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Susceptibilities of Clinical Bacteria Isolates to Two Ethanolic Extracts of *Terminalia schimperiana*

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ABSTRACT: Background/Objectives: The authors evaluated the in vitro antimicrobial effects of the ethanolic extracts of *Terminalia schimperiana* root bark and leaves, which are used locally in the treatment of burns wounds, bronchitis and dysentery. Methods: Five different concentrations of the crude extracts (1.25 mg/ml to 20 mg/ml) were screened against *Staphylococcus aureus* (n=2), *Escherichia coli* (n=2), *Pseudomonas aeruginosa* (n=4), *Salmonella typhi* (n=1), and a reference culture, ATCC 10145 of *P. aeruginosa*, using the agar-well diffusion method on Mueller-Hinton agar. Significant differences in the minimum inhibitory concentrations (MIC) of the extracts were analyzed using Analysis of Variance (ANOVA). Gentamicin was included as a standard antibiotic. Phytochemical analysis of the root bark extract was performed. Results: The results revealed that the extracts exhibited varying but significant activities against *S. aureus*, *P. aeruginosa* and *S. typhi* but not to *E. coli*. Inhibition zone diameters (IZD) ranged from 17.2 mm to 10.0 mm. MIC values ranged from 0.058 mg/ml to 2.089 mg/ml. Inhibition zone diameters of gentamicin ranged from 21.8 mm to 10 mm. However, two *P. aeruginosa* isolates were resistant to gentamicin at all the concentrations tested. Conclusion: The results seem to support the efficacy of the extracts in the folkloric treatment of burns wounds, and bronchitis and dysentery respectively, and show that the in vitro antibacterial activities of the extracts are comparable with that of gentamicin. The authors recommend that the extracts be subjected to more detailed studies in view of their potentials in the treatment of infections caused by resistant bacteria. ©JASEM

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The public is becoming increasingly aware of problems with the misuse of conventional antibiotics, especially the intractable problem of antibiotic resistance. Because one of the solutions to this problem is the need for alternative sources of drugs, the attention of clinical microbiologists have recently turned to antimicrobial plant extracts as sources of antimicrobials. It is very likely that phytochemicals will become a major source of antiinfective agents and many plant sources are now being investigated. One drawback in the effort to harness medicinal plant extracts is the lag in the analysis of local plants. Out of about 500,000 species of plants, only about 2% have been subjected to a complete ethnobotanical and biochemical analysis and less than 10% have been tested for biological activity (Asuzu 2001; Okafor, 2002). In addition, the knowledge of plant medicines has been the exclusive preserve of traditional herbal healers or native doctors. Until recently, native doctors were very reluctant to part with information concerning their medicinal plants or charged prohibitive amounts of money for those who required the knowledge of their plants. However, the situation has changed as many bio-prospectors are now working side by side with herbal healers to gain from their knowledge of medicinal plants. Many researchers are involved in conducting follow-up research to verify the authenticity of information by indigenous peoples concerning the medicinal uses of plants (Taylor, 2005)

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The medicinal activities of plants have been attributed to the presence of a variety of secondary metabolites. Scientists involved in medicinal plant research have found the presence of secondary metabolites such as alkaloids, flavonoids, glycosides, cardiac glycosides, saponins, tannins and essential oils (Vera, 1993; Ofokansi et al., 2003; Nmema et al., 2009). Levan et al., (1979), and Ibrahim et al., (1997), proposed that the presence of tannins, alkaloids, flavonoids and saponins suggest possible antimicrobial activity by plants.

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The use of plants for drug development has its undeniable benefits. In research, using medicinal plants makes research less expensive and more efficient, as laboratory synthesis of new medicines is becoming increasingly costly (Taylor, 2005). In therapy, plant drugs are considered to be more superior since they have a wide range of activities, with little or none of the adverse effects associated with synthetic antimicrobials. They are effective and yet gentle and many have tropisms to specific organs or systems in the body. Plant drugs usually have multiple effects on the body. Their actions often go beyond the symptomatic treatment of disease (Murray, 1995). Plant-derived drugs are easily available wherever they grow naturally and therefore more affordable. Economically, harnessing these forest resources for commercial purposes will be a good source of income. In addition, the herbal products market offers many opportunities for cultivating medicinal plant crops so as to meet the demands of the growing market.

Terminalia schimperiana is a broad leaved, small tree that can reach up to 7─14m. It is native to tropical Africa and in Nigeria is found in the woodland savanna. It is deciduous to semi-evergreen, depending on the climate. The leaves are alternate, simple, elliptic to obvate, entire, 9-15cm long and 3-8cm broad, green above with pale undersides. The flowers are tiny and form spikes at the base of the leaves. The fruit is a samara with a single wing, 6-9cm long which turns brown with age (African Plants Database; Arbornea). In parts of West Africa, *T. schimperiana* is used as a medicinal plant. The leaves are used to treat bronchitis and dysentery and the stem bark is applied to wounds. Herbalists use the root bark to treat burn wounds in Izzi Local Government Area of Ebonyi State where it is known as 'Oshioku' (Personal communication- Mbam, V. 2007). To our knowledge no research has yet been conducted on the antibacterial properties of the ethanolic extracts *T. schimperiana* root bark and leaves.

The aim of the present study is to evaluate the *in vitro* antimicrobial activities of crude ethanolic extracts of *Terminalia schimperiana* root bark and leaves with a view to recommend further research on any extract found with significant activity.

MATERIALS AND METHODS

***Collection and identification of the medicinal plant materials*:** *Terminalia schimperiana* root bark and leaves were selected for study after consultation with a local herbalist, based on their uses in traditional medicine. They were collected from Nsukka in Enugu State and Iboko in Izzi Local Government Area of Ebonyi State. The plants were identified by Plant Taxonomist, Emeritius Professor J. C. Okafor of Department of Botany, Faculty of Biological Sciences, University of Nigeria, Nsukka. Voucher specimens were deposited at Department of Botany Herbarium, University of Nigeria, Nsukka and ascribed number 578.

Drying and pulverization of plant parts: The root bark and leaves were dried for two weeks at room temperature. They were each pulverized in a mill into dry powder and packaged in clean black polythene bags.

***Ethanolic Extraction*:** Fifty grams (50 g) of each pulverized material was introduced into a 1000 ml conical flask and 250 ml (or equivalent w/v concentrations for higher volumes) of 96% Ethanol solvent was added to the powder. The mixture was stirred with a glass rod and macerated for 48 hours at room temperature. Thereafter, the mixture was filtered with Whatman No. 1 filter papers inserted in a funnel to separate the filtrate (extract) from the residue (marc). The marc was rinsed with half the volume of solvent previously used and filtered again. The extract was concentrated using a rotary evaporator under low pressure at 40^{o}C and then dried at room temperature. The extract was stored in labeled, sterile amber bottles and kept in a refrigerator at 4^{o}C, as previously described (Nmema, 2009).

Test organisms: The test organisms included *Staphylococcus aureus* (n=2), *Escherichia coli* (n=2), *Pseudomonas aeruginosa* (n=4), *Salmonella typhi* (n=1), and a reference culture, ATCC 10145 of *P. aeruginosa.* They were collected from the diagnostic laboratories of National Orthopaedic Hospital, Enugu, Departments of Microbiology and Veterinary Microbiology, University of Nigeria, Nsukka, between 2005 and 2007. The isolates were transferred to Microbiology Department, University of Nigeria, Nsukka for confirmation of their identity using standard biochemical tests.

Serial Dilution of Crude Extract: A microgram balance (Mettler H8) was used to weigh out 0.02 g of each crude extract and this was introduced into a sterile Bijou bottle containing 1ml of the solvent (or equivalent w/v concentrations for higher volumes of solvent). The solvents were 25% dimethylsulfoxide (DMSO) for the root bark and propylene glycol for the leaves extract. This was thoroughly mixed using a vortex mixer to give a 20 mg/ml concentration. Serial dilution was carried out by using a sterile pipette to

transfer a measured volume of this dilution into another bijou bottle containing an equal amount of solvent to obtain a 10 mg/ml dilution. This procedure was used to prepare two-fold dilutions of 5 mg/ml, 2.5 mg/ml and 1.25 mg/ml of the crude extracts.

***Sensitivity Test of Organisms with Plant Extracts using Agar-well Diffusion Method*:** The test organisms were sub-cultured on nutrient agar plates and incubated at 37°C for 18-24 hours. The growth from each plate was transferred into test tubes containing 5 ml of 0.9% sterile saline and the volume adjusted in a spectrophotometer to obtain a turbidity which matches that of 0.5 McFarland Standard (containing 1.5×10^8 cfu/ml). A sterile cotton swab was immersed in the bacterial suspension. Excess fluid was expressed by rotating the swab against the inside wall of the test tube. This was then used to swab the surface of the Mueller-Hinton agar plates while rotating the plate anticlockwise until the entire surface has been swabbed.

The sensitivities of the test organisms to the crude extracts were tested using the agar-well diffusion method (Rios et al., 1988). A sterile cork borer with a diameter of 8mm was used to bore wells into the seeded Mueller-Hinton agar plates. A drop of molten agar was placed in each well to seal the bottom. Using a sterile pipette, 0.1ml of each of the dilutions (20 mg/ml, 10 mg/ml, 5 mg/ml, 2.5 mg/ml and 1.25 mg/ml) of each extract was introduced into a labeled well. The same quantity of gentamicin (standard antibiotic) and solvent were used as positive and negative controls respectively. Gentamicin was used in five dilutions (10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml and 0.625 µg/ml. Replicate plates were prepared for each organism. The plates were allowed to stand on the bench for 1 hour at room temperature for proper diffusion to take place and subsequently incubated at 37°C for 24 hours. After incubation, the plates were examined and inhibition zone diameters (IZD) measured with a ruler. The IZD (mm) was recorded by calculating the mean of IZDs for each set of replicate plates.

Determination of Minimum Inhibitory Concentration (MIC) values of the Extracts with Activity: The MIC values of the extracts were determined from the results of the antimicrobial screening. Microsoft Excel was used to plot the graph of mean IZD[2] against log drug concentration. A trend line was fitted into the scatter diagram to obtain intercept on the log drug conc axis (C), after which the MIC values (antilog of C) were obtained (NCCLS, 1999).

Phytochemical Analyses of the ethanolic extract: The ethanolic extract was subjected to phytochemical analysis based on procedures outlined by Harbourne (1973) and Trease and Evans (1989).

RESULTS AND DISCUSSION

***Results of antimicrobial screening of root bark* extract:** Table 1 shows the results of antimicrobial screening of the ethanolic extract of the root bark**.** The extract exhibited varying but significant activities against *S. aureus*, *P. aeruginosa* and *S. typhi* The inhibition zone diameters (IZD) ranged from 17.2 mm to 11.0 mm, the activity decreasing with decreased concentration. However, the extract did not have any effect at the concentration of 1.25 mg/ml. It also did not inhibit the growth of *E. coli* at any of the concentrations tested.

Results of antimicrobial screening of leave extract: Table 2 shows the results of antimicrobial screening of the ethanolic extract of the leaves**.** The extract exhibited varying but significant activities against *S. aureus*, *E. coli* 1, *P. aeruginosa*, and *S. typhi*. The inhibition zone diameters (IZD) ranged from 16.0 mm to 10.0 mm, the activity decreasing with decreased concentration. It may be inferred from this result that the root bark extract shows a higher activity than the leaves extract. However, unlike the root bark extract, the leaves extract was able to inhibit some isolates at the concentrations of 2.5 mg/ml and 1.25 mg/ml. It did not inhibit the growth of *E. coli* 2 at any of the concentrations tested. Out of all the bacteria isolates, *E. coli* showed the lowest susceptibility to the extracts.

***Results of antimicrobial screening of gentamicin standard*:** Table 3 shows the results of antimicrobial screening of the gentamicin standard**.** The antibiotic exhibited varying but significant activities against *S. aureus*, *P. aeruginosa* 3 and 4, *E. coli* and *S. typhi*. The inhibition zone diameters (IZD) ranged from 21.8 mm to 10.0 mm, the activity decreasing with decreased concentration. The antibiotic however, did not inhibit any of the isolates at 1.25 mg/ml. It also did not inhibit *P. aeruginosa* 1 and 2 at any of the concentrations tested. *P. aeruginosa* 1 and 2 were however susceptible to the two extracts even at the lowest concentration (1.25 mg/ml) of the leaves extract.

***Minimum inhibitory concentrations of the extracts and gentamicin*:** Table 4 shows the minimum inhibitory concentrations (MIC) values of the two extracts and gentamicin. MIC values of the root bark

extract ranged from 0.282 mg/ml to 1.622 mg/ml but showed no inhibition against *E. coli*. MIC values of the leaves extract ranged from 0.056 mg/ml to 1.462 mg/ml but showed no inhibition against *E. coli* 2. MIC values of gentamicin ranged from 0.912 mg/ml to 1.995 mg/ml but showed no inhibition against *P. aeruginosa* 1 and 2.

***Results of phytochemical analysis*:** Table 5 shows the phytochemicals present in crude ethanolic extract of *T. schimperiana* root bark. The compounds included an abundance of tannins and saponins, high concentrations of flavonoids, glycosides and carbohydrates, and moderate concentrations of alkaloids and resins. Others included steroids, terpenoids, reducing sugars and acidic compounds. These phytochemicals include compounds purported to confer antimicrobial properties on plants (Levan et al., 1979; Ibrahin et al., 1997).

***Results of ANOVA*:** For *S. aureus,* Analysis of Variance (ANOVA) revealed no significant differences ($P>0.05$) in the mean MIC values of the root bark extract, leaves extract and gentamicin. For *E. coli*, there were significant differences ($P<0.05$) in the mean MIC values of the leaves extract and gentamicin. For *P. aeruginosa*, ANOVA revealed no significant differences ($P>0.05$) in the mean MIC values of the root bark extract, leaves extract and gentamicin. For *S. typhi*, there were no significant differences ($P>0.05$) in the mean MIC values of the root bark and leaves extracts but significant differences ($P<0.05$) in the mean MIC values of these two extracts and gentamicin.

Conclusion: The results obtained in the present study reveal that the ethanolic extracts of *T. schimperiana* root bark and leaves possess significant antibacterial activities against the test organisms. This result seems to support the fact that the extracts are used in the folkloric treatment of burns wounds, and bronchitis and dysentery respectively. The results also show that the in vitro antibacterial activities of the extracts are comparable with that of gentamicin standard. In addition, two isolates resistant to gentamicin were susceptible to the extracts. The authors recommend that the extracts be subjected to further research aimed at developing plant-based antimicrobials against antibiotic-resistant bacteria.

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Table 1: Inhibition zone diameters (IZD) exhibited by ethanolic extract of *T. schimperiana* root bark on the test organisms, measured in millimeters (mm)

Test organism	Inhibition zone diameters				
S. aureus 1	13.0±0.23	11.7±0.34	10.0±0.00	0	0
S. aureus 2	14.5±0.87	13.5±0.50	12.5±0.50	11.7±0.17	0
E. coli 1	0	0	0	0	0
E. coli 2	0	0	0	0	0
P. aeruginosa 1	16.5±0.50	14.5±0.23	13.0±0.00	11.0±0.00	0
P. aeruginosa 2	15.5±0.58	13.5±0.23	10.5±0.50	0	0
P. aeruginosa 3	17.2±0.17	16.3±0.34	15.0±0.00	12.0±0.00	0
P. aeruginosa 4*	16.5±0.50	15.0 ± 0.00	13.5±0.50	0	0
S. typhi	15.0±0.00	13.3±0.67	12.5±0.50	11.5±0.50	0

n = 3, *ATCC Typed Sample

Table 2: Inhibition zone diameters (IZD) exhibited by ethanolic extract of *T. schimperiana* leaves on the test organisms, measured in millimeters (mm)

Test organism	Inhibition zone diameters				
S. aureus 1	14.5±0.50	12.5±0.50	11.0±1.00	0	0
S. aureus 2	15.0±0.23	13.5±0.50	11.5±0.50	0	0
E. coli 1	11.0±0.00	10.0±0.00	0	0	0
E. coli 2	0	0	0	0	0
P. aeruginosa 1	16.0±0.50	14.5±0.50	13.5±0.23	12.0±0.00	10.0±0.58
P. aeruginosa 2	14.0±1.00	12.5±0.50	12.5±0.50	11.0±0.58	10.0±0.00
P. aeruginosa 3	14.0± 1.00	12.3±0.67	10.5±0.50	10.0±0.00	0
P. aeruginosa 4*	14.0±0.56	12.7±0.17	10.3±0.88	0	0
S. typhi	15.7±0.17	13.0±0.00	12.3±0.34	10.3±0.67	0

n = 3, *ATCC Typed Sample

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Table 3: Inhibition zone diameters (IZD) exhibited by gentamicin on the test organisms measured in millimeters (mm)

Test organism	Inhibition zone diameters				
S. aureus 1	13.8±0.17	12.0±0.05	10.5±0.58	0	0
S. aureus 2	15.2±0.17	13.5±0.05	11.3±0.34	10.0±0.23	0
E. coli 1	20.0±0.50	18.0±0.23	15.3±0.67	0	0
E. coli 2	21.0±0.50	19.0±0.00	17.2±0.17	0	0
P. aeruginosa 1	0	0	0	0	0
P. aeruginosa 2	0	0	0	0	0
P. aeruginosa 3	18.0±0.50	11.3±0.67	10.0±0.00	0	0
P. aeruginosa 4*	21.8±0.44	21.0±0.58	18.0±0.50	0	0
S. typhi	17.5±0.05	15.0±0.58	13.5±0.05	0	0

n = 3, *ATCC Typed Sample

Table 4: Minimum inhibitory concentrations (MIC) values

Test organism	**Root bark extract (mg/ml)**	**Leaves extract (mg/ml)**	**Gentamicin (µg/ml)**
S. aureus 1	1.549	0.832	1.585
S. aureus 2	0.689	1.462	1.047
E. coli 1	NI	2.089	0.955
E. coli 2	NI	NI	0.912
P. aeruginosa 1	0.933	0.158	NI
P. aeruginosa 2	1.622	0.056	NI
P. aeruginosa 3	0.282	0.891	1.205
P. aeruginosa 4*	1.250	0.902	1.995
S. typhi	0.689	0.912	1.472

NI- No Inhibition, *ATCC Typed Sample

Table 5: Phytochemical composition of *T. schimperiana* root bark crude ethnanolic extract

Compound	**Concentration**
Alkaloid	+ +
Glycosides	+ + +
Reducing sugar	+
Flavonoids	+ + +
Steroids	+
Terpenoids	+
Acidic compounds	+
Fats and oils	-
Tannins	++++
Saponins	++++
Carbohydrates	+ + +
Resins	+ +

Key
++++Abundantly present
+++Present in very high concentration
++Present in moderately high concentration
+Present in small concentration
-Not present

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