Phytochemical screening and antimicrobial activity of flowers of *Terminalia brownii*

Sebastian M Ikikii, Josiah O Odalo, Aloice O Omondi and John M Kahindo

DOI: [https://doi.org/10.22271/phyto.2022.v11.i5a.14499](https://doi.org/10.22271/phyto.2022.v11.i5a.14499)

Abstract

*Terminalia brownii* is traditionally used in treatment and management of human illness such as arthritis, hepatitis, diabetes, stomach ulcers, abdominal pain and yellow fever. The objective of this study aimed at determining phytochemical, antibacterial and antifungal activity of *Terminalia brownii* flower extracts. Extraction was performed using n-hexane, dichloromethane, dichloromethane: methanol: methanol (1:1), methanol and water. The extracts were screened for the presence of phytochemicals namely alkaloids, flavonoids, tannin, terpenoids, saponins, steroids, phenols and glycosides. Antibacterial activity test of n-hexane, dichloromethane, dichloromethane: methanol (1:1), methanol and water. Crude extracts of *Terminalia brownii* flowers were tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus Aureus* and antifungal activity against *Candida albicans*. Phytochemical screening of dichloromethane: methanol (1:1) and methanol flower extract of *Terminalia brownii* the study revealed the presence of alkaloids, tannin, saponins, phenols and absence of flavonoids and steroids in both dichloromethane: methanol (1:1) and methanol extracts. Glycoside and terpenoids were only found in methanol extract. Antibacterial activity test of n-hexane, dichloromethane, dichloromethane: methanol (1:1), methanol and water crude extract of *Terminalia brownii* flowers against *E. coli*, *P. aeruginosa* and *S. Aureus* showed that all extracts except n-hexane extract exhibited good antibacterial activity against *E. coli*, *S. Aureus* and *P. Aeruginosa* with zone of inhibition ranging between 8.0 ±0.71 and 17.3 ±0.42 mm. Dichloromethane: methanol (1:1) flower extract exhibited the highest inhibitory activity against *E. coli* and *P. Aeruginosa* with zone of inhibition of 17.3±0.42 and 16.0±0.71 respectively. Only Methanol extract exhibited antifungal activity against *Candida albicans* with zone of inhibition of 7.6±0.84 mm. Dichloromethane: methanol flower extract was the most active with minimum inhibitory concentration (MIC) for *E. coli* and *P. aeruginosa* decreasing with decreasing concentration of crude extract starting from 9.25±1.34 and 7.50±0.42 mm for *E. coli* and *P. aeruginosa* respectively. The MIC values were higher in *E. coli* 1.25±0.07 mm. The present study concluded that phytochemicals in dichloromethane: methanol (1:1) and methanol flower extract are almost similar with those identified in the previous studies of roots, stem bark and leaves of *Terminalia brownii*.

Keywords: Crude extract, *terminalia brownie*, phytochemical screening, antimicrobial activity

1. Introduction

Medicinal plants are widely used in the treatment of diseases all over the world. World Health Organization estimate that more than 80% of the world’s population especially those living in developing countries relies on herbal medicine for their basic health care needs [1]. Medicinal properties of plants are dependent on the presence of certain phytochemical compounds which are responsible for their antimicrobial property [2]. Bioactive compounds from plants have gained attention due to their ability in treatment and prevention of diseases [3, 4]. Natural product has been and still remains the major source of all medicine due to their rich, and diverse source of a variety of phytochemicals. The plant *Terminalia brownii* belongs to combretaceae family comprising of about 525 species of herbs, trees, shrubs and 20 genera of liana of the flowering plants in the order Myrtales [5]. The tree is semi deciduous with dark gray coarse bark and its native to Sudan, Ethiopia, Eritrea, Kenya, Tanzania, Democratic Republic of Congo and Djibouti [6]. Conventionally stem bark decoctions of *T. brownii* is used in treatment and management of arthritis, diabetes, stomach ulcers, abdominal pain, heartburn and diarrhea [7, 8].

Previous studies on roots, stem bark and leaves of *T. brownii* have reported they contain phytochemicals with numerous pharmacological activities such anti-inflammatory, antifungal, antibacterial and anti-viral [6, 9, 10] but from review of existing literature, no work has been done on screening of phytochemical and antimicrobial potential of *T. brownii* flowers. Therefore,
this study was performed to determine phytochemical and antimicrobial activity of *T. brownii* flower extract.

2. Materials and Methods

2.1 Sample collection and Extraction

Flowers of *Terminalia brownii* were collected from Mwingi sub-county; Kitui County, Kenya, they were washed, chopped into small pieces, air dried under the shade at room temperature for two weeks and pulverized to a fine homogenous powder using an electric grinder and sequentially extracted through maceration using solvent of increasing polarity: n-hexane, dichloromethane, dichloromethane: methanol (1:1) and methanol as described by [11]. To simulate the traditional method for preparing herbal medicine, 200g of each sample were also extracted using hot water.

2.2 Phytochemical screening

Phytochemical screening tests were carried out on flower’s dichloromethane: methanol (1:1) and methanol crude extract to determine the presence of secondary metabolites such as alkaloids, flavonoids, tannin, terpenoids, saponins, steroids, phenols and glycosides using standard procedure as described by [12, 13].

2.3 Screening for antimicrobial activity

The activity of flower extract of *T. brownii* was tested against *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 10145) *Staphylococcus Aureus* (ATCC 43300) and *Candida albicans*. The test micro-organisms were obtained from Technical University of Mombasa, Biology laboratory. Antimicrobial activities of flower extracts of *T. brownii* was determined by agar well diffusion method. The test organism were prepared in Muller Hinton agar Nutrient broth and incubated at 37 °C for 24hours. After incubation, bacterial and fungal suspensions were aseptically inoculated on Mueller Hinton agar through surface spreading using sterile swabs to obtain uniform inoculum and allowed to dry for 10 minute. Four wells were carefully made on the agar plate using sterile borer. The Crude extracts of flower were dissolved in dimethyl sulphoxide (DMSO) and further diluted with distilled water preparing a working concentration of 50mg/mL, 25mg/mL, 12.5mg/mL and 6.25mg/mL. The wells were carefully loaded with 50 µL of each of diluted crude extract and both positive and negative controls. Distilled water was used as negative control while amoxicillin was used as positive control for bacteria and plate left in laminia flow for 30minutes to allow the system stabilize as the inoculated microorganism get acclimatized to the new environment. The culture plates were incubated at 37°C for 24hrs and activity was expressed as the diameter of the clear zone of inhibition (mm).

2.4 Minimum inhibitory concentration

Minimum inhibition concentration (MIC) of dichloromethane: methanol (1:1) flower crude extract which showed strongest antimicrobial activity against *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 10145) and *S. Aureus* (ATCC 25922) was determined by micro-dilution method as described by [14]. 0.5 McFarland standard broth culture of test organism *E. coli*, *P. aeruginosa* and *S. Aureus* were prepared and incubated at 37 °C for 48 hours. After incubation bacterial suspensions were aseptically inoculated on Mueller Hinton agar through surface spreading using sterile swabs to obtain uniform inoculum and allowed to dry for 10 minute. Three wells were carefully made on the agar plate using sterile borer. Crude extract of methanol and dichloromethane: methanol (1:1) flower were dissolved in DMSO and serially diluted two-fold preparing working concentration of 50mg/mL, 25mg/mL, 12.5mg/mL and 6.25mg/mL. The wells were carefully loaded with 50 µL of each of diluted crude extract and both positive and negative controls. Distilled water was used as negative control while amoxicillin was used as positive control for bacteria and plate left in laminia flow for 30minutes to allow the system stabilize as the inoculated microorganism get acclimatized to the new environment. The culture plates were incubated at 37°C for 24hrs and activity was expressed as the diameter of the clear zone of inhibition (mm).

2.5 Data analysis

Data on antimicrobial activity of flower extract of *T. brownii* was reported as mean± standard deviation. One-way ANOVA was used to determine statistical significance of zone of inhibition and minimum inhibition concentration of the flower extracts of *T. brownii* against the positive and negative control where *p*≤ 0.05 value was considered to be significant.

3. Results

3.1 Phytochemical screening test

The phytochemical test results for the first time screening of dichloromethane: methanol (1:1) and methanol crude extract of *Terminalia brownii* flower revealed presence of alkaloids, tannin, saponins, phenols and absence of flavonoids and steroids in both extracts (Table 1). Glycoside and terpenoids were only found in methanol extract.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytochemicals</th>
<th>Reagent used</th>
<th>DCM: MeOH (1:1)</th>
<th>MeOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Mayers reagent</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>NaOH solution</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Tannin</td>
<td>Distilled water and FeCl₃ solution</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Terpenoids</td>
<td>CHCl₃ and conc. H₂SO₄</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>Distilled water</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>CHCl₃ and conc. H₂SO₄</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Phenols</td>
<td>FeCl₃ solution</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Glycosides</td>
<td>Glacial acetic acid, FeCl₃ and conc. H₂SO₄</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) presence of substance and (-) absent of substances.

3.2 Antimicrobial activity of crude extracts

Antimicrobial screening results showed that all extract except n-hexane showed good antibacterial activity against *E. coli*, *S. Aureus*, and *P. Aeroginosa* with zone of inhibition ranging between 8.0±0.71 and 17.3±0.42 mm (Table 2). However, Dichloromethane: methanol (1:1) flower extract exhibited...
significantly the highest inhibitory activity against E. coli and P. Aeruginosa with zone of inhibition of 17.3±0.42 and 16±0.71 respectively. Further the study revealed that only Methanol extract exhibited antifungal activity against Candida albicans with zone of inhibition of 7.6±0.84 mm.

Table 2: Antimicrobial activity of crude extracts of and flowers of Terminalia brownii

<table>
<thead>
<tr>
<th>Zone of inhibition in mm (mean ± SD)</th>
<th>Extract</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>S. Aureus</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-hexane</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>11±1.13</td>
<td>13±1.41</td>
<td>9.6±0.84</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dichloromethane: Methanol (1:1)</td>
<td>17.3±0.42</td>
<td>16±0.71</td>
<td>13±0.99</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanol</td>
<td>13±1.41</td>
<td>12±0.71</td>
<td>15±1.48</td>
<td>7.6±0.84</td>
<td>-</td>
</tr>
<tr>
<td>water</td>
<td>8±0.71</td>
<td>10±0.28</td>
<td>9.25±1.34</td>
<td>0±0</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water</td>
<td>14±0.64</td>
<td>11.5±0.78</td>
<td>13.2±0.64</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>24±1.41</td>
<td>17.7±0.42</td>
<td>22.7±0.49</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15±0.094</td>
<td>-</td>
</tr>
</tbody>
</table>

(-) mean no activity

3.3 Determination of Minimum Inhibition Concentration

The results of MIC showed that zone of inhibition were directly proportional to concentration starting from 9.25±1.34 and 7.50±0.42 for E. coli and P. aeruginosa respectively (Table 3). The MIC values were higher in E. coli (1.25±0.07) and P. aeruginosa (1.11±0.13) agreeing with [13] that increasing the concentration of antimicrobial substance enhance zone of inhibition.

Table 3: Minimum inhibition concentration (MIC) of dichloromethane: methanol (1:1) crude extract of Terminalia brownii flowers

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Concentration used</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>Distilled water</th>
<th>Amoxicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50 mg/ml</td>
<td>9.25±1.34</td>
<td>7.50±0.42</td>
<td>-</td>
<td>9.30±0.28</td>
</tr>
<tr>
<td>2</td>
<td>25 mg/ml</td>
<td>3.75±0.07</td>
<td>3.90±0.14</td>
<td>-</td>
<td>3.78±0.11</td>
</tr>
<tr>
<td>3</td>
<td>12.5 mg/ml</td>
<td>2.05±0.21</td>
<td>2.20±0.14</td>
<td>-</td>
<td>2.28±0.18</td>
</tr>
<tr>
<td>4</td>
<td>6.25 mg/ml</td>
<td>1.25±0.07</td>
<td>1.11±0.13</td>
<td>-</td>
<td>1.28±0.04</td>
</tr>
</tbody>
</table>

(-) mean no activity

4. Discussion

Phytochemical results of dichloromethane: methanol (1:1) and methanol crude extract of T. brownii flower indicated presence of alkaloids, tannin, saponins, phenols and absence of flavonoids and steroids in both extracts. Glycoside and terpenoids were only found in methanol extract. In a similar study of phytochemical screening, dichloromethane: methanol (1:1) and methanol root and stem bark extract of T. brownii showed presence of tannin, saponins, phenols, terpenoids, steroids, flavonoids and absence alkaloids while glycoside was present in methanol root [15, 16]. The results of the study are consistent with literature report that showed differential distribution of secondary metabolites justifying the use of specific parts of T. brownii in treatment and management of particular illness in herbal medicine. Antimicrobial test of dichloromethane, dichloromethane: methanol (1:1), methanol and water flower extract of T. brownii showed significant antibacterial activity against E. coli, P. aeruginosa, S. Aureus and only methanol extract showed antifungal activity against C. albicans suggesting flower extract of T. brownii contain biologically active compound inhibiting test pathogen. In previous studies of antibacterial activities, dichloromethane: methanol (1:1) and methanol extract of T. brownii roots, stem bark and leaves exhibited good antibacterial activity against E. coli, P. aeruginosa and S. Aureus. The activity of extract was attributed to the presence of phytochemical such alkaloids, flavonoids, tannin, terpenoids, saponins, steroids, phenols and glycosides [17, 18, 19].

Dichloromethane: methanol (1:1) crude extract which showed the highest activity against E. coli (17.3±0.42) and P. aeruginosa (16±0.71) was used in determining minimum inhibitory concentration. Results of MIC showed that decrease in concentration of crude extract, reduced antibacterial activity. E. coli (1.25±0.07) had the highest MIC value compared to P. aeruginosa (1.11±0.13) suggesting that increasing the concentration of antimicrobial substance enhance zone of inhibition.

5. Conclusion

The present study has revealed that flower extract of T. brownii used in this study exhibit antibacterial and antifungal (Methanol extract only) effects against test organism used, which is attributed to the presence secondary metabolites such alkaloids, tannin, saponins, phenols, terpenoids and glycoside hence the extract can be used in the treatment of bacterial and fungal (methanol extract) infections therefore the researcher proposes for isolation of chemical compound responsible for activity against bacteria and fungal for drug development.

6. Recommendation

The study proposes the use of other extracting solvents and subsequent screening of phytochemical and antimicrobial activity against E. coli (ATCC 35218), P. aeruginosa (ATCC 10145) S. Aureus (ATCC 43300) and C. albicans using extracting solvent as a negative control.

7. Acknowledgements

The authors in this study appreciate Technical University of Mombasa (TUM) and Government Chemist –Mombasa for providing laboratory space and providing technical support over the study period. We also acknowledge George Nzai for support in extraction at Government Chemist and Kennedy Agoi for support in antimicrobial assays at TUM. This work forms part of the requirements for the Master’s Degree of TUM by the first author.

Conflicts of interest
There is no conflict of interest among the authors.

8. References


