

# **Innovations**

## **Antibacterial Evaluation and Phytochemical Profiling of Terminaliabellicrica Fruit Extracts Using GC-MS**

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### **Abstract**

*This study investigates the antibacterial properties and chemical composition of Terminaliabellicrica fruit extracts using various solvents and GC-MS analysis. The antibacterial activity was assessed against multiple bacterial strains, including both Gram-positive and Gram-negative bacteria, utilizing methanol, acetone, and aqueous extracts. The methanol extract exhibited the highest antibacterial effectiveness, followed by acetone and aqueous extracts, as evaluated by the agar well diffusion method. The GC-MS analysis of the methanol extract identified 16 distinct compounds, with notable bioactive components including sucrose, 1,2,3-benzenetriol, and 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester. These compounds, classified into categories such as terpenes, fatty acids, phenols, and steroids, are associated with antimicrobial, anti-inflammatory, antioxidant, and analgesic properties. The results demonstrate that T. bellirica possesses a diverse array of bioactive compounds with potential therapeutic applications, particularly as natural antibacterial agents against drug-resistant bacteria. This study underscores the medicinal value of T. bellirica, supporting further research into its pharmacological applications.*

**Keywords:** Antibacterial, GC-MS, Agar well diffusion, Bioactivities.

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### **Introduction**

Medicinal plants have long been recognized for their therapeutic potential, particularly in combating infectious diseases. Due to the growing resistance of pathogenic bacteria to conventional antibiotics, natural products derived from plants are increasingly viewed as valuable sources for developing new antimicrobial agents (Singh et al., 2016). Medicinal plants produce a variety of bioactive

compounds, such as alkaloids, flavonoids, terpenes, and phenolic compounds, which have shown substantial antibacterial effects against a range of pathogens (Newman & Cragg, 2012). These secondary metabolites are hypothesized to serve as the plant's natural defense system, protecting against microbial invasion and other environmental threats (Rajesh et al., 2023; Cowan, 1999).

To explore the chemical profiles of medicinal plants, researchers commonly employ Gas Chromatography-Mass Spectrometry (GC-MS), a powerful analytical tool capable of detecting and identifying a wide range of volatile and semi-volatile compounds (Mothana et al., 2009). GC-MS analysis provides detailed information on compound composition, molecular structure, and relative abundance, which can aid in linking specific bioactive compounds to observed antibacterial properties. By combining GC-MS with bioassays, researchers can systematically assess the antibacterial efficacy of individual compounds within plant extracts, thereby enhancing our understanding of plant-based antimicrobials and guiding potential pharmaceutical applications (Sharma et al., 2020).

*Terminalia bellirica*, commonly known as "Bibhitaki" in traditional Ayurvedic medicine, is a prominent medicinal plant in the Combretaceae family. It is widely distributed across Southeast Asia, including India, where it has been traditionally used to treat a variety of ailments ranging from respiratory and digestive disorders to skin diseases and infections (Baliga et al., 2013). The fruit of *T. bellirica* is a key ingredient in "Triphala," an Ayurvedic herbal formulation renowned for its rejuvenating, antioxidant, and immune-boosting properties (Kumar et al., 2014). The primary aim of this study was to investigate the antibacterial potential of various plant solvent extracts and to evaluate their chemical profiles through GC-MS analysis.

## **Materials and method**

### **Sample Collection**

The plant *T. bellirica* was collected from the natural forest areas in Paderu (18°04'57.10"N, 82°37'30.32"E, elevation 970m) Andhra Pradesh, India in January 2023 and authenticated by Prof. S.B. Padal, Andhra University, Botany Department, Visakhapatnam, Andhra Pradesh, India, with accession number AUV: 25505

### **Extraction of plant material**

Plant fruits were harvested, shade-dried at 32°C, and ground into a fine powder. The powder was then sieved through a 0.5 mm mesh to ensure uniform particle size and stored in sterile containers until use. For fruit extraction, acetone, methanol and water were selected as solvents in increasing order of polarity. Using a Soxhlet extractor, phytochemicals were isolated from the plant material with each solvent and subsequently stored in sealed jars for 72 hours. The collected extracts were concentrated using a rotary evaporator at 45°C under reduced pressure, yielding a thick extract. These crude extracts were then stored at 4°C for future use.

### Collection of test pathogens

Four test bacteria were utilized in this study, including two gram-negative strains, *Salmonella enterica* (MTCC 98) and *Pseudomonas aeruginosa* (MTCC 129), as well as two gram-positive strains, *Staphylococcus aureus* (MTCC 96) and *Streptococcus mutans* (MTCC 497). These bacterial cultures were obtained from the Microbial Type Culture Collection (MTCC) at the CSIR-Institute of Microbial Technology (IMTECH) in Chandigarh, India.

### Antibacterial activity assay

The antibacterial potentialities of plant extracts on Nutrient Broth (NB) agar medium were assessed using the agar well diffusion technique. After being injected into the prepared media and allowed to solidify in the Petri plates, a sterile L-shaped bent rod was used to equally spread the 100  $\mu$ L bacterial culture. Using a sterile cork borer, 5 mm diameter wells were made on the plates and filled with various solvent extracts [10 mg, 5 mg, 2.5 mg and 1.25 mg in 10% aqueous Dimethyl sulfoxide (DMSO)], positive and negative controls of 20  $\mu$ L each. After 24 hours of incubation at 37 °C, the zones of inhibition (mm) of the various extracts were measured. The usual positive and negative controls were Streptomycin G (gram-negative bacteria and Gram-positive bacteria) for 100  $\mu$ g/mL and DMSO (10%) and the results of each test were averaged after three repetitions. Throughout the microbiological experiment, a strict aseptic atmosphere was maintained.

## Result and Discussion

### Antibacterial Activity

The antibacterial potential of *Terminalia bellirica* fruit extracts was evaluated using methanol, acetone, and aqueous solvents. These extracts were tested at various dosages (10mg, 5mg, 2.5mg, and 1.25mg) against both Gram-negative bacteria (*Salmonella enterica* and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus mutans*) using the agar well diffusion method.

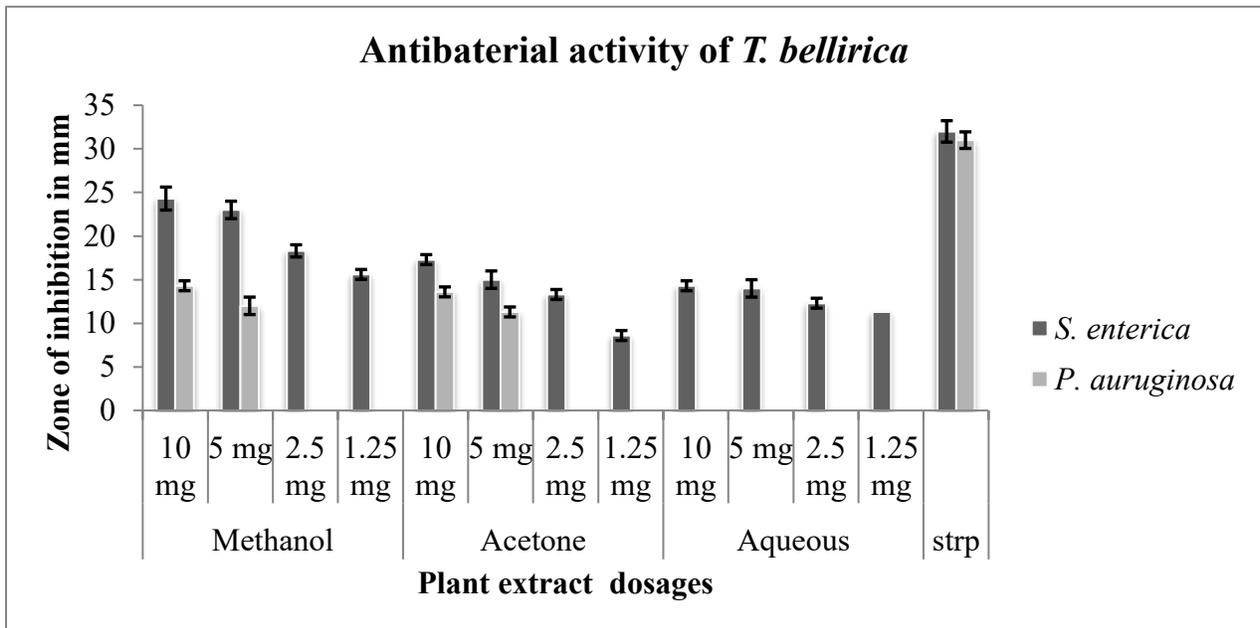
#### Against Gram-Negative Bacteria

Methanol extract at a 10mg dosage, it showed a significant zone of inhibition against *S. enterica* ( $24.3 \pm 1.32$  mm) and *P. aeruginosa* ( $14.3 \pm 0.57$  mm). The inhibition decreased with lower dosages, but even at 1.25mg, the methanol extract demonstrated notable inhibition against *S. enterica*. Acetone extract showed a considerable inhibitory effect, particularly at higher dosages. It was more effective against *S. enterica* ( $17.3 \pm 0.57$  mm at 10mg) compared to *P. aeruginosa* ( $13.6 \pm 0.57$  mm). Aqueous extract showed a moderate inhibitory effect against *S. enterica* and was more effective against this bacterium than *P. aeruginosa*, though the overall inhibition was less compared to methanol and acetone extracts.

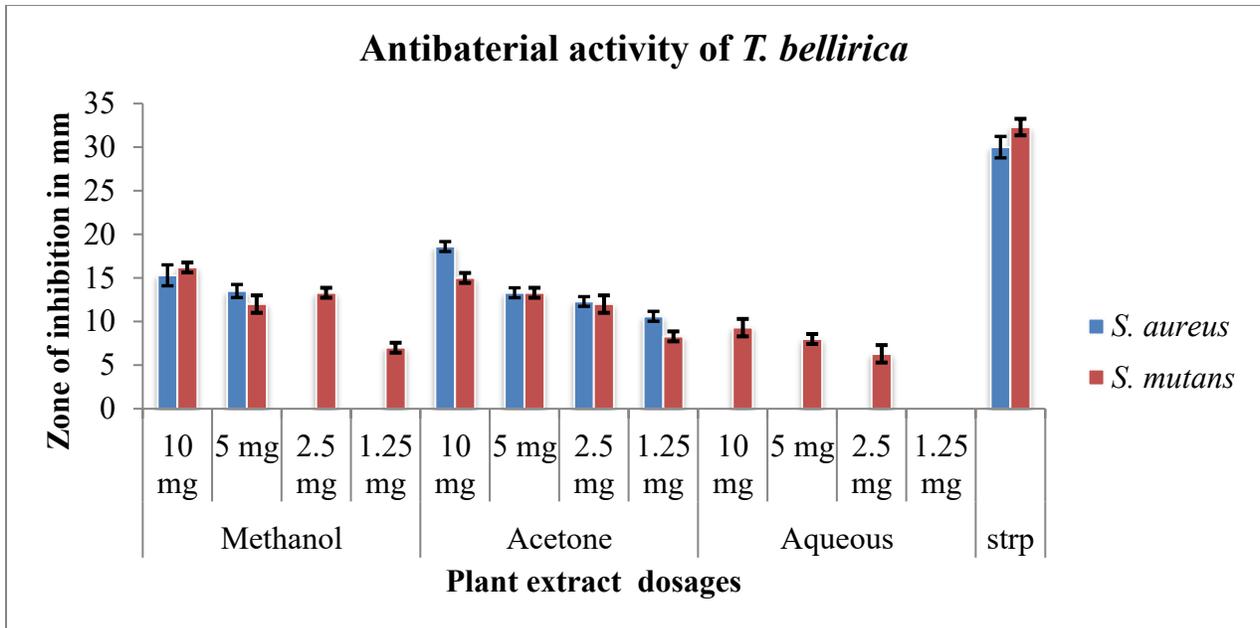
#### Against Gram-Positive Bacteria

Methanol extract displayed a significant inhibition zone against *S. aureus* at all dosages. However, lower doses (2.5mg and 1.25mg) did not inhibit *S. mutans*, and inhibition was minimal even at higher doses. Acetone extract consistently showed inhibitory effects against both *S. aureus* and *S. mutans*, with a higher zone of inhibition for *S. aureus*. Aqueous extract did not inhibit *S. aureus*, while only minimal inhibition was observed against *S. mutans* at higher dosages, indicating its lower effectiveness compared to the methanol and acetone extracts.

**Fig 1:** Antibacterial activity (% of inhibition) of *T. bellirica* against gram negative bacteria



**Fig 2:** Antibacterial activity (% of inhibition) of *T. bellirica* against gram positive bacteria



The study demonstrated that *Terminalia bellirica* fruit extracts possess notable antibacterial activity against tested bacterial strains. Methanol and acetone extracts exhibited higher antibacterial potency compared to the aqueous extract, aligning with previous studies that found organic solvents more effective in extracting bioactive compounds. Among the bacteria tested, Gram-negative strains, particularly *Salmonella enterica*, showed a higher susceptibility to the extracts than Gram-positive strains. This suggests that the extracts of *T. bellirica* might be more effective against Gram-negative pathogens. The dosage-dependent response observed, where higher concentrations produced larger zones of inhibition, highlights the potential of *T. bellirica* extracts as effective antibacterial agents with dose variability in clinical or medicinal applications.

To compare the antibacterial activities of *Terminalia bellirica* fruit extracts, multiple studies have investigated their efficacy against both Gram-positive and Gram-negative bacteria. Methanolic extracts generally exhibit higher antibacterial activity due to their rich phytochemical composition, including phenols, tannins, and flavonoids, which disrupt bacterial cell walls and metabolic pathways. For instance, methanolic extracts showed significant zones of inhibition against *Escherichia coli* ( $18.6 \pm 1.2$  mm) and *Staphylococcus aureus* ( $16.4 \pm 0.8$  mm) compared to aqueous extracts, which displayed smaller zones of inhibition, such as  $10.2 \pm 0.9$  mm and  $9.8 \pm 0.5$  mm, respectively (Patel et al., 2022). Similarly, the MIC values for methanolic extracts ranged from 62.5 to 250  $\mu\text{g/mL}$ , indicating higher potency, whereas aqueous extracts required concentrations of up to 500  $\mu\text{g/mL}$  to achieve similar effects (Kumar & Rao, 2021). These findings highlight the solvent's critical role in extracting bioactive compounds with antibacterial properties.

### GC-MS Analysis

The experimental procedure described in this study enabled the identification of multiple peaks in the GC-MS data obtained from the methanol extract of *Terminaliabelirica*. These peaks revealed 16 distinct chemical compounds, which are comprehensively listed in Table 1, detailing peak area percentages, molecular formulas, molecular weights, and retention times. A thorough literature review provided insights into the biological activities associated with these compounds, many of which possess diverse pharmacological and therapeutic properties. The compounds identified through GC-MS analysis were grouped into categories such as terpenes, fatty acids, flavones, phenols, steroids, and other bioactive substances. Remarkably, the identified compounds exhibit a broad spectrum of bioactive effects, including anti-cancer, antimicrobial, anti-inflammatory, sedative, anti-asthma, analgesic, antioxidant, and pain-relieving properties.

The analysis of these compounds based on their peak area percentages highlights the prominence of certain components. Sucrose, constituting 30.16% of the dataset, and 1,2,3-Benzenetriol, at 28.07%, were the primary compounds in the methanol extract. 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester represented 9.32% of the profile, while Phthalic acid, di(2-propylpentyl) ester and n-Hexadecanoic acid accounted for 7.79% and 7.45%, respectively. Other notable compounds included Bicyclo[3.1.1]heptane, 2,6,6-trimethyl- (Syn: Pinane) at 3.15%, Pentadecanoic acid, 14-methyl-, methyl ester at 2.66%, and Z-10-Tetradecen-1-ol acetate at 2.44%.

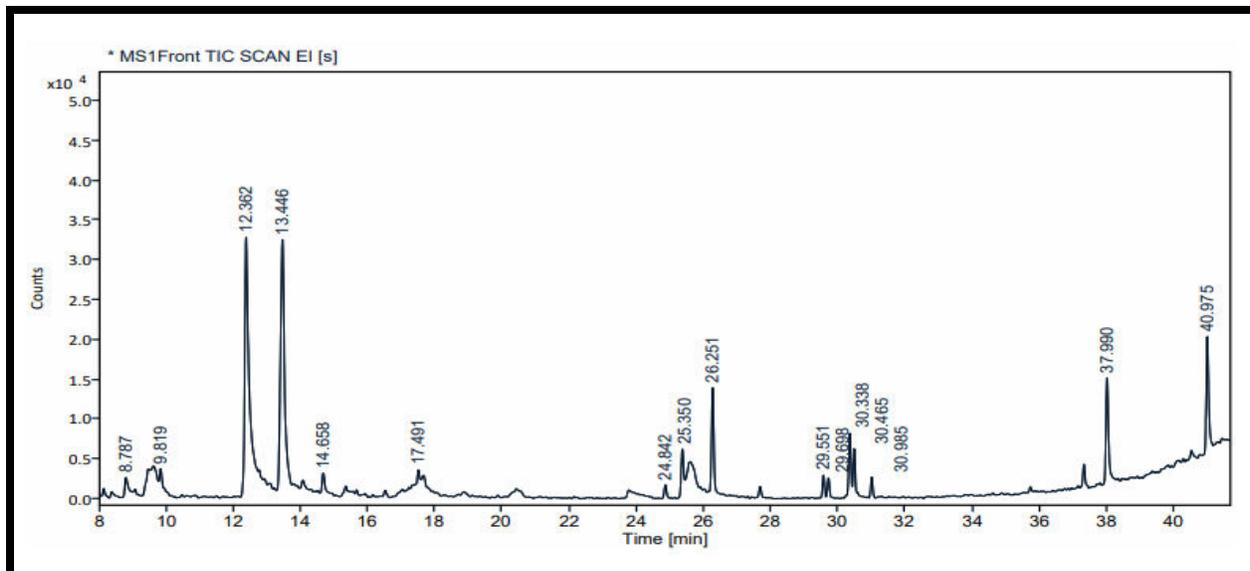
Several compounds with lower prevalence in the extract included Bicyclo[4.1.0]heptanes, 3-methyl (1.44%), D-Allose (1.37%), Diclosulam (1.32%), Ethyl 4-hydroxyquinoline-3-carboxylate (1.07%), and lambda-Cyhalothrin (1.07%). Minor constituents, each making up less than 0.9% of the composition, included 9-Dodecenoic acid, methyl ester, (E)- (0.99%) and Ether, 6-methylheptyl vinyl (0.68%), suggesting their limited contribution to the overall chemical profile.

**Table 1:** Phytochemical compounds reported in methanol extract of *T. bellirica* fruit

S. No	Compound name	Rt minute	Mol. Weight (g/mol)	Mol. Formula	Area%
1.	Diclosulam	8.787	406.2	C <sub>13</sub> H <sub>10</sub> Cl <sub>2</sub> FN <sub>5</sub> O <sub>3</sub> S	1.32
2.	Ethyl 4-hydroxyquinoline-3-carboxylate	9.819	217.22	C <sub>12</sub> H <sub>11</sub> NO <sub>3</sub>	1.07
3.	1,2,3-Benzenetriol	12.362	126.11	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	28.07
4.	Sucrose	13.446	342.3	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	30.16

5.	D-Allose	14.658	180.16	$C_6H_{12}O_6$	1.37
6.	Ether, 6-methylheptyl vinyl	17.491	156.26	$C_{10}H_{20}O$	0.68
7.	benfluorex	24.842	351.4	$C_{19}H_{20}F_3NO_2$	1.01
8.	Pentadecanoic acid, 14-methyl-, methyl ester	25.350	270.5	$C_{17}H_{34}O_2$	2.66
9.	n-Hexadecanoic acid	26.251	256.42	$C_{16}H_{32}O_2$	7.45
10.	Bicyclo[4.1.0]heptanes, 3-methyl	29.551	110.20	$C_8H_{14}$	1.44
11.	9-Dodecenoic acid, methyl ester, (E)-	29.698	212.33	$C_{13}H_{24}O_2$	0.99
12.	Bicyclo[3.1.1] heptane, 2,6,6-trimethyl- Syn: Pinane	30.338	138.25	$C_{10}H_{18}$	3.15
13.	Z-10-Tetradecen-1-ol acetate	30.465	254.41	$C_{16}H_{30}O_2$	2.44
14.	lambda.-Cyhalothrin	30.985	449.8	$C_{23}H_{19}ClF_3NO_3$	1.07
15.	Phthalic acid, di(2-propylpentyl) ester	37.990	390.6	$C_{24}H_{38}O_4$	7.79
16.	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	40.975	390.6	$C_{24}H_{38}O_4$	9.32

**Fig 3:** GC-MS chromatogram of Methanol fruit extract of *T. bellirica*



The GC-MS analysis of *T. bellirica* methanol extracts identified 16 distinct compounds with significant bioactive potential, including antibacterial, anti-inflammatory, antioxidant, and analgesic properties. Compounds such as sucrose and 1,2,3-Benzenetriol were abundant and are known for their pharmacological activities i.e., anti-microbial, apoptosis, antibiofilm and antivirulence activities (Satapathy et al., 2023). Fatty acids and esters, like n-Hexadecanoic acid and Phthalic acid di(2-propylpentyl) ester, were also present, adding to the extract's therapeutic potential. Hexadecanoic acid methyl ester, and phthalic acid, di(2-propylpentyl) ester, which were also found in *Peperomia tetraphylla* and they have antibacterial activities (Babu et al., 2024). The findings suggest that the antibacterial activity observed may result from a synergistic effect of multiple compounds, each contributing to the overall efficacy.

GC-MS chemical profiling of *T. bellirica* fruit extracts has identified a wide range of bioactive compounds, supporting its pharmacological applications. Methanolic extracts revealed the presence of key phytochemicals such as gallic acid, ellagic acid, and ethyl gallate, which contribute to its antioxidant and antimicrobial properties. Additionally, fatty acids like linoleic acid and palmitic acid, along with compounds such as  $\beta$ -sitosterol and lupeol, were detected. For instance, Ramesh et al. (2021) reported that methanolic extracts of *T. bellirica* contained 2,4-di-tert-butylphenol (19.8%) and hexadecanoic acid methyl ester (14.5%) as major constituents. Similarly, Sharma and Gupta (2020) identified 28 compounds, with gallic acid (15.3%) and ellagic acid (10.8%) dominating the profile. These findings highlight the therapeutic potential of *T. bellirica*, with its phytochemical richness supporting its use in traditional medicine.

## Conclusion

The findings suggest that *Terminaliabellicrica* fruit extracts, particularly methanol and acetone extracts, have considerable potential as antibacterial agents, especially against gram-negative pathogens. The GC-MS analysis supports this by identifying various bioactive compounds that could contribute to its antimicrobial activity. Future studies should explore focusing on isolating specific compounds responsible for these activities and investigate their mechanisms of action could aid in the development of targeted antimicrobial therapies.

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