Original Article

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

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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 coriacea (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 150mg/ml) was compared with that of standard amoxicillin (0.5 and 1mg/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 coriacea 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 coriacea, 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 remedies¹. 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
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\(^2\). The ethnomedicinal information obtained from the traditional herbal practitioners may serve as an initial lead for isolation and characterization of bioactive compounds. Phytocstituents 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\(^3\)\(^-\)\(^5\). 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\(^6\). 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\(^7\)\(^,\)\(^8\). 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. \(\text{H}_2\text{SO}_4\) 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 added 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 \(\text{H}_2\text{SO}_4\) and \(\text{CHCl}_3\) was added and shaken well. \(\text{CHCl}_3\) 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. \(\text{H}_2\text{SO}_4\) added from the sides of the test tube. First red, then blue and finally green colour were observed. It indicates presence of Steroids.

#### Test for Cardiac Glycosides:

- **a.** Baljer’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 kidniant test: To 2ml of extract glacial acetic acid and 1 drop of 5% \(\text{FeCl}_3\) and conc. \(\text{H}_2\text{SO}_4\) 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. \(\text{H}_2\text{SO}_4\), boil and filter. Cool the filtrate and shake with equal volume of \(\text{CHCl}_3\). Ammonial layer does not turn to pink colour. Absence of Anthraquinone Glycosides.

- **b.** Modified Borntrager’s test: To 5ml extract and 5ml 5% \(\text{FeCl}_3\) and 5ml dil. \(\text{HCl}\). Heat for 5min in boiling water bath cool and benzene separate the organic layer and add equal volume of dilute ammonia. Ammonial 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. Persiant 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 Alkaldoids:
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. 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 μl were prepared. 25-30 sterile Whatmann filter paper discs. All the bacterial and fungal strains were grown in respective media for overnight at 370 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 0 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 2.43, 2.53, 2.13 and 2.46 cm against Escherichia coli at a concentration of 50, 100 and 150μg/ml respectively.
Table 2: Determination of MIC of methanolic fruit of *T. coreacea* against different bacteria

<table>
<thead>
<tr>
<th>Name of bacteria</th>
<th>Growth in nutrient agar containing different concentrations of extract in mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>+</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>+</td>
</tr>
</tbody>
</table>

Table 3: Antibacterial activity of Amoxicillin and fruits methanolic extracts

<table>
<thead>
<tr>
<th>Micro organisms</th>
<th>Zone of inhibition in cm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extract conc. Mg/ml</td>
</tr>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>2 ± 0.10</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>2 ± 0.16</td>
</tr>
</tbody>
</table>

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.

5. REFERENCES


Conflict of Interest: None
Source of Funding: Nil