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

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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 (1:1), methanol and water. The extracts were screened for the presences 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 aeroginosa, 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. aeroginosa* and *S. Aureus* showed that all extracts except n-hexane extract exhibited 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. Dichloromethane: methanol (1:1) flower extract exhibited the highest inhibitory activity against *E. coli* and *P. Aeroginosa* 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 aeroginosa (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 ºC for 24hours. After incubation, bacterial and fungal suspensions were aseptically inoculated on Mueller Hinton agar through surface spreading using sterile swaps 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 100mg/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 and fluconazole were used as positive control for bacteria and fungal respectively. Plates were kept in lanina 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 ºC for 24hrs and activity was expressed as the diameter of the clear zone of inhibition (mm) around each well indicating antimicrobial activity of the crude extract of *Terminalia brownii* [13].

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. aeroginosa (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. aeroginosa* and *S. Aureus* were prepared and incubated at 37 ºC for 48 hours. After incubation bacterial suspensions were aseptically inoculated on Mueller Hinton agar through surface spreading using sterile swaps 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 lanina flow for 30minutes to allow the system stabilize as the inoculated microorganism get acclimatized to the new environment. The culture plates were incubated at 37ºC 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 $p \leq 0.05$ 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 1: Phytochemical screening of crude extract of dichloromethane: methanol (1:1) and methanol of *Terminalia brownii* flower

S.No.	Phytochemicals	Reagent used	DCM: MeOH (1:1)	MeOH
1	Alkaloids	Mayers reagent	+	+
2	Flavonoids	NaOH solution	-	-
3	Tannin	Distilled water and $FeCl_3$ solution	+	+
4	Terpenoids	$CHCl_3$ and conc. H_2SO_4	-	+
5	Saponins	Distilled water	+	+
6	Steroids	$CHCl_3$ and conc. H_2SO_4	-	-
7	Phenols	$FeCl_3$ solution	+	+
8	Glycosides	Glacial acetic acid, $FeCl_3$ and conc. H_2SO_4	-	+

(+) 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. Aeroginosa* 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.

Distilled water was used as negative control while Amoxicillin and fluconazole as positive control for bacteria and fungal respectively. Dichloromethane: methanol (1:1) extract showed high zones of inhibitions hence it was selected for the determination of minimum inhibitory concentration against *E. coli and P. aeruginosa*.

Table 2: Antimicrobial activity of crude extracts of and flowers of *Terminalia brownie*

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

(-) 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)

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

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

(-) 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. aeroginosa*, *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. aeroginosa* 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) agreeing with [13] 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. aeroginosa* (ATCC 10145) *S. Aureus* (ATCC 43300) and *C. albicans* using extracting solvent as a negative control.

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Conflicts of interest

There is no conflict of interest among the authors.

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