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Full Length Research Paper

Phytochemical and antimicrobial screening of crude extracts from the root, stem bark, and leaves of *Terminalia glaucescens*

Adebayo, E. A.[1] and Ishola, O. R.[2]

[1]Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[2]Department of Biological Sciences, Covenant University, Otta, Ogun State, Nigeria.

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The root, stem-bark and leaves of *Terminalia glaucescens* were investigated for activity against some pathogenic organisms. Phytochemical screening revealed the presence of alkaloids, tannins, saponins, steroids, flavonoids, anthraquinones and phlobatannins (mostly in root and stem -bark). The results of *in-vitro* antimicrobial screening of the crude methanol extract exhibited a wide range of activity on *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus, Proteus mirabilis* and *Candida albicans.* The crude leaf extracts inhibited the growth of *E. coli, S. aureus, S. typhi and C. albicans* at a concentration of 50, 80, 40 and 60 mg/ml respectively, while the stem-bark extract had minimum inhibitory concentration (MIC) of 40 mg/ml on *K. pneumoniae, S. aureus*, *S. typhi* and 30 mg/ml on *C. albicans.* The extract from the root inhibited the growth of *E. coli* at a concentration of 10 mg/ml while *K. pneumoniae, P. mirabilis* and *C. albicans* were inhibited at a concentration of 60 mg/ml. The findings indicated that the extracts from *T. glaucescens,* contained bioactive components that have antimicrobial properties.

Key word: *Terminalia glaucescens,* phytochemical, antimicrobial agents, crude extract, minimum inhibitory concentration.

INTRODUCTION

Terminalia glaucescens is commonly found in Savannah regions. The plant is locally abundant and its common names are: baushe (Hausa), Idi Odan (Yoruba), Edo (Igbo). The tree is up to 20 m high, bole usually short and gnarled. The bark is dark grey, deeply fissured; slash yellowish or reddish rapidly turning darker. Sometimes shoots and young foliage have densely hairy leaves, about 8.5-15 cm long, and 2.5-7.5 cm broad. Flowers are greenish-white, very small and strongly scented with brown hairs at the base of the style. Wood is pale yellow-brown, hard and coarse textured (keay and Onochie, 1964). Ndukwe et al. (2005) reported that *T. glaucescens* has antimicrobial properties and can be used as a chemotherapeutic agent. *T. glaucescens* is reported to having potential for oral infection treatment (Ndukwe et al., 2005), employed in local dental hygiene (Rotimi and Bartlett, 19 88) and found to show impressive activity against the broad spectrum of organisms (Taiwo et al., 1999).

*Corresponding author. E-mail: brogoke2003@yahoo.com. Tel.: +2348038099092.

The plant is also reported to have traditional medicinal uses such as antimalaria, treatment of diarrhea and tooth decay (Ojo et al., 2006). The potential of aqueous extracts of *T. glaucescens* stem -bark against some pathogenic organisms have been extensively investigated, used as basis for selected chewing sticks (Ndukwe et al., 2007; Ojo et al., 2006 and Rotimi and Bartlett, 1988). However, reports on bioactive activities of the methanol extracts from root and leaf of *T. glaucescens* have not been widely investigated. This present study was intended to elucidate the chemical constituents' methanolic extraction of root, stem-bark and leaves with a view of authenticating the plant's antimicrobial potentials.

MATERIALS AND METHODS

Collection and preparation of plant material

Fresh root, stem bark and leaves of *T. glaucescens* were collected from teaching and research farm of Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The samples were air-dried in the laboratory at ambient temperature (30 ± 2°C) for 10 days; pulverized using a mechanical grinder and the obtained powders was

stored until further use.

Preparation of methanol extract

100 g of each plant part (root, stem-bark and leave) were packed in a soxhlet extractor and extracted with methanol. The methanol extracts were evaporated to dryness using a rotary evaporator (Stuart, Barloworld and Model RE 300) to obtain 22.3 g (root), 25.3 g (stem-bark) and 15.7 g (leaves) of crude extracts. The various crude extracts were later subjected to bioassay analyses.

Preparation of crude extract

The stock solutions were prepared by extracting 10 g of each plant part (root, stem-bark and leaves) in 10 ml of dimethylsulphoxide (DMSO) to obtain stock solutions of 1000 mg/ml concentration each. From the stock solution, concentrations (mg/ml) of 250, 200, 150, 100, 50 and 10 were obtained by serial dilution. These concentrations were stored at 15°C until further use.

Test microorganisms

Pure clinical isolates of *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus anthracis, Salmonella typhi, Pseudomonas aeruginosa, Proteus mirabilis* and *Candida albicans* were obtained from the Department of Medical Microbiology, Teaching Hospital, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria. The organisms were subcultured on Nutrient agar slant in bijou bottles and incubated at 37°C for 24h. The organisms were then stored at 4°C until needed.

Phytochemical Screening

The phytochemical analyses of the plant extracts were carried out following the methods of Sofowora (1986), Trease and Evans (19 83), Wallis (1967), Rai and Obayemi (1973) and Elujoba et al. (1986). They are briefly described below.

Alkaloids

About 0.2 g of extract was warmed with 1% of aqueous hydrochloric acid for two minutes. The mixtures were filtered and few drops of Dragendorff's reagent were added. A reddish-brown colour and turbidity with the reagent indicated the presence of alkaloids (Sofowora, 1986).

Flavonoids

Small quantities of the extracts were dissolved in10% of sodium hydroxide (NaOH) and Hydrochloric acid (HCl). A yellow solution that turned colourless on addition of HCl indicated the presence of flavonoids (Wallis, 1967).

Anthraquinones

5 g of the extracts was shaken with 10 ml of benzene. The solution was filtered and 5 ml of 10% NH_4OH solution was added to the filtrate. A pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of anthraquinones (Rai and Obayemi, 1973).

Glycosides

The test method is referred to as Lieberman's test. A small quantity of the extracts was dissolved in 2 ml of acetic anhydride and cooled in ice. Sulphuric acid (conc.) was then carefully added. The colour change from violet to blue to green indicated the presence of a steroidal nucleus (that is algycone portion of the cardiac glycoside) (Elujoba et al., 1989).

Tannins

5 mg of the powdered extracts was stirred with 10 ml of hot distilled water, filtered and ferric chloride was added to the filtrate and observed for blue-black, blue-green or green precipitate (Sofowora, 1986).

Steroids

The test for steroids was done by the Lieberman acid test. A portion of the extract was treated with drops of acetic anhydride. Concentrated H_2SO_4 was carefully added to the side of the test tube. The presence of a brown ring at the boundary of the mixture was taken as positive result (Trease and Evans, 1983).

Saponins

0.1 g of the powdered extract was boiled in 10 ml of distilled water for 5 min and decanted while still hot. The filtrate was used for the following tests (Sofowora, 1986).
(a) Frothing test: 1 ml of filtrate was diluted with 4 ml of distilled water and mixture was shaken vigorously and observed for persistent foam which lasted for at least 15 min.
(b) Emulsion test: This was performed by adding 2 drops of olive oil to the frothing solution and shaken vigorously. Formation of an emulsion indicated a positive test.

Phlobatannins

Deposition of a red precipitate when an aqueous extract of the plant was boiled with 1% aqueous hydrochloric acid indicated the presence of phlobatannins (Sofowora, 1986).

Evaluation of antimicrobial activity

The agar diffusion method as described by Osadebe and Ukwueze (2004) was adopted for the study. Broth cultures of the test isolates (0.1 ml) containing 1.0 X 10^5 CFU/ml of organisms were introduced into a sterile Petri dish and 15 mls of molten Mueller Hinton agar were added. The content was thoroughly mixed and then allowed to solidify. Holes were bored on the plates, using a standard sterile cork borer of 5 mm diameters and equal volumes of the plant extracts (1000 µl) were transferred into the wells with the aid of micropipette. The experiments were carried out in duplicate. The plates were allowed to stand for one hour for prediffusion of the extracts to occur and incubated at 37°C for 24 h (Esimone et al., 1998). At the end of incubation, the plates were observed and zones of inhibition were measured. The average zones of inhibition were recorded.

Determination of minimum inhibitory concentration (MIC)

The determination of the minimum inhibitory concentration (MIC) was carried out according to methods of Ndukwe et al. (2007). The medium used was nutrient agar solution which was prepared according to the manufacturers' standard of 28 g/1000 ml. In this case double strength was prepared by dissolving 28 g in 500 ml of distilled water which was swirled and mixed thoroughly by heating to allow uniform dissolution after which 5 ml of it was dispensed into 30

Table 1. Phytochemical analyses of the root, stem-bark and leaves of *Terminalia glaucescens.*

Phytochemicals	Root	Stem-bark	Leaves
Alkaloids	+	+	+
Flavonoids	-	+	+
Saponins	+	+	+
Steroids	+	+	+
Cardiac glycosides	-	-	-
Phlobatannins	+	+	+
Anthraquinones	+	-	+
Tannins	+	+	+

Key: +, present; -, absent.

Table 2. Antimicrobial activities of *T. glaucescens* (leaf methanolic extract).

Conc. of extract (mg/ml)	Zone of inhibition (mm)							
	EC	KP	SA	BA	ST	PA	PM	CA
250	26	0	28	0	25	0	0	28
200	26	0	26	0	25	0	0	24
150	18	0	20	0	18	0	0	18
100	18	0	14	0	16	0	0	14
50	15	0	0	0	14	0	0	0
10	0	0	0	0	0	0	0	0

EC= *E. coli*, KP= *K. pneumoniae*, SA= *S. aureus*, BA= *B. anthracis*, ST= *S. typhi*, PA= *P. aeruginosa*, PM= *P. mirabilis*, CA= *C.albicans.* *Values are means of duplicate readings.

sets of universal bottles and sterilized in an autoclave at 12°C for 15 min. The agar was allowed to cool to 45°C and each graded solution was then mixed gently with molten double strength nutrient agar in a Petri -dish and allowed to solidify for one hour. Extracts concentrations of (100, 80, 60, 50, 40, 30, 10, 5 and 0.5) mg/ml were prepared by serial dilution. Each plate was divided into eight equal sections and labeled appropriately. The 5 mm diameter filter paper discs (Whatman No. 1) were placed aseptically in the labeled section of the plate using sterile forceps. With a micropipette, 0.1ml of each bacterial suspension was taken and transferred aseptically with care into each appropriate pre-labeled paper disc on the agar plates. The plates were incubated for 24hrs at 37°C after which they were observed for growth or death of the test organisms. The lowest concentration inhibiting growth was taken as the minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

The phytochemical screening of the root, stem-bark and leaves of *T. glaucescens* revealed the presence of alkaloids, saponins, steroids, phlobatannins (root, stem-bark and leaves), Flavonoids (stem-bark and leaves), anthraxquinones (Root and leaves) as shown in Table 1. The presence of saponins, tannins, alkaloids and steroids in the plant's part is an indication that the plant is ofpharmacological importance (Hostettmann and Marston, 1995).

The antimicrobial activities of the leaf methanolic extract against *E. coli, S. aureus, S. typhi* and *C. albicans* was shown in Table 2. Methanolic extract from stem-bark was active against *K. pneumoniae*, *S. aureus, S. typhi* and *C. albicans* (Table 3), while methanolic extract from the root inhibited the growth of *E. coli*, *K. pneumoniae, P. mirabilis* and *C. albicans* (Table 4). Ndukwe et al. (2005) reported that saponins and aglycones present in plant extracts have varied uses as antiulcerogenic, anti-inflamatory, fibrinolytic, antipyretic, analgesic and anti-edematous. The activity of the extracts from the leaves and stem-bark against *S. aureus,* which is the potential causative organism of the tooth decay agreed with previous work (Rotimi and Bartlett, 1988), which reported *T. glaucescens* as phytotherapeutic agent for dental hygiene. The observed antibacterial effects collaborate its traditional uses.

The plant stem bark is used traditionally in treatment of typhoid fever and various stomach related problems (Adetunji, 1999). In this work the extracts of the plant's stem bark inhibited the growth of *E. coli* and *S. typhi* to a high degree. These two bacteria are responsible for various stomach related illnesses; *S. typhi* is the causative organism of typhoid fever, a systemic infection associated with the consumption of contaminated food while, *E. coli* is responsible for a number of food related illnesses

Table 3. Antimicrobial activities of *T. glaucescens* (stem-bark methanolic extract).

Conc. of extract (mg/ml)	Zone of inhibition (mm)							
	EC	KP	SA	BA	ST	PA	PM	CA
250	0	28	22	0	25	0	0	29
200	0	28	18	0	25	0	0	27
150	0	25	18	0	18	0	0	20
100	0	18	18	0	16	0	0	18
50	0	15	15	0	14	0	0	16
10	0	0	0	0	0	0	0	0

EC= *E. coli*, KP= *K. pneumoniae*, SA= *S. aureus*, BA= *B. anthracis* , ST= *S. typhi, P. aeruginosa*, PM= *P. mirabilis*, CA= *C. albicans.* *Values are means of duplicate readings.

Table 4. Antimicrobial activities of the methanolic root extracts of *T. glaucescens.*

Conc. of extract (mg/ml)	Zone of inhibition (mm)							
	EC	KP	SA	BA	ST	PA	PM	CA
250	36	6	0	0	0	0	26	35
200	27	28	0	0	0	0	22	33
150	23	22	0	0	0	0	18	27
100	22	18	0	0	0	0	16	17
50	18	0	0	0	0	0	0	0
10	14	0	0	0	0	0	0	0

EC= *E. coli*, KP= *K. pneumoniae*, SA= *S. aureus*, BA= *B. anthracis* , ST= *S. typhi, P. aeruginosa*, PM= *P. mirabilis*, CA= *C. albicans.* *Values are means of duplicate readings

Table 5. MIC values of methanolic leaf, stem-bark and root extracts of *T. glaucescens.*

Test orgs.	MIC at different conc. (mg/ml) of extracts		
	Leaf	Stem-bark	root
E. coli	50	0	10
K. pneumoniae	0	40	60
S. aureus	80	40	0
B. anthracis	0	0	0
B. anthracis	40	40	0
P. aeruginosa	0	0	0
P. mirabilis	0	0	60
C. albicans	60	30	60

that manifest themselves in the form of diarrhea (Adams and Moss, 1999). The relatively large zone of inhibition exhibited by the extract on *K. pneumoniae, P. mirabilis* and *C. albicans* suggests that it could be used in the treatment of infections commonly associated with the microorganisms. *T. glaucescens* has been listed as one of the medicinal plants in common use in Africa (Ndukwe et al., 2005). The MIC results were presented in Table 5. The crude extract of the leaves inhibited and finally prevented the growths of *E. coli (*50 mg/ml), *S. aureus* (80 mg/ml), *S. typhi* (40 mg/ml) and *C. albicans* (60 mg/ml), while the extract from the stem-bark had minimal inhibittion at 40 mg/ml (*K. pneumoniae, S. aureus* and *S. typhi*) and at 30 mg/ml against *C. albicans.* The MICs for the root were 10 mg/ml against *E. coli* and 60 mg/ml against *K. pneumoniae, P. mirabilis* and *C. albicans*. The findings in this work have justified the potent use of this plant in ethano medicinal treatment of oral infection, malaria, diarrhea etc which are caused by some of these organisms used in this study. Work is still on in the isolate and

characterization of the bioactive compounds in this plant.

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