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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF SOME NIGERIAN MEDICINAL PLANTS

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Abstract

Ten Nigerian plants suggested from their ethnomedical uses to possess antimicrobial and antioxidant activities were studied for their anti-microbial and anti-oxidant properties. Antimicrobial activity was tested against *Escherichia coli* NCTC 10418, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, *Candida pseudotropicalis* and *Trichophyton rubrum* (clinical isolate). *Trichilia heudelotti* leaf extract showed both antibacterial and antifungal activities and was the most active against all the strains of bacteria tested. *Boerhavia diffusa*, *Markhamia tomentosa* and *T. heudelotti* leaf extracts inhibited the gram negative bacteria *E.coli* and *P. aeruginosa* strains whereas those of *M. tomentosa*, *T. heudelotti* and *Sphenoceutrum jollyamum* root inhibited at least one of the fungi tested. At a concentration of 312 µg/ml, hexane and chloroform fractions of *T. heudelotti* extract inhibited 6 and 14% of the fifty mult-idrug resistant bacteria isolates from clinical infectins, respectively. At ≤ 5mg/ml, the $CHCl_3$ (64%) and aqueous (22%) fractions of *T. heudelotti* and those of $CHCl_3$ (34%) and EtOAC (48%) of *M. tomentosa* gave the highest inhibition that was stronger than their corresponding methanol extracts. The corresponding EC_{50} of the extracts on *M. acuminata*, *T. heudelotti*, *E. senegalensis* and *M. tomentosa* were 4.00, 6.50, 13.33, and 16.50 ig/ml using the TLC staining and 1,1-dipheyl-2-picry-hydrazyl (DPPH) free radical scavenging assay. Therefore, leaf extracts of *M. tomentosa* and *T. heudelotti*, especially the latter, possess strong antimicrobial and antioxidant activities and should be further investigated. These activities justified the ethnomedical uses of these plants.

Key words: Antimicrobial, antifungal, antioxidant properties, Nigerian medicinal plants.

Introduction

Out of a total of 25 plants growing in Nigeria and selected based on their local uses, 15 of them have already been examined for their anti-microbial properties (Dalziel, 1956; Aladesanmi et al. 1986; Abbiw, 1989; Igbal et al., 1998; Kokuraro, 1993; Aderinokun et. al, 1999; Oluronke et al., 1999; Okafor et al., 2001; Wu et al., 2001) and hence were dropped. The remaining ten plants were investigated, some of which were used by the traditional healers for diseases such as diabetes, inflammation, loss of appetite, heart troubles, sores and skin infections (Oliver-Bever, 1986) as shown in Table 1. Eleven methanol extracts from these plants were studied for their antibacterial and antifungal properties against reference strains and multi-drug resistant clinical bacteria isolates. Their antioxidant activities were also assessed. This screening is of significant importance because of the urgent need for compounds that would be added to or replace the current antimicrobial agents to which microbes have become largely resistant (Chopra et. al., 1997).

Materials and methods

Plant material and extracts preparation

The dried (500 g) powder of the eleven plant parts was extracted with MeOH in a Soxhlet apparatus. The MeOH extracts of the 2 most antimicrobial active plants, *T. heudelotti* and *M. tomentosa*, were partitioned

successively with n-hexane, $CHCl_3$, EtOAc and H_2O to obtain their corresponding fractions. Each extract or fraction (1.0 g) was taken to make stock solutions for the antimicrobial and antioxidant tests.

Antimicrobial Activity

Disc Diffusion Assay (Murray et al., 1995)

The strains used were; *E.coli* NCTC 10418, *P. aeruginosa* ATCC 10145, *S. aureus* NCTC 6571 and *B. subtilis* NCIB 3610 for bacteria and *C. albicans*, *C. pseudotropicalis* NCYC 6 and *T. rubrum* for fungi. The standard strains were from stocks of culture collections maintained in our laboratory. Bacteria were maintained on nutrient agar slants, and fungi on Sabouraud Dextrose agar slants at 4^oC and subcultured monthly. Each extract (900 mg) was dissolved in 4 ml MeOH/H_2O. Discs of Whatman No 1 filter paper (ϕ 6 mm) were soaked with 2 drops of the extract using a sterile Pasteur pipette and allowed to dry at room temperature.

Two colonies of a 24-hour plate culture of each organism were transferred aseptically into 10 ml sterile distilled water in a test tube and mixed thoroughly, using an electric shaker, for uniform distribution. A sterile cotton swab was then used to spread the resulting suspension uniformly on the surface of oven-dried Isosensitest agar (Oxoid) and Sabouraud dextrose agar plates (Sterillin) for bacteria and fungi, respectively. These were incubated for an hour at 37^oC and 25^oC for bacteria and fungi, respectively. Sterile forceps were used to place each of the discs on the agar plates aseptically and the plates were then refrigerated for 30 min at 4^oC following which, the inoculated plates were incubated at 37^oC for 24 hours for bacteria strains and at 25^oC for 72 hours for the fungal strains. Antimicrobial activity was evaluated by noting the zone of inhibition against the test organisms. Those extracts showing any inhibition at all were noted for further tests for the quantitative assessment of their activity.

Agar diffusion assay

Dilutions of 40, 20, 10 and 5 mg/ml were prepared from 225 mg/ml stock solutions of the four extracts that inhibited the microorganisms. Volumes (20 ml) of molten nutrient agar were seeded with 1 ml portions of overnight cultures of microorganisms and poured into sterile Petri dishes (85 mm) and allowed to set. Holes of diameter 9 mm were made in the agar plates using a sterile metal cup-borer. Two drops of each extract and control were put in each hole under aseptic condition, kept at room temperature for 1 hour to allow the agents to diffuse into the agar medium and incubated accordingly (Reeves et al., 1979). Chloramphenicol (100 µg/ml) and acriflavine (6.3 mg/ml) were used as positive controls for bacteria and fungi respectively, MeOH/H_2O was the negative control. The plates were then accordingly incubated at 37^oC for 24 hours for the bacterial strains and at 25^oC for 72 hours for the fungal strains. The zones of inhibition were measured and extracts that gave significant activity against gram-negative isolates (*E. coli* and *P. aeruginosa*) were further tested against multi-drug resistant bacteria including uropathogenic *E. coli* and *P. aeruginosa* wound isolates.

Determination of the antibiotic susceptibility of bacteria isolates

Forty-eight clinical isolates of bacteria from wound, stool and sputum of patients diagnosed with wound, gastro-intestinal tract and respiratory tract infections and apparently healthy individuals and two *Bacillus* species from the environment were used. The organisms were isolated on blood agar prepared with 5% human blood on Nutrient agar (Oxoid) and identified using their morphological characteristics and standard biochemical tests (Barrow and Feltham, 1993).

The Disc diffusion method (NCCLS, 2003) was used for the determination of microbial sensitivity. The antibiotic discs employed were: Nalidixic acid, Ofloxacin, Augmentin, Tetracycline, Nitrofurantoin, Chloramphenicol and Cephalothin all at 30 µg, Amoxycillin and Cotrimoxazole at 25 µg, Erythromycin at 15 µg, Gentamicin, Ciprofloxacin and Penicillin-V at 10 µg. The zones of inhibition were measured and interpretation was in accordance with manufacturer's instructions (AB, Biodisc; PDM Interpretative chat).

Determination of Minimum Inhibitory Concentrations (MICs) of the fractions of *M. tomentosa* and *T. heudelotti* on multi drug resistant clinical isolates

The MIC were determined by the agar dilution method on Nutrient Agar (Oxoid) using a stock solution (50 mg/ml) of each fraction and extract dissolved in 50% MeOH/H_2O (Murray et al., 1995). The final plate concentrations were 5.0, 2.5, 1.25, 0.625 and 0.3125 mg/ml for the extract/fractions and 0.5, 0.25, 0.125, 0.0625, 0.03125mg/ml for chlorocresol (BDH), the +ve control. The hexane and $CHCl_3$ fractions were dissolved in Tween 80 (Sigma-Aldrich Co., USA) and the rest in 50% MeOH/H_2O and these vehicles were the –ve

controls. Bacteria were grown for 18 hours in Nutrient broth (Oxoid) and culture suspensions of 10^8 cfu/ml were applied to the surface of the nutrient agar plates containing dilutions of the fractions, chlorocresol, Tween 80 and methanol employing a multipoint inoculator. Plates were incubated at 37^{o}C for 24 hrs, after which all plates were observed for growth. The minimum dilution of fractions completely inhibiting the growth of each organism was taken as the MIC.

Antioxidant assay
Rapid radical scavenging screening

With the aid of a capillary tube, stock solutions from the 10 extracts were spotted on silica gel thin layer chromatographic (TLC) plates and developed with the solvent systems of n-hexane 50:50, 30:70; $CHCl_3$ 100%; $CHCl_3$-EtOAc 60:40; $CHCl_3$-MeOH 80:20, 60:40; EtOAc-MeOH 90:10; EtOAc-MeOH-H_2O 100:17:13 and BuOH-AcOH-H_2O 100:10:10, depending on the plant extract. After development, the chromatograms were dried and sprayed with a 0.4 mM solution of the stable radical, DPPH. Yellow spots formed against purple background were taken as positive results. The time (duration) for the development of the yellow colour indicated whether the antioxidant activity was strong or not (Mensor et al., 2001; Burits and Bucar, 2000).

DPPH photometric assay

The free radical scavenging activities of each extract and ascorbic acid were analysed by the DPPH assay (Sanchez-Moreno, et al 1998). A 1.0 ml of the test extract, at gradient final concentrations of 0.5 - 500 µg/ml, was mixed with 2 ml of 0.3 mM DPPH solution in MeOH in a cuvette. The absorbance was taken at 517 nm after 20 minutes of incubation in the dark at room temperature. The experiment was done in triplicates. The percentage antioxidant activity was calculated as follows:
%Antioxidant Activity [AA] = 100 - [{(Abs $_{sample}$ – Abs $_{blank}$) X 100}/Abs $_{control}$]. Where Abs $_{sample}$ was the absorbance of sample solution (2.0 ml) + DPPH solution (1.0 ml, 0.3 mM), Abs $_{blank}$ was the absorbance of Methanol (1.0 ml) + sample solution (2.0 ml), Abs $_{control}$ was the absorbance of DPPH solution (1.0 ml, 0.3 mM) + methanol (2.0 ml).

Results

As shown in Table 2, among the 10 plants studied, only *M. tomentosa* and *T. heudelotti* leaf MeOH extracts showed both antifungal and antibacterial activities. The leaf MeOH extract of *B. diffusa* gave only antibacterial activity whereas that of *S. jollyanum* root showed only antifungal properties (Table 2). In addition, *T. heudelotti* extract showed significant activity against the gram–negative bacteria, *P. aeruginosa* (MIC = 10mg/ml) and *E. coli* (MIC = 20mg/ml). The result of the susceptibility study showed a high level of antibiotic resistance by the clinical and environmental isolates (Table 3). There was a high level of antibiotic resistance to the commonly used antibiotics such as penicillin V (100%), cephalothin (98%), tetracycline (82%), and augmentin (77%). Some resistance was also demonstrated against fluoroquinolones e.g., ofloxacin (6.3%), ciprofloxacin (21%) and erythromycin (100%). Generally, 64% of the isolates were resistant to more than 50% of the antibiotics tested (Table 3). The activity of the crude MeOH extracts and partition fractions of *M. tomentosa* and *T. heudelotti* against the multi-drug resistant isolates are given in Table 3. At concentrations of ≤ 1.25 mg/ml, the $CHCl_3$ fractions of *M. tomentosa* and *T. heudelotti* showed the best activity, with the latter inhibiting the growth of most of the multi-drug resistant bacteria at a concentration of 312µg/ml. The intensity of the spots and reaction time of the DPPH radical scavenging capacities of the ten MeOH extracts using the TLC method showed that *M. tomentosa*, *T. heudelotti* and *M. accuminata* gave the highest antioxidant activities (Table 4). The EC_{50} of the extracts using DPPH photometric assay were *M. acuminata* 4.00, AA 4.65, *T. heudelotti* 6.50, *E. senegalensis* 13.33, *M. tomentosa* 16.50, *P. barteri* 66.67 and *S. jollyamum* 133.34 µg/ml (Figure 1).

Discussion

All the plants investigated have been reported to be used in ethnomedicine (Table 1).. Studies have been carried out to test the hypoglycaemic, antifungal and antioxidant activities of *B. diffusa* extracts (Chude et. al., 2001, Agrawal et. al., 2004, Amarnath and Pari, 2004). Similarly, Abo and Ashidi (1999) examined the antimicrobial and antifungal effects of the plant. Our results confirm the insignificant antimicrobial activity. As shown in Table 2, *B. diffusa* leaf extract has little activity against the bacteria and no activity against the fungi tested and therefore validates the traditional uses of the plant in treating boils and abscess. The root and leaf

Table 1: Local Therapeutic uses of some Nigerian medicinal plants.

Botanical Name (family)	Local Nigerian Names	Plant part tested	Claimed therapeutic use
Boerhavia diffusa Linn. (Nyctaginaceae)	Etiponla	Leaves	Diabetes, anti inflammatory, Abscess, boils
Ekebergia senegalansis A Juss (Meliaceae)	Ijebo, Ayape, oromu	Leaves	Antiepileptic, antimalaria
Gossypium arboretum (Malvaceae)	Owu	Bark	Male contraceptive
Markhamia tomentosa (Benth) K.Schum (Bignoniaceae)	Iru aya	Leaves	Anti snake venom/bite, sore eyes, heart pain, scrotal elephantiasis
Massularia acuminata (G. Don) Bullock (Rubiaceae)	Pako Ijebu	Leaves	Cure of mouth infections
Pleioceras barteri Baill (Apocynaceae)	Pariomo da	Root	Abortifacient, emmenagogue
Pleioceras barteri Baill (Apocynaceae)		Leaves	
Plumbago zeylanica Lin.-Holl. (Plumbaginaceae)	Inabiri	Root	Appetite stimulant, antiseptic skin disease, scabies, ulcers.
Psidium guajava Lin.-Holl. (Myrtaceae)	Guofa	Bark	Leaf used as antiseptic, antidiarrhoea.
Sphenoceutrum jollyanum Pierre (Menispermaceae)	Akerejupon	Root	Chewing sticks, stomachic
Trichilia heudelotti (Oliver) Planch (Meliaceae)	Akorere, rere	Leaves	Sores, heart troubles, pile

Table 2. In-vitro Antimicrobial activity of the methanolic extracts of some Nigerian medicinal Plants.

	Diameter of zone of inhibition in mm exclusion of the 9 mm hole diameter							
Extracts	Concentration (mg/ml)	*E. coli* NCTC 10418 (G - ve)	*B. subtilis* NCIB 3610 (G + ve)	*P. aeruginosa*. ATCC 10145 (G - ve)	*S. aureus* NCTC6571 (G + ve)	*C. albicans*	*C. pseudotropicalis*	*T. rubrum*
Boerhavia diffusa leaves (methanol extract)	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.	0.0	1.0	0.0	0.0	0.0
	20	0.0\	0.0	0.0	1.0	0.0	0.0	0.0
	40	0.0	2.0	0.0	2.0	0.0	0.0	0.0
	225	0.0	6.0	3.0	4.0	0.0	0.0	0.0
Markhamia tomentosa (methanol extract	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	20	0.0	0.0	0.0	7.0	0.0	0.0	0.0
	40	0.0	5.0	0.0	10.0	0.0	0.0	0.0
	225	1.0	8.0	10.0	16.0	0.0	3.0	0.0
Sphenoceutrum jollyanum Root (methanol extract)	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	40	0.0	0.0	0.0	0.0	0.0	2.0	1.0
	225	0.0	0.0	0.0	0.0	5.0	6.0	3.0
Trichila heudelotti leaves (methanol extract)	5	0.0	0.0	0.0	5.0	0.0	0.0	0.0
	10	0.0	0.0	4.0	6.0	0.0	0.0	0.0
	20	5.0	0.0	6.0	6.0	0.0	0.0	0.0
	40	6.0	2.0	14.0	15.0	0.0	0.0	2.0
	225	16.0	6.0	20.0	18.0	3.0	1.0	2.0
Chloramphenicol	(100µg/ml)	11.0	12.0	0.0	11.0	N.T.	N.T.	N.T.
Acriflavin	6.3 mg/ml	N.T.	N.T.	N.T.	N.T.	18.0	21.0	30.0
Methanol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The methanolic extracts of *Ekebergia senegalansis* leaf, *Gossypium arboretum* bark, *Massularia acuminata* leaf, *Pleioceras barteri* root and leaf, *Plumbago zeylanica* root and *Psidium guajava* bark were inactive to all the organisms tested. N.T.: Not tested.

Table 3: Antibiotic resistant properties of organisms and the MIC values of the methanolic extracts and their partition fractions of *M. tomentosa* and *T. heudelotti* leaves. 178

Bacteria species	Source	Antibiotics resistant Properties of Organisms													% resistance of standard antibiotic	MIC (µg/ml) Chlorocressol	MIC(mg/ml) of extracts									
		Nal	Ofl	Aug	Tet	Amx	Cot	Nit	Gen	CL	CI	PV	CE	EM			**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Acinetobacter spp	clinical	R	S	R	R	R	R	S	R	S	S	R	R	R	69.2	125.00	>5	>5	5	5	>5	>5	5	>5	>5	2.5
A. baumanii	clinical	R	S	R	R	R	S	S	R	R	S	R	R	R	69.2	125.00	>5	>5	>5	>5	>5	>5	2.5	>5	>5	>5
A. baumanii	clinical	S	S	R	R	R	S	S	S	S	S	R	R	R	46.2	125.00	>5	>5	>5	>5	>5	>5	5	>5	>5	>5
A. haemolyticus	clinical	S	S	S	S	S	S	S	S	S	S	R	R	R	23.1	62.50	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
A. haemolyticus	clinical	R	S	R	R	S	S	R	ND	S	R	R	R	R	53.8	62.50	5	>5	2.5	5	>5	0.312	0.312	>5	>5	2.5
Citrobater freundii	clinical	S	S	S	S	S	S	S	S	S	S	R	R	R	23.1	125.00	>5	>5	5	>5	>5	>5	5	> 5	>5	5
C. freundii	clinical	S	S	R	R	R	S	S	R	S	S	R	R	R	53.8	125.00	>5	>5	5	>5	>5	>5	5	>5	>5	5
Bacillus spp	clinical	R	S	S	S	S	S	S	S	R	R	R	R	R	46.2	62.50	>5	2.5	>5	>5	5	1.25	0.312	>5	>5	>5
Bacillus spp	clinical	S	S	R	R	R	R	R	S	S	S	R	R	R	61.5	62.50	>5	>5	>5	>5	5	>5	>5	>5	>5	>5
Bacillus spp	clinical	S	S	R	R	R	R	S	S	S	S	R	R	R	53.8	62.50	5	>5	>5	>5	>5	2.5	>5	>5	>5	>5
Bacillus spp	clinical	R	S	S	R	S	S	S	S	S	R	R	R	R	46.2	62.50	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
Bacillus spp	env.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	62.50	>5	>5	5	>5	5	>5	1.25	>5	>5	>5
Bacillus spp	env.	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	62.50	>5	>5	5	>5	5	>5	1.25	>5	>5	>5
Bacillus subtilis	NCIB3610	S	S	R	R	S	S	S	S	S	S	R	R	R	53.8	125.00	>5	0.312	>5	>5	>5	2.5	5	5	>5	>5
Escherichia coli	clinical	R	R	R	R	R	R	R	R	R	R	R	R	R	100.0	125.00	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
E. coli	Clinical	R	S	R	S	S	R	S	R	S	S	R	R	R	53.8	250.00	2.5	1.25	>5	>5	5	>5	2.5	>5	>5	>5
E. coli	Clinical	R	S	R	R	R	R	S	R	S	S	R	R	R	69.2	125.00	5	2.5	2.5	>5	>5	>5	2.5	>5	>5	>5
E. coli	clinical	S	S	S	R	S	S	S	R	S	S	R	R	R	38.4	62.50	2.5	>5	1.25	5	>5	>5	0.312	>5	>5	>5
E. coli	clinical	R	R	R	R	R	S	S	S	R	R	R	R	R	76.9	125.00	>5	>5	5	>5	>5	>5	2.5	>5	>5	>5
E. coli	clinical	S	S	R	R	S	R	R	R	R	S	R	R	R	69.2	62.50	5	>5	2.5	>5	>5	>5	>5	>5	>5	2.5
E. coli	clinical	R	S	R	R	R	S	S	R	S	S	R	R	R	61.5	125.00		2.5	1.25	1.25	2.5	0.312	>5	0.312	>5	>5	>5
E. coli	Clinical	S	S	S	S	R	S	S	S	S	S	R	R	R	30.8	250.00		>5	0.625	2.5	>5	>5	>5	0.312	>5	>5	2.5
E. coli	NCTC10418	R	S	R	R	R	S	S	S	R	S	R	R	R	61.5	125.00		>5	>5	>5	>5	>5	>5	5	5	>5	>5

Proteus vulgaris	Clinical	S	S	R	R	R	R	S	R	S	S	R	R	R	61.5	125.00	>5	5	>5	2.5	>5	>5	1.25	>5	>5	2.5
P. vulgaris	Clinical	S	S	R	R	R	S	S	R	S	S	R	R	R	53.8	125.00	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
P. vulgaris	Clinical	S	S	R	R	R	R	R	R	R	R	R	R	R	84.6	125.00	>5	1.25	>5	>5	>5	>5	>5	>5	>5	>5
P. vulgaris	Clinical	S	S	R	R	R	R	S	S	S	S	R	R	R	53.8	125.00	>5	>5	>5	>5	>5	0.312	1.25	>5	>5	2.5
P. mirabilis	NCIB67	R	S	R	R	R	R	R	R	R	R	R	R	R	92.3	125.00	>5	2.5	>5	>5	>5	5	5	>5	>5	>5
Pseudomonas spp	Clinical	R	R	R	R	R	R	S	R	R	R	R	R	R	92.3	62.50	>5	5	5	>5	>5	>5	2.5	>5	>5	>5
Pseudomonas spp	Clinical	S	S	R	R	S	S	S	S	S	S	R	R	R	38.4	62.50	>5	>5	5	>5	>5	>5	>5	>5	>5	>5
Pseudomonas spp	Clinical	R	S	R	R	R	R	R	R	R	S	R	R	R	84.6	62.50	>5	1.25	5	>5	>5	>5	2.5	>5	>5	>5
Pseudomonas spp	Clinical	S	S	R	R	R	R	S	S	S	S	R	R	S	46.2	500.00	>5	>5	5	>5	>5	>5	>5	>5	>5	>5
Pseudomonas aeruginosa	ATCC10145	R	S	S	S	S	S	S	S	S	S	R	R	R	30.8	500.00	>5	5	5	>5	>5	>5	5	>5	>5	>5
Salmonella spp	Clinical	R	S	R	R	R	S	S	R	S	S	R	R	R	61.5	125.00	>5	>5	>5	>5	5	>5	>5	>5	>5	>5
Salmonella spp	Clinical	R	S	R	R	R	R	S	S	S	S	R	R	R	61.5	125.00	>5	>5	>5	>5	5	>5	>5	>5	>5	>5
Salmonella spp	Clinical	S	S	S	R	S	R	S	R	R	S	R	R	R	53.8	125.00	>5	>5	>5	>5	5	>5	>5	>5	>5	>5
Salmonella spp	Clinical	R	S	R	R	R	S	R	R	R	S	R	R	R	76.9	125.00	>5	>5	>5	>5	5	>5	>5	>5	>5	>5
Salmonella enterica var. choleraesius	Clinical	S	S	S	S	S	S	S	S	S	S	R	S	R	15.4	62.50	2.5	>5	1.25	1.25	5	>5	0.625	>5	2.5	2.5
Salmonella enterica var. typhimurium	Clinical	S	S	R	R	S	S	S	S	S	S	R	R	R	38.4	125.00	>5	>5	>5	>5	>5	>5	5	>5	>5	>5
Staphylococcus aureus	Clinical	S	S	R	R	R	R	S	R	S	S	R	R	R	61.5	62.50	>5	>5	5	>5	>5	>5	2.5	>5	>5	>5
S. aureus	Clinical	S	S	R	R	R	R	R	R	R	S	R	R	R	76.9	62.50	>5	>5	>5	>5	>5	>5	2.5	>5	>5	>5
S. aureus	Clinical	S	S	R	R	R	R	S	R	S	S	R	R	R	61.5	62.50	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
S. aureus	Clinical	R	S	R	R	S	S	S	R	R	R	R	R	R	69.2	62.50	>5	1.25	>5	>5	5	>5	0.312	>5	>5	>5
S. aureus	Clinical	R	S	R	R	R	R	S	S	S	S	R	R	R	61.5	62.50	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
S. aureus	Clinical	S	S	R	S	S	R	S	R	S	S	R	R	R	46.2	62.50	>5	>5	5	>5	>5	>5	2.5	>5	>5	2.5
S. aureus	Clinical	R	S	S	R	S	S	S	R	S	S	R	R	R	46.2	62.50	1.25	1.25	1.25	>5	>5	>5	0.312	>5	>5	2.5
S. aureus	NCTC6571	S	S	S	S	S	S	S	S	S	S	R	R	R	23.1	125.00	1.25	>5	1.25	5	>5	0.312	5	>5	>5	>5
Staphylococcus epidermidis	clinical	R	S	R	R	R	R	S	R	S	R	R	R	R	76.9	62.50	1.25	0.625	2.5	>5	1.25	0.625	1.25	>5	>5	>5
S. epidermidis	clinical	S	S	R	R	R	R	S	R	R	S	R	R	R	69.2	62.50	1.25	0.625	2.5	>5	1.25	0.625	1.25	>5	>5	>5
Vibrio cholerae	clinical	R	S	R	R	R	S	S	R	S	S	R	R	R	61.5	500.00	>5	>5	5	>5	>5	>5	1.25	>5	>5	>5

Keys: **1, 2, 3, 5**: n-hexane, chloroform, ethylacetate and aqueous partition fraction of *Markhamia tomentosa* methanol (MeOH) extract; **4**: MeOH extract of *M. tomentosa*;
6, 7, 8, 10: n-hexane, chloroform, ethylacetate and aqueous partition fraction of *Trichilia heudelotti* methanol (MeOH) extract; **9**: MeOH extract of *T. heudelotti*;
Antibiotics: Nal: Nalidixic acid; Ofl: Ofloxacin; Aug: Augmentin; Tet: Tetracycline; Amx: Amoxycillin; Cot: Cotrimoxazole (Trimethoprim/sulpha-methoxazole); Nit: Nitrofurantoin; Gen: Gentamycin; CL: Chloramphenicol; CI: Ciprofloxacin; PV: Penicillin V; CE: Cephalothin; EM: Erythromycin. R: Resistant; S: Sensitive; ND: Not Determined

Table 4: Radical scavenging abilities of the methanolic extracts from Ten Nigerian medicinal plants using rapid DPPH TLC screening.

Plants (parts)	Reaction speed	Intensity of spots
Sphenocentrium jollyanum (root)	Slow	++
Pleioceras barteri (root)	Slow	++
Pleioceras barteri (leaf)	-	-
Massularia accuminata (leaf)	Fast	+++
Boerhavia diffusa (leaf)	Slow	+
Plumbago raylanica (root)	-	-
Markhamia tomentosa (leaf)	Fast	+++
Gossypium arberum (stem)	-	-
Ekebergia senegalensis (leaf)	Slow	++
Trichilia heudoleotii (leaf)	Fast	+++

Keys: -: no yellow colouration,
+: weak intensity of yellow colouration (15 – 30 mins before colour development)
++: intermediate intensity (1 – 15 mins before colour development)
+++: strong intensity (immediate reaction)

extracts of *S. jollyanum* have antiviral properties (Moody and Roberts, 2002a, b), the extracts of various morphological parts possess significant antioxidant, *in-vitro* antipyretic and analgesic activities (Oke and Hamburger, 2002; Mutho et al., 1998). The present results suggest that *S. jollyanum* possess significant antifungal activity (Table 2). Thus, this plant could be a source of useful antifungal agent. The other six plants investigated (Table 2) did not show any significant antibacterial or antifungal activity; however, activity has been reported for the Ethiopian *P. zeylanica* root on some pneumonia causing pathogens (Lemma et al., 2002).

The observation of weak and moderate antioxidant activities of *B. diffusa* and *S. jollyanum* extracts (Table 4) is in consistent with those reported (Mutho et al., 1998; Chude et. al., 2001, Oke and Hamburger, 2002; Agrawal et. al., 2004, Amarnath and Pari, 2004). The leaf extract of *M. tomentosa* showed good anti-staphylococcal activity as well as activities against *P. aeruginosa* and *B. subtilis*. It also showed encouraging activities against the fungus *C. pseudotropicalis* thereby demonstrating activity against a broad spectrum of organisms (Table 2). Extract of *T. heudelotti* leaves showed better antifungal and antibacterial activities than *M. tomentosa* (Table 2). *T. heudelotti* revealed an interesting activity against *S. aureus* (MIC = 5 mg/ml), *P. aeruginosa* (MIC = 10 mg/ml) and some activities against *E. coli* (MIC = 20 mg/ml), *B. subtilis* and *T. rubrum* (MIC = 40 mg/ml). Leaf extract of *T. heudelotti* also showed weak activities against the remaining 2 fungi tested (Table 2).

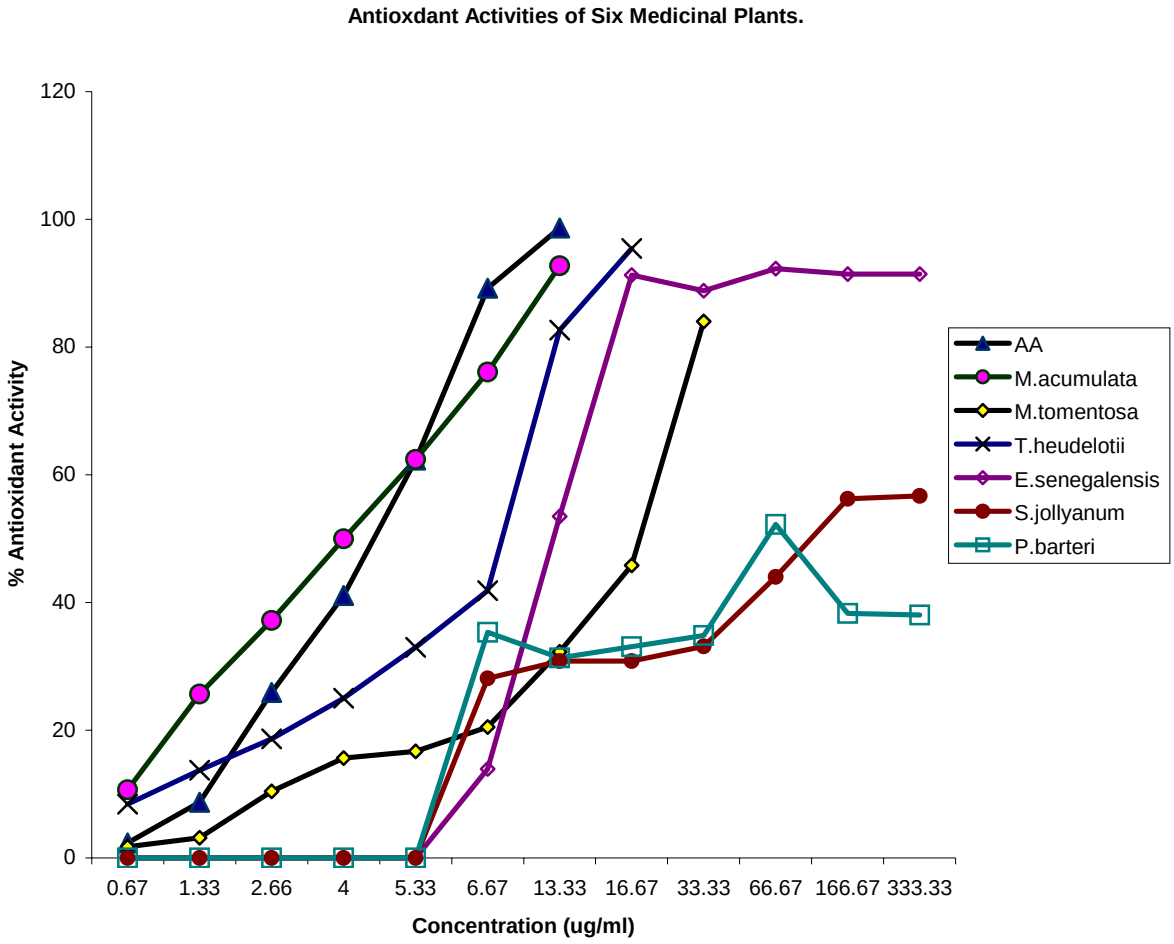

Figure 1: Antioxidant activity of six medicinal plants

The extracts of the two most active plants *M. tomentosa* and *T. heudelotti* were further fractionated to ascertain the properties of the active ingredients. These were tested against 50 multidrug resistant isolates from clinical and environmental sources including the organisms from the family Enterobacteriaceae which are commonly involved in clinical infections and are known to be highly versatile at acquiring resistance characteristics (Lamikanra et al., 1989). Two versatile pathogens, *P. aeruginosa*, a major nosocomial pathogen with low intrinsic susceptibility to antimicrobial agents and very high ability to acquire resistance, and *S. aureus* were also among those tested The organisms studied covered gram positive and gram-negative isolates (Table 3) and the demonstrated activities of the fractions showed that these plants have a wide spectrum of activity.

The bacteria used in Table 3 were not only resistant to the routinely used antibiotics in Nigeria such as Penicillin V and Erythromycin (100%); Cephalothin (98%), Tetracycline (82%), Augmentin (77%) and Cotrimoxazole but also showed resistance to the newer generation antibiotics like the fluoroquinolones e.g. ofloxacin (6.3%) and ciprofloxacin (21%). Sixty-four (64%) percent of the isolates was resistant to more than 50% of the antibiotics tested (Table 3). The resistance properties of these isolates showed the worsening situation of antibiotics resistance in the Nigerian environment and lead credence to the search for substances that could be added to or replace the antibiotics in current clinical use, which are becoming less useful with every

passing hour (Chopra et al., 1997, Okeke and Sosa, 2003). The better activities observed for the extracts and fractions of the two most active plants may provide an answer to this phenomenon.

The partition fractions, n-hexane, $CHCl_3$, EtOAc, H_2O together with the very active crude MeOH extracts of *M. tomentosa* and *T. heudelotti* showed MIC of < 5mg/ml against clinical and environmental isolates, especially against microbes that showed > 60% resistance against commonly used antibiotics (Table 3). Hence, extracts and active fractions of these plants may therefore be considered as alternative antimicrobial agent(s) against possible multiple antibiotic resistant strains that are widespread in the communities, especially in ethnomedicine (Chambers, 2003). At ≤ 1.25 mg/ml, the orders of activity presented for the fractions and extracts of *M. tomentosa* and *T. heudelotti* were MeOH < n-hexane = H_2O < EtOAc < $CHCl_3$; and EtOAc = MeOH = H_2O < n-hexane < $CHCl_3$ respectively (Table 3). The better activities of the fractions over the mother MeOH extracts showed that definite compounds present in these fractions were responsible for these activities. The *M. tomentosa* and *T. heudelotti* $CHCl_3$ fractions showed good anti-staphylococcal activity and MIC values of ≤ 5 mg/ml against multidrug resistant wound isolate of *Ps. aeruginosa*. The *M. tomentosa* $CHCl_3$ fractions also showed better activities against *E. coli, B. subtilis*, *P. vulgaris* and *Salmonella enterica var. choleraesius*, while that of *T. heudelotti* gave additional activities against *A. haemolyticus* and *Staphylococcus epidermidis* (Table 3).

Aladesanmi and Odediran (2000) had reported the isolation of antimicrobial phenolic acids from the EtOAc fraction of *T. heudelotti*. However, activities at < 5mg/ml given by the $CHCl_3$ fraction against 66% of the multidrug resistant organisms suggest the possibility of some other active constituent(s), whose activities are synergistic with the phenolic acids of the fractions. The frequent occurrence of variations in the MIC values within species and between related organisms suggests that resistances to *M. tomentosa* and *T. heudelotti*, when they occur, are due to intrinsic properties rather than acquired characters of the species. For these reasons, it would be useful if the extracts of the plants or active principles can be exploited for development into antimicrobial chemotherapeutic agents. Similarly there is the need to characterize the active principles in *M. tomentosa*. These plant extracts and fractions may serve as a good source of some cheap and highly effective antimicrobial agents for bacteria infections caused by multiresistant organisms.

The generation of the reactive oxygen species (ROS) beyond what the ability of the body can cope with leads to oxidative stress (Sies 1985, Gutteridge and Halliwell 1994, Maxwell 1995). Free radical oxidative stress has been implicated in the pathogenesis of a variety of human diseases like: artherosclerosis, diabetes mellitus, hypertension, inflammation, cancer and AIDS (Halliwell and Gutteridge 1989). The use of DPPH scavenging assays in assessing the cell membrane integrity/cell membrane stabilising capacities of plant constituents has given explanations as to the possible ways by which phytomedicines could help to reduce diseases caused by infections, inflammation and oxygen radicals generation affecting the cell membrane (Sadique et al 1989, Tsuda, 1998, 2000). The models of scavenging DPPH free radicals used are the rapid screening and the photometric assay methods commonly employed for evaluating antioxidants activities based on their abilities to donate hydrogen ion (Kumazawa, et al 2002). The DPPH is a free radical stable at the room temperature. The methanolic solution gives a purple colouration which when reduced by an antioxidant molecule gives rise to a yellow solution. On comparison with ascorbic acid (AA), a standard antioxidant drug, the antioxidant activities of the extracts in decreasing order were *M. acuminata* > AA > *T. heudelotti* > *E. senegalensis* > *M. tomentosa* > *P. barteri* > *S. jollyamum*. The EC_{50} values of *M. acuminata*, AA, *T. heudelotti* and *E. senegalensis* antioxidant activities were higher than that of 14.16 $\pm$ 0.20 μg/ml reported for rutin, a pure standard antioxidant compound (Mensor et al 2001). However, only *M. tomentosa* and *T. heudelotti* gave both strong radical scavenging abilities (Table 4) and antimicrobial activities (Tables 2, 3). It is therefore desirable to isolate and characterise the antioxidant agents from these two plants, *T. heudelotti* and *M. tomentosa* and determine whether or not the same constituents are responsible for both the antimicrobial and antioxidant activities.

Conclusion

The methanolic extracts of *E. senegalensis, G. arboreum, M. acuminata, P. zeylanica, P. barteri* and *P. guajava* have no antibacterial and antifungal activities. The leaf extracts of *M. tomentosa* and *T. heudelotti* showed relatively high antimicrobial activities complemented with impressive antioxidant activities. *M. acuminata* with the highest antioxidant activity showed no antimicrobial activity. The antimicrobial activities were found in the $CHCl_3$ partition fractions of *T. heudelotti* and *M. tomentosa* leaves.The former would be a better choice for antimicrobial activity, the later, *M. acuminata* leaf, would be good as an antioxidant agent. These results showed that the active plants *M. tomentosa* and *T. heudelotts*, especially the latter, could be further exploited for chemotherapeutic agents that could be used against infections caused by multiple antibiotic resistance strains very common in Nigeria. Alternatively, the extracts and active fractions of these plants may be used directly in ethnomedicine as antimicrobial agents.

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