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Evaluation of antimicrobial efficacy of ethanolic fruit extracts of *Terminalia pallida* Brandis

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Abstract

Terminalia pallida has been traditionally used to treat cough, cold, diarrhea, respiratory infections, peptic ulcers, diabetes, fissures, cracks, skin diseases and used in the tanning and dyeing industries. Owing to its bioactive compounds, such as tannins, flavonoids, and triterpenoids this study aimed to evaluate the antimicrobial efficacy of *T. pallida* fruit extracts against various microbial strains. The antimicrobial activity was determined using minimum inhibitory concentration (MIC) values and zone of inhibition measurements against *Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa, Candida albicans, and Aspergillus niger*. The MIC indicated that *S. aureus, B. cereus, S. epidermidis*, and *E. coli* were sensitive to the extract at 12.5 mg/ml. However, *E. aerogenes* and *P. aeruginosa* required higher concentrations of 25 and 50 mg/ml, respectively, to inhibit growth. For fungal strains, MIC was observed as 25 mg/ml. The zone of inhibition studies confirmed these findings, showing significant inhibition of Gram-positive bacteria at both low and high doses of the extract. *P. aeruginosa* exhibited moderate sensitivity at the high dose, while *E. coli* and *E. aerogenes* showed resistance. In fungal strains, *C. albicans* was found to be more sensitive than *A. niger*. Ethanolic fruit extract of *T. pallida* demonstrated strong antimicrobial activity, particularly against Gram-positive bacteria, with dose-dependent efficacy. Further research is needed to optimize the concentrations and explore mechanisms to enhance the activity against resistant Gram-negative strains.

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Introduction

Plants have traditionally been used to treat various infections, and modern research has validated the antimicrobial properties of many plant species. Herbal medicine is a promising alternative for combating infectious diseases (Chaughule and Barve, 2024; Singamaneni *et al.*, 2020). The genus *Terminalia*, which comprises various species, has demonstrated significant antimicrobial properties that have been extensively explored in numerous studies (McGaw *et al.*, 2001). Extracts from *Terminalia* species have shown effectiveness against different pathogens, including multiple microbes such as bacteria, fungi, protozoa, and viruses (Fyhrquist *et al.,* 2014). The literature on *Terminalia* species shows significant antimicrobial properties, especially in *T. ferdinandiana, T. bellarica, and T. chebula*, against drug-resistant bacteria such as MRSA (Methicillin-resistant Staphylococcus aureus) and fungi such as Candida (Konczak, 2014). These properties are attributed to bioactive compounds, such as tannins, flavonoids, terpenoids, and phenolic acids, found in various plant parts. Therefore, we hypothesized that *T. pallida* fruits which are used as a substitute for *T. chebula*, may exhibit similar antimicrobial effects. This hypothesis justifies screening *T. pallida* fruits for antimicrobial activity, potentially contributing to new, natural treatments for resistant infections (Dwivedi, 2007; Gurib-Fakim, 1994; Kesharwani *et al.,* 2017; Latheef, 2007).

Terminalia pallida Brandis, commonly known as the pale-leaved *Terminalia*, is a prominent species of the Combretaceae family. *T. pallida* is native to the arid and semi-arid regions of South India, particularly Andhra Pradesh and Tamil Nadu (Anonymous, 1976). It is a semi-evergreen tree that grows to a height of 40 feet. It is endemic to the Eastern Ghats, particularly on the hilltops of dry deciduous forests. This species is mainly found in Chittoor and Kadapa districts (Kameswara Rao, 2003). The tree leaves are thick, simple, alternate, ovate to elliptic pale green leaves and their flowers are pale yellow, appearing as simple terminal and axillary spikes. The fruits were glossy, light green and faintly ridged when dry. *T. pallida* have a generation age of 29 years (Gupta, 2002). According to the IUCN Red List, the number of *T. pallida* is decreasing in the wild, and it has been given a vulnerable status and is recognized for its potential antimicrobial, anti-inflammatory, and antioxidant properties (Dokuparthi *et al.,* 2014; Sarvan Kumar *et al.,* 2021).

Infectious diseases continue to pose a significant global health burden, with diseases caused by *Mycobacterium, Pseudomonas,* and *Candida* leading to high morbidity and mortality rates, particularly in low- and middle-income countries (WHO, 2008). Despite advances in modern medicine, including the development of vaccines and antibiotics, challenges remain owing to the emergence of drug-resistant pathogens and limited access to healthcare (WHO, 2012). Current treatment options for infectious diseases often rely on antibiotics, antivirals, and antifungals; however, the overuse and misuse of these drugs have led to increasing resistance, rendering some treatments ineffective. Additionally, the rapid pace of urbanization, climate change, and increased global connectivity have facilitated the spread of infectious diseases, creating new challenges for global public health systems (Prestinaci, 2015). The objective of this research is to evaluate the antimicrobial efficacy of Terminalia pallida fruit extracts against various bacterial and fungal strains. The study aims to determine the minimum inhibitory concentration (MIC) and assess the zone of inhibition for these strains, with particular interest in optimizing concentrations to enhance efficacy against microbes and understanding the potential of T. pallida as an antimicrobial agent.

Material and methods

Plant material

Terminalia pallida Brandis fruits were collected from Seshachalam Hills, Andhra Pradesh, in January 2023 and authenticated by the Botanical Survey of India, Hyderabad.

Extraction

Dried fruits were powdered, macerated with 80%

alcohol, and fractionated with n-hexane. Subsequently, it was dried using a rotary evaporator (Vedh Instruments, 2020 Model, India) (Sanagala *et al.,* 2024; Dokuparthi *et al.,* 2021).

Reagents and chemicals

All chemicals and reagents were procured from Sigma-Aldrich (laboratory grade, with the highest purity available).

Antimicrobial activity screening

We evaluated the anti-microbial properties of ethanolic fruit extract of *T. pallida* using various bacterial and fungal strains. *E. coli* (MTCC-452), *S. aureus* (MTCC-96), *S. epidermidis* (MTCC-3615), *E. aerogenes* (MTCC-8100), *B. cereus* (MTCC-430), *P. aeruginosa* (MTCC-424, *C. albicans*, and *A. niger*. All microbial type cultures collection (MTCC) from Pune, India. The MIC values of *T. pallida* ethanolic fruit extract was evaluated using a microdilution method in a 90-well microtiter plate.

Preparation of solvent

A stock solution of 20% DMSO was prepared by mixing 2 ml of 100% DMSO with 8 ml of distilled water. The mixture was maintained at room temperature (37°C) until use.

Preparation of sample

T. pallida ethanolic fruit extract (100 mg) was weighed and dissolved in 1 ml of 20% DMSO. The solution was thoroughly stirred and vortexed until the extract was dissolved entirely.

Microtiter plate setup

A 90-well microtiter plate was used for this assay. A culture medium (100 µL) was added to all wells designated for each sample. Next, 100 µL of 20% DMSO was added to each well and mixed thoroughly. Serial dilutions were performed by transferring 100 µL from the first well to the second well, continuing this process to the sixth well, and discarding 100 µL from the sixth well to maintain consistent volumes. The seventh well was used as a negative control, containing 100 µL of medium and 5 µL of the culture. The eighth well served as the solvent control, containing 100 µL of medium, 100 µL of 20% DMSO, mixed thoroughly, and 5 µL of culture after discarding 100 µL from this well.

The microtiter plate was covered with aluminum foil to prevent contamination and was incubated at 37°C for 24 h. After the 24-hour incubation period, the wells were examined using a spectrophotometer at 600 nm for microbial growth to determine sensitivity. The MIC was determined by identifying the lowest extract concentration that inhibited the visible growth of microorganisms. All steps were conducted under sterile conditions to avoid contamination. Care was taken to ensure that the microtiter plate was properly covered and undisturbed during the incubation period for accurate results (Kowalska-Krochmal and Dudek-Wicher, 2021; Elshikh and Mickymaray, 2019).

Zone of inhibition determination

The antimicrobial activity of ethanolic fruit extract of *T. pallida* was evaluated using the agar well diffusion method to determine the zone of inhibition against selected strains.

Preparation of medium plates

Sterilized LB agar media for bacterial strains and Sabouraud Dextrose Agar (SDA) media for fungal strains were prepared, and approximately 25 mL was poured into each 90 mm disposable petri plate under sterile conditions within a laminar flow hood. The agar was then allowed to solidify. All materials, including the cork borer, pipettes, and forceps, were sterilized before use. All steps were performed under aseptic conditions to prevent contamination, including using a laminar flow hood and wearing sterile gloves.

Preparation of wells

Four wells were created in each plate at the center of the four zones using a sterile cork borer. The cork borer was sterilized before each well to avoid cross-contamination. Active bacterial culture (40 µL) was dispensed onto the surface of each LB agar plate. The

culture was evenly spread using a sterile disposable L-shaped spreader. The culture was allowed to adsorb on agar for a few minutes. Different loading materials (40 µL) were dispensed into each of the four wells using a sterile pipette tip.

1. Well 1: 25mg/mL concentrated sample of ethanolic fruit extract of *T. pallida*
2. Well 2: 12.5mg/mL concentrated sample of ethanolic fruit extract of *T. pallida*
3. Well 3: Solvent control (20% DMSO)
4. Well 4: Positive control (100 µg/mL Cefixime/15 µg/mL Ketoconazole)

Incubation

The plates were then incubated at 37°C for 24-48 hours under sterile conditions. After the incubation period, the zones of inhibition around each well were measured to determine the antimicrobial efficacy of the ethanolic fruit extract of *T. pallida*. Strict sterile conditions were maintained throughout the procedure to ensure that the observed zones of inhibition were due to the plant extract and not contamination (Manandhar *et al.,* 2019; Eloff 1998; Al Aboody; 2020).

Results and discussion

MIC determination

The differences in absorption measured at 600 nm using a spectrophotometer before and after treatment with ethanolic fruit extract of *T. pallida* are presented in Table 1. Negative values denote increased microbial growth, whereas positive values indicate reduced microbial growth.

The MIC values indicate the lowest extract concentration required to inhibit microbial growth. All tested bacterial strains, except *E. aerogenes* and *P. aeruginosa*, exhibited a minimum inhibitory concentration (MIC) of 12.5 mg/ml. *E. aerogenes* had an MIC of 25 mg/ml and *P. aeruginosa* had an MIC of 50 mg/ml. Strains with MIC values of 12.5 mg/ml were more sensitive to the extract than those with higher MIC values (Table 1). Both fungal strains showed MIC at 25 mg/ml, but *C. albicans* was found to be more sensitive than *A. niger*.

Table 1. MIC of ethanolic fruit extract of *T. pallida* on microbial strains.

	S. aureus (S1) MTCC-96	*B. cereus (S2)* MTCC-430	*S. epidermidis (S3)* MTCC-3615	*E. coli (S4)* MTCC-452	*E. aerogenes (S5)* MTCC-8100	*P. aeruginosa* (S7) MTCC-424	*C. albicans*	*A. niger*
Solvent Control	- 0.14±(0.04)	-0.12±0	-0.13±0.01	-0.03±0.04	0±0.02	-0.13±0.17	-0.07±0.01	-0.04±0.04
Negative control (No culture)	-0.23±0.04	-0.78±0.1	-0.62±0.09	-0.72±0.48	-0.61±0.84	-0.88±0.76	-0.35±0.04	-0.09±0.05
T. pallida (1.56mg/ml)	-0.37±0.27	-0.18±0.08	-0.12±0.02	-0.52±0.46	-0.45±0.6	-0.66±0.37	-0.38±0.01	-0.45±0.03
T. pallida (3.125mg/ml)	-0.18±0.09	0±0.1	0.09±0.24	-0.28±0.29	-0.26±0.17	-0.65±0.08	-0.69±0.29	-0.62±0.11
T. pallida (6.25mg/ml)	-0.08±0.02	-0.11±0.05	-0.01±0.07	-0.03±0.01	0.01±0.02	-0.23±0.23	-0.33±0.21	-0.11±0.02
T. pallida (12.5mg/ml)	0.28±0.05	0.62±0.09	0.15±0.15	0.16±0.18	-0.05±0.55	-0.17±0.3	-0.06±0.01	-0.05±0
T. pallida (25mg/ml)	0.43±0.08	0.48±0.1	0.37±0.19	0.43±0.06	0.02±0.51	-0.05±0.64	0.29±0.01	0.39±0.02
T. pallida (50mg/ml)	0.15±0.07	0.17±0.01	0.21±0.09	0.33±0.09	0.08±0.12	0.09±0.13	0.17±0.11	0.21±0.07

All the values are expressed as Mean± SD in duplicates.

Although the degree of sensitivity varies, ethanolic fruit extract of *T. pallida* is effective against a range of Gram-positive and Gram-negative bacteria. Gram-positive bacteria, notably *S. aureus, B. cereus,* and *S. epidermidis*, showed higher sensitivity to the extract at lower concentrations (12.5 mg/ml). In contrast, some Gram-negative bacteria, such as *E. aerogenes* and *P. aeruginosa*, were less sensitive to the extract, requiring higher concentrations (25 mg/ml and 50 mg/ml, respectively) for effective growth inhibition. It is also apparent that, the extract has broad-spectrum antimicrobial and antifungal properties; its efficacy is more against bacterial strains than fungal strains. *T. pallida* is more potent against Gram-positive and

some Gram-negative bacteria, such as *E. coli*.

Zone of inhibition

The antimicrobial effect of *the* ethanolic fruit extract of *T. pallida*, as indicated by the zone of inhibition, was observed in various microbial strains. In the solvent control treatment, no inhibition zones were observed for any strains, indicating that the solvent alone did not exhibit antimicrobial activity.

The experiment demonstrated a dose-dependent response, with higher doses of ethanolic fruit extract of *T. pallida* resulting in larger zones of inhibition, indicating more potent antimicrobial activity.

Table 2. Antimicrobial effect of ethanolic fruit extract of *T. pallida*.

Treatment	*S. aureus* (S1)	*B. cereus* (S2)	*S. epidermidis* (S3)	*E. coli* (S4)	*E. aerogenes* (S5)	*P. aeruginosa* (S7)	*C. albicans*	*A. niger*
Solvent Control	0	0	0	0	0	0	0.1±0.14	0.05±0.07
Low Dose TP	0.30±0	0.10±0.17	0.37±0.32	0	0	0.37±0.06	0.35±0.07	0.1±0.14
High Dose TP	0.50±0	0.67±0.15	0.60±0.53	0	0	0.57±0.06	0.65±0.07	0.35±0.07
Positive Control	1.50±0	1.17±0.29	1.07±0.92	0	0	0.47±0.06	1.6±0.14	1.10±0.14

All the values are expressed as Mean± SD in triplicates.

When treated with a low dose of Ethanolic fruit extract of *T. pallida*, *S. aureus* showed a zone of inhibition of 0.30±0 cm, *B. cereus* had a smaller inhibition zone of 0.10±0.17 cm, and *S. epidermidis* exhibited a zone of inhibition of 0.37±0.32 cm. Additionally, *S. aureus* showed a zone of inhibition of 0.37±0.06 cm, and *P. aeruginosa* has showed a zone of inhibition of 0.37±0.06 cm. The remaining strains, *E. coli*, *E. aerogenes*, exhibited no inhibition zones at low doses.

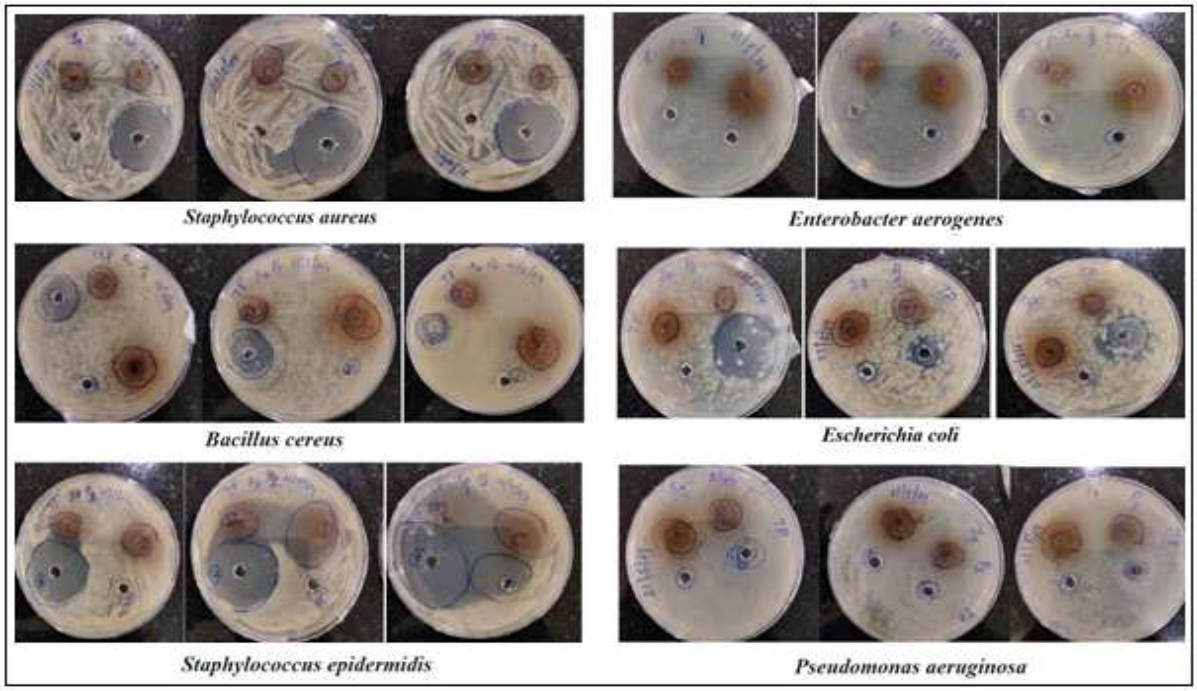

Fig. 1. Effect of ethanolic fruit extract of *T. pallida* against various microbial strains.

A high dose of the ethanolic fruit extract of *T. pallida* demonstrated increased antimicrobial activity. *S. aureus* had an inhibition zone of 0.50±0 cm, *B. cereus* showed a zone of 0.67±0.15 cm, and *S. epidermidis* had an inhibition zone of 0.60±0.53 cm. Notably, *P. aeruginosa* exhibited an inhibition zone of 0.57±0.06 cm at the high dose. At the high dose, *S. aureus* also had an increased inhibition zone of

0.50±0 cm. However, *E. coli* and *E. aerogenes* showed no inhibition zones, even at high doses.

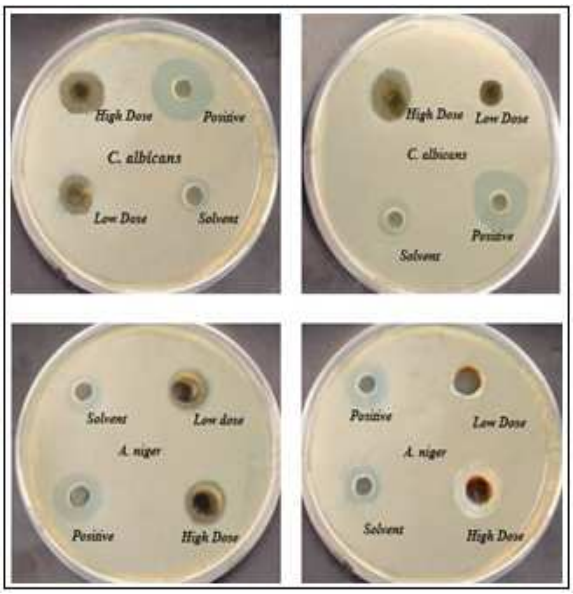

Fig 2. Effect of ethanolic fruit extract of *T. pallida* against various fungal strains.

Ethanolic fruit extract of *T. pallida* exhibit significant antibacterial properties, particularly against Gram-positive bacteria. The efficacy of the extract was dose-dependent, with higher concentrations yielding substantial inhibitory effects. However, its effectiveness against Gram-negative bacteria is variable, with some strains like *P. aeruginosa* showing moderate sensitivity while others like *E. coli* and *E. aerogenes* are resistant.

These findings suggest that *T. pallida* fruit extract has potential as a natural antibacterial agent, particularly for treating infections caused by Gram-positive bacteria (Table 2, Fig 1&2).

The antifungal study on *C. albicans* and *A. niger* reveals a dose-dependent effect. For *C. albicans*, the low dose shows moderate activity (0.35), while the high dose increases to 0.65. In comparison, *A. niger*, the low dose shows low activity (0.10), and the high dose improves to 0.35, though still less effective than against *C. albicans*. Further studies are needed to optimize the concentration of the extract and to investigate its mechanisms of action to enhance its efficacy against resistant strains. These antimicrobial effects can be attributed to bioactive compounds such as tannins, flavonoids, terpenoids, and phenolic acids found in various parts of plants, including the leaves, fruits, and bark. Specific compounds, such as gallic acid, ellagic acid, chebulagic acid, and quercetin, have been identified as key bioactive components of the *Terminalia* genus.

These phytochemicals have shown significant antibacterial, antifungal, antiviral, and antiparasitic activity. Gallic acid and ellagic acid are known for their potent antioxidant and antibacterial properties, whereas flavonoids such as quercetin exhibit broad-spectrum antibacterial activity against various pathogens. Tannins and phenolic acids contribute to antimicrobial efficacy by disrupting the microbial cell walls and inhibiting microbial enzymes. The diverse phytochemical composition of *Terminalia* species underlies their extensive use in traditional medicine and their potential for developing natural antimicrobial agents.

Conclusion

The ethanolic fruit extract of *T. pallida* showed strong antibacterial properties, particularly against Gram-positive bacteria, such as *S. aureus, B. cereus*, and *S. epidermidis*, with effective inhibition at 12.5 mg/ml. Gram-negative bacteria, such as *E. aerogenes* and *P. aeruginosa*, require higher concentrations, indicating lower sensitivity. These results also suggest its potential as a natural antifungal agent, particularly against *C. albicans*. The efficacy of the extract was dose-dependent, suggesting its potential use as a natural antimicrobial agent. Further research is needed to enhance its effectiveness against resistant Gram-negative strains such as *E. coli* and *E. aerogenes*.

Conflict of interest

The authors declare that they have no conflict of interest.

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