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Full Length Research Paper

Phytochemical evaluation and antibacterial profile of *Treculia africana* Decne bark extract on gastrointestinal bacterial pathogens

S. O. Ogbonnia[1]*, N. V. Enwuru[2], E. U. Onyemenem[1], G. A. Oyedele[2] and C. A. Enwuru[3]

[1,3]Department of Pharmacognosy, Faculty of Pharmacy University of Lagos, Nigeria.
[2]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy University of Lagos, Nigeria.
[3]Nigeria Institute of Medical Research, PMB 2013, Yaba, Lagos, Nigeria.

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Treculia africana **Decne (Fam. Moraceae) is a highly valued economic plant, as well as an important medicinal plant widely used in the traditional herbal medicine for the treatment of several ailments of both microbial and non-microbial origins. It was, therefore, investigated for activity in *vitro* on pathogenic bacterial isolates of gastrointestinal tract. Fresh plant materials were collected from the Forestry Division in Oyo State and the aqueous ethanol (70%) extracts of the powdered bark were obtained by maceration method. The bacterial organisms tested were *Salmonella typhi* (ATCC24682), *Shigella dysentriae* (ATCC23513), *Escherichia coli* (Clinical isolate), *Pseudomonas aeruginosa* (ATCC12462) and *Staphylococcus aureus* (ATCC23815). Susceptibility testing and phytochemical screening of the plant extracts were performed by standard procedures. Aqueous ethanol extract of *T. africana* was effective on the tested organisms. The mean Minimum Inhibition Concentration (MIC) of the extract ranged from 3.125 to 25 mg/ml for different organisms tested. The extract exhibited minimum bactericidal concentration (MBC) of 50 mg/ml on *S. dysentriae* and *P. aeruginosa* only, while other tested bacteria strains required higher concentrations. Phytochemical screening revealed the presence of steroidal saponin glycosides as the major component, anthraquinone glycoside and polyphenols. Our results offer a scientific basis for the traditional use of *T. africana*. The aqueous ethanol extract of the bark was effective *in vitro* in this study on gastrointestinal bacteria pathogens, and thus could be explored for further pharmaceutical use.**

Key words: *Treculia africana*, antibacterial, pathogen, phytochemistry.

INTRODUCTION

The curative potentials of plants have long been recognized and to date plants remain the main source of drugs in the Nigerian traditional medicine. Plants with curative potentials are known as medicinal plants and have been employed in the treatment of various ailments, and some are employed as poisons to kill since pre-historic times (Herbert, 1989). Some plants have been employed in the traditional herbal medicine for only curative purposes while others have served as drugs as well as food and condiments in food for hundreds of years in many countries of the world and were found to have the advantage of having little or no side effects (Hassain, 2002). *Treculia africana and* other species of *Treculia* are amongst such plants that have both food and medicinal values.

T. africana Decne (Fam., Moraceae) commonly known as African Breadfruit and locally called *Ukwa* in Igbo vernacular is one of the most cherished economic plants, and is also a highly valued medicinal plant widely utilized in most preparations in the traditional herbal medicine. Its seeds are a veritable source of highly nutritious *ukwa* food which is found to have an excellent polyvalent dietetic value and have also biological value of its proteins exceeding that of soybean (Enibe, 2006).

The crude extracts from different parts of the plant have been used in the folk medicine in the treatment of various ailments. It is used either singly or in combination with

*Corresponding author. E-mail: steveogbonnia@yahoo.com.

other herbs in the traditional herbal preparations by different communities to treat various diseases. Decoctions from different plant parts are used as an anti-inflammatory agent and in the treatment of whooping cough. The crushed leaves juice is applied on the tongue as a treatment for thrush in children; the latex is applied as an antibacterial agent in eardrops, and as chewing stick.

The *T. africana* leaves decoctions were reported used in Trinidad and Bahamas to lower blood pressure (Morton, 1987), and is used also in some communities as an effective treatment in stomach upset and other gastro intestinal infections. Most of these claimed uses are yet to be scientifically verified and evaluated.

The purpose of this study was, therefore, to evaluate the traditional use of the bark of *T. africana* as an anti-bacterial agent in the treatment of lower abdominal pains caused by some bacterial pathogens of gastrointestinal tract.

MATERIALS AND METHODS

Collection and preparation of plant material

The stem bark of *T. africana* (*Fam*. Moraceae) was collected fresh in the early hours in the Month of October, 2005, from the tree in the Onigambari Forest Reserve, Oyo State and was identified at the Forest Research Institute of Nigeria (FRIN) Ibadan. Herbarium specimen was prepared and assigned a voucher no 107532 and was deposited with FRIN. The identified stem barks (1kg) were washed with tap water, cut into small pieces and dried at an ambient temperature (40 – 50°C), then milled to a coarse powder. The powder (400 g) was extracted by maceration in a 3 L beaker with 1600 mL of an aqueous ethanol (70%) for 72 h with constant stirring and filtered first through glass wool and then through Whatman no 4 filter paper. The extract was concentrated under reduced pressure in a rotary evaporator and yielded a constant dry weight of 22.24 g (5.56%).

Phytochemical evaluation of the crude extracts

The phytochemical screening was carried out using the methods as described by: Farnsworth (1966), Eaton (1989), Silva et al. (1993), Harborne (1998) and Houghton and Raman (1998).

Alkaloids

The extract (1.0 g) was dissolved in 10 mL purified water and filtered. To 5 mL of the filtrate, 2 mL of 10% HCl were added and boiled in a water bath for about 3 min. To 2 mL of the alkaloid salt solution, 3 drops of Mayer's reagent or Dragendorff's reagent were added.

Carbohydrates

The Molisch test was used for all monosaccharide and Fehling's solutions (I and II) test was used for detection of all reducing sugars (Eaton, 1989).

Cardiac glycosides

These were tested using Legal test and Kedde test for the detection of the presence of lactone ring and the Killer-Kiliani test for the detection of the presence deoxysugar, Farnsworth (1966).

Cyanogenetic glycosides

These were identified by suspending 0.5 g extract in 10 mL sterile water, and filtered. Sodium picrate paper was added to the filtrate and heated to boil.

Anthraquinone

The extract was also tested for free aglycone, and also for the bonded anthraquinones aglycone in the glycoside. Five grams of the extract was added to 10 mL of water, boiled and filtered and allowed to cool. Then 2 mL of the filtrate was shaken with 5 mL of chloroform. The chloroform layer separated and concentrated to about 2 mL and 2-3 mL ammonia solution added. The bonded aglycone was tested using modified Bőrntrager's test Houghton and Raman (1998).

Saponins

The extract was subjected to frothing test for the identification of saponin. Haemolysis test was further performed on the frothed extract in water to remove false positive results. Steroidal and triterpenes saponins were distinguished using the Liebermann-Burchard test (Farnsworth, 1966).

Flavonoids

The presence of flavonoids was determined using magnesium powder and a few drops of HCl test (Silva et al., 1993).

Bacterial isolates

The human pathogenic test organisms were: *Salmonella typhi* (ATCC 24682), *Shigella dysentria* (ATCC 23513), *Escherichia coli* (clinical isolate), *Pseudomonas aeruginosa* (ATCC 12462), and *Staphylococcus aureus* (ATCC 23815). The bacteria strains were re-identified and characterized prior to use. The strains were maintained on Mueller-Hinton agar plates and suspended in Mueller-Hinton broth prior to use for antimicrobial susceptibility testing.

Determination of antimicrobial activity

The agar well diffusion method (Perez et al., 1990; Alade and Irobi, 1993; Abioye et al., 2004) was used. The dried extract was reconstituted with sterile distilled water to obtain a stock solution of 250 mg mL^{-1} from which various concentrations of 250, 125, 62.5, 31.25 and 15.625 mg mL^{-1} were prepared. Overnight broth culture of the respective bacteria strains were adjusted to turbidity equivalent to 0.5 McFarland standard. Mueller–Hinton agar plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacteria strains. Five wells (6 mm in diameter) were made equidistance in each of the plates using a sterile cork borer. Up to 100 µl (0.1 ml) of each concentration of the extract were respectively introduced into the wells using sterile automatic pipettes, with the stock solution in the center well. It was allowed to diffuse at room temperature for 2 h. The plates were incubated at 37°C for 24 h. The solvent control and the control antibiotic discs, gentamycin disc (10 µg) for gram-positive bacteria and ofloxacine disc (30 µg) for gram-negative bacteria were used.

Table 1. The antibacterial susceptibility pattern of bark extract of *Treculia africana* using agar well diffusion method.

Microorganisms	Mean diameter of zone of inhibition in mm (mean ± SEM)	
	Methanol extract (250 mgmL^{-1})	Standard Drug control OFL (30 µg mL^{-1}) for GNB and G (10 µg mg mL^{-1}) for GPB
Gram positive		
Staph. aureus (ATCC 24632)	16.00±0.70	19.67±0.41
Gram negative		
P. aeruginosa (ATCC 12462)	21.67±0.41	26.67±0.4l
S. dysentriae (ATCC 23513)	19.33±0.41	31.00±0.00
S. typhi (ATCC 24632)	19.33±0.41	31.00±0.00
E. coli (Clinical isolate)	18.33±0.41	12.33±0.41

Values of inhibitory zone diameter were expressed as mean± SEM for *Traculia africana* at 250 mg/ml. OFL = oxafloxacin, G = gentamycin, GNB =gram-negative bacteria and GPB = gram-positive bacteria.

Diameters of the inhibition zones were measured. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extract.

Determination of antimicrobial susceptibility (MIC and MBC)

The aqueous ethanol extract of *T. africana* showed good activity in all bacteria strains in the solid agar well diffusion test. Those concentrations given an inhibitory zone of 11.00 mm diameter were chosen to assay for the minimum inhibitory concentration (MIC) with the agar dilution method according to NCCLS (1997) guideline.

The inoculum was prepared from an overnight broth cultures and adjusted to turbidity equivalent to 0.5 McFarland standards. Two fold serial dilutions of the stock solution of the extract were made to obtain concentration ranges of 50 to 0.0488 mg mL^{-1}. A 4 mL of each dilution was incorporated in 16 mL of the appropriate melted agar medium and poured into each of the eleven Petri dishes. Each Petri dish was divided into five sections. A loopful of the diluted culture of each test organisms was inoculated by streaking on the surface of each sectioned Petri dish. The Petri dishes were incubated at 37^{o}C for 24 h. A control was also set which contained only nutrient agar and the test organism. The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of each organism.

The Minimum Bactericidal Concentration (MBC) was determined by sub-culturing the plates that does not show bacterial growth from the results obtained in MIC determination. The organisms were sub-cultured on Muller-Hinton agar and incubated at 37^{o}C for 24 h, after which the viable cells were counted. The MBC was determined as the lowest concentration of the antimicrobial agent giving 99.9% reduction in the number of colony forming unit (cfu) from that of the original inoculum.

Statistics

The results were expressed as mean ± standard error of mean (S.E.M) and the test doses compared with the control by analysis of variance (ANOVA). Differences were considered significant if $p < 0.05$.

RESULTS

The result of the susceptibility profile of the various test organisms is presented in Table 1. The aqueous ethanol extract of the bark of *T. africana* inhibited all the bacteria tested with a measurable zone of inhibition. The standard positive control showed inhibition diameter ranging from 12.33 ± 0.41 – 31 ± 0.00 mm (ofloxacin-30 µg) against gram-negative bacteria and 19.67 ± 0.41 (Gentamycin-10 µg) against gram-positive bacteria.

The extract had higher inhibitory activity on gram-negative bacteria than in gram-positive bacteria. *Pseudomonas aeruginosa* (ATCC12462) was the most sensitive strain to the extract and exhibited the maximum zone of inhibition diameter of 21 mm at 250 mg/ml with ofloxacine exhibiting 26 mm at 30 µg/ml. *S. aureus (*ATCC 23815) was the lowest sensitive strain to the extract, exhibiting zone diameter of 16 mm at 250 mg/ml with gentamycin exhibiting 19 mm at 10 µg/ml.

The MIC and MBC results of *T. africana* bark extract are presented in Table 2. The mean MIC of the extract ranged from 3.125 to 25 mg/ml. The MBC of the extract was 50 mg/ml on *S. dysentriae* (ATCC 23513) and *P. aeruginosa* (ATCC 12462), other strains including *S. typhi* (ATCC 24632), *E.coli* (clinical isolate) and *S. aureus* (ATCC 23815) required higher concentrations.

The phytochemical screening showed a conspicuous absence of alkaloids but presence of glycosides including saponin glycosides with steroidal nucleus as the major constituent, anthraquinone glycosides and polyphenols (Table 3).

DISCUSSION

Plant product drugs and herbal remedies have been employed since prehistoric times to treat human and animal diseases, and several countries still rely on plants and herbs as the main sources of drugs. *T. africana* and other species of *Treculia* are widely employed in the traditional herbal medicine because of their multiple physiological and pharmacological activities. The scientific evaluation studies on the effectiveness of *T. africana* stem bark *in vitro* on gastrointestinal bacterial pathogens has given credence to the ethnobotanical use of the plant

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Treculia africana* extract against test bacteria organisms.

Microorganisms	Mean MIC and MBC of *Treculia africana* (mg/ml)		
	MIC range (mg mL^{-1})	Specific MIC (mg mL^{-1})	MBC (mg mL^{-1})
Gram positive			
S. aureus (ATCC 24632)	6.25-25	12.5	> 50
Gram negative			
E. coli (Clinical isolate)	6.25-25.0	12.5	> 50
S. typhi (ATCC 24632)	6.25-25.0	12.5	> 50
P.aeruginosa (ATCC 12462)	12.50-50.0	25.0	> 50
S. dysentriae (ATCC 23513)	3.13-12.50	6.25	50

Table 3. Phytochemical screening of the aqueous ethanol extracts of *T. africana* bark.

Phytochemical	Ocurrence
Alkaloids	-
Cardiac glycosides	+
Anthraquinone glycoside	+ +
Free aglycone	+
Saponin glycosides	+++
Steriodal nucleus	+++
Triterpenoid nucleus	-
Cyanoglycoside	-
Polyphenols	++
Flavonoids	-
Red cell Haemolysis	+++

- = Absent, + = slightly present, ++ = fairly present, +++ = abundantly present.

part in the treatment of gastrointestinal tract problems and the accompanied stomachache.

The aqueous ethanol extract of stem-back of *T. africana* was found to exert an antibacterial activity against the five bacteria strains used in the study. All the bacteria strains were inhibited at the extract concentrations used in the susceptibility test except *P. aeruginosa* which was not susceptible at a very low concentration. The study showed that gram-negative bacterial strains used (*P. aerugiosa, S. typhi, S. dysentriae* and *E. coli*) were generally more susceptible to the extract's activity than gram-positive bacteria strain (*S. aureus*). *P. aeruginosa exhibited* the highest zone of inhibition at the concentration used followed by *S. typhi*, *S. dysentriae* and *E.*coli. This report is a welcome development since multi-drug resistant bacteria are on increase in both hospital and community environments in Nigeria against orthodox antibiotics (Chamber, 1979; Lamikanra and Ndep, 1993; Chamber, 2001; Brown and Anicelus, 2004). This includes *S. aureus* reported to be highly resistant to ampicillin, cephalexin, methicillin and vancomycin; and is also resistant to gentamycin, rifampicin and chloramphenicol (Onanuga et al., 2005; Donaldson and Gosbell, 2006). The reason for the differential sensitivity between gram-positive pattern and gram-negative bacterial strains could not be ascribed to their morphological differences or adduced to their chemical compositions. Gram-negative bacteria have an outer phospholipids membrane with the structural lipopolysaccharide components, which make their cell wall impenetrable to antimicrobial agents (Nikaido and Vaara, 1985), while the gram-positive bacteria should be more susceptible having only an outer peptidoglycan, which is not effective permeability barrier (Scherer and Gerhardt, 1971). In spite of the permeability difference, the aqueous ethanol extract of *T. africana* exerted a broader spectrum of inhibitory activity on gram-negative bacteria than on gram-positive bacteria strain. The higher activity of the extract against *P. aeruginosa*, *S. typhi, S. dysentriae* and *E. coli* which are at times responsible for the pathogenesis of urinary and enteric infections provides a scientific evidence for the efficacy of *T. africana* in treating such infections (Ramesh et al., 2001).

The study showed that *P. aeruginosa* had a higher MIC value which translated that a higher concentration of the extract is required to inhibit the organism's growth, while *S. dysentria* had a lower MIC value and would require a very low extract concentration to inhibit its growth .The minimum bactericidal concentration (MBC) value of the extract was high for *P. aeruginosa* and *S. dysentria*.. The other bacteria test organisms only had a viable colony count after they have been re-cultured in nutrient agar. This could be explained that higher concentrations of the extract above those used in the experiment would be required to inhibit the growth of the organisms. Hence, the extract was bacteriostatic to *E. cloi, S. typhi* and *S. aureus* at the concentration used but was bactericidal against *P. aerugionsa* and *S. dysentria*. The variation in the quantity of the active ingredient required to effect inhibition may not matter much since medicinal plant have been reported to have little or no side effects (Hassain, 2002).

Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extract and the one

that predominates over the others. It may also be used to search for bioactive lead agents that could be used in the partial synthesis of some useful drugs (Yakubu et al., 2005). Phytochemical screening of the plant stem bark revealed the presence of saponins as the major active secondary metabolite, while anthraquinones and polyphenols other than flavonoids as minor constituents. Saponins are characterized by their surface active properties and they dissolve in water to form foamy solutions and because of surface activity some drugs containing saponins have a very long history of usage (Dean, 1999). Saponins have been implicated as a bioactive antibacterial agent of plants containing them (Mandal et al., 2005; and Manjunatha, 2006). The exhibited antibacterial properties of *T. africana* can be attributed to the presence of steroidal saponins and polyphenols in the bark. Polyphenols were earlier reported to have some antibacterial activities (Tomas-Barberan et al., 1990), and might have complimented or potentiated the saponins in the antibacterial activities exhibited by *T. africana* bark extract. More work is recommended.

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