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Antibacterial Activity of Methanolic Extract of *Moringa oleifera* Lam. Leaf on ESBL Producing Bacterial Isolates from Urine of Patients with Urinary Tract Infections

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ABSTRACT

The study was carried out to determine the effects of methanolic extract of *Moringa oleifera* on bacterial isolates from urine of patients with urinary tract infection (UTI). One hundred and fifty urine samples were collected for this study at Ekiti State Teaching Hospital, Ado Ekiti, Nigeria, between March 2015 and June 2015. Ethical clearance was obtained in order to carry out the study. Microscopical examination of the urine smear (wet preparation) revealed presence of yeast (48.0%), bacteria (88.9%), white blood cells (10%) and epithelial cells (14%). A total of 89 bacteria were isolated belonging to 40 different bacteria species. The Gram positive bacteria isolated include *Corynebacterium accolens, Arthrobacter mysorens, Rhodococcus equi, Staphylococus aureus, Luteococcus sanguinis, Aerococcus viridians, Actinomyces urogenitalis, Helicobacillus massiliensis, Branchibus cervicis, Arthrobacter cretinolyticus, Streptococcus rubneri* among others. While the Gram negative bacteria were *Cetobacterium somerae, Escherichia coli, Klebisiella pneumoniae, Yersinia frederikseni, Enterobacter aerogenes, Vibrio mimicus, Acinetobacter baumanii, Pantoea agglomerans, Proteus mirabilis, Pseudomonas aeruginosa, Citrobacter freundii, Proteus vulgaris* among others. The bacteria isolated showed multi-drug resistance to the antibiotics tested. None of the bacterial isolates showed susceptibility to all the antibiotics tested, as they showed resistance to between 2 to 8 out of the 8 antibiotics tested per organism. All the bacteria tested showed evidence of ESBL production, and all of them were susceptible to the methanolic extract of dried leaf of *Moringa oleifera*. The qualitative analysis for phytochemical constituents of the methanolic extract of *Moringa oleifera* indicated the presence of saponnins, flavonoids, steroids and cardiac glycosides. The methanolic extract of dried leaf of *Moringa oleifera* was found to possess potent phytochemicals with high inhibitory activities on bacteria of UTIs origin.

Key words: Antibacterial activity, *Moringa oleifera* Lam, Methanolic extract, Phytochemicals, Urinary tract infections.

INTRODUCTION

Human urine can support bacterial growth due to its favourable chemical composition. A urinary tract infection (UTI) is an infection that affects part of the urinary tract. When it affects the lower urinary tract it is known as a simple cystitis (a bladder infection) and when it affects the upper urinary tract it is known as pyelonephritis (a kidney infection). Symptoms of UTI are: a burning feeling when you urinate; a frequent or intense urge to urinate, even though little comes out when you do; pain or pressure in your back or lower abdomen; cloudy, dark, bloody, or strange-smelling urine; feeling tired or shaky; as well as fever or chills (Nicolle, 2008).

Urinary tract infection (UTI) occurs when there is an anatomical or functional break in the host defence system, therefore allowing for the adherence, multiplication and persistence of microorganisms in that part of the urinary tract (Brook *et al*., 2010). Urinary tract infection is one of the most important causes of morbidity in the world population, affecting all age groups. Anatomically urinary tract is divided into an upper portion composed of kidneys, renal pelvis, and urethers and a lower portion made of urinary bladder and urethra (Foxman, 1990). UTI is an inflammatory response of the urothelium to bacterial invasion that is usually associated with bacteriuria and pyuria. UTI may involve only the lower urinary tract or both the upper and the lower tract. Urinary tract infection may be asymptomatic or symptomatic; asymptomatic which is defined as true bacteriuria is the absence of specific symptoms of the urinary tract infection while symptomatic is define as bacteriuria with symptoms (McCormick *et al*., 2008).

Moringa oleifera commonly referred to as *Moringa*. It is an exceptionally nutritious vegetable tree with a variety of potential uses. These leaves have high medicinal value (Fahey, 2005). Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess anti tumor, antipyretic, antiepileptic, anti inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are

being employed for the treatment of different ailments in the traditional system of medicine (Nikkon *et al*., 2003).

The aim of this study is to look into the possibility of the future use of *Moringa oleifera* in treating urinary tract infections. The objectives of the study are to: (a) isolate, characterise and identify bacteria that are associated with the urinary tract infections, (b) investigate the bacterial isolates' susceptibility to antibacterial agents, (c) determine the phytochemical constituents of *Moringa oleifera*, and (d) determine the effect of *Moringa oleifera* extracts on microorganisms that are drug resistant.

MATERIALS AND METHODS

Study area and population

This study was conducted at the Ekiti State University Teaching Hospital (EKSUTH), Ado-Ekiti Ekiti State, Nigeria over a period of three month (March-June, 2015). One hundred and fifty patients with urinary tract infections during the study period were randomly recruited. Approval was sought and collected from the Research/Ethics Committee of the Ekiti State University Teaching Hospital before the commencement of the research work.

Sampling Techniques

Sterile universal bottles were used to collect the urine samples from patients in EKSUTH and the samples transported from the hospital to the Microbiology laboratory of Afe Babalola University Ado-Ekiti (ABUAD), using a cold box, for microbiological investigations.

Sample processing

Using a micropipette, 0.1ml of well-mixed un-centrifuged urine was inoculated on plate count agar and Cysteine lactose electrolyte deficient (CLED) agar using the pour plate method. The plates were incubated aerobically at 37℃ for 24 hours and counts were expressed in colony forming units per millimetre (CFU/ml).

Ten (10) ml of each urine sample was centrifuged at 1000g for 5 minutes, the supernatant was discarded and a drop of the well mixed sediment was transferred unto a microscope slide and covered with a cover slip. It was examined microscopically at high magnification (x100) for the presence of pus cells, red blood cells, epithelial cells, casts, crystals and yeast cells. Another drop of the urine sample was transferred to a microscopic slide and it was allowed to dry after which it was Gram-stained. It was examine microscopically at high magnification (x100) for the presence of bacteria, polymorphonuclear cells e.t.c.

Inoculation, isolation, characterization and identification of isolates.

All isolates were characterised using standard microbiological and biochemical tests as described by Barrow and Feltham (1993), and Cheesbrough (2006). Bacterial isolates were identified with the help of online Gideon informatics (1994-2014), with reference to Barrow and Feltham (1993) and Garrity *et al* (2005).

Antibiotic susceptibility test

All the isolated organisms were tested for antibiotic susceptibility using Kirby-Bauer disc diffusion method on Mueller-Hinton agar. This was carried out by making an even spread of the pure isolates on prepared Mueller-Hinton agar using sterile swab sticks and aseptic placement of the antibiotics discs using sterile forceps. The plates were incubated aerobically at 37˚C for 24 hours after which the zones of inhibition were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2013). Antibiotics used were; Ceftazidime (CAZ) (30µg), Cefuroxime (CXM) (30µg), Ceftriazone (CRO) (30µg), Augmentin (AUG) (30µg), Ofloxacin (OFL) (5µg), Gentamicin (GEN) (10µg), Nalidixic acid (NAL) (30µg), Nitrofurantoin (NIT) (200µg), Amoxycillin (25µg), Tetracycline (25µg) for gram negative isolates and Cefixime (30µg), Cloxacillin (CLO) (5µg), Augmentin, (AUG) (30µg), Cotrimoxazole (COT) (25µg), Erythromycin (ERY) (5µg), Gentamicin (GEN) (10µg), Streptomycin (STR) (10µg), Tetracycline (TET) (10µg) and Chloramphenicol (CHL) (10µg) for gram positive isolates.

Assay for ESBL production

Assay for production of extended spectrum β lactamase production by the bacterial isolates from urine sample was determined phenotypically by the double disc method as described by Clinical and Laboratory Standards Institute [2013]. Antibiotics used are: Ceftazidine (30µg) and Ceftazidine/Clavulanic acid(30/10µg); Cefuroxime (30µg) and Cefurozime/Clavulanic acid(30/10µg); Ceftriazone (30µg) and Cetriaxone/Clavulanic acid (30/10µg). ESBL production by an organism was determined by calculating the difference in the diameter of the zone of inhibition produced when an antimicrobial agent is used singly and when it is combined with clavulanic acid. ESBL production is shown by a difference equal to or greater than 5mm. (eg, ceftazidime zone =16; ceftazidime/clavulanic acid zone=21), Clinical and Laboratory Standards Institute [2013].

Plant sample collection

The Moringa leaves used in this research work were obtained from ABUAD Moringa plantation, in the teaching and research farm of Afe Babalola University Ado-Ekiti (ABUAD), Nigeria.

Preparation of Moringa (*Moringa oleifera* extracts)

The leaves were gotten fresh and air dried for weeks. After drying, the leaves were grinded to fine powder using the electric blender. The powdered Moringa (100g) was soaked into 500ml of methanol, left for 72 hours at room temperature after which it was filtered using filter paper. The extract was obtained by drying off the methanol with the use of rotary evaporator.

Antimicrobial activity of Moringa extracts using Agar-well diffusion method

Susceptibility of the isolated organisms to moringa extracts was determined by agar well diffusion technique using Mueller-Hinton agar. Seven millimetre (7mm) diameter wells were prepared on agar containing a suspension of each isolated organisms. The methanolic extracts were diluted, using DMSO as diluents and different concentrations (500-100mg/ml) were added to the wells. The plates were left at ambient temperature for 15 minutes and then incubated at 37˚C for 24hours, after which the zones of inhibition were observed and recorded.

Phytochemical analysis

Tests for the presence of the following plant secondary metabolites: alkaloids, saponins, tannins, flavonoids, steroids and cardiac glycosides were carried out on the methanolic extracts of the leaf of *Moringa oleifera* as described by Sofowora (2008).

Test for alkaloids: A volume 2ml of the extract was added to 1ml of aqueous hydrochloric acid. A few drops of saturated picric acid solution were added. A cream coloured precipitate obtained indicated the presence of alkaloid.

Test for tannins: To 2ml of the plant extract was dissolved in 10ml of distilled water and filtered. 2 ml of the extract was added to 2ml $FeCl_3$. If a blue-black precipitate was obtained, this indicated the presence of tannins.

Test for saponins (Frothing test): From the extract, 0.5ml was added to 5ml distilled water. Frothing presence indicated presence of, saponnins.

Test for flavonoids: About 2ml of plant extract was weighed in a test tube and dissolved diluted NaOH and diluted HCl were added. The presence of yellow solution that colourless indicates the presence of flavonoids (Sofowora, 2008).

Test for steroids: A volume of 10ml of plant extract was dissolved in 10ml chloroform and filtered, and 2ml of the filtrate was added to 2ml acetic anhydride and Conc H_2SO_4. Blue-green ring obtained the presence of terpenoids.

Test for Cardiac glycosides: A volume of 2ml of extract was added to 1ml glacial acetic acid, 1ml $FeCl_3$ and 1ml Concentrated H_2SO_4. Absence of green-blue colour indicated that cardiac glycosides were absent.

RESULTS

Urine samples were obtained from 150 Urinary tract infection patients attending Ekiti State University Teaching hospital Ado-Ekiti, Ekiti state, aged 30.83±13.39 (16-72) years, and made up of 19 males and 31 females.

Microscopical examination of the urine smear (wet preparation) revealed presence of yeast (48.0%), bacteria (88.9%), White Blood Cells (10%) and epithelial cells (14%). Macroscopical and microscopical appearances of some selected urine samples are presented in Table 1.

A total of 89 bacteria were isolated belong to 40 different bacteria species. The Gram positive bacteria are; *Corynebacterium accolens, Arthrobacter mysorens, Rhodococcus equi, Staphylococus aureus, Luteococcus sanguinis, Actinomyces urogenitalis, Aerococcus viridians, Helicobacillus massiliensis, Branchibus cervicis, Arthrobacter cretinolyticus, Streptococcus rubneri* among others. The Gram negative bacteria are; *Cetobacterium somerae, Escherichia coli, Klebisiella pneumoniae, Yersinia frederikseni, Enterobacter aerogenes, Vibrio mimicus, Acinetobacter baumanii, Pantoea agglomerans Proteus mirabilis, Proteus vulgaris* among others (Figure 1).

The gram positive bacteria isolates were highly susceptible to Ofloxacin (81.81%), but showed varied resistance to other antibiotics tested with cloxacillin giving the least susceptibility of 11.36% (Figure 2). The gram negative bacteria isolates from urine samples showed varied resistance to all the antibiotics tested, with Augmentin (Amoxcillin/clavulate) showing 100% resistance to the isolates (Figure 2). None of the bacterial isolates showed susceptibility to all the antibiotics tested, as they showed resistance to between 2 to 8 out of the 8 antibiotics tested per organism (Figure 3).

All the bacterial isolates tested positive for Extended Spectrum Beta Lactamases (ESBL) production. The same organisms were susceptible to Imipenem and indication of lack of Carbapenenase production (Tables 2 and 3). All the Extended Spectrum Beta Lactamases producing isolates were susceptible to the methanolic extract of *Moringa oleifera* (Tables 4).

The analysis of the phytochemical components of Moringa oleifera indicated the presence of saponnins, flavonoids, steroids and cardiac glycosides, but no alkaloids and tannins.

DISCUSSION

Urinary Tract Infection (UTI) is one of the major infections accounting for about 8.3 million visits to doctors yearly. This study is designed to investigate the in-vitro susceptibility pattern of bacteria associated with UTI to the extract of *Moringa oleifera*. *M. oleifera* has been known for its antibacterial and antifungal activities and possesses a lot of macro and microelements nutrients (USDA, 2003; Katayon *et al*., 2005; Kebreab *et al*., 2005; Farooq *et al*., 2007).

Bacteriological studies were carried out on 150 urine samples and the following bacterial species were isolated namely; *Cetobacterium somrae*, *Escherichia coli, Luteococcus sanguinis, Enterobacter aerogenes, Rhodococcus equi, Corynebactrium accolens, Staphylococcus pettenkoferi, Staphylococcus aureus, Branchibus cervicis, Pantoea agglomerans, Acinetobacter baumanii, proteus spp, Vibrio mimicus, Arthrobacter cretinolyticus, Actinomyces urogenitalis, Klebsiella pneumoniae* among others. *E. coli, Enterobacter aerogenes* and *Cetobacterium somrae* were the most common pathogen isolated in patients with UTI in this study. Earlier study indicated *E. coli* as the most common pathogen associated with UTI (Ebie *et al.*, 2001; Njoku *et al.*, 2001). Onifade *et al.* (2005) and Aiyegoro *et al.* (2007) also reported that *E. coli* was the most commonly isolated pathogen in significant bacteriuria. In a similar study by Nwanze *et al.* (2007) the most common isolates were *Escherichia coli* (51.2%), *S. aureus* (27.3%), and *Klebsiella pneumoniae* (12.8%). This same pattern was also reported by Kolawole *et al.* (2009).
Several studies have demonstrated that the geographical variability of pathogens occurrence in cases of UTIs is limited by the predominance of Gram negative, usually Enterobacteriaceae and particularly *E. coli* and *Enterobacter* spp., in various regions of the world and the resistance patterns of these organisms can vary significantly between hospital, countries and continents (Teppa and Roberts, 2005; Fatima and Ishrat, 2006).
The bacterial isolates show both resistivity and susceptibility to the antibiotics used. The gram negative bacteria were resistant to Augmentin (100%), Cefuroxime (90.91%), Cefixine (59.1%) while they were susceptible to Gentamicin (63.63%), Ofloxacin (65.15%) and Nitrofurantoin (77.27%). All the Gram positive bacteria were resistant to Ceftazidime (81.82%), Cefuroxime (75%), Erythromycin (77.28%) and Cloxacillin (88.64%) while they were susceptible to Ofloxacin (81.81%) and Gentamicin (68.18%).
This study has demonstrated high antimicrobial potency of methanolic extracts of *M. oleifera* against urinary tract isolates. This tends to correspond with the work of Arun and Purnachandra (2011), who in their work on; the phytochemical screening and antibacterial activity of *Moringa oleifera* against *Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* from urinary tract infected patients

showed that the antibacterial activity of the extract on the organisms increased as the concentration of the extract increased.

CONCLUSION

Based on the results obtained from this study, various multidrug resistant bacteria belonging to different genera, could be associated with Urinary tract infections. It was interesting to observe that methanolic extract of *Moringa oleifera* had antibacterial activities on all the multidrug resistant bacteria tested. A hope for solution to the menace in medical treatment caused by multidrug resistant bugs may not be farfetched. Further work should be carried out to isolate, purify and possibly characterize the active constituents responsible for the activity of this plant. As well as elucidating their possible mechanisms of actions. Traditional use of medicinal plants is therefore encouraged.

Table 1: Macroscopy and microscopy analysis of urine samples

S/N	SAMPLE CODES	MACROSCOPY	MICROSCOPY (Wet prep)	MICROSCOPY (Gram stained)
1	7	Clear, yellow	Bacteria, yeast cells	Multiple bacteria
2	8	Clear, yellow	Yeast cells, epithelial	Epithelial cells, bacteria(4)
3	10	Clear, deep amber	Yeasts, epithelial	Epithelial cells, bacteria(3)
4	11	Clear, deep yellow	Yeast, WBCs	Bacteria (6)
5	12	Clear, light yellow	WBCs, yeast cells	Multiple bacteria
6	13	Turbid, milky	Epithelial cells, crystals	Epithelial cells, bacteria (3)
7	14	Amber, clear	Casts, phosphate	Polymorphonuclear cells
8	19	Turbid, yellow	Casts, yeast cells	Multiple bacteria
9	20	Milky, turbid	Epithelial cells, yeast	Multiple bacteria
10	21	Light yellow, turbid	Phospahate, casts	Multiple bacteria
11	22	Golden yellow, turbid	Epithelial cells, yeast	Bacteria (27)
12	23	Turbid, yellow	Bacteria	Multiple bacteria
13	24	Turbid, yellow	Yeast cells	Bacteria (6)
14	25	Turbid, deep amber	WBCs, phospahate	Bacteria (3)
15	26	Clear, amber	Bacteria	Multiple bacteria
16	27	Clear, white	Bacteria	Multiple bacteria
17	28	Clear, white	Yeast cells	Polymorphonuclear cells
18	29	Cloudy, amber	Epithelial cells, casts	Multiple bacteria, epithelial cell
19	30	Clear, deep amber	Epithelial cells, cast	Epithelial cells, bacteria (5)
20	32	Yellow, clear	Yeast cells, casts	Bacteria (13)
21	35	Deep amber, turbid	WBCs, epithelial	Bacteria (5)
22	36	Clear, yellow	Epithelial cells,yeast	Multiple bacteria
23	37	Amber, turbid	Casts, yeast cells	Bacteria (7)
24	38	Deep amber, turbid	Bacteria, yeast cells	Bacteria (12)
25	42	Amber, clear	Yeast cells	Epithelial cells

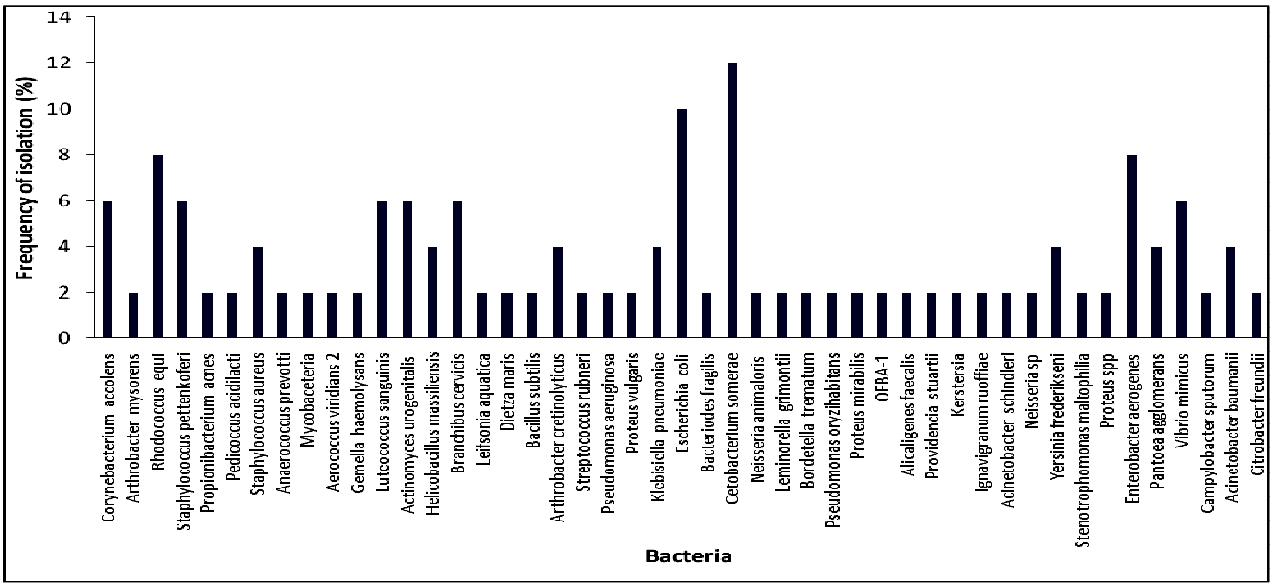

FIGURE 1: Frequency of occurrence of bacteria isolated from urine of UTI patients

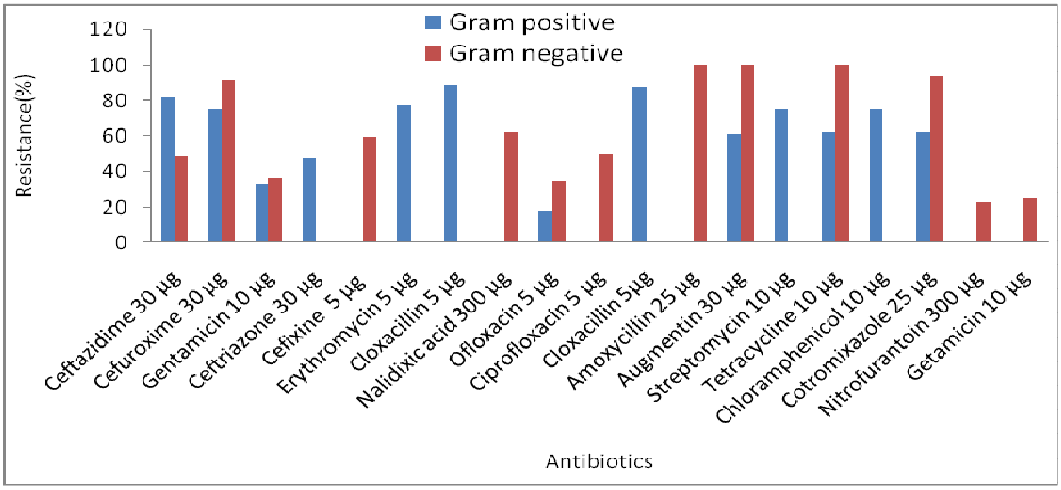

Figure 2: Antibiotic resistance patterns of bacterial isolates from urine of UTI patients

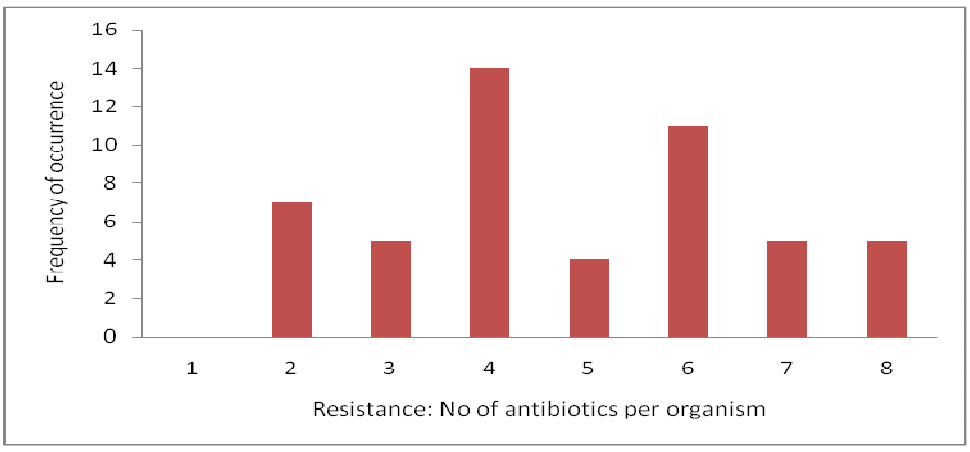

Figure 3: Multiple drug resistant patterns of bacterial isolated form UTIs

Table 2: Extended Spectrum Beta Lactamases production table for Gram positive bacterial isolates

CODES		CRO	CRO + CLAV	CXM	CXM + CLAV	CAZ	CAZ + CLAV	IPM
	QUANTITY (µg)	30	30/10	30	30/10	30	30/10	10
2d	*Rhodococccus equi*	12^{a}	20^{a}	15^{b}	21^{b}	9	12	32 S
15a	*Corynebacterium accolens*	9^{a}	16^{a}	13^{b}	18^{b}	7^{c}	14^{c}	27 S
5c	*Staphylococcus pettenkofin*	13^{a}	25^{a}	11^{b}	16^{b}	5^{c}	10^{c}	25 S
23a	*Rhodococcus equi*	7^{a}	13^{a}	9^{b}	14^{b}	12^{c}	17^{c}	24 S
4d	*Staphylococcus aureus*	0^{a}	12^{a}	7^{b}	14^{b}	5^{c}	13^{c}	30 S
2b	*Rhodococccus equi*	15^{a}	21^{a}	10^{b}	15^{b}	12^{c}	23^{c}	31 S
4b	*Rhodococcuss equi*	0^{a}	12^{a}	10	12	17^{c}	25^{c}	36 S
23c	*Branchibus cervicis*	5^{a}	11^{a}	7^{b}	13^{b}	9^{c}	21^{c}	33 S
32a	*Actinomyces urogenitalis*	10	10	7^{b}	13^{b}	5	7	23 S
23a	*Rhodococcus equi*	12	17	15	17	13^{c}	18^{c}	34 S
5b	*Staphylococcus pettenkoferi*	9^{a}	15^{a}	7^{b}	14^{b}	9	10	27 S
11d	*Corynebacterium accolens*	8^{a}	16^{a}	7	10	8^{c}	16^{c}	28 S
17a	*Corynebacterium accolens*	10^{a}	15^{a}	10	10	0^{c}	7^{c}	23 S
7c	*Streptococcus rubneri*	12^{a}	17^{a}	10^{b}	15^{b}	12^{c}	21^{c}	30 S
14c	*Aerococcus viridians*	16^{a}	21^{a}	5^{b}	13^{b}	6	8	26 S
35b	*Dietza maris*	10	10	12^{b}	21^{b}	10^{c}	15^{c}	34 S
	Staphylocccocus aureus ATC25923	9^{a}	14^{a}	14	18	12^{c}	17^{c}	31 S

^{abc***}Zones of inhibition values within rows with the same superscript indicate Extended Spectrum Beta Lactamases production
*Values are zones of inhibition in millimetres(mm)
CAZ - Ceftazidime, CXM – Cefuroxime , CRO – Ceftriazone, CLAV – Clavulanic acid, IPM - Imipenem

Table 3: Extended Spectrum Beta Lactamases production table for Gram negative bacterial isolates

CODES		CRO	CRO+ CLAV	CXM	CXM +CLAV	CAZ	CAZ+CLAV	IPM
	QUANTITY (µg)	30	30/10	30	30/10	30	30/10	10
8c	*Providencia stuartii*	0	0	10^{b}	18^{b}	17^{c}	19^{c}	32 S
31b	*Cetobacterium somrae*	0^{a}	21^{a}	0^{b}	13^{b}	10^{c}	15^{c}	30 S
33c	*Klebsiella pneumonia*	17	17	10	10	0^{c}	15^{c}	30 S
23ci	*Neisseria animaloris*	10	12	0^{b}	10^{b}	10	12	23 S
27e	*Pantoea agglomerans*	9^{a}	21^{a}	0^{b}	8^{b}	20^{c}	25^{c}	32 S
27d	*Escherichia coli*	17^{a}	26^{a}	10^{b}	18^{b}	6^{c}	15^{c}	32 S
32c	*Yersinia frederikseni*	15	15	0^{b}	15^{b}	12	13	30 S
4ai	*Pseudomonas aeruginosa*	16^{a}	26^{a}	5^{b}	13^{b}	6	10	27 S
11bi	*Citrobacter freundii*	10^{a}	15^{a}	10	10	0^{c}	7^{c}	32 S
4di	*Klebsiella pneumonia*	7^{a}	17^{a}	9	11	0^{c}	12^{c}	23 S
10ai	*Escherichia colii*	16^{a}	21^{a}	5^{b}	13^{b}	6	8	34 S
9ai	*Proteus vulgaris*	16^{a}	27^{a}	8^{b}	17^{b}	6^{c}	12^{c}	25 S
34e	*Cetobacterium somrae*	7^{a}	17^{a}	10	12	0^{c}	15^{c}	27 S
7a	*Cetobacterium somrae*	10^{a}	15^{a}	0^{b}	10^{b}	10	12	32 S
4ai	*Pseudomonas aeruginosa*	9	10	13^{b}	18^{b}	12^{c}	24^{c}	30 S
40b	*Vibrio mimcus*	9^{a}	16^{a}	13^{b}	18^{b}	7^{c}	14^{c}	31 S
36d	*Cetobacterium somerae*	17^{a}	21^{a}	9	10	0^{c}	15^{c}	32 S
Type	*Proteus mirabilis* ATCC12453	9^{a}	19^{a}	13^{b}	18^{b}	7^{c}	14^{c}	28 S

^{abc***}Zones of inhibition values within rows with the same superscript indicate Extended Spectrum Beta Lactamases production
*Values are zones of inhibition in millimetres (mm)

CAZ - Ceftazidime, CXM – Cefuroxime , CRO – Ceftriazone, CLAV – Clavulanic acid IPM - Imipenem

Table 4: Antimicrobial activity of methanolic extract of *Moringa oleifera* on bacterial isolates from urine

CODES	ORGANISMS	500mg/ml	400mg/ml	300mg/ml	200mg/ml	100mg/ml	Control
	Gram-positive						
2d	*Rhodococccus equi*	13	13	11	1	0	0
15a	*Corynebacterium accolens*	16	15	11	3	0	0
5c	*Staphylococcus pettenkofin*	15	5	3	3	0	0
23a	*Rhodococcus equi*	12	8	4	0	0	0
4d	*Staphylococcus aureus*	13	12	5	3	0	0
2b	*Rhodococccus equi*	15	13	12	3	0	0
4b	*Rhodococcuss equi*	10	12	9	3	0	0
23c	*Branchibus cervicis*	10	10	7	5	0	0
32a	*Actinomyces urogenitalis*	12	9	7	6	0	0
23a	*Rhodococcus equi*	12	11	8	5	0	0
5b	*Staphylococcus pettenkoferi*	15	11	9	4	0	0
11d	*Corynebacterium accolens*	16	12	8	7	0	0
7c	*Streptococcus rubneri*	17	12	9	8	0	0
17a	*Corynebacterium accolens*	15	9	7	8	0	0
14c	*Aerococcus viridians*	13	8	8	7	0	0
35b	*Dietza maris*	12	10	11	7	0	0
ATCC 25923	*Staphylocccocus aureus*	14	13	8	5	0	0
	Gram-negative						
8c	*Providencia stuartii*	14	13	11	1	0	0
31b	*Cetobacterium somrae*	13	11	11	5	0	0
33c	*Klebsiella pneumonia*	17	16	11	3	0	0
23ci	*Neisseria animaloris*	15	3	3	3	0	0
27e	*Pantoea agglomerans*	12	8	5	0	0	0
27d	*Escherichia coli*	15	13	12	8	0	0
32c	*Yersinia frederikseni*	10	12	9	3	0	0
4ai	*Pseudomonas aeruginosa*	13	12	9	7	0	0
11bi	*Citrobacter freundii*	10	10	8	7	0	0
4di	*Klebsiella pneumonia*	9	7	6	3	0	0
10ai	*Escherichia colii*	13	10	7	5	0	0
9ai	*Proteus vulgaris*	12	11	9	7	0	0
7a	*Cetobacterium somrae*	15	11	10	9	0	0
40b	*Vibrio mimcus*	15	13	10	10	0	0
34e	*Cetobacterium somrae*	16	13	11	9	0	0
36d	*Cetobacterium somerae*	16	12	10	8	0	0
ATCC 12453	*Proteus mirabilis*	15	11	10	1	0	0

*Values are zones of inhibition in millimetres (mm)

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