may be explained by variations in extraction methods, solvent type, and environmental conditions in which the plants grow.

As described by Manzo et al. (2017) and Okoli et al. (2023), alkaloids are highly influenced by the choice of solvent; aqueous solvents tend to be less effective in extracting alkaloids than certain organic solvents. Although this study used 70% ethanol, which is sufficiently polar and generally capable of extracting a wide range of compounds, it is possible that the pH conditions, extraction duration, or solvent-to-sample ratio were not optimal to extract alkaloids at levels detectable by qualitative tests. In addition, environmental factors such as climate, soil type, and altitude (Rinjani has distinct ecological characteristics) may influence alkaloid biosynthesis, so that *Legundi* plants from this area may naturally produce very low amounts of alkaloids or none at all. Clinically, the absence of alkaloids does not reduce the antibacterial relevance of this plant, as the literature also indicates that evidence of a specific contribution of *Legundi* alkaloids to antibacterial activity remains limited (W. Khan et al., 2019). Rather, the consistent presence of flavonoids, saponins, tannins, and steroids in all plant parts examined in this study is likely the main driver of its antibacterial effects.

The phytochemical screening results showed that terpenoids were only detected in the stem bark and fruit and were absent in the leaves and roots. Interestingly, in the antibacterial assay against *Klebsiella pneumoniae*, the stem bark and leaves were the two parts that produced a "very strong" category at 100% concentration, while the roots and fruits fell into the "strong" to "very strong" categories, even though all parts showed a trend of increasing inhibition zone diameter with increasing extract concentration.

The literature explains that terpenoids possess lipophilic properties that allow them to interact directly with bacterial membranes, disrupting their integrity and permeability (Ajayi et al., 2019; Bhattarai et al., 2023; Chhetry et al., 2022; Sudhan et al., 2021). Extracts rich in terpenoids have been reported to produce significant inhibition zones against various Gram-negative bacteria, including *K. pneumoniae*, due to their ability to penetrate the outer lipid bilayer and modify membrane fluidity.

The antibacterial assay results showed a highly consistent pattern: the higher the extract concentration (from 20% to 100%), the larger the inhibition zone diameter against all three test bacteria, for roots, stem bark, fruits, and leaves alike. This pattern is consistent with the dose–response relationship frequently reported in antibacterial phytotherapy studies (Kamoldeen et al., 2023; Raja et al., 2021). From a pharmacodynamic perspective, increasing extract concentration implies a higher number of bioactive molecules interacting with bacterial cells. This increases the likelihood of membrane integrity disruption, inhibition of key enzymes, interference with nutrient transport, and inhibition of the biosynthesis of essential macromolecules, ultimately leading to lysis or death of bacterial cells (Abubakar et al., 2025; Mandal & Sircar, 2016; Valentin et al., 2025).

In addition, several studies have shown a correlation between total phenolic content (including flavonoids and tannins) and the size of antibacterial inhibition zones (Girsang et al., 2024). Although this study did not measure total phenolic content, the trend of increasing inhibition zone diameters with increasing extract concentration suggests that phenolic components in *Legundi* play an important role in its antibacterial activity, either alone or in combination with other metabolites.

In all antibacterial tests, the positive control, amoxicillin, produced inhibition zones much larger than those of *Legundi* extracts, with a "very strong" category and diameters of approximately 23–40 mm (depending on the test bacterium). Meanwhile, *Legundi* extracts at the highest concentration (100%) generally produced inhibition zones categorized as "strong" to "very strong", but still did not surpass amoxicillin. This difference is understandable for several reasons: first, amoxicillin is a pure antibiotic with a single specific mechanism of action and high potency against target bacteria; second, *Legundi* extract is a complex mixture of many compounds, each present at relatively lower concentrations and acting through more diverse but individually weaker mechanisms than a single antibiotic; third, the bioavailability and diffusion of active compounds within the agar matrix may differ between a pure antibiotic and a crude plant extract (Mottaghipisheh, 2024; Osagie et al., 2023).

This indicates that, in its crude extract form, *Legundi* is not yet suitable to be considered a direct substitute for synthetic antibiotics, but is more realistically positioned as a candidate for adjunctive or complementary therapy. In the future, optimization of extraction methods (for example, purification of flavonoid/terpenoid-rich fractions, selection of more specific solvents, or advanced extraction techniques) may increase the concentration of active compounds and bring its effectiveness closer to that of standard antibiotics (Armansyah et al., 2022; Mottaghipisheh, 2024).

One of the important findings from the literature is that combinations of several secondary metabolites often yield stronger antibacterial effects than single compounds alone (Gupta et al., 2025; Shamsudin et al., 2022). This pattern is clearly reflected in the present results, where all plant parts containing a combination of flavonoids, saponins, tannins, and steroids were able to inhibit the growth of *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae*. Flavonoids function by damaging membranes and disrupting enzymatic function and nucleic acid synthesis; saponins alter membrane permeability; tannins precipitate proteins and damage the cell wall; while steroids can modify membrane structure and lipid interactions. These complementary mechanisms likely explain why at high concentrations (80–100%), *Legundi* extracts achieved “very strong” inhibition categories against several bacteria, particularly *S. aureus* and *K. pneumoniae*.

Other studies on various plants have also shown that extracts containing multiple classes of secondary metabolites simultaneously tend to have stronger and broader-spectrum antibacterial activity, including against common pneumonia pathogens (Ahmed et al., 2019; Gupta et al., 2025). Thus, the data from this study support the concept that a multicomponent approach using plant extracts such as *Legundi* can be an effective strategy to inhibit respiratory pathogens.

In general, the literature indicates that Gram-positive bacteria tend to be more susceptible to plant extracts than Gram-negative bacteria, because Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides (LPS) that acts as a major barrier to the diffusion of many antimicrobial agents (Aminu et al., 2023; Ikoyi et al., 2023). The findings of this study show that *Staphylococcus aureus* (Gram-positive) is indeed highly sensitive to *Legundi* leaf and fruit extracts, with inhibition zones categorized as “strong” to “very strong” at high concentrations. *Streptococcus pneumoniae* also showed a favorable response, although its inhibition zones were generally slightly smaller than those of *S. aureus*, consistent with differences in cell wall structure and virulence factors between these bacteria.

Klebsiella pneumoniae, a Gram-negative bacterium, also showed “strong” to “very strong” inhibition zones, particularly with stem bark and leaf extracts at 80–100% concentrations. This suggests that the combination of secondary metabolites in *Legundi* is sufficiently potent to overcome the outer membrane barrier of Gram-negative bacteria, especially at higher concentrations. These findings are in line with several reports indicating that plant extracts rich in terpenoids and saponins can disrupt or increase the permeability of the outer membrane of Gram-negative bacteria, thereby enhancing the susceptibility of bacteria to other antibacterial compounds (Leouifoudi et al., 2015; Pane, 2024). Thus, the response pattern observed in this study supports the theory that differences in cell wall structure influence bacterial susceptibility, but that *Legundi* extracts, with their rich composition of secondary metabolites, are still capable of inhibiting important Gram-negative respiratory pathogens such as *K. pneumoniae* when used at sufficiently high concentrations (Al-Kuraishy et al., 2018).

When the four plant parts are compared, the leaves tend to produce the most consistently high inhibition zones against *S. aureus* and *S. pneumoniae*, and “strong” to “very strong” categories against *K. pneumoniae* at high concentrations. This is consistent with the literature, which states that the leaves of *Vitex trifolia* L. generally contain higher concentrations of flavonoids and tannins than other plant parts and therefore exhibit more prominent antibacterial activity (Buah, 2023; Rasyid et al., 2020; Suyasa et al., 2022).

Practically, leaves also have advantages in terms of raw material availability and sustainability, as they can be harvested periodically without damaging the plant, whereas large-scale collection of roots or stem bark may threaten plant survival and disturb the ecosystem.

The literature also emphasizes that the use of leaves as the main raw material in phytotherapeutic preparations is often considered more environmentally friendly and economical, especially for industrial-scale development (Ante et al., 2021; Idowu et al., 2020; Oni et al., 2022). Considering antibacterial effectiveness, secondary metabolite content, availability, and sustainability, *Legundi* leaves appear to be the primary candidate for further development as a raw material for antibacterial phytotherapeutic preparations.

Clinically, the three test bacteria *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae* are important pathogens in respiratory tract infections, including pneumonia. The Government of Indonesia, through the 2023–2030 national action plan, has designated pneumonia in children as a priority issue and emphasized the importance of rational antibiotic use to curb resistance (Ministry of Health, Republic of Indonesia, 2023). In this context, exploration of plant-based antibacterials such as *Legundi* is highly relevant. Several studies have reported that *Vitex trifolia* L. has the potential to inhibit various respiratory pathogens and is associated with conditions such as asthma and pneumonia (Ács et al., 2018; Zulkifli et al., 2021). There is even evidence that *Legundi* leaves exhibit in vitro antituberculosis activity, further reinforcing its role as a plant with broad-spectrum antibacterial potential in respiratory infections.

The findings of this study, which demonstrate significant antibacterial activity against the three major pneumonia-causing bacteria, strengthen the position of *Legundi* as a phytopharmaceutical candidate to support respiratory tract infection therapy. Although it cannot yet replace synthetic antibiotics, *Legundi* extract has potential for use as a complementary therapy to reduce bacterial load, decrease the required antibiotic dose, and indirectly help lower the risk of resistance development (Elghaffar et al., 2022; Manandhar et al., 2019; Zazharskyi et al., 2020).

CONCLUSIONS

All plant parts (roots, stem bark, leaves, and fruits) exhibited the ability to inhibit the growth of the three test bacteria, with inhibition zone diameters generally increasing in line with rising extract concentrations (20–100%). The presence of flavonoids, saponins, tannins, and steroids in all plant parts, as well as terpenoids in the stem bark and fruits, is strongly suspected to contribute synergistically to the observed antibacterial activity. These compounds act through mechanisms such as disruption of the cell membrane, protein precipitation, and interference with bacterial enzyme function and or cell wall integrity. The favorable activity against pneumonia-causing bacteria supports the use of *Legundi* as a phytopharmaceutical candidate for the treatment of respiratory tract infections. These findings are consistent with the literature reporting the role of *Vitex trifolia* in managing respiratory disorders and related infections.

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