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In Vitro Antibacterial Activity of Leaf and Bark Extracts of *Ficus trimenii* Using Different Solvent

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ABSTRACT

Ficus trimenii is a medicinal plant native to Sri Lanka and is traditionally used in Ayurvedic medicine for the treatment of skin infections, including rashes and eczema, wound cleansing, prevention of certain communicable diseases, and management of fractures. This study aimed to screen the phytochemical constituents and evaluate the antibacterial activity of different solvent extracts obtained from the leaves and bark of *F. trimenii* against *Staphylococcus aureus* and *Escherichia coli*. Fresh and healthy leaves and bark were collected from the Peradeniya Royal Botanical Garden and separately macerated with methanol, acetone, and petroleum ether for 48 hours. Antibacterial activity was evaluated using the agar well diffusion method, with cloxacillin and ciprofloxacin used as reference antibiotics for *S. aureus* and *E. coli*, respectively. The minimum inhibitory concentration (MIC) was determined using the microbroth dilution method. Preliminary phytochemical screening was also conducted for all extracts. All extracts exhibited antibacterial activity against both bacterial strains, except the petroleum ether bark extract, which showed no inhibition zone. The petroleum ether leaf extract demonstrated the strongest antibacterial activity, with an MIC of 1.74 mg/mL against both *S. aureus* and *E. coli*. Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, polyphenols, sterols, and tannins in the leaf and bark extracts. These findings indicate that methanol, acetone, and petroleum ether extracts of *F. trimenii* leaves and bark possess notable in vitro antibacterial activity against *S. aureus* and *E. coli*. The results provide scientific support for the traditional medicinal use of *F. trimenii* and indicate the need for further in vivo studies to confirm its antibacterial potential.

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ABSTRAK

Ficus trimenii merupakan tanaman obat yang berasal dari Sri Lanka dan secara tradisional digunakan dalam pengobatan Ayurveda untuk mengatasi infeksi kulit, termasuk ruam dan eksim, membersihkan luka, mencegah beberapa penyakit menular, serta membantu penanganan patah tulang. Penelitian ini bertujuan untuk mengidentifikasi kandungan fitokimia dan mengevaluasi aktivitas antibakteri dari berbagai ekstrak pelarut daun dan kulit batang *F. trimenii* terhadap *Staphylococcus aureus* dan *Escherichia coli*. Daun dan kulit batang yang segar dan sehat dikumpulkan dari Peradeniya Royal Botanical Garden, kemudian dimaserasi secara terpisah menggunakan metanol, aseton, dan petroleum eter selama 48 jam. Aktivitas antibakteri diuji menggunakan metode difusi sumuran agar, dengan kloksasilin dan siprofloksasin sebagai antibiotik pembanding masing-masing untuk *S. aureus* dan *E. coli*. Konsentrasi hambat minimum atau minimum inhibitory concentration (MIC) ditentukan menggunakan metode mikrodilusi kaldu. Skrining fitokimia awal juga dilakukan terhadap seluruh ekstrak. Semua ekstrak menunjukkan aktivitas antibakteri terhadap kedua jenis bakteri, kecuali ekstrak kulit batang dengan petroleum eter yang tidak menunjukkan zona hambat. Ekstrak daun dengan petroleum eter menunjukkan aktivitas antibakteri paling kuat dengan nilai MIC sebesar 1,74 mg/mL terhadap *S. aureus* dan *E. coli*. Hasil skrining fitokimia menunjukkan adanya alkaloid, flavonoid, glikosida, polifenol, sterol, dan tanin pada ekstrak daun maupun kulit batang. Temuan ini menunjukkan bahwa ekstrak metanol, aseton, dan petroleum eter dari daun dan kulit batang *F. trimenii* memiliki aktivitas antibakteri in vitro yang nyata terhadap *S. aureus* dan *E. coli*. Hasil penelitian ini memberikan dukungan ilmiah terhadap penggunaan tradisional *F. trimenii* sebagai tanaman obat dan menunjukkan perlunya penelitian lanjutan secara in vivo untuk mengonfirmasi potensi antibakterinya.

Kata Kunci: *Ficus trimenii*; ekstrak daun; ekstrak kulit batang; aktivitas antibakteri; konsentrasi hambat minimum (MIC); skrining fitokimia.

1. Introduction

Antimicrobial activity refers to the ability of a substance or agent to inhibit or kill microorganisms such as bacteria, viruses, fungi, or parasites. Antimicrobial agents can work through various mechanisms, including disrupting cell walls, interfering with protein synthesis, inhibiting nucleic acid synthesis and disrupting cell membranes [1]. Antimicrobial resistance (AMR) occurs when microorganisms such as bacteria, viruses, fungi, and parasites evolve to withstand antimicrobial agents, making standard treatments ineffective. Bacterial AMR was estimated to be directly responsible for 1.27 million global deaths in 2019 and contributed to 4.95 million deaths [2]. Antimicrobial resistance requires a coordinated effort at the global, national, and local levels to ensure the continued effectiveness of antimicrobial agents in treating infectious diseases.

In recent decades, there has been a growing focus on naturally occurring antimicrobials, especially medicinal plants with antimicrobial properties. This interest has prompted increased scientific research into their possible applications in contemporary medicine, as medicinal plants contain various bioactive compounds known as phytochemicals, including alkaloids, flavonoids, terpenoids, and phenolic acids, which possess antibacterial properties [3].

Ficus trimenii plant is a native plant in Sri Lanka and India that belongs to the Moraceae family [4]. The leaves are thick ovate and dark green colour. Also, the leaves have a parallel vein structure (**Figure 1**).

Figure 1. Plant of *Ficus trimenii*

It is commonly used in ayurvedic medicine for the treatment of skin infections such as rashes, eczema, wound cleansing, preventing some communicable diseases and fractures. Particularly, this plant's bark and leaf are used for wound cleansing and skin infections. Some of the other Ficus species possess anti-inflammatory, antimicrobial, antidiabetic, antiarthritic, anticancer, hepatoprotective, neuroprotective, and wound-healing properties; however, substantial gaps remain in the phytochemical and pharmacological characterization of numerous Ficus taxa [5]. Despite the widespread traditional use of *F. trimenii* in managing skin infections, scientific investigation has largely failed to verify its therapeutic claims. To date, no studies have evaluated the antibacterial activity or phytochemical composition of *F. trimenii* leaves and bark, representing a significant knowledge gap. Thus, the novelty of the present study lies in providing a systematic first evaluation of the antibacterial potential of the leaves and bark of *F. trimenii*. Further, scientific validation of this traditionally important medicinal plant is essential to determine its potential as a source of novel antibacterial agents, particularly in the context of increasing antimicrobial resistance and the growing demand for evidence-based herbal medicines. Therefore, this study aims to evaluate the antibacterial activity of methanol, acetone, and petroleum ether extracts of the leaves and bark of *F. trimenii* against *Staphylococcus aureus* and *Escherichia coli*, using cloxacillin and ciprofloxacin as standard antibiotics, while also providing the first phytochemical assessment of these plant materials.

2. Methods and Materials

Material

Methanol, acetone, petroleum ether, sterile water, Muller-Hinton Agar, Muller Hinton Broth, Ciprofloxacin, Cloxacillin tablets were used for this study. Equipment included a rotary evaporator, incubator, analytical balance, hot air oven, and autoclave.

Collection of plant material and preparation of plant extracts

Fresh and healthy leaves and bark were collected from the Peradeniya Royal Botanical Gardens in March. The plant was identified and authenticated (Ref. No.: 2024/334) by the National Herbarium, Royal Botanic Garden, Peradeniya as *Ficus trimenii King ex Trimen*. Extraction was performed using three solvents with different polarities to obtain a broader range of phytochemicals from the plant material. An amount of 200 g of fresh leaves and bark was washed and cleaned using tap water, followed by distilled water. The cleaned plant materials were chopped into smaller size

pieces, and these pieces were shade dried. An amount of 175 g of dried leaves and bark was obtained. It was then milled into a fine powder using a clean and sterile grinder and sieved using a 45 µm sieve. The powder was tightly packed in an airtight container with desiccant (silica gel) and stored at 4 °C a refrigerator for further use. Methanol around 250 mL was added to the 25 g coarse powders of leaves and bark materials separately and kept for two days (48 hours) at room temperature with intermittent shaking. Then the supernatant was decanted and filtered through Whatman No 1 filter paper (diameter: 24 cm, pore size 11 µm) under reduced pressure. The solvent was removed under reduced pressure using a rotary evaporator at 45 °C. The resulting crude extract was stored at 4 °C a refrigerator until further use [6]. The above procedure was repeated for acetone, and petroleum ether solvent extracts.
Yield in percentage of crude extract will be calculated as:

$$\text{Yield in percentage of crude extract} = \frac{\text{Dried crude extract weight}}{\text{Dried raw materials weight}} x\ 100\%$$

Phytochemical screening of plant extracts

Methanol, acetone, and petroleum ether extracts from the bark and leaves of *F.trimenii* were analyzed for phytochemical compounds such as alkaloids, flavonoids, phenolic compounds, saponins, tannins, glycosides, polyphenols, gums and mucilage, and sterols. The analysis followed methods described in previous studies by Usman et al. (2009) and Needhiraja et al. (2024) on various medicinal plants in the Ficus family [7], [8].

Test for alkaloids

Wagner's test

A few drops of Wagner's reagent were added into each 1 mL of crude extract. The alkaloids were indicated by the presence of brown of reddish-brown precipitate.

Test for flavonoids

Lead acetate test

For each 1 mL of crude extract, a few drops of 10% lead acetate solution were added. The flavonoids were indicated by the white or yellow precipitate formation.

Test for tannins

Ferric Chloride test

An amount of 50 mg of extract was dissolved in 5 mL of distilled water and few drops of neutral 5% ferric chloride solution were added into this. The presence of tannins was indicated by dark green colour.

Test for phenolic compounds

Gelatin test

An amount of 50 mg of extract was dissolved in 5 mL of distilled water and 4 to 5 drops of 1% gelatine solution containing 10% NaCl were added. The presence of phenolic compounds was indicated by the white colour precipitation.

Test for saponins

Froth test

Few amounts of each powdered crude extract were added to 2-3 mL of distilled water and shaken vigorously. The saponins were indicated by the formation of stable honeycomb froth presenting at least for 30 minutes.

Test for glycosides

Keller- killiani's test

1 mL of Glacial acetic acid and 1 mL of 5% ferric chloride solution were added into each 1mL of crude extract. 2 mL of conc. H_2SO_4 were added carefully to the side of the test tube. Glycosides were indicated by the reddish-brown colour appearance at the junction of the two layers.

Test for polyphenols

1 mL of 1% ferric chloride and 1 mL of 1% potassium ferricyanide were added into 1 mL of each crude extract. The presence of polyphenols was indicated by the fresh reddish blue colour.

Test for gum and mucilage

The amount of 100 mg of the extract was dissolved in 10 mL of distilled water and 2 mL of absolute alcohol were added to this with constant stirring. The presence of gums and mucilage were indicated by cloudy or white colour precipitate.

Test for Sterols

Salkowski's test

A small amount of crude extract was mixed with 2 mL of chloroform, and 2 mL of conc. H_2SO_4 was added carefully, and the mixture was shaken gently. The presence of cholesterol was indicated by the reddish-brown colour.

Evaluation of antibacterial activity

The in vitro antibacterial activity of methanol, acetone, and petroleum ether extracts of *F. trimenii* leaves and bark was evaluated using the agar well diffusion method. All the procedures were done under aseptic conditions. Reference antibiotics such as cloxacillin and ciprofloxacin were used as the positive control. Methanol, acetone and petroleum ether were used as negative control. All the experiments were carried out in triplicate.

Preparation for test samples and positive control

Methanol, acetone, and petroleum ether extracts of leaves and bark were dissolved in methanol, acetone, and petroleum ether, respectively. An amount of 100 mg of methanol extract of leaves was dissolved in 1 mL of the respective solvent and shaken well to prepare a 100 mg/mL stock solution. A series of concentrations (50 mg/mL and 25 mg/mL) was prepared by serial dilution. The same procedure was followed to prepare the other extracts [9].

500 mg of ciprofloxacin and cloxacillin tablets were separately transferred into sterile containers with sterile caps. Then, 5 mL of sterile distilled water was added to the container and stirred until fully dissolved (100 mg/mL). A stock solution was prepared. From the stock solution, 1 mL was transferred into a sterile flask, and sterile distilled water was added to 200 mL to prepare a 5 µg/mL solution [10].

Bacterial strain collection

Staphylococcus aureus (Gram-positive; aerobes, ATCC 25923) and *Escherichia coli* (Gram-negative; aerobes, ATCC 25922) were obtained from Teaching Hospital Jaffna.

Preparation of inoculum

Fresh overnight bacterial strains cultured on Muller Hinton Agar plates at 37˚C were used for plate inoculation. The inoculum density was 1×10^8 cells/mL, compared with the 0.5 McFarland standard solution of $BaSO_4$. Microbial inoculum was prepared by dissolving loop full of colonies of test microbes in 10 mL of physiological saline until the turbidity is at 0.5 McFarland standard. (Equivalent to 1×10^8 CFU/ mL) [11].

Agar well diffusion assay

A sterile swab was dipped into the bacterial suspension of *S. aureus* within 15 minutes after adjusting the turbidity of the inoculum suspension. The swab was rotated several times, pressing firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. Then it was inoculated onto the dried surface of a Muller Hinton agar plate by streaking the swab over the entire sterile agar surface. The procedure was repeated two more times and the plate was rotated for 60˚ each time to ensure an even distribution of inoculum. The top of the plate was replaced and allowed to sit for 3-5 minutes, but no longer than 15 minutes, to absorb any excess surface moisture before preparing wells. The above procedure was carried out for bacterial suspension of *E. coli*. Five wells, each consisting of a 6 mm diameter, were made in each agar Petri dish of solidified agar medium (keeping a distance of more than 24 mm between the centers of two wells). Wells were cut in the agar with the help of a sterilized cork-borer (hollow stainless- steel gel cutter). An amount of 100 µL of 100 mg/mL, 50 mg/mL, and 25 mg/mL of sample, positive control, and negative control were incorporated into the wells in the inoculated agar plate. The plates were kept upright in an incubator at 37 °C for 15 minutes after incorporating the samples into the wells. Plates were incubated aerobically for 24 hours at 37℃. All the tests were done in triplicate. After incubation, zones of inhibition caused by extracts, negative controls, and positive controls were measured to the nearest "mm" using a transparent scale/sliding caliper [12].

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of plant extracts was determined by broth microdilution susceptibility testing using 96-well plates.

An amount of 50 µL sterile Muller Hinton Broth was placed into each well. A stock concentration of 100 mg/mL was prepared using the respective solvents. From that stock solution, 50 µL of sample was placed in the first row of the first well of the 96-well plate. A serial dilution was done along the row and 50 µL of mixture solution was removed from the final wells of the row to obtain a concentration gradient from 100 mg/mL to 0.049 mg/mL. 30 µL of MHB was added to each well and 10 µL of *E. coli* bacterial suspension (1×10^8 CFU/mL) was added to each well. The final bacterial suspension concentration was 11×10^7 CFU/mL. After adding the bacterial inoculum and adjusting the final well volume to 90 µL, the first well had a final extract concentration of 27.8 mg/mL, followed by two-fold serial dilutions to obtain a final concentration range of 27.8–0.014 mg/mL.

The growth control contained MHB and the bacterial suspension without plant extract and confirmed bacterial growth under the test conditions. The sterility control contained MHB without bacterial inoculum and confirmed the medium's sterility. A solvent control containing the corresponding extraction solvents, methanol, acetone, and petroleum ether, was included to determine whether the solvent itself affected bacterial growth. Ciprofloxacin or cloxacillin (5 µg/mL) served as the positive antimicrobial control. All tests were done in triplicate.

The micro well plates were incubated overnight at 37℃. After the incubation, 10 µL (1.5 mg/mL) of resazurin dye was added to each well as an indicator. The micro well plates were incubated again for 1-2 hours at 37℃. MIC was defined as the lowest concentration that retained the blue color of resazurin, indicating no microbial growth. The above procedures were repeated with other extracts and an *S. aureus* bacterial suspension [13].

Data analysis

The results of preliminary phytochemical tests indicated the presence of phytochemicals as "+" and the absence of phytochemicals as "–". Each experiment was done in triplicate. The diameter of the zone of inhibition (in millimeters) was used to screen the antimicrobial activity of samples. Results were expressed as Mean ± standard deviation. The MICs were analyzed using one-way ANOVA followed by the Tukey test in SPSS version 23 to determine statistical significance between extracts for a particular bacterial strain. $P \leq 0.05$ was considered statistically significant[14].

3. Results and Discussion

Yield in percentage of crude extracts

The yield in percentage of methanol, acetone and petroleum ether extracts of bark and leaves of *F. trimenii* is tabulated in following **Table 1**.

Table 1. Yield in percentage of methanol, acetone and petroleum ether extracts of bark and leaves of *F. trimenii.*

Extracts	Weight of powdered leaves/barks (g)	Mean weight of crude extract (g)	Yield in percentage (% w/w)
Leaves			
Methanol	25	0.68	2.72
Acetone	25	0.50	2.0
Petroleum Ether	25	0.40	1.6
Bark			
Methanol	25	0.67	2.68
Acetone	25	0.76	3.04
Petroleum Ether	25	0.56	2.24

The highest yield in percentage was observed for the acetone extract of bark. Meanwhile, the lowest yield in percentage was observed for the petroleum ether extract of leaves. Increasing the polarity of the extracting solvent improved yield (%), except for the acetone extract of bark.

The yield in percentages in the extraction reveal the efficiency of different solvents in isolating bioactive compounds from leaves and bark. For the leaves, methanol yielded 2.72% of the crude extract, which is higher than acetone's yield of 2.00% and petroleum ether's lower yield of 1.60%. This suggests that methanol is the most effective solvent for extracting compounds from the leaves. Similarly, for the bark, acetone achieved the highest yield at 3.04%, followed closely by methanol at 2.68% and petroleum ether at 2.24%. Overall, these results indicate that while methanol is generally effective for both plant parts, acetone particularly excels in extracting from bark, highlighting the importance of solvent choice in maximizing yield. Shehzad *et al. al* indicated that the highest yield in percentage was observed for the methanol extract of leaves of *F. bengalensis,* which is similar to this study[15]. Further, the percentage yield of the methanol extract of leaves was higher than that of the bark. The highest extractive value of the methanol extract of leaves may be due to better solubility of the bioactive components in organic solvents.

Preliminary phytochemical screening

Phytochemical screening of the plant extract was done to identify the main classes of secondary phytochemical compounds responsible for antibacterial activity. Observations are tabulated in **Table 2**.

Table 2: Preliminary phytochemical screening of methanol, acetone and petroleum ether extracts of leaf and bark of *F. trimenii*

Phyto chemicals	**Name of the Tests**	**Leaves**			**Bark**		
		Methanol	Acetone	Petroleum Ether	Methanol	Acetone	Petroleum Ether
Alkaloids	Wagner's test	-	-	+	-	+	+
Flavonoids	Lead acetate test	+	-	+	+	+	-
Saponins	Froth test	-	-	-	-	-	-
Tannins	Ferric chloride test	+	+	+	+	+	+
Phenolic Compounds	Gelatine test	+	+	-	+	+	-
Glycosides	Keller-killiani's test	+	-	-	+	+	-
Sterols	Salkowski's test	+	+	+	+	+	+
Polyphenols		+	+	-	+	+	-

Methanol extracts of leaves showed positive results for flavonoids, tannins, phenolic compounds, glycosides, sterols, and polyphenols, while alkaloids and saponins were absent. Methanol extracts of bark showed positive results for flavonoids, tannins, phenolic compounds, glycosides, sterols, and polyphenols, while alkaloids and saponins were absent. Cokera & Adeniyi-Aogo screened the phytochemical presence in the methanol extract of leaves of *F. mucuso and F. vogeli*, which showed positive results for sterols, alkaloids, tannins, saponins, terpenoids, anthraquinones, and flavonoids [10]. However, there are no studies done on the phytochemical screening of the methanol extract of the leaves and bark of *F. trimenii.*

Acetone extracts of leaves gave positive results for tannins, phenolic compounds, sterols and polyphenols, while alkaloids, flavonoids, saponins, and glycosides were absent. Acetone extracts of bark showed positive results for alkaloids, flavonoids, tannins, phenolic compounds, glycosides, sterols, and polyphenols, while saponins were absent.

Petroleum ether extracts of leaves revealed the presence of some secondary metabolites such as alkaloids, flavonoids, tannins, and sterols. Meanwhile, phenolic compounds, saponins, polyphenols, and glycosides were negative. Petroleum ether extracts of bark showed alkaloids, tannins, and sterols. Meanwhile, phenolic compounds, flavonoids, glycosides, saponins, and polyphenols were negative.

These findings illustrate the diverse range of secondary metabolites present in *F. trimenii*, highlighting how different solvents can effectively extract various

phytochemicals, with certain compounds being more prevalent depending on the extraction method used.

Evaluation of antibacterial activity

The in vitro antibacterial activity of methanol, acetone, and petroleum ether extracts of *F. trimenii* leaves and bark was evaluated against two clinically important pathogenic bacterial species, *E. coli* and *S. aureus*. The test was done in triplicate for each extract concentration, negative control, and positive control. Zones of inhibition of methanol, acetone, and petroleum ether extracts of leaves and barks of *F. trimenii* are shown in **Table 3**.

Table 3. Zone of inhibition of methanol, acetone and petroleum ether extracts of leaves and bark of *F. trimenii* against *S. aureus* and *E. coli*

Test sample	Concertation (mg/mL)	(M±SD) Leaves		(M±SD) Bark	
		S. aureus	*E. coli*	*S. aureus*	*E. coli*
Methanol	100	16.67±3.26	17.33±0.94	17.67±4.09	16.33±0.47
	50	15.00±3.29	11.00±1.63	11.67±4.57	11.33±1.24
	25	13.33±3.39	6.00±0.81	10.33±4.77	8.67±0.94
Acetone	100	14.67±4.90	15.33±0.47	18.00±1.63	23.00±1.41
	50	10.00±1.63	11.66±1.24	14.00±0.81	17.67±2.05
	25	-	9.67±1.69	12.33±0.47	11.67±2.35
Petroleum Ether Extraction	100	19.33±1.69	19.00±0.00	-	-
	50	13.66±2.05	17.00±0.81	-	-
	25	9.33±0.94	12.67±0.94	-	-
Cloxacillin	0.005	25.94±0.91	-	25.94±0.91	-
Ciprofloxacin	0.005	-	32.00±1.67	-	32.00±1.67
Negative control	NA	NA	NA		NA

Note: NA-no activity

The leaves extracts of methanol, acetone, and petroleum ether produced zones of inhibition 16.67±3.26 mm, 14.67±4.90 mm, and 19.33±1.69 mm against *S. aureus*, respectively. For *E. coli*, the inhibition zones were 17.33±0.94 mm, 15.33±0.47 mm, and 19.00±0.00 mm at a concentration of 100 mg/mL. The bark extracts of methanol and acetone produced zones of inhibition of 17.67±4.09 mm and 18.00±1.63 mm against *S. aureus*, respectively. For *E. coli*, the inhibition zones were 16.33±0.47 mm and 23.00±1.41 mm at a concentration of 100 mg/mL. As the concentration of extracts increases, the zone of inhibition expands, indicating a direct correlation between bioactive compound levels and antibacterial activity.

Among the plant extracts, the petroleum ether leaves extract at 100 mg/mL showed the highest antibacterial activity against *S. aureus*. The acetone bark extract at 100 mg/mL showed the highest antibacterial activity against *E. coli*. The results revealed that the methanol, acetone, and petroleum ether extracts of the leaves of *F. trimenii*, and the methanol and acetone extracts of the bark of *F. trimenii*, efficiently suppress the growth of the pathogens *S. aureus* and *E. coli* with variable potency. However, the petroleum ether bark extract of *F. trimenii* has not been shown to suppress the growth of *S. aureus* and *E. coli*. Differences in Antibacterial activity could be due to differences in chemical composition, functional groups present in active constituents, the ratio of active

constituents, synergistic interactions with major and minor constituents, and the mechanism of action of bioactive constituents. In addition, activity depends on extraction time, temperature, and the time-lapse between extraction and analysis of the components, which affect the quality and quantity of phytochemicals in the extract [16].

Labu *et al*. investigated the antibacterial activity of the methanol leaf extract of *Ficus nervosa* (L.) and its fractions obtained using ethyl acetate, petroleum ether, chloroform, and carbon tetrachloride, along with methanol and aqueous extracts. Among the tested extracts and fractions, the carbon tetrachloride fraction exhibited a higher zone of inhibition than methanol, measuring 19.5 ± 0.15 mm against *S. aureus* and 19.3 ± 0.52 mm against *E. coli*. Furthermore, compared with the previous study, the methanol extract in the present study showed a larger zone of inhibition. This difference may be attributed to the fractionation of the methanol extract using solvents of different polarities, which may have resulted in the distribution of phytochemicals with varying polarities among the different solvent fractions.*[17]*. Kassaw *et al*. studied the antibacterial activity of the methanol extract of *Ficus palmata* and showed a similar zone of inhibition for *S. aureus* but less *for E. coli*. This might be attributed to the presence of different phytoconstituents[18]. Ramakrishnaiah *et al*. indicated that the ethanol extract of the bark of *Ficus religiosa* exhibited a zone of inhibition of 2.4 mm at 400 mg/mL against *E. coli*. Compared with this study, this zone of inhibition is low, possibly due to differences in solvent and species [19]. However, the agar well diffusion method is suitable for initial screening purposes. Nevertheless, it has limitations, as the diffusion characteristics of various solvents and extracts in agar may differ.

Minimum Inhibitory Concentration (MIC) of the Plant Extracts

MIC is defined as the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism. The growth of the tested organism was detected using resazurin dye by the appearance of a pink color, while the absence of microorganisms was indicated by a violet color. The well where violet color persisted until the transition into a pink color was identified as the well containing the sample at the minimum inhibitory concentration (MIC) [20] MIC of methanol, acetone and petroleum ether extracts of leaves and methanol and acetone barks of *F. trimenii* were found against the *S. aureus* and *E. coli*. MIC was not assessed for the petroleum ether bark extract, as no zone of inhibition was observed at a concentration of 100 mg/mL. The MIC values of extracts are given in **Table 4**.

Table 4. Minimum Inhibitory Concentrations of *Ficus trimenii* Leaf and Bark Extracts against *S. aureus* and *E. coli*

Bacterial Strain	**Minimum Inhibitory Concentration (mg/ml)**				
	Methanol		Acetone		Petroleum Ether
	Leaves	Bark	Leaves	Bark	Leaves
E. coli	3.47^{a}	3.47^{a}	3.47^{a}	1.74^{b}	1.74^{b}
S. aureus	1.74^{b}	1.74^{b}	1.74^{b}	3.47^{a}	1.74^{b}

Note: The values are represented with different superscripts; in each row, the values differ significantly ($p<0.05$).

The MIC values against *S. aureus* ranked as follows: acetone extract of bark at 3.47 mg/mL > methanol extract of leaves, methanol extract of bark, acetone extract of leaves, petroleum ether extract of leaves at 1.74 mg/mL. Since lower MIC values indicate stronger antibacterial activity, the extracts with an MIC of 1.74 mg/mL demonstrated greater antibacterial activity than the acetone bark extract, which had an MIC of 3.47 mg/mL.

The MIC values against *E. coli* ranked as follows: Acetone extract of leaves at 3.47 mg/mL > methanol extract of leaves, methanol extract of bark, acetone extract of bark, and petroleum ether extract of leaves at 1.74 mg/mL. Since lower MIC values indicate stronger antibacterial activity, the extracts with an MIC of 1.74 mg/mL demonstrated greater antibacterial activity than the extracts, which had an MIC of 3.47 mg/mL.

A study conducted by Tikent *et al*. reported that hydroethanolic leaf extracts of *Ficus carica* L. exhibited MIC values of 128 and 64 µg/mL against *S. aureus* and *E. coli*, respectively [21]. Further, Sadiq et al evaluated the MIC of hydro methanol extract of the bark of *Ficus Platyphylla*, showed 250 and 500 mg/mL for *S.aureus* and *E.coli* respectively [22]. These MIC values were higher than those observed in the present study, indicating comparatively weaker antibacterial activity. The observed differences may be attributed to differences in the extraction solvent used, as well as geographical and environmental differences in the plant material.

Statistical analysis showed no significant differences in the MICs of the extracts for *S. aureus*, except for the acetone bark extract. Similarly, for *E. coli*, there were no significant differences among the MICs of methanol leaf and bark extracts and acetone leaf extract, while the acetone bark and petroleum ether leaf extracts also did not differ significantly. Previous studies have reported MIC values (78.12 µg/mL) for crude extracts of *F. chlamydocarpa* and *F. cordata* (which belong to the Ficus species) against both *S. aureus* and *E. coli*, which is lower than the MIC value reported in this study [23].

This study has several limitations that should be considered when interpreting the findings. First, the antibacterial evaluation is preliminary in vitro screening, so the observed activity cannot be directly extrapolated to therapeutic efficacy in vivo. Second, the extracts demonstrated antibacterial activity at relatively high concentrations, which may limit their practical applicability and indicate the need for further investigation at lower concentrations. Qualitative phytochemical screening also provides only preliminary information about the chemical constituents and does not establish their concentration or biological relevance. Finally, this study did not isolate or structurally characterize the active antibacterial compounds. Further research should therefore include quantitative phytochemical analysis, bioassay-guided fractionation, isolation and identification of active compounds, and in vivo evaluation to confirm the antibacterial potential of *Ficus trimenii.*

4. Conclusion

This study demonstrated that the methanol, acetone, and petroleum ether extracts of leaves, as well as the methanol and acetone extracts of bark of *F. trimenii* exhibit antibacterial activity against *S. aureus* and *E. coli*. Among them, the acetone extract of bark and the petroleum ether extract of leaves of *F. trimenii* were more effective against *E. coli*. The leaf and bark extracts are more effective against *S. aureus*. Phytochemicals such as alkaloids, flavonoids, glycosides, polyphenols, and tannins may be responsible for their antibacterial action. The extracts showed preliminary in vitro antibacterial activity; however, further fractionation and characterization are needed to identify the active compounds responsible for this activity.

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Conflict of interest:

Authors declare no conflict of interest.

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