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COMPARATIVE EVALUATION OF ANTIPSEUDOMONAS ACTIVITIES OF GENTAMICIN AND AQUEOUS EXTRACT OF *Terminalia schimperiana* ROOT BARK

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ABSTRACT

The author conducted a comparative evaluation of antipseudomonas activities of gentamicin and aqueous extract of *Terminalia schimperiana* root bark, a local remedy used in the treatment of burns wounds. Five different concentrations of gentamicin (0.625 µg/ml -10 µg/ml) and aqueous extract of *Terminalia schimperiana* root bark (1.25 mg/ml -20 mg/ml) were screened against *Pseudomonas aeruginosa* clinical isolates (n=14) and a reference culture, ATCC 10145 of *P. aeruginosa*, using the agar-well diffusion method. Significant differences in the minimum inhibitory concentrations (MIC) of gentamicin and aqueous extract were analysed using Analysis of Variance (ANOVA). Results revealed that 7 *P. aeruginosa* isolates were resistant to gentamicin at all the concentrations tested, while 8 isolates were susceptible to gentamicin at concentrations of 2.5 µg/ml, 5.0 µg/ml and 10 µg/ml, inhibition zone diameters (IZDs) ranging from 15.3 mm to 23.0 mm. All the isolates were susceptible to the aqueous extract, IZD values ranging from 12.0 mm to 22.5 mm. MIC values for gentamicin ranged from 0.2 µg/ml to 1.995 µg/ml, while MIC values for aqueous extract ranged from 0.054 mg/ml to 1.622 mg/ml. Analysis of variance (ANOVA) revealed no significant difference (P=0.32) in the MIC values of gentamicin and the aqueous extract. The aqueous extract inhibited 7 isolates resistant to gentamicin, suggesting that it possesses better antipseudomonas properties than gentamicin. The author recommend that the aqueous extract of *T. schimperiana* root bark be subjected to more detailed studies in view of its potential in the healing of wound and burns infections caused by resistant bacteria.

KEYWORDS: *Pseudomonas aeruginosa*, *Terminalia schimperiana*, gentamicin, comparative evaluation.

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INTRODUCTION

Strains of pathogenic bacteria have increasingly exhibited multiple drug resistance to a variety of first line antibiotics which have rendered these antibiotics ineffective against them. With few exceptions, the introduction of a new antibiotic has been followed within a few years by the first cases of resistance (Wain *et al*., 1997). Perhaps one of the most resistant of bacterial pathogens is *Pseudomonas aeruginosa*, a gram-negative aerobic rod (Nmema *et al*., 2003). *Pseudomonas aeruginosa* has emerged as an important cause of infections, especially in persons with compromised host defence mechanisms such as loss of the integrity of skin and mucous membrane or underlying immune deficiency. It causes urinary tract infections (UTI), respiratory tract infections, dermatitis, skin and soft tissue infections, eye and ear infections, bacteremia and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients (Qarah *et al*., 2003). *P. aeruginosa* shows a particular propensity for

the development of resistance which may be natural or acquired, with increasing multiple resistance emerging over time. Strains of this pathogen have notoriously shown multi-drug resistance to the mainstream antibiotics used in Nigeria (Kesah *et al*., 1999; Ozumba, 2003; Olayinka *et al*., 2004).

The challenges posed by emergence of these resistant mutants include many incidences of therapeutic failure and the need to provide alternative therapeutic agents, especially from natural sources. Various research efforts aimed at evaluating the antimicrobial activities of plant extracts have yielded encouraging results (Iwalokun *et al.,* 2001; Fakeye *et al*., 2002; Ofokansi *et al*., 2003; Nmema *et al*., 2003) while further studies are ongoing to develop effective antimicrobials from plants.

Terminalia schimperiana Hochst (synonym.: *T. glaucescens* Planch. ex Benth; Igbo: Oshioku) is a tree belonging to the family *Combretaceae*. *Terminalia* is a genus of large trees comprising around 100 species distributed in tropical regions of the world. There are 11 species found in West Africa. Species of *Terminalia* are most widely used for medicinal purposes and some like *T. catappa,* have edible fruits. Trees of this genus are known as a source of secondary metabolites-cyclic triterpenes and their glycoside derivatives, flavonoids, tannins and other aromatic compounds (McGraw *et al*., 2001; Adjanahoun *et al*., 1991).

T. schimperiana is native to tropical Africa. In Nigeria, it is found in the woodland savanna. It is a broad leaved, small tree that can reach up to 7─14m. 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, 2007; Arbonnier, 2004). In parts of West Africa, *T. schimperiana* is used as a medicinal plant [Sofonara, 1982]. 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, 2007). When applied to wounds, it produces a burning sensation, similar to what is experienced when iodine is applied to wounds. The twigs may be chewed to promote oral hygiene. The leaves are used to treat bronchitis and dysentery.

In the past, herbal healers were very reluctant to part with information concerning their medicinal plants, but today, many researchers are involved in conducting follow-up research to verify the authenticity of various indigenous herbal medicines in collaboration with herbal healers. The aim of the present study is to evaluate the antibacterial activity of *Terminalia schimperiana* aqueous extract in comparison with gentamicin, a first line antibiotic.

MATERIALS AND METHODS

Collection and extraction of root bark

Terminalia schimperiana root bark was collected from Iboko in Izzi LGA of Ebonyi State, and authenticated by Professor Okafor (Emeritus), Department of Botany, University of Nigeria, Nsukka. The material was washed in water to remove soil and other debris, cut into pieces and dried for two weeks at room temperature. It was then pulverized in a mill into dry powder, packaged in a clean black polythene bag and kept for further studies. The aqueous extract was prepared by introducing fifty grams (50 g) of the pulverized material into a 1000 ml conical flask and adding 250 ml of distilled water (or equivalent w/v concentrations for higher volumes). The mixture was stirred with a glass rod and macerated for 48 hours at 4^{o}C in a refrigerator to prevent deterioration of the extract with time. The mixture was then 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 water again, filtered, and the filtrates pooled together. The aqueous extract was air dried in open trays at room temperature and the extracts stored in labeled, sterile amber bottles and stored in a refrigerator at 4^{o}C as previously described (Nmema *et al*., 2003).

Standardization of aqueous extract

Five dilutions of the aqueous extract were prepared as follows: 0.02 g of crude extract was weighed with a microgram balance (Mettler H8) and introduced into a sterile Bijou bottle containing 1ml of the sterile distilled

water (or equivalent w/v concentrations for higher volumes of solvent). This was thoroughly mixed to give a 20 mg/ml concentration. Serial dilution was carried out by using a sterile pipette to transfer 2 ml 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 extract as previously described (Nmema *et al*., 2003).

Serial dilution of gentamicin solution
An ampoule containing 280 mg/2 ml of gentamicin was procured from a local pharmacy.
280 mg/2 ml of gentamicin is equivalent to140mg/ ml. 10 µg/ml of gentamicin was prepared as follows:

First, the dilution formula $C_1V_1 = C_2V_2$ was used to calculate the amount of water needed to prepare a concentration of 1mg/ml of gentamicin.

C_1=140 mg/ ml C_2 = 1 mg/ ml V_1 = 1 ml V_2 = ?

$$V_2 = \frac{C_1 V_1}{C_2} = \frac{140 \times 1}{1} = 140 \text{ ml}$$

Therefore 139 ml of sterile distilled water was added to 1ml of gentamicin stock solution to give 1 mg/ml, which is equivalent to 1000 µg/ml. Then 1 ml of the1000 µg/ml solution was added to 99 ml of distilled water to produce 10 µg/ml of gentamicin. Starting with the 10 µg/ml concentration, serial dilution was carried out to obtain concentrations of 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml and 0.625 µg/ml.

Test organisms: Clinical isolates of *Pseudomonas aeruginosa* (n=14) and a reference culture, ATCC 10145 of *P. aeruginosa* were included in the study.

Antimicrobial sensitivity screening with aqueous extract and gentamicin
The sensitivities of the test organisms to the crude extracts were tested using the agar-well diffusion method. A sterile cork borer with a diameter of 8mm was used to bore wells into Mueller-Hinton agar plates whose surfaces had been swabbed with standardized suspensions of the test organisms (0.5 McFarland Standard). Using a sterile pipette, 0.1ml of each concentration of the extract was introduced into a labeled well. Similarly, 0.1 ml of each concentration of gentamicin was introduced into an appropriately labeled well (Rios *et al*., 1988). Replicate plates were prepared for each organism. The plates were kept on the bench for 1 hour at room temperature for proper diffusion to take place and subsequently incubated at 37^{o}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 concentrations of gentamicin and aqueous extract
To determine the MIC values of gentamicin and aqueous extract, 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 (C) on the log drug concentration axis, after which the MIC values (antilog of C) were obtained (NCCLS, 1999).

Statistical Analysis
The values obtained in the experiments were expressed statistically as mean $\pm$SEM. Analysis of Variance (ANOVA) was used to determine significant differences in the minimum inhibitory concentrations (MIC) of gentamicin and the aqueous extract based on 95% confidence limit (P=.05).

RESULTS AND DISCUSSION

Activity of graded concentrations of gentamicin on the test organisms

Table 1 shows the results of the antimicrobial susceptibility screening with gentamicin. Seven (7) *P. aeruginosa* isolates were resistant to gentamicin at all the concentrations used. Eight (8) *P. aeruginosa* isolates were susceptible at concentrations of 2.5 µg/ml, 5.0 µg/ml and 10 µg/ml with inhibition zone diameters (IZDs) ranging from 10.0 mm to 23.0 mm. However, these susceptible isolates were resistant to the concentrations of 1.25 µg/ml and 0.625 µg/ml.

Activity of crude aqueous extract of *T. schimperiana* root bark on the test organisms

The results on Table 2 show that the aqueous extract possesses a very high activity against *P. aeruginosa* isolates, with some isolates susceptible to the concentration of 1.25 mg/ml. The inhibition zone diameters at 20 mg/ml concentration ranged from 22.5 mm to 14.0 mm while that of 1.25 mg/ml concentration ranged from 16.5 mm to 12.0 mm. Only the concentrations of 2.5 mg/ml and 1.25 mg/ml recorded 8 and 11 resistant strain respectively. It is also interesting to note that seven (7) *P. aeruginosa* isolates which were resistant to all the concentrations of gentamicin (Table 1) were susceptible to *T. schimperiana* extracts up to 5.0 mg/ml, with *P. aeruginosa* Strain 3 showing susceptibility up to 1.25 mg/ml (Table 2).

Minimum inhibitory concentrations (MIC) of gentamicin and aqueous extract on the test organisms.

Table 3 shows the MIC values of gentamicin and aqueous extract on the test organisms. The results shown on Table 3 and Figure 3 clearly show that the seven *P. aeruginosa* isolates numbered 3, 4, 5, 9, 10, 11 and 12 were found to be resistant to (or not inhibited by) gentamicin at all the concentrations used. For the eight gentamicin-susceptible isolates, the MIC values of gentamicin ranged from 0.2 µg/ml to 1.995 µg/ml, indicating a good activity. However the aqueous extract inhibited all the isolates, with varying MIC values ranging from 0.095mg/ml to 1.622 mg/ml. Analysis of variance (ANOVA) revealed no significant differences (P=0.32) in the MIC values of gentamicin and the aqueous extract.

DISCUSSION

The increasing resistance of *P. aeruginosa* isolates to gentamicin and other first and second line antibiotics has been reported by previous workers in Nigeria. In a previous research, 32% gentamicin resistance was reported among 125 *P. aeruginosa* isolates from paediatric patients in Lagos University Teaching Hospital (LUTH) (Kesah et al., 1999). A decade later, 83% resistance to gentamicin and multiple resistances to other antibiotics were reported among 12,458 urinary isolates of *P. aeruginosa* [Jombo et al., 2008]. The increasing antibiotic-resistance among bacteria has been attributed to exchange of resistance factors among bacteria of related genera (Mazodier & Davies, 2013; Davies, 1997). In Nigeria, other factors including abuse of antibiotics, self-prescription, incomplete dosage and easy accessibility of antibiotics in local pharmacies have been cited (Oyetunde et al., 2013; Ekwocha et al., 2013).

MIC values have inverse relation to drug activity, meaning that the lower the MIC, the higher the activity of an antimicrobial agent. It then follows that the aqueous extract compares favorably with gentamicin in its anti-pseudomonas activity. This is supported by results of Analysis of variance (ANOVA) revealing no significant differences (P=0.32) in the MIC values of gentamicin and the aqueous extract. However, the aqueous extract inhibited 7 isolates resistant to gentamicin, suggesting that it possesses better anti-pseudomonas properties than gentamicin.

The aqueous extract of *T. schimperiana* contains alkaloids, flavonoids, anthraquinones, saponins, tannins, steroids, terpenoids, phlobatanins and carbohydrates, while cardiac glycosides are lacking (Oshomo & Idu, 2011). Earlier workers have proposed that the presence of flavonoids, alkaloids, tannins and saponins in plants confer antimicrobial activity on the plants (McGraw et al., 2001; Levan et al., 1979; Ibrahim et al., 1997). The presence of these antimicrobial compounds in the aqueous extract may be a confirmation of the antibacterial activity demonstrated in this study.

The high susceptibility of the isolates to the aqueous extract of *T. schimperiana* root bark seems to lend credence to the claims of its wound healing property expressed by indigenous people. Since drug-resistant *P. aeruginosa* strains are often incriminated in persistent wound and burns infections, the aqueous extract may be exploited in the development of novel drugs for treatment of burns wound infections. The author therefore recommends that the aqueous extract of *T. schimperiana* root bark be subjected to more detailed studies in view of its potential in the healing of wound and burns infections caused by resistant bacteria.

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Table 1: Inhibition zone diameters (mm) exhibited by gentamicin on the test organisms

Test organism	Concentration of gentamicin in µg/ml				
	10	5	2.5	1.25	0.625
P. aeruginosa 1	22.0±0.50	20.0±0.50	17.7±0.34	0	0
P. aeruginosa 2	23.0 ±0.23	22.5±0.05	19.8±0.44	0	0
P. aeruginosa 3	0	0	0	0	0
P. aeruginosa 4	0	0	0	0	0
P. aeruginosa 5	0	0	0	0	0
P. aeruginosa 6	22.0 ±0.50	19.0±0.00	17.0±0.23	0	0
P. aeruginosa 7	22.2 ±0.17	19.0±0.58	18.0±0.00	0	0
P. aeruginosa 8	21.0 ±0.50	20.5±0.50	16.0±0.58	0	0
P. aeruginosa 9	0	0	0	0	0
P. aeruginosa 10	0	0	0	0	0
P. aeruginosa 11	0	0	0	0	0
P. aeruginosa 12	0	0	0	0	0
P. aeruginosa 13	18.0±0.50	11.3±0.67	10.0±0.00	0	0
P. aeruginosa 14	14.3±0.34	12.2±0.17	10.0±0.23	0	0
P. aeruginosa 15*	21.8±0.44	21.0±0.58	18.0±0.50	0	0

n = 3- Values represent means ± standard error of the mean, *ATCC Typed Sample

Table 2: Inhibition zone diameters (mm) exhibited by aqueous extract of *T. schimperiana* root bark on the test organisms

Test organism	Concentration of extract in mg/ml				
	20	10	5	2.5	1.25
P. aeruginosa 1	17.0±0.58	15.0±0.50	13.5±0.50	0	0
P. aeruginosa 2	22.5±0.50	22.5±0.50	21.0±1.00	18.5±0.89	16.5±0.50
P. aeruginosa 3	18.2±0.17	15.5±0.50	14.0±0.00	12.5±0.50	12.0±0.00
P. aeruginosa 4	15.8±0.44	13.7±0.34	12.0±0.00	0	0
P. aeruginosa 5	16.0±0.56	14.0±0.00	12.5±0.23	0	0
P. aeruginosa 6	17.5±0.50	15.5±0.23	14.0±0.00	12.0±0.00	0
P. aeruginosa 7	22.5±0.87	20.5±0.50	19.5±0.50	17.5±0.23	15.7±0.34
P. aeruginosa 8	21.2±0.17	19.5±0.23	18.0±0.00	16.0±0.00	14.5±0.50
P. aeruginosa 9	15.5±0.50	14.5±0.50	12.5±0.50	0	0
P. aeruginosa 10	15.0±1.00	12.7±0.93	10.5±0.50	0	0
P. aeruginosa 11	15.0±0.00	13.0±0.50	10.3±0.34	0	0
P. aeruginosa 12	14.0±0.23	12.7±0.17	10.5±0.50	0	0
P. aeruginosa 13	15.8±0.44	14.5±0.50	12.5±0.50	10.0±0.50	0
P. aeruginosa 14	15.5±0.23	14.5±0.50	13.0±0.00	11.7±0.17	0
P. aeruginosa 15*	17.5±0.50	15.0±0.58	13.5±0.50	0	0

n = 3, *ATCC Typed Sample

Table 3: Minimum inhibitory concentrations of gentamicin and aqueous extract on the test organisms.

Test organism	Gentamicin (µg/ml)	Aqueous extract (mg/ml)
P. aeruginosa 1	0.200	1.496
P. aeruginosa 2	1.109	0.054
P. aeruginosa 3	NI	0.178
P. aeruginosa 4	NI	1.549
P. aeruginosa 5	NI	1.549
P. aeruginosa 6	1.160	0.912
P. aeruginosa 7	0.691	0.095
P. aeruginosa 8	0.692	0.105
P. aeruginosa 9	NI	1.038
P. aeruginosa 10	NI	1.622
P. aeruginosa 11	NI	1.585
P. aeruginosa 12	NI	1.472
P. aeruginosa 13	1.205	0.933
P. aeruginosa 14	0.759	0.759
P. aeruginosa 15*	1.995	1.472

Key: NI - No inhibition

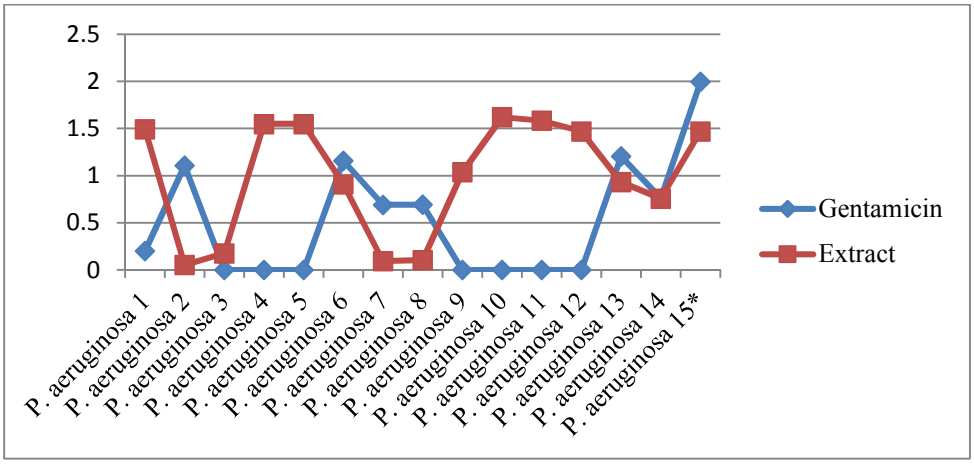

Figure 3: Graph comparing MIC of gentamicin and aqueous extract on the test organisms.