



Original Article

# Phytochemical Investigation and Evaluation of Antimicrobial Activity of Methanolic Extract of *Terminalia coriacea*

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ABSTRACT

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The goal of this study was to determine preliminary phytochemical investigation and evaluation of antibacterial activity of methanolic extract of *Terminalia coreacea* (Leathery Murdah) belonging to family Combretaceae toward two phytopathogenic bacteria. The antibacterial activity of the extract was done on standard and two wild pathogenic bacterial strains *Bacillus subtilis* and *Escherichia coli*. The antimicrobial activity screening was done by the agar cup plate method using sterile top agar. Zone of inhibition of extract (50, 100 and 150 mg/ml) was compared with that of standard amoxicillin (0.5 and 1 mg/ml) prepared in DMSO. The extract shows potential antibacterial properties comparable with that of standard amoxicillin against the organisms tested. The methanolic extract of *Terminalia coreacea* displayed a concentration related antibacterial activity. The results show the inhibition of bacterial growth was more pronounced on *Escherichia coli* as compared to the other tested organisms.

**Keywords:** Antibacterial activity, *Terminalia coreacea*, Cup plate method, Amoxicillin.

## 1. INTRODUCTION

Medicinal plants are widely used for treatment of diseases all over the world. According to WHO report above 80% of the world populations are taking interest in indigenous medicinal plant remedies<sup>1</sup>. Therefore it is essential to investigate traditional medicine with a view to identify and exploit safe and effective remedies for ailments of both microbial and non microbial origin. The use of

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phytochemicals, antimicrobial properties, could be of great significance in therapeutic approaches. 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<sup>2</sup>. The ethnobotanical information obtained from the traditional herbal practitioners may serve as an initial lead for isolation and characterization of bioactive compounds. Phytocostituents are the natural bioactive compounds, exhibit potential therapeutic properties; work with nutrients and fibres to form an integrated part of defensive system in which alkaloids, flavonoids, saponins, terpenoids, phenolics, tannins etc considered as major constituents in crude drugs. These plant derived compounds are considered to be active against human pathogenic microorganisms. In the present study *Terminalia coreacea*, a potential medicinal plant from Sheshachalam hills of Chittoor district, Andhra Pradesh, was selected and screened against all test pathogens.

## 2. MATERIAL AND METHODS

### Plant material

The plant material was collected from Tirumala hills of Chittoor district and identified with the help of regional floras<sup>3-5</sup>. The ethnomedicinal properties of the species were recorded based on interviews conducted with elder people from tribal communities, inhabited in and around the forests. The fruit of *Terminalia coreacea*(Roxb.) (Combretaceae) was collected from Tirumal hills, Chittoor district. The collected plant material was identified and authenticated by Dr.Madhava Chetty (Assistant Professor, S.V.University, Tirupati, India) with a voucher number 985.

### Microorganisms

Standard cultures of *Bacillus subtilis*, *Escherichia coli* were obtained from Food and Drug laboratory, Hyderabad. The microorganisms were identified by staining techniques. The organisms were maintained by sub culturing at regular intervals in nutrient agar medium.

### Antimicrobial agent

The reference standard amoxicillin was procured as gift sample from Hindustan antibiotics Ltd., Pune.

### Phytochemistry

The fruit of *Terminalia coreacea* (Roxb.) Wight & Arn. was washed to render it free from dust. The material was then dried under shade for about 5-7 days and weighed. About 1kg of the dried fruit was grinded into a coarse powder using a mechanical grinder and passed through sieve no. 40 to get the powder of desired coarseness. The powdered material was then preserved in an air tight container for future use and successively extracted with methanol and water (1:4) using soxhlet apparatus for 6 hours<sup>6</sup>. The extracts were filtered, concentrated under reduced pressure to dryness; the extract was weighted to calculate percentage of yield and subjected for phytochemical screening using standard

procedures<sup>7, 8</sup>. The positive reaction was observed for different groups of phytochemical compounds. Alkaloids, phenols, anthocyanins, anthracene glycosides, saponins and steroids were recorded as most predominant chemical derivatives followed by flavones, catecholic compounds, proteins, gallic tannins, etc (Table-1).

### Test for carbohydrate

#### a. Molisch's Test:

To 2-3ml of the extract, add few drops of  $\alpha$ -naphthol and conc.  $H_2SO_4$  from the sides of the test tube was added. Violet ring is not observed at the junction. It indicates absence of carbohydrates.

#### b. Fehling's Test:

To equal volumes of Fehling's A and B reagents, 2-3ml of test solution was add and heated on a water bath for 5mins. Brick red precipitate is not observed. It indicates absence of carbohydrates.

### Test for Amino Acids:

#### a. Ninhydrin test:

Heat 3ml of test solution & add 3 drops of ninhydrine solution. Purple colour was not observed.

### Test for Steroids and Triterpenoids:

a. Salkowski Reaction: To 2ml of the extract, 2ml of  $H_2SO_4$  and  $CHCl_3$  was added and shaken well.  $CHCl_3$  layer appears red and acid layer appears yellow fluorescence. It indicates the presence of Steroids.

b. Liebermann Burchard reaction: To 2ml of extract was mixed with chloroform, 1-2ml; of acetic anhydride and 2 drops of conc.  $H_2SO_4$  added from the sides of the test tube. First red, then bkue and finally green colour were observed. It indicates presence of Steroids.

### Test for Cardiac Glycosides:

a. Baljet's test: A thin section of the drug is dipped in Sodium Picrate solution. Yellow to orange colour was obtained. Presence of Cardiac Glycosides.

b. Legal's test: To aqueous or alcoholic extract 1ml of Legal's reagent was added. Pink to red colour appeared. It indicates Presence of Cardiac Glycosides.

c. Keller killiani test: To 2ml of extract glacial acetic acid and 1 drop of 5%  $FeCl_3$  and conc.  $H_2SO_4$  was added. Reddish brown colour appeared at the junction of 2 liquid layers and upper layers bluish green. It indicates presence of Cardiac Glycosides.

### Test for Anthraquinone Glycosides:

a. Borntrager's test: To 3ml of the extract add dil.  $H_2SO_4$ , boil and filter. Cool the filtrate and shake with equal volume of  $CHCl_3$ . Ammonical layer does not turn to pink colour. Absence of Anthraquinone Glycosides.

b. Modified Borntrager's test: To 5ml extract and 5ml 5%  $FeCl_3$  and 5ml dil. HCl. Heat for 5min in boiling water bath cool and benzene separate the organic layer and add equal volume of dilute ammonia. Ammonical layer did not shows pinkish-red colour. Absence of Anthraquinone Glycosides.

**Test for Saponin Glycosides:**

- a. Foam test: Shake the drug extract vigorously with water. Persistent foam was not observed. Absence of saponin glycosides.

**Test for Flavonoids:**

- a. Shinoda test: To the extract add 5ml of 95% ethanol and few drops of conc. HCl and 0.5gm of magnesium turnings. Dark pink to red colour is observed. Presence of flavonoids.
- b. Alkaloid reagent test: Treat the extract with few ml of NaOH solution followed by drop wise addition of dil. HCl acid. Yellow colour is observed which later changes to colourless. Presence of flavonoids.
- c. Zink HCl test: Treat the extract with zinc dust and conc. HCl. Dark red colour is observed. Presence of flavonoids.

**Test for Alkaloids:**

- a. Dragendroff's test: To the test solution, add Dragendroff's reagent. Orange brown ppt. is formed. Presence of alkaloids.
- b. Mayer's test: To the test solution, add Mayer's reagent. Cream coloured ppt. is formed. Presence of alkaloids.
- c. Hager's test: To the test solution, add Hager's reagent. Yellow ppt. is formed. Presence of alkaloids.
- d. Wagner's test: To the test solution, add Wagner's reagent. Reddish brown ppt. is obtained. Presence of alkaloids.

**Antimicrobial assay**

The antimicrobial activity was performed by employing the pour plate as well as disc diffusion methods<sup>9, 10, 11</sup>. The crude extracts of each sample dissolved in dimethyl sulfoxide (DMSO) and the concentrations of 50 to 150 mg/ml of each sample was applied to µml were prepared. 25-30 sterile Whatmann filter paper discs. All the bacterial and fungal strains were grown in respective media for overnight at 37 C. The suspension of microorganisms adjusted to 105 to 107CFU/ml in broth of the media. In this method (Pour-plate method) 100 suspension of bacterial suspension microorganisms were prepared in the nutrient broth were inoculated in the nutrient agar in petri dishes at room temperature in sterile condition and mixed thoroughly to ensure uniform growth. This was allowed to stand for 15 minutes so that the medium got solidified. The sterile filter paper discs of 5 mm diameter containing different concentrations of crude extracts were aseptically placed on the agar plates. All the seeded petri dishes were incubated at 27±2 ° C for twenty-four hours in case of bacteria and 48-72 hours for fungal species. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The appearance of bacterial free zone around the disc is known as inhibitory zone, is considered as positive response. The diameter of the zones of inhibition around each disc measured and recorded at the end of the incubation period. The diameters of the inhibition zones were measured and expressed in millimetres. Each test

was performed in three replicates and repeated twice. Model values were selected.

**3. RESULTS AND DISCUSSION**

The present study was designed to evaluate the phytochemical and antimicrobial activity of methanolic extract of fruit of *Terminalia coreacea*. Phytochemical studies (Table-1) revealed that methanol extracts exhibited positive reaction for maximum no of secondary metabolites. In vitro antimicrobial studies of *Terminalia coreacea* (Table-3) revealed that crude drug extracts had significant antimicrobial activity against the test pathogens. The methanol extract of fruit exhibited zone of inhibition 2, 2.16, 2.4, 1.93 and 2.4 cm against *Bacillus subtilis*, and 2.4, 2.43, 2.53, 2.13 and 2.46 cm against *Escherichia coli* at a concentration of 50, 100 and 150µg/ml respectively.

**Table 1: Preliminary phytochemical screening report of methanolic extract of *Terminalia coreacea* (Roxb.)**

Sl.no	Name of the Test	Results	Sl.no	Name of the Test	Results
<b>Test For Carbohydrates</b>			<b>Test For Tannins</b>		
a.	Molisch Test	-	a.	Ferric Chloride Test	+
b.	Fehling's Test	-	b.	Vanillin Hcl Test	+
c.	Benedict's Test	-	c.	Bromine Water Test	+
<b>Test For Amino Acids</b>			d.	Acetic Acid Solution+ Test	+
a.	Ninhydrin Test	-	e.	Lead Acetate Solution+ Test	+
b.	Test For Tyrosine	-	f.	Gelatin Solution Test	+
c.	Test For Cysteine	-	g.	Dilute Iodine Solution+ Test	+
<b>Test For Steroids And Triterpenoids</b>			h.	Potassium Dichromate+ Test	+
a.	Salkowaski Reaction	+	i.	Dilute HNO <sub>3</sub> Test	+
b.	Liebermann Burchard+ Reaction	+			
<b>Test For Cardiac Glycosides</b>					
a.	Baljet's Test	+			
b.	Legal's Test	+			
c.	Keller Killiani Test	+			
<b>Test For Anthraquinone Glycosides</b>					
a.	Bortrager's Test	-			
b.	Modified Bortrager's- Test	-			
<b>Test For Saponin Glycosides</b>					
	Foam Test	-			
<b>Test For Flvanoids</b>					
a.	Shinoda Test	+			
b.	Alkaline Reagent Test	+			
c.	Zinc Hcl Test	+			
<b>Test For Alkaloids</b>					
a.	Dragendroff's Test	+			
b.	Mayer's Test	+			
c.	Hager's Test	+			
d.	Wagner's Test	+			

Note: + Indicates Presence of results, - Indicates Absence of results

**Table 2: Determination of MIC of methanolic fruit of *T. coreacea* against different bacteria**

Name of bacteria	Growth in nutrient agar containing different concentrations of extract in mg/ml					
	0	5	15	25	50	100
<i>B. subtilis</i>	+	+	+	+	-	-
<i>E. coli</i>	+	+	+	-	-	-

**Table 3: Antibacterial activity of Amoxicillin and fruits methanolic extracts**

Micro organisms	Zone of inhibition in cm.				
	Extract conc. Mg/ml			Amoxicillin conc. Mg/ml	
	50	100	150	0.5	1
<i>B. subtilis</i>	2 ± 0.10	2.16 ± 0.07	2.4 ± 0.13	1.93 ± 0.05	2.4 ± 0.09
<i>E. coli</i>	2.4 ± 0.16	2.43 ± 0.07	2.53 ± 0.07	2.13 ± 0.09	2.46 ± 0.09

#### 4. CONCLUSION

The present work revealed that *Terminalia coreacea* has rich and diversified phytochemical compounds like alkaloids, coumarins, gallic-tannins and other compounds. The plant parts were also rich in phenols and flavonoids. These antimicrobial agents with its significant inhibition activity against various clinical isolates suggest conducting further studies for isolation and characterization of active principles.

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