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Susceptibility pattern of *Escherichia coli* from urinary tract infections to antibiotics and methanol extracts of *Olax subscorpioidea* and *Sida corymbosa*

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Abstract
Antibiotic resistance in urinary tract infections (UTIs), of which *Escherichia coli* causes about 80% of cases is on increase, causing mortality, morbidity and increased health care costs. Clinical isolates of *E. coli* (13) from UTIs were tested for susceptibility to standard antibiotics and extracts of *Olax subscorpioidea* and *Sida corymbosa*. Methanol extracts of the plants were screened at 20, 40, 80 and 100 mg/ml against the isolates using agar-well diffusion method while antibiogram was determined by Kirby-Bauer disc diffusion method. The minimum inhibitory concentrations (MICs) of the plants` extracts and two antibiotics were determined by agar dilution method. The isolates were mostly susceptible to ofloxacin and 100% resistance to augmentin. Extracts of the plants showed good and dose-dependent activities, even on the multidrug resistant *E. coli* isolates. The zones of inhibition of the extracts ranged 9-16 mm while the MICs ranged 0.5-10 mg/ml on the isolates. This study has shown that MDR *E. coli* in UTIs are still prevalent and that the roots of *O. subscorpioidea* and stem of *S. corymbosa* extracts have good antibacterial activities against the isolates. The results justified the traditional use of the plants to treat infections generally and the potential utilization in the treatment of UTIs.

Keywords: Urinary tract infection; Antibiotics; *Escherichia coli*; *Olax subscorpioidea*; *Sida corymbosa*

INTRODUCTION

Urinary tract infections (UTIs) are one of the most frequent infectious diseases around the world with enteric bacteria being the most frequent cause, though the distribution of pathogens that cause UTI is changing [1]. *Escherichia coli* is the cause of 80-85% of UTIs [2], causing community-acquired and large portion of nosocomial UTIs, accounting for substantial medical costs, morbidity and mortality worldwide [3,4]. Antibiotic resistance of urinary tract pathogens has been known to increase worldwide, especially to commonly used antimicrobials [5]. Various studies have shown that *E. coli* and other uropathogens have become more resistant to commonly used antibiotics worldwide but the resistance pattern vary according to geographical area [6-9]. Plants are an important source of potentially useful agents for the development of new chemotherapeutic agents and testing plant extracts for antimicrobial activity could

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be a good method to identify new antimicrobial drugs [10].

Olax subscorpioidea Oliv. (Olacaceae), is a shrubby plant which is practically confined to the tropics especially Africa. It is known as "Ifon" in Yoruba [11]. Its ethanol extract was reported to show considerable antibacterial activity [12]. It was reported by Orabueze et al. [13] to exhibit antimicrobial properties against periodontal disease causing organisms, thus up-holding their folkloric use in oral disorder management while Victoria *et al.* [14] reported the ulcer healing property of the plant and the phytochemical constituents of its methanol extract to be glycosides, terpenoids, alkaloids and steroids. Kazeem *et al.* [15] also reported the antidiabetic potential of *O. subscorpioidea* in Wistar rats while Ishola *et al*. [16] reported antinociceptive and anti-inflammatory effect of leaf extract of *O. subscorpioidea* in rodents, thus confirming its folkloric uses in the treatment of painful inflammatory conditions.

Sida corymbosa R. E. Fries (Malvaceae) is a shrubby semi-woody perennial to 1.25 m high, of roadsides, tracks, waste places and overgrazed pasture, from Sierra Leone to Southern Nigeria, and occurring in Central America [17]. It is commonly known as iseketu in Yoruba land. John-Africa *et al*. [18] demonstrated the anti-ulcer and wound healing activities of *S. corymbosa* and its constituents had anti-haemorrhagic properties in rats, thereby providing scientific validation for the ethnomedical use of the plant in bleeding control [19]. Ekpendu [20] documented the use of the plant in the Benue area of Nigeria as an ulcer remedy. In this study, we hereby report the antibacterial potential of root of *O. subscorpioidea* and stem of *S. corymbosa* against uropathogenic *E. coli* from tertiary hospitals in comparison to standard antibiotics.

EXPERIMENTAL

Collection of plant materials. Fresh plants of *Olax subscorpioidea* and *Sida corymbosa* were collected and identified at the Botanical Garden, University of Ibadan, Ibadan. Authentication of the plants was done at the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, where the herbarium specimen (FHI 109962) was deposited for future references.

Extraction procedure. The roots of *Olax subscorpioidea* and stem of *Sida corymbosa* were air dried in an enclosed area accessible to ventilation under room temperature. They were then grinded into small particles. The components of the blended plants were extracted with methanol using Soxhlet apparatus. The filtrates were concentrated and dried inside oven at 40^{o}C and stored at 4^{o}C for subsequent use.

Collection of clinical isolates. The organisms used were bacterial isolates of *Escherichia coli* from urinary tract infections obtained from Teaching Hospitals, which include University College Hospital (UCH), Ibadan; Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife; LAUTECH Teaching Hospital (LTH), Osogbo and Ogbomoso. They were all maintained on agar slants at 4^{o}C prior to use. Standard bacterial organisms used were collected from Pharmaceutical Microbiology Laboratory of the Faculty of Pharmacy, University of Ibadan, Ibadan, which include *Bacillus anthracis* (ATCC 14186), *Pseudomonas aeruginosa* (ATCC 15442), *Proteus mirabilis* (ATCC 15290), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922).

Susceptibility testing of isolates to standard antibiotics. The susceptibility testing of the isolates to standard antibiotics was performed using Kirby-Bauer disc diffusion method. The antibiotics used include Nitrofurantoin (300 µg), Cefuroxime (30 µg), Ceftaxidime (30

µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg), and Augmentin (30 µg).

Susceptibility testing of isolates to plants` extracts. The antibacterial screening of the extracts was done using agar-well diffusion method according to Oyedeji *et al*. [21] with slight modifications. The test bacteria were inoculated into tubes of nutrient broth and incubated at 37^{o}C for 18 hours before use. Each of the overnight broth culture was adjusted to 0.5 McFarland standard. 0.2 ml of the standardized cell suspensions were used to seed 20 ml sterile molten Mueller-Hinton agar in McCartney bottles and these were poured into sterile plates after gentle swirling and allowed to set. A sterile cork borer (6mm diameter) was used to bore wells into which 4 drops of each of the methanol extract were introduced at 20, 40, 80 and 100 mg/ml. Gentamicin (10 µg/ml) was introduced into a well to serve as positive control and methanol as negative control. The plates were then incubated at 37^{o}C for 24 hours and diameters of zone of inhibition around wells were measured and recorded.

Determination of minimum inhibitory concentrations (MICs). The MIC of each extract was determined using agar-dilution method. Methanol and sterilized distilled water were used as diluents to prepare the stock concentrations, which were serially diluted to obtain concentrations of 10, 5, 2.5, 1, 0.5, 0.25, 1.25 and 0.0625 mg/ml in the agar, and each of the test isolates was inoculated on the plates by streaking. The MICs of two antibiotics was also determined using the above method.

RESULTS AND DISCUSSION

The percentage extraction yield of root of *Olax subscorpioidea* was two times higher than that of stem of *Sida corymbosa*. The extraction yield was 6.8% for *O. subscorpioidea* and 3% for *S. corymbosa* (Table 1) with each showing different macroscopic characteristics.

This study reported the preliminary antimicrobial activities of the extracts on five standard organisms. The highest zone of inhibition was seen with extract of *O. subscorpioidea* against *Staphylococcus aureus* (20 mm) and the lowest zone against *Bacillus anthracis* (10 mm) and *Pseudomonas aeruginosa* (10 mm) while extract of *S. corymbosa* showed highest zone of inhibition against *Proteus mirabilis* (20 mm) and the lowest zone against *Bacillus anthracis* (10 mm) (Table 3). This indicated that both Gram positive and Gram-negative organisms were susceptible to the extracts, which is in line with what was reported by Odeita *et al*. [22]. The result of susceptibility testing of the clinical isolates to the extracts showed that all the isolates were susceptible to the extract of *O. subscorpioidea* while four isolates showed complete resistance to the extract of *S. corymbosa*. The ranges of zone of inhibition of the extracts on the susceptible isolates were 10-16 mm and 9-13 mm for *O. subscorpioidea* and *S. corymbosa* respectively (Table 4). These results revealed that the antibacterial activity of *O. subscorpioidea* extract is higher than that of the *S. corymbosa* on the test isolates. The results of the antimicrobial activity screening of the extracts on the five standard organisms were more susceptible than the clinical isolates. Incessant use of antibiotics has been reported to be an important risk factor for extension of resistance to antimicrobial agents [23].

The result of the antibiotic susceptibility testing of the isolates revealed that the test isolates exhibited highest sensitivity to ofloxacin (53.85%) and highest resistance to augmentin (100%) followed by ceftazidime and cefuroxime (76.92%), and nitrofurantoin, gentamicin and ciprofloxacin (53.85%) (Table 2). This result is in line with the result of Idowu and Okanlawon [6]. It was also reported by Sabir *et al*. [24] that

uropathogenic *E. coli* exhibited resistance to ceftazidime (73.8%), gentamicin (59.8%) and ciprofloxacin (54.2%). These results indicated that many of the isolates were multidrug resistant and there is possible high incidence of antibiotic resistance that may render many of these antibiotics inefficient for empirical prescription to treat UTIs.

The minimum inhibitory concentrations (MICs) of the extracts against the isolates were 0.5-2.5 mg/ml for *O. subscorpioidea* and 5-10 mg/ml for *S. corymbosa* against some of the clinical isolates. The result showed a little difference, but comparable result to that of Ayandele and Adebiyi [12] in which the MIC of *O. subscorpioidea* recorded against *E. coli* isolated in hospital from urine was 5 mg/ml. The MICs of two antibiotics, ciprofloxacin and gentamicin, were determined to be 0.125-0.25 µg/ml and 0.5-1 µg/ml respectively. The result is in line with CLSI report [25]. Comparison of the susceptibility pattern of clinical isolates of *E. coli* to standard antibiotics and methanol extracts of root of *O. subscorpioidea* and stem of *S. corymbosa* showed the extracts to be more effective compared to the antibiotics against which many of the isolates were resistant. Most of the MDR uropathogenic *E. coli* were found to be sensitive to the extracts. For instance, isolates *E. coli* 1 and *E. coli* 16 showed resistance to all the antibiotics while the extracts were effective against these isolates with inhibition zones ranging from 10 to 15 mm.

Table 1: Extraction yield and macroscopic characteristics of extracts of the plants

Extracts	% Yield	Macroscopic Characteristics
O. subscorpioidea	6.8	Brown crystalline substance
S. corymbosa	3	Dark coloured substance

Table 2: Susceptibility of clinical isolates of *E. coli* to selected antibiotics

Isolates	Diameter of zones of inhibition of antibiotics (mm)						
	Nit	Cpr	Caz	Crx	Gen	Ofl	Aug
E. coli 1	R	R	R	R	R	R	R
E. coli 4	S	S	R	R	S	S	R
E. coli 7	R	R	R	S	S	S	R
E. coli 8	R	R	R	R	R	R	R
E. coli 9	R	I	R	R	R	S	R
E. coli 10	S	R	R	R	S	R	R
E. coli 13	R	R	R	R	R	R	R
E. coli 14	I	I	S	S	S	I	R
E. coli 15	S	R	I	R	R	S	R
E. coli 16	R	R	R	R	R	R	R
E. coli 17	S	S	S	S	S	S	R
E. coli 18	S	S	R	R	S	S	R
E. coli 21	R	I	R	R	R	I	R

S: Sensitive, I: Intermediate, R: Resistance, Nit: Nitrofurantoin (300 µg), Cpr: Ciprofloxacin (5 µg), Caz: Ceftazidime (30 µg), Crx: Cefuroxime (30 µg), Gen: Gentamicin (10 µg), Ofl: Ofloxacin (5 µg), Aug: Augmentin (30 µg).

Table 3: Susceptibility of standard organisms to extracts of the plants

Organisms	Diameter of zone of inhibition of extracts (mm)		
	O. subscorpioidea	*S. corymbosa*	Control
Bacillus anthracis	10	11	28
Pseudomonas aeruginosa	10	12	15
Proteus mirabilis	18	20	24
Staphylococcus aureus	20	15	24
Escherichia coli	14	14	20

Control = Gentamicin (10µg)

Table 4: Susceptibility of clinical isolates to extracts of the plants

	Diameter of zones of inhibition of extracts (mm)								
	20 mg/ml		40 mg/ml		80 mg/ml		100 mg/ml		Control
Isolates	OS	SC	OS	SC	OS	SC	OS	SC	
E. coli 1	10	R	11	11	12	12	14	12	R
E. coli 4	12	9	13	10	15	11	16	12	22
E. coli 7	R	10	10	10	12	11	13	11	21
E. coli 8	10	R	12	R	13	R	15	R	R
E. coli 9	R	R	R	R	11	R	13	R	R
E. coli 10	10	R	10	10	11	11	12	11	20
E. coli 13	R	R	10	R	14	R	14	R	R
E. coli 14	10	R	10	R	11	R	11	R	20
E. coli 15	11	10	13	12	13	13	14	13	R
E. coli 16	10	11	13	12	14	13	15	13	R
E. coli 17	10	10	11	12	12	12	15	13	20
E. coli 18	R	R	R	10	10	11	12	11	22
E. coli 21	11	10	11	11	12	12	13	12	R

OS: *Olax subscorpioidea*, SC: *Sida corymbosa*, R: Resistance, Control: Gentamicin (10 µg)

Table 5: MIC of extracts of *Olax subscorpioidea* and *Sida corymbosa* on selected isolates

	Conc. (mg/ml)		Conc. (µg/ml)	
Isolates	OS	SC	Gen	Cpr
E. coli 4	0.5	5	0.5	0.25
E. coli 17	2.5	10	1	0.125

OS: *Olax subscorpioidea*, SC: *Sida corymbosa*, Gen: Gentamicin, Cpr: Ciprofloxacin

CONCLUSION

This study showed the occurrence of antibiotic resistance of *Escherichia coli* from urinary tract infections and that the extracts of the two plants showed antimicrobial activities against the test isolates. Although the two plants showed enough antimicrobial activity to justify the traditional uses, the roots of *Olax subscorpioidea* had higher activity than the stem of *Sida corymbosa.* The two plants` extracts can be potential sources of antimicrobial agents in the treatment of urinary tract infection, especially those caused by *E. coli.*

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