



ISSN (E): 2277- 7695  
 ISSN (P): 2349-8242  
 NAAS Rating: 5.03  
 TPI 2019; 8(6): 1065-1068  
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 www.thepharmajournal.com  
 Received: 01-04-2019  
 Accepted: 03-05-2019

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## Evaluation of antimicrobial and anthelmintic activity of roots of *Terminalia paniculata*

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### Abstract

**Objective:** The objective of the study was to evaluate the antimicrobial and anthelmintic effect of the ethanol root extract of *Terminalia paniculata* Roth., (Family: Combretaceae).

**Methods:** *Terminalia paniculata* roots were shade dried, powdered, and extracted by Soxhlet extraction procedure using ethanol. Standard procedures were used for preliminary phytochemical studies of the ethanolic extract. *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 441, *Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 424 were chosen for antibacterial activities. Adult Indian earthworms *Pheritima posthuma* was used for anthelmintic activity.

**Results:** The results of antimicrobial susceptibility reveals ethanolic extract has promising antibacterial activity against gram-negative bacteria (*E. coli* and *P. aeruginosa*) as compared to gram-positive bacteria (*S. aureus* and *B. subtilis*). The ethanolic extract of *Terminalia paniculata* root revealed significant anthelmintic activity in a dose-dependent manner.

**Conclusion:** An ethanolic extract of *Terminalia paniculata* root possesses antimicrobial and anthelmintic activities.

**Keywords:** *Terminalia paniculata*, piperazine citrate, antimicrobial, anthelmintic activity

### 1. Introduction

Medicinal plants are widely used for the treatment of diseases all over the world. According to a WHO report above 80% of the world, the population is taking interest in indigenous medicinal plant remedies [1]. It is estimated that about 75% of the biologically active plant-derived compounds, presently in use worldwide, have been derived through follow up researchers to verify the authenticity of data from folk and ethnomedicinal uses. So there is a great scope for new drug discoveries based on traditional plant uses [2].

*Terminalia paniculata* Roth., Syn. *Pentaptera paniculata* (Family: Combretaceae) is a large deciduous tree, 20-30 m in height with a clear bole of about 10 m and brown to dark brown rough bark peeling off in thin flakes; leaves simple, upper alternate, lower opposite, oblong or elliptic, acute or acuminate, pale brown with two glands near the base of the midrib below, main nerves 10-15 pairs; flowers reddish brown, sessile, in rusty pubescent spikes; fruits reddish brown-winged, one wing broad and the other two narrow, widely distributed in India [3-4].

Ethnobotanical knowledge is very ancient in India. Traditionally, flower juice and bark of *Terminalia paniculata* have been used as a remedy for cholera, diabetes, inflamed parotid glands, menstrual disorders, cough, microbes, wounds, ulcers, worm, skin disease, leprosy and anemia [3, 4, 5].

Several pharmacological and biological tests that have been reported on this plant are evident from kinds of literature. *Terminalia paniculata* is also used to treat cough, bronchitis, cardiac debility, hepatitis, and diabetes [6] and has spermicidal activity [7, 8]. The methanolic extracts of stem bark, leaf, and fruit possess antibacterial, antifungal, and antioxidants activity [9, 10]. The antioxidant activity with 2, 2-diphenyl-1-picrylhydrazyl (DPPH) has been evaluated [11]. The leaves and acetone and methanol fruit are reported to have marked the Anti-HIV-1 effect [12, 13]. The aqueous bark extract possesses a protective effect against CCl<sub>4</sub>-induced liver injury in rodents [14].

Following phytochemical have been reported on this plant in the literature. Ellagic acid was isolated from the methanol extract of *Terminalia paniculata* leaves and heartwood [12]. 3,3'-di-O-methyl ellagic acid and 3,4,3'-O-trimethyl flavellagic acid was isolated from methanol extract of heartwood [15, 16].  $\beta$ -sitosterol, terminic acid, a dihydroxytriterpene carboxylic acid isolated from this plant [3, 5].

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An extensive literature survey does not reveal the antimicrobial and anthelmintic activity of root. So, the present study explored the antimicrobial and anthelmintic activity of an ethanol extract from *Terminalia paniculata* root.

## Materials and methods

### Plant material

The root parts of the plant *Terminalia paniculata* were collected from the forests of the Purba-Medinipur district of West Bengal during August 2018 and identified by the taxonomists of the Botanical Survey of India, Shibpur, Howrah, West Bengal, India. A voucher specimen has been kept in our research laboratory for further reference. After authentication, fresh roots were collected in bulk, washed with potable water to remove adhering dirt followed by rinsing with distilled water and were then shade dried for two weeks. The dried root plant materials were pulverized using a mechanical grinder to obtain a coarse powder.

### Preparation of extracts

The powdered plant material (500 g) was extracted with 1 liter of ethanol (95% v/v) for 48 hrs using a Soxhlet extractor. The extract obtained was evaporated under vacuum to remove the solvent completely and concentrated to obtain a dark reddish semisolid residue (7.60 g).

### Preliminary phytochemical tests

Standard procedures [17-18] were used for preliminary phytochemical studies of the ethanolic extract to know the nature of phytoconstituents present within the extract.

### Antimicrobial activity

Ethanolic extracts were tested against a panel of 4 pathogenic bacterial strains including *Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 441, *Escherichia coli* MTCC 443 and *Pseudomonas aeruginosa* MTCC 424 were purchased from Institute of Microbial Technology, Sector 39, Chandigarh, India.

### Anthelmintic activity

Adult Indian earthworms *Pheritima posthuma* was used for anthelmintic activity. Selected earthworms are 4-5 cm in length and 0.1-0.2 cm in width. The earthworms were washed with normal saline to remove all the fecal matter. Ethanolic extract was suspended in 1% Tween-80 in normal saline.

### Evaluation of Antimicrobial activity

#### Determination of minimum inhibitory concentration (MIC)

##### Broth dilution method

The minimum inhibitory concentration (MIC) was determined using the broth dilution method as described by Stalons *et al.* [19]. Concentrations of extracts (1-9 mg mL<sup>-1</sup>) dilutions were prepared using tubes containing 9 ml of double strength broth. In all test tubes, test antimicrobial compound is added except uninoculated (negative control) and control (positive) tube. The positive control tube is to check for the suitability of the test microorganism and the viability of the inoculums. The final volume was adjusted in all tubes by using sterile water. The tubes were inoculated with the suspension of standardized inocula (0.5 McFarland standard) and incubated at 37°C for 24 h. MIC was recorded as the lowest concentration of extract showing no visible bacterial growth.

## Determination of the zone of inhibition

### Disc diffusion method

The disc diffusion assay was used to screen for antibacterial activity as described by Drew *et al.* [20]. The standard inoculum was introduced onto the surface of the sterile agar plates and a sterile glass spreader was used for even distribution over the media. Blank sterile paper discs (6 mm) were placed on the inoculated Mueller-Hinton agar surface and impregnated with 50 µL of the different extracts. A concentration of 10 µg/disc of Streptomycin (Sigma Aldrich, India), was used as a standard. The procedure was repeated for all the selected bacterial species used. The plates were incubated at 37 °C for 24 h. All tests were performed in triplicate and the antibacterial activity was expressed as the mean diameter of inhibition zones (mm) produced by the extracts.

### Evaluation of anthelmintic activity

The anthelmintic activity was evaluated on adult Indian earthworms *Pheritima posthuma* due to its anatomical and physiological resemblances with the intestinal roundworm parasites of the human beings. The method of Dash *et al.* and Ghosh *et al.* [21-22], was followed for the screening of anthelmintic activity. Each group was treated with vehicle (1% Tween-80 in normal saline), piperazine citrate 15 mg/ml and ethanolic extract of 10, 15, 20 and 25 mg/ml in normal saline containing 1% Tween-80. Observations were made for the time taken to paralyze and death of individual worms up to 4 h of the test period. Paralysis was said to occur when the worms did not revive in saline. Death was concluded when the worms lost their motility followed by fading of their body color.

## Results

### Preliminary phytochemical tests

The ethanol extract of *Terminalia paniculata* exhibits flavonoids, terpenoids, saponins, tannins, phenol and quinones (Table 1).

### Antimicrobial activity

#### Broth dilution method

The MIC of ethanolic extract of the root of *Terminalia paniculata* was 5 mg/ml against *S. aureus*, 4 mg/ml against *B. subtilis*, 6 mg/ml against *E. coli* and 6 mg/ml against *P. aeruginosa*. The MIC determination was performed in triplicate for each organism.

#### Disc diffusion method

The ethanolic extract exhibited (Table 2) potent antibacterial activity against *S. aureus* (10.22±0.25 mm), *B. subtilis* (11.66±0.30 mm), *E. coli* (14.66±0.33 mm) and *P. aeruginosa* (13.30±0.32 mm). The same for the standard drug was found to be 8±0.25 mm, 9±0.23 mm, 12±0.22 and 10±0.25 mm against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* respectively.

### Evaluation of anthelmintic activity

The ethanolic extract of *Terminalia paniculata* root revealed significant anthelmintic activity. The results are depicted in Table 3 shows the time taken for paralysis and death of worms after treatment with the extract at the selected concentration.

## Discussion

Plants have their unique place, due to no side effects. Whereas synthetic drugs are found to have some or other side effects. [23]. Active phytochemical compounds having the pharmacological activities obtained from the medicinal plants were a potent effect on human health. Based on the preliminary phytochemical tests reveals the presence of various secondary metabolites such as flavonoids, terpenoids, saponins, tannins, phenol, and quinones on *Terminalia paniculata*.

The ethanolic root extracts of *Terminalia paniculata* was produced antibacterial activity against all the tested organisms. The results of antimicrobial susceptibility reveal ethanolic extract have promising antibacterial activity against gram-negative bacteria (*E. coli* and *P. aeruginosa*) as compare to gram-positive bacteria (*S. aureus* and *B. subtilis*).

The ethanolic extract showed the anthelmintic activity in a dose-dependent manner at 10 to 25 mg/ml. Tannins, the secondary metabolite, occur in several plants have been reported to show anthelmintic properties by several researchers [24, 25]. Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or, binds to the glycoprotein on the cuticle of the parasite [26], and cause death. Anthelmintic drugs exert their activity by paralyzing the worms by causing damage to the outer mucopolysaccharides layer.

## Conclusion

The ethanolic root extracts of *Terminalia paniculata* possesses antimicrobial and anthelmintic activities. Further studies are required to identify the actual chemical constituents that are present in the crude extract of this plant which is responsible for antimicrobial and anthelmintic activity.

## Acknowledgment

The authors are thankful for the principal Netaji Subhas Chandra Bose Institute of pharmacy, Tatla, Chakdaha, Nadia, West Bengal, India, for encouragement to carry out this study.

## Abbreviation used

*S. aureus*: *Staphylococcus aureus*; *B. subtilis*: *Bacillus subtilis*; *E. coli*: *Escherichia coli*; *P. aeruginosa*: *Pseudomonas aeruginosa*; mm: Millimetre; µg: Microgram; cm: Centimeter; mg: Milligram.

## Authors contribution

Suman Acharyya: All fieldwork, laboratory experiments, preparation of the manuscript. Abhik Si: Supporting laboratory experiments. Prasenjit Bhuniya: Supporting laboratory experiments.

## Conflicts of interest

We the authors announced that we have no conflicts of interest.

**Table 1:** Preliminary phytochemical screening of ethanol extract of *Terminalia paniculata*.

S. No.	Tests	Inference
1	Glycosides	-
2	Alkaloids	-
3	Flavonoids	+
4	Steroids	-
5	Terpenoids	+
6	Saponins	+
7	Tannins	+
8	Phenol	+
9	Quinone	+

(+): Present; (-): Absent.

**Table 2:** Antibacterial activity of ethanol extracts of *Terminalia paniculata* against tested bacterial strain.

Microorganisms	Diameter of inhibition zone (mm)		MIC (mg/ml)
	Ethanol extract	Streptomycin	Ethanol extract
<i>S. aureus</i>	10.22±0.25	8±0.25	5
<i>B. subtilis</i>	11.66±0.30	9±0.23	4
<i>E. coli</i>	14.66±0.33	12±0.22	6
<i>P. aeruginosa</i>	13.30±0.32	10±0.25	6

Note: The control disc used for solvent had no zone of inhibition, so their data was omitted from the above data. Inhibition zones including the diameter of the paper disc (6

mm). Results are expressed as the mean ± SEM of triplicate measurements.

**Table 3:** Anthelmintic activity of ethanolic extracts of *Terminalia paniculata* root.

Treatments	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Piperazine citrate (Standard)	15	31.53±1.35	53.43±1.14
Ethanolic extract	10	90.53±1.12	130 ±1.58
	15	76.33±1.26	115.50±1.12
	20	63.46±2.17	100.47±2.25
	25	43.33±1.28	87.33±1.67

Results are expressed as Mean ± SEM, n=6, student't' test vs control.

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