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Antibacterial Activity of Leaf, Stem and Flower Extracts of Songgolangit Plant Against *Staphylococcus aureus*, *Escherichia coli*, and *Pectobacterium sp.*

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Abstract

The increasing prevalence of antibiotic-resistant bacteria has encouraged the exploration of natural sources of antibacterial compounds, including *Tridax procumbens L.* This study aimed to evaluate the antibacterial activity of leaf, stem, and flower extracts of *T. procumbens* against *Staphylococcus aureus*, *Escherichia coli*, and *Pectobacterium sp.* Plant samples were collected in Denpasar, Bali, dried, powdered, and extracted by maceration using 96% ethanol, n-hexane, and ethyl acetate. Antibacterial activity was assessed using the disc diffusion method at extract concentrations of 10–50%, with 1% DMSO as the negative control. Inhibition zone diameters were analyzed using analysis of variance followed by the least significant difference test at the 5% significance level. Leaf and stem extracts showed no antibacterial activity against any tested bacteria. Antibacterial activity was observed only in the flower extracts against *S. aureus*, with the n-hexane extract producing the largest inhibition zone (10.0 mm at 50% concentration), followed by the ethyl acetate extract, while the ethanol extract was inactive. No extracts inhibited *E. coli* or *Pectobacterium sp.* These findings indicate that n-hexane flower extract possesses limited antibacterial activity against *S. aureus*, highlighting its potential as a source of antibacterial compounds and the need for further purification and characterization of its bioactive constituents.

Keywords

Antibacterial, *Escherichia coli*, *Pectobacterium sp.*, *Staphylococcus aureus*, *Tridax procumbens.*

1. Introduction

The increasing emergence of antibiotic-resistant bacteria has become a major global challenge that threatens the effectiveness of conventional antibacterial treatments. The continuous use and misuse of antibiotics have accelerated the development of resistant strains, creating an urgent need to explore alternative antibacterial agents from natural resources. Medicinal plants have attracted considerable attention because they contain diverse bioactive compounds that may serve as potential sources of new antimicrobial agents (Atanasov et al., 2021; Murray et al., 2022). Among these plants, songgolangit (*Tridax procumbens* L.) has received interest due to its traditional medicinal applications and reported pharmacological activities.

Songgolangit (*T. procumbens*) is a herbaceous plant belonging to the Asteraceae family and is widely distributed in tropical and subtropical regions. Although frequently regarded as a weed, this plant has been traditionally utilized for therapeutic purposes because of its rich phytochemical composition. Previous studies have reported that *T. procumbens* contains various secondary metabolites, including flavonoids, alkaloids, saponins, tannins, sterols, and terpenoids, which contribute to biological activities such as antioxidant, anti-inflammatory, and antimicrobial effects (Mundada & Shivhare, 2010; Rahman et al., 2024). These compounds are considered capable of inhibiting bacterial growth through several mechanisms, including disruption of bacterial cell membranes, inhibition of metabolic enzymes, and interference with essential cellular processes.

The antibacterial potential of *T. procumbens* has been reported against various pathogenic bacteria. However, its effectiveness varies depending on several factors, including plant organ, extraction solvent, extract concentration, environmental conditions, and the bacterial species tested. Previous studies demonstrated that *T. procumbens* extracts could inhibit pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli*, although differences in antibacterial activity were observed among extraction methods and bacterial strains (Kumar & Naidu, 2011; Bharathi et al., 2012; Syed et al., 2020). These variations indicate that the presence of bioactive compounds does not solely determine antibacterial activity but is also influenced by their distribution within plant tissues and their extractability using different solvents.

Plant organs represent an important factor affecting phytochemical composition and biological activity. Leaves, stems, and flowers have different physiological functions, resulting in variations in secondary metabolite accumulation. Flowers, for example, may contain specific defensive compounds associated with protection against environmental stress and biological threats. Therefore, comparative evaluation of different plant organs is necessary to determine which part of *T. procumbens* provides the highest antibacterial potential. Furthermore, extraction solvent polarity plays a crucial role in determining the type and concentration of compounds obtained. Polar solvents tend to extract phenolic and flavonoid compounds, while semi-polar and non-polar solvents are more effective for extracting compounds such as terpenoids and sterol derivatives. Differences in solvent properties may consequently influence antibacterial activity (Ameer et al., 2017; Atanasov et al., 2021; Pan et al., 2023).

Bacterial characteristics also influence the antibacterial response of plant extracts. *Staphylococcus aureus* is a Gram-positive bacterium associated with various human infections and has demonstrated high adaptability and antimicrobial resistance (Akinduti et al., 2022; Gherardi, 2023). In contrast, *E. coli* and *Pectobacterium* sp. are Gram-negative bacteria possessing an additional outer membrane that may restrict the penetration of antibacterial compounds. *Pectobacterium* sp. is also recognized as an important plant pathogen responsible for soft rot disease in various horticultural crops, causing significant economic losses (Charkowski, 2018). Therefore, evaluating antibacterial activity against both Gram-positive and Gram-negative bacteria provides broader information regarding the effectiveness and spectrum of plant-derived antibacterial compounds.

Figure 1. Songgolangit Plant

Songgolangit (*Tridax procumbens L.*) is a herbaceous plant belonging to the Asteraceae family and is widely distributed in tropical and subtropical regions, as shown in Figure 1. Although frequently regarded as a weed, this plant has been traditionally utilized for therapeutic purposes because of its rich phytochemical composition. Previous studies have reported that *T. procumbens* contains various secondary metabolites, including flavonoids, alkaloids, saponins, tannins, sterols, and terpenoids, which contribute to biological activities such as antioxidant, anti-inflammatory, and antimicrobial effects (Mundada & Shivhare, 2010; Rahman et al., 2024).

Although previous studies have investigated the antibacterial properties of *T. procumbens*, most research has focused on specific plant organs, particularly leaves, or evaluated limited extraction conditions (Yang et al., 2018). Information regarding the comparative antibacterial activity of leaf, stem, and flower extracts using solvents with different polarities against both human and plant pathogenic bacteria remains limited, particularly for *T. procumbens* collected in Denpasar, Bali. Therefore, this study aims to compare the antibacterial activity of leaf, stem, and flower extracts of *T. procumbens* prepared using ethanol, ethyl acetate, and n-hexane against *S. aureus*, *E. coli*, and *Pectobacterium* sp. The results are expected to provide insights into the potential utilization of *T. procumbens* as a natural antibacterial source and contribute to the development of plant-based antimicrobial agents.

2. Literature Review

2.1. Phytochemical Compounds and Antibacterial Activity of Tridax

The exploration of medicinal plants as alternative antibacterial agents has increased due to the global concern regarding antimicrobial resistance (Al-Dhabi et al., 2015; Ashraf et al., 2023). Among medicinal plants, *Tridax procumbens L.* (*Asteraceae*) has received considerable attention because of its diverse phytochemical constituents and biological activities (Gbadamosi et al., 2024). Several studies have demonstrated that *T. procumbens* contains bioactive compounds such as flavonoids, alkaloids, tannins, saponins, terpenoids, phenolic compounds, and sterol derivatives, which are associated with antimicrobial effects (Singh et al., 2025). These secondary metabolites can inhibit bacterial growth through multiple mechanisms, including disruption of bacterial cell membranes, inhibition of essential enzymes, and interference with cellular metabolism.

Recent investigations have confirmed the antibacterial potential of *T. procumbens* extracts against pathogenic bacteria. Ikala et al. (2024) reported that methanolic leaf extracts of *T. procumbens* exhibited antibacterial activity against multidrug-resistant Escherichia coli and Staphylococcus aureus, with phytochemical profiling revealing various bioactive compounds. Similarly, recent phytochemical analyses identified compounds such as flavonoids, terpenoids, alkaloids, and fatty acid derivatives that may contribute to antimicrobial activity (Singh et al., 2025).

However, the antibacterial activity of *T. procumbens* is not uniform and depends on several factors, including plant origin, extraction method, solvent characteristics, and bacterial species. Therefore, evaluating different plant organs and extraction solvents is important to identify the most effective source of antibacterial compounds. Recent studies have suggested that *T. procumbens* remains a promising natural resource for antibacterial discovery, although further purification and characterization of active compounds are required (Farhan et al., 2024).

2.2. Effect of Plant Organs and Extraction Solvents on Antibacterial Activity

The structural differences between bacterial groups influence the antibacterial effectiveness of plant-derived compounds. Gram-positive and Gram-negative bacteria possess different cell envelope characteristics that affect their susceptibility to antimicrobial substances. Staphylococcus aureus is a Gram-positive bacterium with a thick peptidoglycan layer but without an external membrane barrier. This structure allows several phytochemicals, including flavonoids, phenolics, and terpenoids, to interact more easily with bacterial cell components. In contrast, Gram-negative bacteria such as *Escherichia coli* and *Pectobacterium sp.* possess an additional outer membrane containing lipopolysaccharides, which can reduce the penetration of antibacterial compounds. Differences in bacterial structure often result in variation in inhibition responses to plant extracts. Plant secondary metabolites may inhibit bacterial growth through membrane disruption, protein denaturation, enzyme inhibition, and interference with nucleic acid synthesis (Khameneh et al., 2019; Bouyahya et al., 2022). However, Gram-negative bacteria generally show higher resistance because their outer membrane functions as a selective permeability barrier that limits the entry of hydrophobic and hydrophilic compounds (Oulahal & Degraeve, 2022).

Recent studies on *T. procumbens* have demonstrated differences in antibacterial responses among bacterial species. Ikala et al. (2024) reported that *T. procumbens* extract exhibited antibacterial activity against multidrug-resistant *S. aureus* and *E. coli*, although the inhibition level varied between bacterial strains. Similarly, Gopinathan et al. (2024) found that the ethanol extract of *T. procumbens* showed antibacterial potential, confirming that plant extracts may exhibit selective activity depending on bacterial characteristics. These findings support the importance of evaluating antibacterial activity against both Gram-positive and Gram-negative bacteria to determine the spectrum of activity of plant-derived compounds. Furthermore, bacterial resistance mechanisms, including changes in membrane permeability, efflux pumps, and enzymatic degradation, may influence antibacterial outcomes. Therefore, testing *S. aureus*, *E. coli*, and *Pectobacterium sp.* provides valuable information regarding the effectiveness of *T. procumbens* extracts against bacteria with different structural characteristics.

2.3. Antibacterial Susceptibility of Gram-Positive and Gram-Negative Bacteria

The structural differences between Gram-positive and Gram-negative bacteria influence the antibacterial activity of plant extracts. Gram-positive bacteria, such as *Staphylococcus aureus*, possess a thick peptidoglycan layer without an outer membrane, allowing antibacterial compounds to interact more easily with bacterial cell components (Egan et al., 2020; Riu et al., 2022). In contrast, Gram-negative bacteria, including *Escherichia coli* and *Pectobacterium* sp., have an additional outer membrane containing Lipopolysaccharides (LPS), which acts as a permeability barrier and limits the penetration of various antimicrobial compounds. These structural differences contribute to variations in bacterial susceptibility toward plant-derived antibacterial agents (Yan et al., 2024).

Plant secondary metabolites, including flavonoids, phenolic compounds, and terpenoids, exhibit antibacterial effects through several mechanisms, such as disruption of membrane integrity, inhibition of bacterial enzymes, interference with nucleic acid synthesis, and alteration of cellular metabolism (Zhang et al., 2025). Flavonoids can damage bacterial membranes and increase membrane permeability, resulting in leakage of intracellular components and inhibition of bacterial growth. However, Gram-negative bacteria generally show greater resistance because their outer membrane reduces the accessibility of bioactive compounds to intracellular targets (Nascimento & Costa, 2025). The selection of bacterial strains is therefore essential in evaluating antibacterial potential. Differences in cell structure may explain why plant extracts often demonstrate stronger activity against *S. aureus* compared with Gram-negative bacteria such as *E. coli* and *Pectobacterium* sp.

3. Methods

This study employed an experimental laboratory design to evaluate the antibacterial activity of *Tridax procumbens L.* extracts derived from different plant organs and extraction solvents. The research was conducted from January to March 2026 at the Biotechnology Laboratory, Faculty of Agriculture, Udayana University; the Genetic Resources and Molecular Biology Laboratory, Postgraduate Building, 3rd Floor, Sudirman Campus, Denpasar; and the Genetics Science Laboratory. Plant samples were collected from the Experimental Garden of the Faculty of Agriculture, Udayana University, located at Pedungan, South Denpasar, Denpasar City, Bali. Healthy *T. procumbens* plants without visible damage or disease symptoms were selected as samples. The evaluated plant organs included leaves, stems, and flowers, which were analyzed separately to determine differences in antibacterial potential.

The collected plant materials were sorted, thoroughly washed with running water, cut into smaller pieces, and dried at room temperature (approximately 25 °C) until completely dry. The dried samples were then ground into powder to increase extraction efficiency. Each plant organ was extracted separately using three solvents with different polarity levels, namely 96% ethanol, ethyl acetate, and n-hexane. Extraction was performed using the maceration method with a plant powder-to-solvent ratio of 1:10 (w/v) for 5 × 24 hours. During the maceration process, the samples were stirred every 24 hours to enhance the diffusion of bioactive compounds into the solvent. The obtained filtrates were concentrated using a vacuum rotary evaporator at 40 °C and 95 rpm until thick extracts were produced. The extracts were subsequently dissolved using 1% Dimethyl Sulfoxide (DMSO), which was also used as the negative control.

The antibacterial activity of the extracts was evaluated using the disc diffusion method on Nutrient Agar (NA) medium. The tested bacterial strains included *Staphylococcus aureus*, *Escherichia coli*, and *Pectobacterium sp.* Bacterial cultures were rejuvenated before testing and evenly inoculated onto NA plates. Sterile paper discs containing different extract treatments, including ethanol extract (KE), ethyl acetate extract (KEA), and n-hexane extract (KNH), were prepared at concentrations of 10%, 20%, 30%, 40%, and 50%. Discs containing 1% DMSO were used as the negative control (KO). After incubation for 24 hours, antibacterial activity was determined by measuring the diameter of the clear inhibition zone surrounding each disc using a millimeter scale. The measured inhibition zone diameter (mm) was used as the primary response variable to represent antibacterial effectiveness.

All treatments were performed with replication, and inhibition zone values were presented as mean measurements. Data showing antibacterial responses were analyzed using one-way Analysis of Variance (ANOVA), followed by the Least Significant Difference (LSD) test at a 5% significance level when significant differences were observed ($p < 0.05$). Statistical analysis was performed using statistical software. Treatments against *E. coli* and *Pectobacterium sp.* were not subjected to further statistical analysis because all treatments produced inhibition zones of 0.0 mm, indicating no detectable antibacterial activity under the experimental conditions.

4. Results

4.1. Activity Based on Plant Organs and Test Bacteria

Songgolangit leaf and stem extracts did not produce inhibition zones at any solvent combination and concentration against S. *aureus*, *E. coli*, or *Pectobacterium sp.* These results indicate that under the extraction and testing conditions used, the concentration or composition of active compounds in these two organs was insufficient to produce a measurable inhibitory effect. Antibacterial activity was only found in the flower extract against *S. aureus.* The n-hexane flower extract began to produce inhibition zones at a concentration of 10%, while the ethyl acetate extract began to show inhibition zones at a concentration of 30%. The ethanol extract did not produce inhibition zones at any concentration. All flower extracts remained inactive against *E. coli* and *Pectobacterium sp.*

Table 1. Antibacterial Activity of *Tridax procumbens* Organ Extracts

Plant Organ	Treatment	***Staphylococcus aureus*** **(mm)**	***Pectobacterium*** **sp. (mm)**	***Escherichia coli*** **(mm)**
Flower	K0	0.0f	0.0	0.0
	KEA1	0.0f	0.0	0.0
	KEA2	0.0f	0.0	0.0
	KEA3	1.5e	0.0	0.0
	KEA4	4.0cd	0.0	0.0
	KEA5	4.7c	0.0	0.0
	KNH1	1.2e	0.0	0.0
	KNH2	3.5d	0.0	0.0
	KNH3	7.0b	0.0	0.0
	KNH4	9.3a	0.0	0.0
	KNH5	10.0a	0.0	0.0
	KE1	0.0f	0.0	0.0
	KE2	0.0f	0.0	0.0
	KE3	0.0f	0.0	0.0
	KE4	0.0f	0.0	0.0
	KE5	0.0f	0.0	0.0
Leaf	KEA1	0.0	0.0	0.0
	KEA2	0.0	0.0	0.0
	KEA3	0.0	0.0	0.0
	KEA4	0.0	0.0	0.0
	KEA5	0.0	0.0	0.0
	KNH1	0.0	0.0	0.0
	KNH2	0.0	0.0	0.0

Plant Organ	Treatment	***Staphylococcus aureus*** **(mm)**	***Pectobacterium*** **sp. (mm)**	***Escherichia coli*** **(mm)**
	KNH3	0.0	0.0	0.0
	KNH4	0.0	0.0	0.0
	KNH5	0.0	0.0	0.0
	KE1	0.0	0.0	0.0
	KE2	0.0	0.0	0.0
	KE3	0.0	0.0	0.0
	KE4	0.0	0.0	0.0
	KE5	0.0	0.0	0.0
Stem	KEA1	0.0	0.0	0.0
	KEA2	0.0	0.0	0.0
	KEA3	0.0	0.0	0.0
	KEA4	0.0	0.0	0.0
	KEA5	0.0	0.0	0.0
	KNH1	0.0	0.0	0.0
	KNH2	0.0	0.0	0.0
	KNH3	0.0	0.0	0.0
	KNH4	0.0	0.0	0.0
	KNH5	0.0	0.0	0.0
	KE1	0.0	0.0	0.0
	KE2	0.0	0.0	0.0
	KE3	0.0	0.0	0.0
	KE4	0.0	0.0	0.0

Table 1 presents the antibacterial activity of leaf, stem, and flower extracts of Tridax procumbens prepared using ethanol, ethyl acetate, and n-hexane solvents against *Staphylococcus aureus*, *Escherichia coli*, and *Pectobacterium sp.* The results showed that antibacterial activity was only detected in flower extracts against S. aureus, while all leaf and stem extracts exhibited no inhibitory effect against the tested bacteria. Among the flower extracts, the n-hexane extract demonstrated the strongest antibacterial activity, producing the largest inhibition zone of 10.0 mm at a concentration of 50% (KNH5), followed by 9.3 mm at 40% concentration (KNH4) and 7.0 mm at 30% concentration (KNH3).

The ethyl acetate extract also showed antibacterial activity, although with lower inhibition values, ranging from 1.5 mm to 4.7 mm at concentrations of 30–50%. In contrast, the ethanol extract did not produce any inhibition zone against S. aureus at any of the tested concentrations. Statistical analysis using the LSD test at the 5% significance level indicated significant differences among treatments with different inhibition responses, as shown by different superscript letters in the *S. aureus* column. The highest antibacterial response was observed in the n-hexane flower extract, indicating that non-polar compounds extracted from the flower organ may contribute to antibacterial activity against *S. aureus.* Meanwhile, no inhibition zones were observed against *E. coli* and *Pectobacterium sp.* in all treatments, suggesting that the tested extracts did not exhibit detectable antibacterial activity against these Gram-negative bacteria under the experimental conditions.

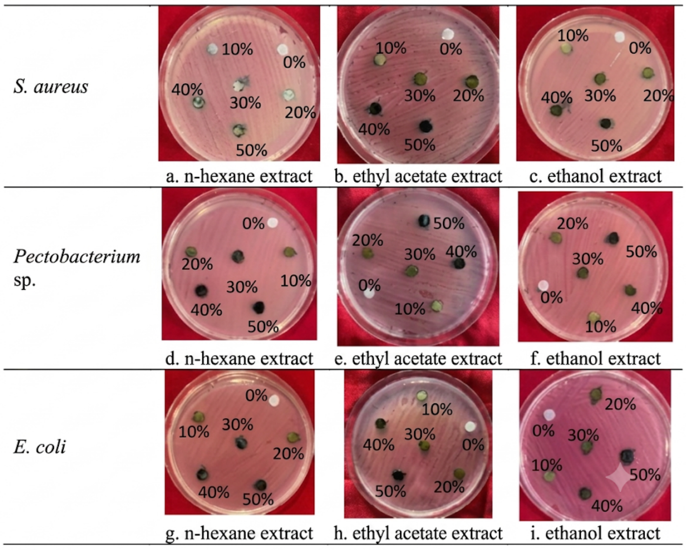

Figure 2. Antibacterial Activity of Songgolangit Leaf Extract Against Bacterial Pathogens

Figure 2 shows the antibacterial activity of *Tridax procumbens* extracts using different solvents against *Staphylococcus aureus*, *Pectobacterium* sp., and *Escherichia coli.* Visible inhibition zones were only observed in flower extracts against *S. aureus*, with the strongest activity shown by the n-hexane extract at higher concentrations. The ethyl acetate extract showed lower inhibition, while the ethanol extract showed no antibacterial activity. No inhibition zones were detected against *Pectobacterium* sp. and *E. coli* in all treatments, indicating that the extracts exhibited selective antibacterial activity depending on bacterial species and solvent type.

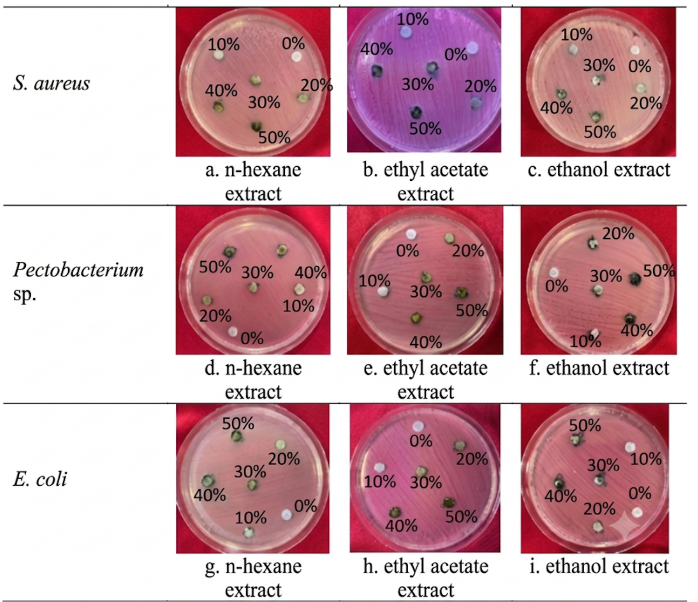

Figure 3. Antibacterial Activity of Songgolangit Leaf Extract

Figure 3 shows the antibacterial activity of Songgolangit leaf extracts prepared using n-hexane, ethyl acetate, and ethanol against *Staphylococcus aureus*, *Pectobacterium* sp., and *Escherichia coli* at concentrations of 10–50%. No inhibition zones were observed for any solvent, concentration, or bacterial species, with all treatments producing inhibition zones of 0.0 mm. These results indicate that the leaf extract did not exhibit detectable antibacterial activity under the tested conditions, regardless of solvent polarity or extract concentration.

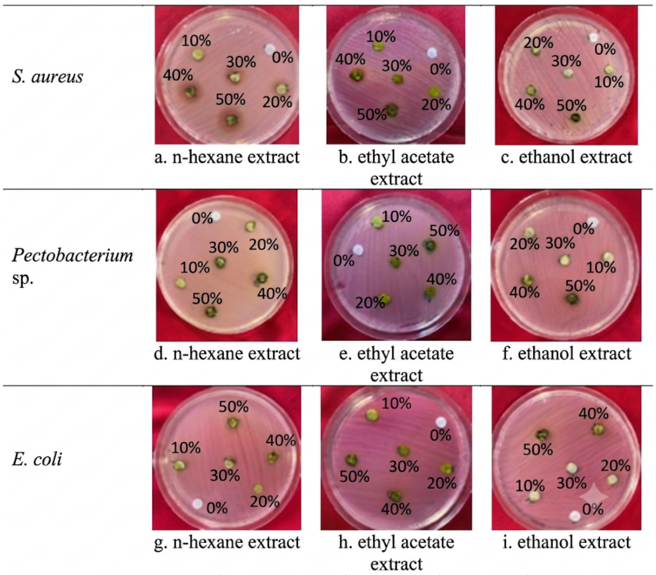

Figure 4. Antibacterial Activity of Songgolangit Flower Extract

Figure 4 shows that Songgolangit flower extracts exhibited antibacterial activity only against *Staphylococcus aureus.* The n-hexane extract showed activity from a concentration of 10%, with the inhibition zone increasing to 10.0 mm at 50%. The ethyl acetate extract showed activity from 30% concentration and reached 4.7 mm at 50%, whereas the ethanol extract produced no inhibition zones at any concentration. All flower extracts were inactive against *Escherichia coli* and *Pectobacterium* sp.

4.2. Effect of Solvent Type and Concentration

The n-hexane flower extract showed the highest activity, with an increase in the inhibition zone diameter from 1.2 mm at 10% concentration to 10.0 mm at 50%. The values at 40% and 50% concentrations were in the same statistical group, so the increase from 9.3 to 10.0 mm was not significantly different. The ethyl acetate extract produced lower activity, at 1.5 mm at 30% concentration, 4.0 mm at 40%, and 4.7 mm at 50%. The ANOVA results yielded an F-value of 42.02 with a p-value of 8.93×10^{-17}, indicating a highly significant effect of solvent type and extract concentration on the inhibition zone diameter of *S. aureus.* The pattern of increasing inhibition zone diameter was consistent with increasing extract concentration, particularly in the n-hexane fraction.

The higher activity of the n-hexane extract indicates that the main antibacterial compounds in the flower are likely nonpolar or semipolar, such as sterols, terpenoids, and certain lipid components. The ethyl acetate extract still extracted some of the active compounds, while ethanol did not produce measurable activity under these experimental conditions. These findings support reports that the distribution of secondary metabolites differs between organs and that flower parts may harbor more dominant antibacterial components (Andriana et al., 2019; Amutha et al., 2019).

The lack of inhibition zones in Gram-negative bacteria may be influenced by the lipopolysaccharide-rich outer membrane, which functions as a selective barrier. This structure can reduce the penetration of active compounds compared to S. aureus, which lacks an outer membrane. Furthermore, differences in bacterial strain, media conditions, inoculum density, extract stability, and the ability of compounds to diffuse within the cell also influence test results. The results of this study differ from several reports demonstrating the activity of *T. procumbens* leaf extract or ethanol extract against test bacteria (Bharathi et al., 2012; Syed et al., 2020). Differences may stem from growth location, harvest time, plant organ, drying method, extraction time, solvent type, and active compound concentration. Because this study did not use standard antibiotics as positive controls, the relative potency of the extracts against standardized antibacterial agents could not be directly determined.

5. Discussion

The antibacterial activity of *Tridax procumbens* extracts in this study was strongly influenced by plant organ, solvent type, extract concentration, and bacterial species. The results demonstrated that only flower extracts exhibited antibacterial activity, particularly against *Staphylococcus aureus*, whereas leaf and stem extracts showed no detectable inhibition against *S. aureus*, *Escherichia coli*, or *Pectobacterium* sp. These findings indicate that the distribution and concentration of antibacterial phytochemicals may vary among different plant organs. Plant secondary metabolites are not uniformly accumulated throughout the plant because their biosynthesis is affected by physiological function and environmental factors. Flowers may contain specific defensive metabolites associated with protection against microbial threats and reproductive tissues, which could explain the higher antibacterial potential observed in this study (Yang et al., 2018; Amutha et al., 2019).

The antibacterial activity of flower extracts was dependent on solvent polarity. The n-hexane extract showed the strongest inhibitory effect against *S. aureus*, with the inhibition zone increasing from 1.2 mm at 10% concentration to 10.0 mm at 50%. In comparison, ethyl acetate extract showed moderate activity, while ethanol extract showed no inhibition. This result suggests that the antibacterial compounds responsible for activity in *T. procumbens* flowers may have relatively low polarity. Non-polar solvents such as n-hexane are effective in extracting compounds including terpenoids, sterols, and lipid-associated bioactive molecules, which have been reported to contribute to antibacterial mechanisms through membrane disruption and interference with cellular processes (Khameneh et al., 2019; Bouyahya et al., 2022). Although many studies have reported antibacterial properties of ethanol extracts of *T. procumbens*, the effectiveness of extraction depends on the plant organ and chemical composition obtained during extraction (Ameer et al., 2017).

Previous studies have confirmed the antibacterial potential of *T. procumbens*, although differences in activity patterns have been reported. Andriana et al. (2019) demonstrated antibacterial activity of *T. procumbens* extracts, while Syed et al. (2020) reported that leaf extracts exhibited inhibitory effects against bacterial pathogens. Similarly, Bharathi et al. (2012) and Ikala et al. (2024) found that *T. procumbens* leaf extracts were active against *S. aureus* and *E. coli*. However, the absence of activity in leaf extracts observed in the present study may be related to differences in geographical origin, plant maturity, environmental conditions, extraction procedures, and secondary metabolite concentration. Variations in growing conditions can modify phytochemical accumulation and subsequently affect biological activity (Yang et al., 2018). Therefore, the antibacterial potential of *T. procumbens* cannot be generalized across all plant organs and extraction methods.

The selective activity against *S. aureus* but not against *E. coli* and *Pectobacterium* sp. may be associated with structural differences between Gram-positive and Gram-negative bacteria. *S. aureus* possesses a thick peptidoglycan layer but lacks the outer membrane found in Gram-negative bacteria, allowing easier penetration of hydrophobic antibacterial compounds. In contrast, Gram-negative bacteria contain an outer membrane rich in lipopolysaccharides that acts as a permeability barrier and reduces susceptibility to many plant-derived compounds (Egan et al., 2020; Riu et al., 2022; Laoth et al., 2026). Previous studies have also emphasized that flavonoids and other plant phenolics often show different antibacterial effectiveness depending on bacterial envelope characteristics (Nascimento & da Costa, 2025; Yan et al., 2024).

The lack of activity against *Pectobacterium* sp. is also noteworthy because this bacterium is associated with soft rot diseases in plants. The resistance of *Pectobacterium* sp. to the tested extracts may result from differences in membrane structure, metabolic adaptation, or insufficient concentration of active compounds reaching antibacterial levels (Charkowski, 2018). Although *T. procumbens* contains various bioactive compounds with antimicrobial potential, including flavonoids, phenolics, and terpenoids, their biological effects depend strongly on extraction efficiency and target bacterial species (Rahman et al., 2024; Farhan et al., 2024).

6. Conclusion

The study concludes that Songgolangit leaf and stem extracts did not exhibit antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, or *Pectobacterium sp.* under the tested conditions, while antibacterial activity was only detected in the flower extract against *S. aureus*. Among the tested treatments, the n-hexane flower extract showed the strongest antibacterial potential, producing the highest inhibition zone of 10.0 mm at a concentration of 50%, followed by the ethyl acetate extract with an inhibition zone of 4.7 mm at the same concentration, whereas the ethanol extract showed no inhibitory effect. These findings indicate that solvent polarity and extract concentration play important roles in determining the antibacterial activity of Songgolangit extracts, suggesting that non-polar compounds in the flower extract may contribute to the observed antibacterial effect.

However, the antibacterial activity remains limited and requires further improvement before practical application. The main limitation of this study is that it only evaluated crude extracts under limited concentration ranges and bacterial isolates without comparison to standard antibiotic controls. Future research should focus on fractionation and identification of active compounds from the n-hexane flower extract, optimization of extract concentrations, determination of Minimum Inhibitory Concentration (MIC), and evaluation using positive antibiotic controls and a broader range of bacterial strains to better understand the antibacterial potential and mechanism of action of Songgolangit extract.

References

Akinduti, P. A., Emoh-Robinson, V., Obamoh-Triumphant, H. F., Obafemi, Y. D., & Banjo, T. T. (2022). Antibacterial activities of plant leaf extracts against multi-antibiotic resistant Staphylococcus aureus associated with skin and soft tissue infections. *BMC Complementary Medicine and Therapies*, *22*(1), 47-56. https://doi.org/10.1186/s12906-022-03527-y.

Al-Dhabi, N. A., Arasu, M. V., & Rejiniemon, T. S. (2015). In vitro antibacterial, antifungal, antibiofilm, antioxidant, and anticancer properties of isosteviol isolated from endangered medicinal plant Pittosporum tetraspermum. *Evidence-Based Complementary and Alternative Medicine*, *7*(3), 56-76. https://doi.org/10.1155/2015/164261Digital.

Ameer, K., Shahbaz, H. M., & Kwon, J. H. (2017). Green extraction methods for polyphenols from plant matrices and their byproducts: A review. *Comprehensive Reviews in Food Science and Food Safety*, *16*(2), 295-315. https://doi.org/10.1111/1541-4337.12253.

Amutha, R., Sudha, A., & Pandiselvi, P. (2019). Tridax procumbens (coat buttons)-A gift of nature: An overview. In *Pharmacological benefits of natural products* (pp. 193–212). Cham: Springer.

Andriana, Y., Xuan, T. D., Quy, T. N., Minh, T. N., Van, T. M., & Viet, T. D. (2019). Antihyperuricemia, antioxidant, and antibacterial activities of Tridax procumbens L. *Foods*, *8*(1), 21-36. https://doi.org/10.3390/foods8010021.

Ashraf, M. V., Pant, S., Khan, M. H., Shah, A. A., Siddiqui, S., Jeridi, M., ... & Ahmad, S. (2023). Phytochemicals as antimicrobials: prospecting Himalayan medicinal plants as source of

alternate medicine to combat antimicrobial resistance. *Pharmaceuticals*, *16*(6), 881-892. https://doi.org/10.3390/ph16060881.
Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: advances and opportunities. *Nature reviews Drug discovery*, *20*(3), 200-216. https://doi.org/10.1038/s41573-020-00114-z.
Bharathi, V., Varalakshmi, B., Gomathi, S., Shanmugapriya, A., & Karpagam, T. (2012). Antibacterial activity of Tridax procumbens Linn. *International Journal of Pharmaceutical Sciences and Research*, *3*(4), 364–367.
Bouyahya, A., Chamkhi, I., Balahbib, A., Rebezov, M., Shariati, M. A., Wilairatana, P., ... & El Omari, N. (2022). Mechanisms, anti-quorum-sensing actions, and clinical trials of medicinal plant bioactive compounds against bacteria: A comprehensive review. *Molecules*, *27*(5), 14-24. https://doi.org/10.3390/molecules27051606.
Charkowski, A. O. (2018). The changing face of bacterial soft-rot diseases. *Annual Review of Phytopathology*, *56*(1), 269–288. https://doi.org/10.1146/annurev-phyto-080417-050112.
Egan, A. J., Errington, J., & Vollmer, W. (2020). Regulation of peptidoglycan synthesis and remodelling. *Nature Reviews Microbiology*, *18*(8), 446-460. https://doi.org/10.1038/s41579-020-0366-3.
Farhan, M. S., Jaafar, F. R., & Oraibi, A. I. (2024). Unveiling the dual anti-viral and anti-bacterial potential of Tridax procumbens: integrating system biology and molecular modelling for therapeutic insights. *Pharmacia*, *71*(9), 1-14. https://doi.org/10.3897/pharmacia.71.e134884.
Gbadamosi, A. S., Oseni, M. O., Adetukonbo, B. A., & Oseni, O. A. (2024). Biochemical characterization, antimicrobial potency, and metallic nanoparticle generation from tridax procumbens extracts. *Asian Plant Research Journal*, *12*(4), 78-94. https://doi.org/10.9734/aprj/2024/v12i4264.
Gherardi, G. (2023). Staphylococcus aureus infection: Pathogenesis and antimicrobial resistance. *International Journal of Molecular Sciences*, *24*(9), 81-94. https://doi.org/10.3390/ijms24098182.
Gopinathan, N., Balaji, B., Gokul Kumar, K., & Senthil Raj. (2024). Assessing the antibacterial activity of *Tridax procumbens* in ethanol extract. *Virdi International Journal*, 7(4), 66-88.
Ikala, R. O., Mshelia, M. B., Innocent, O. O., Gberikon, M. G., & Ebah, E. E. (2024). Phytochemical profile and in vitro antibacterial activity of *Tridax procumbens* leaf extract on multidrug-resistant *E. coli* and *S. aureus* clinical isolates. *Medical and Health Sciences European Journal*, *8*(5), 33–45.
Khameneh, B., Iranshahy, M., Soheili, V., & Fazly Bazzaz, B. S. (2019). Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrobial Resistance & Infection Control*, *8*(1), 118-136. https://doi.org/10.1186/s13756-019-0559-6.
Kumar, O. A., & Naidu, L. M. (2011). Antibacterial potential of Tridax procumbens L. against human pathogens. *Pharmasm*, *2*(2), 1-16.
Laoth, G. L., Wirya, G. N. A. S., & Temaja, I. G. R. M. (2026). In vitro evaluation of bacillus subtilis and pseudomonas fluorescens against klebsiella variicola in kepok banana. *Research Horizon*, *6*(2), 861-872. https://doi.org/10.54518/rh.6.2.2026.1076.
Mundada, S., & Shivhare, R. (2010). Pharmacology of tridax procumbens: A review. *International Journal of Pharmacology and Technology Research*, *2*(2), 1391–1394.
Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., ... & Tasak, N. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet*, *29*(15), 629-655. https://doi.org/10.1016/S0140-6736(21)02724-0.
Nascimento, J. B., & da Costa, J. G. M. (2025). Flavonoids: a review of antibacterial activity against gram-negative bacteria. *International Journal of Microbiology*, *20*(1), 996-1021. https://doi.org/10.1155/ijm/9961121Digital.
Oulahal, N., & Degraeve, P. (2022). Phenolic-rich plant extracts with antimicrobial activity: an alternative to food preservatives and biocides?. *Frontiers in Microbiology*, *12*(5), 753-768. https://doi.org/10.3389/fmicb.2022.753518.
Pan, H., Nie, S., Li, C., Zhang, L., Sun, X., Elateeq, A. A., ... & Fu, Y. (2023). A green and novel strategy based on CO2-responsive surfactant-functionalized multi-walled carbon nanotubes with microwave-ultrasound assistant to improve extraction and enrichment of phytochemicals from plant waste. *Industrial Crops and Products*, *19*(5), 116-124. https://doi.org/10.1016/j.indcrop.2023.116294.
Rahman, Y., Suada, I. K., Wijaya, I. N., Wirawan, G. P., Phabiola, T. A., & Darmawati, I. A. P. (2024). Phytochemical and antioxidant activity of the ethanol extracts of *Songgolangit*

plant (Tridax procumbens L.). *Tropical Journal of Phytochemistry and Pharmaceutical Sciences*, *3*(5), 314–318. https://doi.org/10.26538/tjpps/v3i5.10.

Riu, F., Ruda, A., Ibba, R., Sestito, S., Lupinu, I., Piras, S., ... & Carta, A. (2022). Antibiotics and carbohydrate-containing drugs targeting bacterial cell envelopes: an overview. *Pharmaceuticals*, *15*(8), 942-956. https://doi.org/10.3390/ph15080942.

Singh, N., Chhangani, N., & Bissa, S. (2025). Antibacterial potential and phytochemical analysis of two ethnomedicinally important plants. *Current Research in Microbial Sciences*, *8*(4), 100-117. https://doi.org/10.1016/j.crmicr.2024.100297.

Syed, A., Benit, N., Alyousef, A. A., Alqasim, A., & Arshad, M. (2020). In-vitro antibacterial, antioxidant potentials and cytotoxic activity of the leaves of Tridax procumbens. *Saudi Journal of Biological Sciences*, *27*(2), 757–761. https://doi.org/10.1016/j.sjbs.2019.12.024.

Yan, Y., Xia, X., Fatima, A., Zhang, L., Yuan, G., Lian, F., & Wang, Y. (2024). Antibacterial activity and mechanisms of plant flavonoids against gram-negative bacteria based on the antibacterial statistical model. *Pharmaceuticals*, *17*(3), 292-304. https://doi.org/10.3390/ph17030292.

Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. *Molecules*, *23*(4), 762-776. https://doi.org/10.3390/molecules23040762.

Zhang, Z., Cao, M., Shang, Z., Xu, J., Chen, X., Zhu, Z., ... & Zhang, J. (2025). Research progress on the antibacterial activity of natural flavonoids. *Antibiotics*, *14*(4), 334- 344. https://doi.org/10.3390/antibiotics14040334.

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