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Susceptibility of clinical isolates of uropathogenic bacteria from Southwest Nigeria to antibiotics and extracts of *Brachystegia eurycoma* Harms (Leguminosae)

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Abstract
Resistance of uropathogens to antibiotics has been on increase and responsible for increased mortality and morbidity among patients. Clinical isolates (22) of uropathogenic bacteria comprising *Escherichia coli, Klebsiella Pneumoniae, Proteus mirabilis* and *Staphylococcus aureus* were tested for susceptibility to standard antibiotics and extracts of *Brachystegia eurycoma*. Methanolic extracts of leaves, stem and root barks of the plant were screened at 20mg/ml against the organisms using agar well diffusion method. Susceptibility of the isolates to plant extracts and standard antibiotics (amoxicillin, gentamicin, ofloxacin, chloramphenicol, tetracycline, augmentin, nalidixic acid, nitrofurantoin, erythromycin and cotrimoxazole) was done using agar disc diffusion methods respectively. Minimum Inhibitory Concentrations (MICs) of the extracts were determined by agar-dilution method on some of the uropathogens. Ofloxacin was the most active against *E. coli, K. pneumoniae* and *P. mirabilis* while gentamicin and chloramphenicol were the most active on *S. aureus.* The plant's extracts showed good activity on most of the uropathogens, even on the multidrug resistant (MDR) isolates of *S. aureus* and *P. mirabilis*. The MIC of *B. eurycoma extracts* on *E. coli, K. pneumonia* and *S, aureus* varied from 0.06 to 1.0mg/ml. This study showed that MDR uropathogens were still prevalent in Southwest Nigeria and that extracts of *B. eurycoma* contains bioactive compounds having good antibacterial activity, especially on the MDR clinical isolates. The overall results indicated varied patterns of sensitivity and resistance to antibiotics and extracts, warranting judicious and rational use of antibiotics in the routine treatment of UTI to prevent recurrence and development of resistant strains.

Keywords: Uropathogens; Resistance; Antibiotics; *Brachystegia eurycoma* extracts; MIC.

INTRODUCTION

Urinary tract infection (UTI) is a serious health problem and it has been estimated that about six million patients visit outpatient departments and about 300,000 are treated in the wards every year for UTI worldwide (Akortha and Ibadin, 2008). Due to indiscriminate use of antimicrobial drugs, microorganisms have developed resistance to many antibiotics and that has created immense clinical problems in the treatment of infectious diseases (Davis, 1994). Various studies have shown that uropathogens (*E. coli, K. pneumonia, P. mirabilis, S. aureus* and others) have become less susceptible to commonly used antibiotics worldwide but vary according to geographical areas (Winstenley, *et al*., 1997, Gupta, 2003, and Okesola and Aroundegbe, 2011). The emergence of multidrug resistant

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uropathogens is responsible for increased morbidity and mortality, and outbreaks of UTI in many communities including South West Nigeria (Okesola and Aroundegbe, 2011). Therefore there is urgent need to search for new and effective antimicrobial agents to treat MDR uropathogens mediated UTI. Medicinal plants have formed the basis for new drug development and approximately 80% of the world populations depend on traditional medicine for their primary health care (Cragg *et al.*, 1999 and Newman *et al.*, 2007). Screening bioactive extracts of medicinal plants used to treat infections locally is a sure way of discovering new antimicrobial agents (Cowan, 1999; Idowu *et al*., 2006; Kinghorn *et al.*, 2011)

Brachystegia eurycoma Harms (Leguminosae), found in Southern Nigeria and Cameroon (Keay, 1989) is a large crowded forest tree of about 60m high and commonly known among Nigerian tribes as: akolodo (Yoruba), okwen (Edo), eku (Ishan), akpakpa (Ijaw), achi (Igbo), ukung (Efik), etare (Ekoi) and kepuruk (Boki). Apart from the seeds that are popular as soup(Uhegbu *et al*., 2009), various parts of *B. eurycoma* are used by the Nigerian natives to cure infections like scabies, asthma, tuberculosis, bronchitis, catarrh, sore-throat, phlegm and guinea-worm (Burkill, 1997). Adekunle (2000) reported antifungal activity and phytochemical constituents of the plant to be tannins, and flavonoids while Igwe and Okwu (2013) reported antibacterial constituents of the seed oil. We hereby report the antibacterial potentials of *B. eurycoma* in comparison to standard antibiotics against uropathogens: *S. aureus*, *E. coli*, *K. pneumoniae* and *P. mirabilis* from a tertiary hospital in Southwest Nigeria.

EXPERIMENTAL

Collection of plant materials. Fresh leaves, stem bark and root bark of *Brachystegia eurycoma* were collected at the Botanical Garden, University of Ibadan. Identification and authentication of the plant were carried out at the Forest Research Institute of Nigeria (FRIN) where voucher specimen was deposited with herbarium number FHI 109551.

Extraction procedure. The leaves, stem bark and root bark of *Brachystegia eurycoma* were dried at room temperature for about three weeks and grinded to coarse powder. Cold extractions were carried out as follows. Powdered leaves (100g) were soaked in 500 ml of methanol and 80g of the stem bark and root bark of *B. eurycoma* were soaked in 400ml of methanol, with intermittent shaking for 48hrs. The extracts were then filtered using a clean sterile muslin cloth and then Whatman No.1 filter paper, and stored at 4^0C for subsequent use.

Collection of clinical isolates. Clinical isolates of *E. coli, S. aureus, P. mirabis and K. pneumoniae* were collected from the Medical Microbiology Laboratory of Ladoke Akintola Teaching Hospital Osogbo, Osun State. Originally the samples were collected from patients diagnosed with UTI showing significant bacteriuria. Confirmation of identity of the isolates was done by standard bacteriological procedures (Cellimore, 2000; Cheesbrough 2003). The isolates were maintained on Mueller Hinton agar slope and stored at 4^oC prior to use. Typed strains of *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were included as reference organisms.

Susceptibility testing of isolates to standard antibiotics. The agar disc-diffusion method of Kirby Bauer was employed following the procedure recommended by Clinical and Laboratory Standard Institute (CLSI, 2005). The following antibiotic discs were used: Augmentin (30µg); Amoxicillin (25µg); Erythromycin (5µg); Tetracycline (10µg); Cloxacillin (5µg); Cotrimoxazole (25µg); Gentamicin (10µg); Chlroramphenicol (30µg); Nitrofurantoin (300µg); Nalidixic

acid (30µg) and Ofloxacin (30µg). The agar plates were observed for susceptibility (zone of inhibition measured in millimeters) or resistance.

Susceptibility testing of isolates to plant extracts. This was done using the agar well diffusion method of Perez et al., 1990 as follows: 0.2ml of overnight broth culture of each of the clinical isolates standardized by McFarland procedure to containing $10^6 – 10^8$ cfu/ml was transferred to and spread on solidified Muller Hinton agar. A sterile cork borer of 6 mm diameter was used to cut uniform wells in the agar and each well was filled with three drops of each extract at different concentrations. Control experiment was setup with methanol (40%) and gentamicin (10µg). Plates were incubated at 37^{o}C for 24 h and zones of inhibition were measured in millimeter. All tests were done in triplicates.

Determination of minimum inhibitory concentrations (MICs). The MIC of each extract was determined using agar dilution method of Andrews (2001). Each extract was serially diluted to obtain concentrations of 20.0, 10.0, 5.0, 2.5, 1.25, 0.625, 0.3, 0.15, 0.075 mg/ml in the agar and each of the test isolates was inoculated on the plates by streaking. Plates were incubated at 37°C for 24 hours, after which they were observed for growth. The least concentration in which there was no growth was noted as the MIC.

RESULTS AND DISCUSSION

Percentage yields on extraction (Table1) was highest in root bark (4.15%), followed by leaves (3.87%) and stem bark (1.25%) with each showing different macroscopic characteristics. From Table 2, showing the results of susceptibility testing, *S. aureus* was totally resistant to amoxicillin, erythromycin, cloxacillin, cotrimoxazole and augmentin indicating that all the uropathogenic *S. aureus* were multidrug resistant which is similar to that reported by Saravanan *et al.* (2013). However 4 out of the 5 *S. aureus* isolates showed susceptibility to gentamicin and chloramphenicol. In South India, a similar result of 80% susceptibility was observed for gentamicin and penicillin (Rajaduraipandi *et al*., 2006; Saravanan *et al*., 2009). According to Kitara *et al*., 2011, resistance to beta-lactam ampicillin was highest of all the antibiotics at 81.5% for inpatients and 60.7% for outpatients. The resistance was attributed to the presence of beta-lactamase producing *S. aureus* in hospital environment and 'selection pressure' due to the use of the beta-lactam antibiotics for treating UTI.

All the Gram-negative uropathogens showed 100% resistance to amoxicillin, cotrimoxazole and augmentin. *E. coli* and *P. mirabilis* showed 100% resistance to tetracycline. Generally, in this study *P. mirabilis* was the most resistant uropathogens, with 3 out 5 isolates 100% resistant to all antibiotics used. The most active of all the antibiotics used in this study was ofloxacin followed by nalidixic acid and nitrofurantoin (Table 3). Our results are in conformity to that reported by Kibret and Abera (2011), Mohsen et al., (2010) and. Bahashwan and El Shafey (2013). *P. mirabilis* has been implicated in meningitis, empyema, osteomyelitis and gastroenteritis. Also, it frequently causes nosocomial infections of the urinary tract (46%), surgical wounds (24%) and lower respiratory tract (30%). Less frequently, *Proteus species* cause bacteraemia (17%), most often in elderly patients (Mansy, 2001).
Root extracts of *Brachystegia eurycoma* showed highest activity to *K. pneumoniae* 4 with 18mm diameter zone of inhibition followed by *S. aureus* 1, *E. coli* 2 and *K. pneumoniae* 1 with 16mm; the least active of the plant extracts was the leaf extract which showed activity to only *S. aureus* 2 (13mm), *S. aureus* 5 (12mm), *K. pneumoniae* 2 (12mm) and *P. mirabilis* 4 (11mm). All the plant extracts were active against *P. mirabilis*

4 but inactive on other *P. mirabilis* used in this study (Table 4). The results obtained from this study agreed with the work of Igwe and Okwu (2013) who reported that the volatile oil obtained from the extracts of *Brachystegia eurycoma* showed antibacterial activity against *Escherichia coli, Salmonella typhi and Staphylococcus aureus.*

Comparison of the susceptibility pattern of clinical isolates to the antibiotics and crude extracts of *Brachystegia eurycoma* showed that the extracts are very effective compared to standard antibiotics against most of which the organisms have developed resistance. Most of the MDR uropathogens were found sensitive to plant extracts. For instance, *P. mirabilis* 4 which was resistant to most of the antibiotics used in this study was sensitive to the leaf, stem bark and root extracts of *B. eurycoma* with 12mm, 10mm and 13mm diameter zone of inhibition respectively.

Table 1: Extraction yield and macroscopic characteristics of *Brachystegia eurycoma* extracts

Extracts	% Yield	Macroscopical Characteristics
Leaves	3.87	A dark brown colored substance
Stem bark	1.25	A dark brown and sticky substance
Root bark	4.13	A brown crystalline powder

Table 2: Susceptibility of clinical isolates of *S. aureus* (Gram positive organism) to antibiotics

Organism	Aug	Amx	Ery	Tet	Cxc	Gen	Cot	Chl
S. aureus 1	R	R	R	R	R	12	R	20
S. aureus 2	R	R	R	R	R	14	R	15
S. aureus 3	R	R	R	15	R	20	R	16
S. aureus 4	R	R	R	R	R	20	R	R
S. aureus 5	R	R	R	R	R	R	R	21
S. aureus ATCC 25923	R	R	R	R	R	12	12	12

R: Resistance, Aug: Augmentin (30µg), Amx: Amoxicillin (25µg), Ery: Erythromycin (5µg), Tet: Tetracycline (10µg), Cxc: Cloxacillin (5µg), Cot: Cotrimoxazole (25µg), Gen: Gentamicin (10µg), Chl: Chlroramphenicol (30µg).

Table 3: Susceptibility of clinical isolates of *E. coli, K. pneumoniae and P. mirabilis* (Gram negative organisms) to selected antibiotics

Organisms	Diameter of Zones of Inhibition of Antibiotics (mm)							
	Amx	Cot	Nit	Gen	Nal	Ofl	Aug	Tet
E. coli 1	R	R	22	14	20	22	R	R
E. coli 2	R	R	20	14	18	20	R	R
E. coli 3	R	R	22	R	R	9	R	R
E. coli 4	R	R	R	12	14	28	R	R
E. coli 5	R	R	10	12	20	25	R	R
E. coli ATCC	R	R	16	R	18	22	R	R
K. pneumoniae 1	R	R	R	R	R	27	R	R
K. pneumoniae 2	R	R	R	R	12	18	R	R
K. pneumoniae 3	R	R	14	R	20	22	R	R
K. pneumoniae 4	R	R	14	17	15	22	R	10
K. pneumoniae 5	R	R	22	20	16	22	R	R
P. mirabilis 1	R	R	R	PS	PS	PS	R	R
P. mirabilis 2	R	R	R	R	R	R	R	R
P. mirabilis 3	R	R	R	R	R	R	R	R
P. mirabilis 4	R	R	R	R	R	18	R	14
P. mirabilis 5	R	R	R	R	R	R	R	R

R: Resistance, PS: Partially sensitive, Aug: Augmentin (30µg), Amx: Amoxicillin (25µg), Cot: Cotrimoxazole (25µg), Gen: Gentamicin (10µg), Nit: Nitrofurantoin (300µg), Nal: Nalidixic acid (30µg), Ofl: Ofloxacin (30µg), Tet: Tetracycline (30µg).

Table 4: Susceptibility of clinical isolates of *S. aureus, E. coli, K. pneumoniae and P. mirabilis* to leaf, stem and bark extracts of *Brachystegia eurycoma*

Organisms	Diameter of zones of inhibition of extracts (mm)			
	BEL	BES	BER	Control
S. aureus 1	R	15	16	12
S. aureus 2	13	R	12	25
S. aureus 3	R	11	R	15
S. aureus 4	R	11	10	R
S. aureus 5	12	13	14	22
S. aureus ATCC 25923	R	11	R	16
E. coli 1	R	12	12	22
E. coli 2	R	14	16	14
E. coli 3	R	12	13	22
E. coli 4	R	R	R	30
E. coli 5	R	12	12	15
E. coli ATCC 25922	R	12	13	20
K. pneumoniae 1	R	13	16	25
K. pneumoniae 2	11	R	R	30
K. pneumoniae 3	R	R	R	R
K. pneumoniae 4	R	12	13	R
K. pneumoniae 5	R	14	18	20
P. mirabilis 1	R	R	R	R
P. mirabilis 2	R	R	R	R
P. mirabilis 3	R	R	R	R
P. mirabilis 4	12	10	13	30
P. mirabilis 5	R	R	R	R

BEL: *Brachystegia eurycoma* Leaf, BES: *Brachystegia eurycoma* Stem bark, BER: *Brachystegia eurycoma* Root bark, *R*: Resistant.

Table 5: MIC of extracts of *Brachystegia eurycoma* on selected test organisms

Organisms	BES	BER
	Concn. (mg/ml)	
S. aureus 4	0.06	0.5
E. coli ATCC 25922	0.06	1.0
K. pneumoniae 5	0.5	1.0

BES: *Brachystegia eurycoma* Stem bark, BER: *Brachystegia eurycoma* Root bark, ND: Not determined

This implies that *B. eurycoma* contain antimicrobial compounds that are active against multidrug resistant organisms. Adekunle (2000) reported the presence of flavonoids and tannins (two types of phytochemical constituents known for antimicrobial property) in the barks. This study has clearly justified the folkloric use of the plant in treating infections, and demonstrated that the extracts of stem bark and root bark of *Brachystegia eurycoma* possess very promising antimicrobial activity even against some MDR uropathogenic bacteria.

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