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Evaluation of antibacterial activity of leaf and root extracts of *Sansevieria zeylanica* on UTI among students in University of Benin, Edo state

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Abstract

Introduction: *Sansevieria zeylenica* is a plant rich in phenolic compounds, which may be responsible for its antibacterial activities against Uropathogens. Urinary tract infections usually develop in the lower urinary tract and if not properly treated, they ascend to the upper urinary tract, causing severe damage to the kidneys. The aim of this study was to evaluate the antimicrobial properties of *Sansevieria zeylenica* leaf and rootextract against isolate of UTI among students of University of Benin, Edo State.

Materials and methods: Two hundred participants attending University of Benin were randomly enrolled in this study. Urine specimen was collected and analyzed for the detection of Uropathogens using standard microbiological procedure while the leaf and root of *Sansevieria zeylenica* extraction was carried out with methanol using the Soxhlet apparatus. Subsequently, the antimicrobial activity against the isolates was determined using Agar well diffusion and the minimum inhibitory concentration (MIC) of the extracts against the isolates was determined using the microdilution method.

Results: Out of 200 participants studied, 23 (11.5%) had growth of four bacteria species associated with urinary tract infection; *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The activity of both extracts was greater at 200mg/ml against *E. coli* and *P. vulgaris* isolates, but at 400mg/ml against *S. aureus* with

Minimum Inhibitory Concentrations of the leaf extract as 8mg/ml, 4mg/ml, 8mg/ml, and >16mg/ml against *E. coli*, *S. aureus*, *P. vulgaris*, and *P. aeruginosa*, respectively. The combination of leaf extract and Ampicillin increases the activity of Ampicillin, producing a greater zone of inhibition (26mm) against *P. vulgaris* compared to Ampicillin alone (21mm) and leaf extract alone (16mm) giving rise to a synergistic effect.
Conclusion: *Sansevieria zeylanica* extracts in this study can control Urinary Tract Infection and may be combined with conventional antibiotics as a treatment for Uropathogens. Though, the molecular mechanism behind its antibacterial activity would require further investigation.

Keywords: Antibacterial activity, Antibiotics, methanol, *Sansevieria zeylanica*, Uropathogens.

Introduction

Sanseveira zeylanica, also known as bowstring hemp is a species of plant belonging to the genus *Sanseveira* and family *Asparagus* (*Asparagacear*) (Evans 2005). There are more Sansevierians in Africa than anywhere in the world (Carlquist and Schneider, 2007).

In Nigeria, the leaves and roots of *Sansevieria* species are used traditionally to treat diseases such as diarrhea, eczema, gonorrhea, hemorrhoids and piles (Adeyemi *et al.*, 2009).Many studies have shown that medicinal plants are sources of nutrient and non-nutrient compounds, many of which show antioxidant and antimicrobial properties, which are known to protect the human body against both cellular oxidation reactions and pathogens (Nascimento *et al.*, 2000). *Sanseveira* species are known to exhibit anti-inflammatory, antioxidant, and antimicrobial properties (Aliero *et al.*, 2008), the leaf and rhizome extracts of this plant have the ability to inhibit the growth of many disease causing bacteria and this ability serves a potential of broad spectrum antimicrobial effect which makes the plant suitable for producing antimicrobial drugs (Igbinosa and Aiyegoro, 2009).

Urinary tract infection (UTI) affects close to 150 million people worldwide (Sihra *et al*, 2018). Recurrent UTI (rUTI) affects mostly young female, but it has been estimated that up to 50% of women experience UTI at least once in their lives (Silverman *et al.*, 2013).However, UTI is mainly associated with patients that have history of urinary tract abnormalities, suppressed immune systems, long-term catheter use, and recent urinary procedures. Many recent studies propound a genetic susceptibility to the recurrent symptomatic UTI. Numerous genes seem to contribute and have been strongly associated with UTI-prone patients (Murray *et al*., 2021).

Plant medicine serves an alternative that has been an effective complementary practical approach for treating rUTI, especially as a prophylactic therapy of antibiotics, as both have a synergistic effect (Belkaid and Hand, 2014). Plant medicine is a realistic option and a better choice for the long-term prevention of rUTI as they are cheap, readily available, safe to use, with fewer reported side effects, and do not cause bacterial resistance (Belkaid and Hand, 2014).The antimicrobial effects of plant extracts involves multiple mechanisms which include directly killing microbes, interfering with microbial adhesion to epithelial cells and biofilm formation, inhibiting the immunomodulators, or boosting body oxidant status (Belkaid, and Hand, 2014). Against this background, this study was conducted to determine the antibacterial activity of methanolic extracts of *Sansevieria zeylanica* against UTI isolates from university of Benin, Edo State, Nigeria.

Materials and Methods

Study Population

A total of two hundred students residing in the Ugbowo campus of the University of Benin, Edo State, Nigeria, were recruited for this study. Each student gave written consent for their voluntary participation in the study. The University of Benin Ethical Committee approved the protocol for this study.

Collection of Urine Specimen

Voided midstream (clean-catch) urine specimen was collected from each student into a sterile screw-capped plastic universal container. Guidelines on proper collection of urine specimen to prevent contamination were given to each student.

Microbiological Analysis

A standardized loop was used to inoculate 0.001ml of properly mixed uncentrifuge urine specimen onto the surface of cysteine lactose electrolyte deficient (CLED) medium and blood agar. The agar plates were incubated aerobically at 37^0C, and colonies were counted after 24hours. Urinary tract infection was indicated at colony count $\geq 10^5$ colony forming unit/ml, and isolates identified by standard microbiological procedure (Barrow and Feltham, 2003).

Collection of Plant Specimen

Leaves and roots of *Sansevieria zeylanica* were collected from the garden opposite the laboratory complex of the School of Clinical Medicine, Igbinedion University, Okada (IUO), Edo State and confirmed at the Department of Biological Sciences, IUO.

Preparation of Extracts

After drying, 132.23g and 54.81g of the leaves and roots, respectively of *S. zeylanica* were pulverized and the methanolic extraction was carried out with the aid of the Soxhlet apparatus. The extracts were filtered with Whatman No 1 filter paper, concentrated to dryness at 40^0C, and stored at 4^0C until further use.

Antimicrobial Activity Test

Agar well diffusion method described by White and Reeves (1987) was used to determine the susceptibility of the bacterial isolates to the plant extracts. The minimum inhibitory concentration (MIC) of the extracts against the isolates was determined using the microdilution method as previously described (Eloff, 1998). Varying concentrations of the extracts ranging from 0.125mg/ml to 16mg/ml were prepared in Muller-Hinton broth medium. A 0.1ml of the standardized suspension of each bacterial isolate was inoculated into each tube and incubated aerobically at 37^0C for 24 hours. The tube with the lowest concentration without visible growth, when compared with the control (tube containing broth medium and extract only), was regarded as the MIC.

Antibiotic-Extract Combination Synergism Test

The susceptibility of the isolates to selected antibiotic discs impregnated with the plant extracts was determined, and the zone of inhibition was measured and compared with diameters produced by antibiotic alone and extract alone. When the zone of inhibition produced by the antibiotic-extract combination was greater than the diameter produced by antibiotic alone or extracts alone, a synergistic effect was indicated as described by Kingsley *et al* (2013).

Results

Prevalence of Urinary Tract Infection

In the present study, a total of 200 urine samples were collected out of which only 23(11.5%) yielded the growth of uropathogens (Figure 1). Four bacteria species associated with urinary tract infection were *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Figure 2).

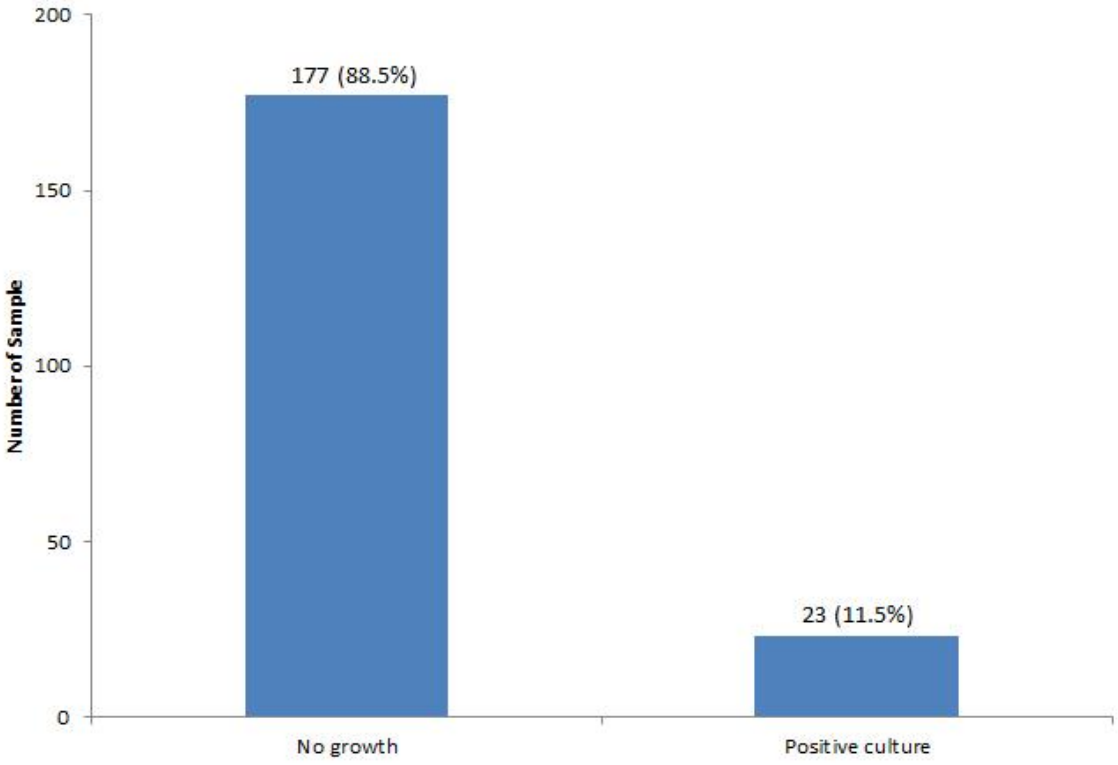

Figure 1: Prevalence of Urinary Tract Infection among the Study Population.

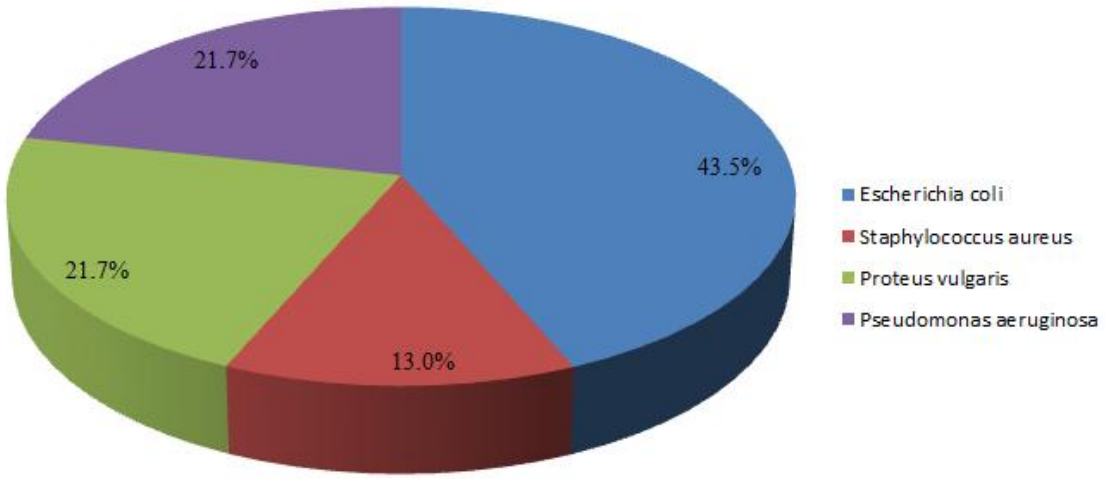

Figure 2: Frequency of Isolated Uropathogens

Antimicrobial Activity of Methanolic Leaf and Root Extracts of *Sansevieria zeylanica*

The antimicrobial activity of the methanolic extracts of the leaf and root of *S. zeylanica* was tested against four bacterial isolates each representing bacteria groups isolated in this study using the agar well diffusion method. Both the leaf and root extracts showed varying degrees of antimicrobial activity at concentration between 100mg/ml and 400mg/ml (zone of inhibition of 0mm to 20mm) (Table 1).The activity of both extracts was greater at 200mg/ml against *E. coli* and *P. vulgaris* isolates, but at 400mg/ml against *S. aureus*.

The Minimum Inhibitory Concentrations (MICs) of the leaf extract were 8mg/ml, 4mg/ml, 8mg/ml, and >16mg/ml against *E. coli*, *S. aureus*, *P. vulgaris*, and *P. aeruginosa*, respectively (Table 2).

Table 1: Antimicrobial Activity of the Methanolic Extracts of the Leaf and Root of *S. zeylanica* with zone of inhibition in millimetre (mm).

Bacteria Isolate	Leave Extract Conc. (mg/ml)			Root Extract Conc. (mg/ml)			AMP	NA
	400	200	100	400	200	100	(10µg)	(30µg)
E. coli	15	15	11	9	13	9	0	0
S. aureus	20	18	9	16	15	10	0	0
P. vulgaris	10	16	9	12	19	10	21	25
P. aeruginosa	0	0	0	0	0	0	0	0

Conc. – Concentration, AMP – Ampicillin, NA – Nalidixic acid.

Table 2: Minimum Inhibitory Concentration (MIC) of the Methanolic Leaf and Root Extracts of *S. zeylanica*.

Bacteria Isolate	*S. zeylanica* Extract (mg/ml)	
	Leave	Root
E. coli	8	16
S. aureus	4	8
P. vulgaris	8	8
P. aeruginosa	>16	>16

Synergistic Effect of Antibiotics and Plant Extracts

The combined effect of selected antibiotics and *S. zeylanica* extracts are shown in Tables 3 and 4. Ampicillin and the leaf extract produced a greater zone of inhibition (26mm) against *P. vulgaris* compared to Ampicillin alone (21mm) and leaf extract alone (16mm) indicatinga synergistic effect.

Table 3: Combined Effect of the Methanolic Leaf Extract and Selected Antibiotics.

Bacteria Isolate	LE	AMP	AMP+LE	NA	NA+LE
	Zone of Inhibition (mm)				
E. coli	15	0	10	0	12
S. aureus	18	0	13	0	10
P. vulgaris	16	21	26	25	13
P. aeruginosa	0	0	0	0	0

LE – 200mg/ml Leave extract, AMP – 10µg/disc Ampicillin, AMP+LE - 10µg/disc Ampicillin and 200mg/ml Leave extract combination, NA – 30µg/disc Nalidixic acid, NA+LE - 30µg/disc Nalidixic acid and 200mg/ml Leave extract combination.

Table 4: Combined Effect of the Methanolic Root Extract and Selected Antibiotics.

Bacteria Isolate	RE	AMP	AMP+RE	NA	NA+RE
	Zone of Inhibition (mm)				
E. coli	9	0	7	0	7
S. aureus	15	0	12	0	11
P. vulgaris	19	21	21	25	16
P. aeruginosa	0	0	0	0	0

RE – 200mg/ml Root extract, AMP – 10µg/disc Ampicillin, AMP+RE - 10µg/disc Ampicillin and 200mg/ml Root extract combination, NA – 30µg/disc Nalidixic acid, NA+RE - 30µg/disc Nalidixic acid and 200mg/ml Root extract combination

Discussion

Urinary tract infections (UTIs) are the second most common human infections worldwide (UDHHS, 2004). With the spread of Multidrug-resistant (MDR) pathogens worldwide, the treatment of UTIs has become difficult resulting in poor prognosis, hence, the need to search for new antimicrobial agents effective against urinary tract pathogens. The present study is aimed at determining the antimicrobial effect of methanolic leaf and root extracts of *S. zeylanica* on uropathogens. *Escherichia coli* is a major pathogen associated with UTI as observed in the present study (43.5%), this is in tandem with a previous study among students of a tertiary institution in Nigeria (Ojo and Anibijuwon, 2010). The high prevalence of *E. coli* as a causative agent of UTI is often linked to faecal contamination of the urethra, especially in females (Nicolle, 2001).

The methanolic leaf and root extracts of *S. zeylanica* showed good antimicrobial activity against tested isolates of *E. coli*, *Staphylococcus aureus*, and *Proteus vulgaris* with zone of inhibition ranging from 9mm to 20mm. Previous studies have reported the antimicrobial effect of Sansevieria species on these pathogens (Deepa *et al*., 2011; Tkachenko *et al*., 2017). Ampicillin and Nalidixic acid are common antibiotics used in the treatment of UTIs, however, it is not uncommon to have uropathogens resistant to these antimicrobial agents (Ojo and Anibijuwon, 2010; Oladehinde *et al*., 2011). Although isolates of *E. coli* and *S. aureus* tested were resistant to Ampicillin and Nalidixic acid, these pathogens were greatly susceptible to the methanolic extracts of *S. zeylanica*. The resistance of the isolates to these antibiotics may be due to the extensive use of these antimicrobial agents over the years.

Pseudomonas aeruginosa showed resistance to both extracts of *S. Zeylanica* and selected antibiotics. In the study of Deepa *et al* (2011), the methanolic leaf and root extracts of *Sansevieria roxburghiana* showed antimicrobial activity against *P. aeruginosa* (zone of inhibition of 12mm). The variations in results could be due to one, differences in the strain of *P. aeruginosa* tested and site of isolation, and two, the presence or high amount of certain bioactive substance(s) in *S. roxburghiana* which may be absent or reduced in *S. zeylanica*. The resistance of strains of *P. aeruginosa* to certain plant extracts has been reported (Ghesmati, 2008).The emergence of multidrug-resistant (MDR) and extensively drug-resistant Pseudomonas aeruginosa strains associated with human infections particularly UTIs is a public health concern (Breidenstein *et al*., 2011; Poole, 2011).

Escherichia coli, *S. aureus*, and *P. vulgaris* growths were inhibited at MIC values of 8mg/ml, 4mg/ml, and 8mg/ml, respectively with the methanolic leaf extract. The MIC of the leaf extract against *S. aureus* in the present study corresponds with the report of Ugbomoiko *et al* (2022). Ampicillin and the methanolic leaf extract showed a synergistic effect against *P. vulgaris*. In their study, Kingsley *et al* (2013) reported that Sansevieria species are capable of producing a synergistic effect when combined with certain antibiotics.

In contrast, an antagonistic relationship was observed between Nalidixic acid and extracts of *S. zeylanica* against all the tested bacterial isolates. Darwish *et al* (2002) reported certain plant extracts capable of antagonizing the antibacterial activity of Nalidixic acid. In the same vein, the root extract of *S. zeylanica* has been reported to have an antagonistic effect when combined with Gentamycin (Ugbomoiko *et al*., 2022).This antagonistic effect of extracts of *S. zeylanica* on Nalidixic acid may be due to the presence of bioactive substance(s) in the plant inhibitory to the action of the antimicrobial agent. Further studies are needed to verify this point.

Conclusion

To the best of our knowledge, this is the first time the antimicrobial effect of extracts of *S. zeylanica* on pathogens associated with UTI is reported. The inhibitory effect of the extracts on both the Gram -positive and -negative bacteria indicates a broad spectrum activity, and the action on the latter is remarkable as these bacteria are major expressers of various resistance genes. Finally, the antimicrobial activity of the extracts of *S. zeylanica* against common uropathogens as observed in this study substantiates the traditional use of this plant in the treatment of UTIs.

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