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Antimicrobial Efficacy of *Chromolaena odorata* Extract against Bacteria Isolated from Patients with Urinary Tract Infection in Ondo State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOO designed the study and the statistical analysis. Author OM wrote the protocol, the first draft of the manuscript, performed the statistical analysis and managed the literature searches. Author BSA managed the analysis. All authors read and approved the final manuscript

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ABSTRACT

Aim: This study is designed to isolate and identify the microorganisms associated with urinary tract infection in infected patients, determine the antimicrobial resistance pattern on the microbial isolates and the antimicrobial efficacy of solvents leaf extract of *Chromolaena odorata* on the resistant isolates.
Methodology: Six hundred (600) mid-stream urine samples were collected from infected patients in some selected hospitals in Ondo State. The phytochemical screening of the plant was determined. The antibiotics sensitivity and antimicrobial activity of the plant extract was determined using plate assay and agar well diffusion methods.
Results: Out of 600 urine samples analyzed, 472 (78.68%) showed significant growth of uropathogens comprising 197 (32.80%) male and 275 (45.83%) female. The isolated microorganisms comprise 5 Gram-positive bacteria (GPB), 9 Gram-negative bacteria (GNB) and 4 *Candida* sp. GNB account for 284(58.79%), GPB 158(32.71%) and *Candida sp* 41(8.48%).

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Escherichia coli showed high prevalence 90(18.63%) while *Klebsiella ozaenae* showed the least 11(2.27%). The phytochemical constituents of *C. odorata* extracts contained tannins, flavonoids, saponins, steroids and terpernoids and bacterial isolates showed resistant to all the antibiotics. *Pseudomonas mendocina* and *P. putida* showed the least resistant to *C. odorata* extracts. The MIC of the leaf extracts ranged from 5.0 mg/ml to 50.0 mg/ml. The methanol extract had the highest antimicrobial activity than n-hexane and chloroform extracts on the uropathogens.

Conclusion: The high therapeutic potentials of *C. odorata* extract against the uropathogens could be used as alternative phytotherapy in herbal medicine to the commercial antibiotics in the treatment of urinary tract infections.

Keywords: Urinary tract infection; bacteruria; antimicrobial resistance; Chromolaena odorata; solvents.

1. INTRODUCTION

Urinary tract infection is defined as the presence or multiplying of microorganisms in the tract through which urine flows from the kidneys via the bladder to outside [1]. The urinary tract represents a system that collects, store and release urine. From the microbiological perspective, urinary tract infection (UTI) can occur anywhere along the urinary tract (which includes bladder, kidneys, ureters and urethra) [2]. Urinary tract infection may involve only the lower urinary tract or both the upper and the lower tracts. Majority of UTIs are not life-threatening and do not cause any irreversible damage [3]. However, when the kidneys are involved, there is a risk of irreparable tissue damage with an increased risk of bacteremia [4]. Nowadays, drug resistance is a huge growing problem in treating infectious diseases like malaria, tuberculosis, diarrheal diseases, urinary tract infections (UTIs) etc. UTIs is a serious public health issue, particularly in the developing world where apart from the high level of poverty, ignorance and poor hygienic practices, there is also a high prevalence of fake and spurious drugs of questionable quality in circulation [5]. Therefore, this study focus on the type of pathogens responsible for UTIs and their susceptibility patterns which could help the clinicians to choose the right empirical treatment, to determine the antimicrobial efficacy of *C. odorata* on the resistant isolates which could serve as an alternative source of biological treatment against resistance microorganisms in herbal medicine.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The retrospective study was conducted from April 2014 to December 2014. A total of 600 urine samples were collected from patients with urinary tract infections (UTI) in some selected Government Hospitals in Ondo State in a sterile transparent sample bottle and then transported to Laboratory, Department of Microbiology, Federal University of Technology, Akure, Nigeria for further analyses.

2.2 Isolation of Microorganisms

The samples (urine) were cultured on sterilized molten MacConkey agar, and CLED (Cystine-Lactose-Electrolyte-Deficient) agar plates and then incubated at 37°C for 24-28 hours. The pure cultures were obtained by continuous streak on sterilized agar plates. Urine specimens with significant bacteruria (10^5 cfu/ml) were recorded as positive.

2.3 Collection of Plant Material

The fresh leaves of the plant, *C. odorata* was collected in a forest in Arigidi-Akoko in Akoko North West Local Government, Ondo State and then washed with sterile distilled water to remove the dirts. The identification of the leaves was authenticated at the Department of Crop Science and Pest Management, Federal University of Technology Akure, Ondo State.

2.4 Preparation of Extracts from Leaves of *C. Odorata*

This was carried out according to the method of Adeshina et al. [5]. The leaves were grinded to powder, then transferred into a grease-free closed airtight container. Four hundred grams (400 g) of the sample was weighed into three (3) separate clean containers containing 750 ml of methanol, chloroform and n-hexane. The mixture was allowed to stand for 72 hours with frequent stirring, sieved using three folds of sterile muslin cloth and then filtered using Whatman filter No. 1

paper. The filtrates were collected into a sterile beaker and concentrated in – vacuousing rotary evaporator. The weight of the dried extracts was measured and reported as percentage recovery. The extract was reconstituted with 30% Dimethyl Sulphoxide (DMSO) and sterilized by filtration using Millipore membrane filter (0.2 µm).

Percentage recovery = {(Weight of extract recovered after extraction/Initial weight of plant sample) x100%}

2.5 Qualitative Analysis of Phyto-chemicals in *Chromolaena odorata*

The phytochemical screening of the *Chromolaena odorata* was carried out according to Trease and Evans [6]. The phytochemicals screened were tannins, alkaloids, flavonoids, steroids, terpenoids, anthraquinone and saponins.

2.6 Data Analysis

Some data obtained were analysed by one way analysis of variance (ANOWA) and Duncan's New Multiple Range Test using SPSS 16.0 version. Differences were considered significant at $p<0.05$.

3. RESULTS

3.1 Prevalence of Infection in Each Location

Table 1 shows the prevalence of the urinary tract infection in each location. The number of samples collected from female shows high positive prevalence when compared to the male. The highest total male positive(21) and the lowest (5) were recorded from State Hospital/Mother and Child Hospital Ondo and General Hospital Igbekebo while the highest female positive (24) and lowest (6) were recorded from General Hospital Irele and General Hospital Ikare respectively. The highest total positive prevalence (42) was obtained from the State Hospital/Mother and Child Hospital Ondo 7.00% while General Hospital Ikare had the lowest total positive prevalence (11) with 1.83%.

Table 1. Prevalence of the urinary tract infection in each location

Location	No Examined	M	F	TMP	TFP	TP	TP (%)
GHIA	17	7	10	5	6	11	1.83
GHOA	39	16	23	10	22	32	5.33
GHI	34	20	14	15	13	28	4.67
GHIO	37	10	27	9	19	28	4.67
GHIDA	37	13	24	12	20	32	5.33
FMCO	18	6	12	6	10	16	2.67
SGHO	35	20	15	11	13	24	4.00
SGHIR	37	5	32	5	24	29	4.83
GHB	43	16	27	12	22	34	5.67
SHMCH	54	23	31	21	21	42	7.00
GHIOJ	35	11	24	9	17	26	4.33
GHIG	17	6	11	5	8	13	2.17
GHID	37	15	22	13	13	26	4.33
GHIJ	36	18	18	16	18	34	5.67
SSHA	39	20	19	15	19	34	5.67
GHO	35	19	16	10	9	19	3.17
GHIGB	35	16	19	16	15	31	5.17
GHIL	15	8	7	7	6	13	2.17

KEY: GHIA- General Hospital Ikare, GHOA- General Hospital Okeagbe, GHI- General Hospital Ipe, GHIO- General Hospital Iwaro-Oka, GHIDA- General Hospital Idanre, FMC – Federal Medical Centre Owo, GHO- General Hospital Okitipupa, SGHIR- General Hospital Irele, GHIB- General Hospital Bolorunduro, SHMCH- State Hospital/Mother and Child Hospital Ondo, GHIOJ- General Hospital Ile-Oluji, GHIG- General Hospital Igbekebo, GHID- General Hospital Idoani, GHIJ- General Hospital Iju, SSHA- State Specialist Hospital Akure, GHO- General Hospital Ore, GHIGB- General Hospital Igbara-Oke, GHIL- General Hospital Igbokoda, M –Male, F – Female, TMP – Total male positive, TFP- Total female positive, TP – Total positive

Table 2. Prevalence of uropathogens isolated from male and female patients and their different age groups

	Male				Female		Age group							
IC	**NI**	**%I**	**M**	**%M**	**F**	**%F**	**0-10**	**11- 20**	**21-30**	**31-40**	**41-50**	**51-60**	**61-70**	**71-80**
EC	90	18.63	23	13.45	67	21.47	5	8	15	16	11	25	9	1
KP	50	10.35	22	12.86	28	8.97	1	5	8	11	9	14	2	-
SA	39	8.07	11	6.43	28	8.97	1	3	5	9	5	10	5	1
PM	24	4.96	9	5.26	15	4.80	2	3	4	4	2	5	3	1
PP	18	3.72	8	4.67	10	3.20	2	2	3	4	2	3	2	-
SE	48	9.93	21	12.28	27	8.65	2	7	9	12	8	6	2	2
EA	26	5.38	10	5.84	16	5.12	1	4	6	4	6	4	1	-
PMe	17	3.51	8	4.67	9	2.88	1	4	3	2	4	2	1	-
PV	20	4.14	8	4.67	12	3.84	-	1	4	3	8	3	-	1
SF	26	5.38	9	5.26	17	5.44	2	2	8	6	-	4	2	2
PF	21	4.34	11	6.43	10	3.20	1	2	6	3	5	3	-	1
PA	27	5.59	11	6.43	16	5.12	2	-	8	7	1	5	2	2
ML	25	5.17	11	6.43	14	4.48	1	2	6	7	3	4	1	1
KO	11	2.27	4	2.33	7	2.24	-	1	3	3	2	2	-	-
CA	19	3.93	5	2.92	14	4.48	-	-	7	5	3	2	2	-
CG	9	1.86	-	-	9	2.88	-	1	3	3	-	1	-	1
CT	7	1.44	-	-	7	2.24	-	-	2	1	2	1	1	-
CK	6	1.24	-	-	6	1.92	-	-	1	4	-	1	-	-

KEY: EC – Escherichia coli, KP - Klebsiella pneumoniae, SA - Staphylococcus aureus, PM - Proteus mirabilis, SE - Staphylococcus epidermidis, EA - Enterobacter aerogenes, PMe - Pseudomonas mendocina, PV - Proteus vulgaris, SF – Streptococcus faecalis, PF - Pseudomonas. fluorescens, PA - Pseudomonas aeruginosa,PP – Pseudomonas putida ML - Micrococcus letus, KO - Klebsiella ozaenae CA- Candida albicans, CG- Candida glabrata, CT- Candida tropicalis, CK- Candida krusei, IC- Isolate code, NI – Number of isolate, M- Male, F – Female, %M- Percentage male, %F- Percentage female

3.2 Prevalence of Uropathogens Isolated from Male and Female Patients and Their Different Age Group

Table 2 shows the prevalence of uropathogens isolated from male and female patients and their different age group. Four hundred and eighty three (483) isolates comprising 171 male and 213 female were isolated from the samples. *Escherichia coli* showed the highest prevalence of 18.63% while *Candida krusei* was the least prevalence of 1.24%. In male, *Escherichia coli, Klebsiella pneumonia* and *Staphylococcus epidermis* showed the highest prevalence of 13.45%, 12.86% and 12.28% with no trace of *Candida glabrata, Candida tropicalis* and *Candida krusei* while in female, *Escherichia coli* recorded the highest prevalence of 21.47% with the lowest of 1.92% by *Candida krusei.* All the bacteria isolate were predominant across the age group with few exceptions. *Klebsiella ozaenae* were absent in patients in age group 1-10, 61-70 and 71-80. *Candida glabrata* and *Candida krusei* were absent in age group 1-10, 41-50 and 61-70 respectively. The higher number of *E. coli* (25), *Klebsiella pneumoniae* (14) and *Staphylococcus aureus* (10) were isolated from age 51-60, *Staphylococcus epidermis* (12) age 31-40 while the lowest (1) was obtained most at age 0-10.

3.3 Percentage Yield of the Leaf Extract after Solvent Extraction

Table 3 shows the percentage yield of the leaf extract after solvent extraction. The highest percentage yield of the leaf extract obtained using methanol and chloroform after recovery showed no significant difference while the n-hexane showed the least yield (5.75%).

3.4 Qualitative Analysis of Phytochemical Content of the Plant Extract

Table 4 shows the phytochemical contents of the plant extract using solvents. Tannins, flavonoids, saponins, terpenoids and steroids were present while the alkaloids and cardiac glycosides component were absent in all the plant extract using methanol, n-hexane and chloroform.

3.5 Antibiotic sensitivity of Gram-Positive Bacteria

Table 5 shows the antibiotic sensitivity patterns of gram-positive bacteria. Out of 39 *Staphylococcus aureus* isolated, 36 showed high resistance (92%) to Cefuroxime. Likewise *Staphylococcus aureus* out 48, 41 (85%) showed resistant while *Staphylococcus epidermidis* showed the least resistant to erythromycin with 3(6%). *Micrococcus leteus* showed the least resistant 2(8%) to cotrimoxazole. *Proteus vulgaris* and *Streptococcus faecalis* showed high resistant 18(90%) and 17 (65%) to ofloxacin out of 20 and 26 respectively.

3.6 Antibiotic Sensitivity Pattern of Gram-Negative Bacteria

Table 6 shows the antibiotic sensitivity pattern of gram-negative bacteria. Varied antibiotics resistant was exhibited among the isolates. *E. coli* showed high resistant to amoxicillin 56(62%), followed by ofloxacin 50(56%) while the least resistant 24(27%) to cotrimoxazole. *Pseudomonas aureginosa* and *Pseudomonas putida* showed 70-100% resistant to chloramphenicol and streptomycin. *Klebsiella pneumoniae* and *Proteus murabilis* showed high resistant to ciprofloxacin. *Pseudomonas mendocina* and *Pseudomonas fluorescens* showed 100% resistant to chloramphenicol. *Klebsiella ozaenae* had the least antibiotic resistant when compared to other isolates. Out of 26 isolates,

Table 3. Percentage yield of the leaf extract after solvent extraction

Amount recovered (g)	Solvent used	Percentage yield (%)
26	Methanol	6.5[a]
23	N-Hexane	5.8[b]
24	Chloroform	6.0[a]

Each value is expressed as mean ± standard error (n = 3). Values with different superscript within a row are significantly different at (p ≤ 0.05)

3.7 Antimicrobial Activity of Leaf Extract of *Chromolaena odorata* on Some Selected Resistant Isolates

Table 7 shows the antimicrobial activity of leaf extract of *Chromolaena odorata* on some selected resistant isolates at varied concentration (30 mg/ml and 50 mg/ml). At 50 mg/ml concentrates, methanolic extract had the highest zone of inhibition of 14.33 mm on *Klebsiella ozaenae, Pseudomonas mendocina* showed no zone of inhibition, there was significant difference in the zone of inhibition of the leaf extracted with n-Hexane and chloroformon the isolates while at 30 mg/ml concentrate, the methanolic extract had the highest zone of inhibition 11.33 mm on

Table 4. Qualitative analysis of phytochemical content of the plant extract

Phytochemicals (mg/ml)	Solvents		
	Methanol	n-Hexane	Chloroform
Alkaloids	-	-	-
Tannins	+	+	+
Flavonoids	+	+	+
Anthraquinones	-	-	-
Saponin	+	+	+
Terpernoids	+	+	+
Steriods	+	+	+
Cardiac glycosides			
Legal	-	-	-
Lieberman	-	-	-
Salkowski	-	-	-
Keller-killiani	-	-	-

KEY: + = Present, - = Absent

Table 5. Antibiotic sensitivity patterns of gram-positive bacteria

Antibiotics	(%) Resistance of gram-positive bacteria to the antibiotics used				
	SA(39)	SE(48)	ML(25)	PV(20)	SF(26)
NIT	32(82%)	30(63%)	9(36%)	7(35%)	10(38%)
CEF	36(92%)	41(85%)	7(28%)	7(35%)	11(42%)
GEN	31(79%)	39(81%)	7(28%)	4(20%)	16(62%)
ERY	11(28%)	3(6%)	6(24%)	6 (30%)	8(31%)
CIP	31(79%)	32(67%)	8(32%)	13(65%)	10(38%)
OFL	27(69%)	25(53%)	4(16%)	18(90%)	17(65%)
STR	30(77%)	33(69%)	9(36%)	15(75%)	14(54%)
AMP	30(77%)	29(60%)	11(44%)	6(30%)	13(50%)
AMO	33(85%)	15(31%)	11(44%)	17(85%)	10(38%)
COT	28(72%)	22(46%)	2(8%)	9(45%)	13(50%)

KEY: *NIT-Nitrofurantoin, CEF-Cefuroxime, GEN-Gentamycin, ERY-Erythromycin, CIP-Ciprofloxacin, OFL-Ofloxacin, STR-Streptomycin, AMP-Ampicillin, AMO-Amoxicillin, COT-Cotrimoxazole, SA-Staphylococcus aureus, SE-Staphylococcus epidermidis, ML-Micrococcus leteus, PV-Proteus vulgaris, SF-Streptococcus faecalis, R-Resistant*

Proteus mirabilis and *Proteus mirabilis.* The highest zone of inhibition using n-hexane and chloroform extract of *Chlomolaena odorata* were 5.66 mm and 7.33 mm on *Klebsiella ozaenae* while *Pseudomonas fluorescens* and *Pseudomonas mendocina* showed the least zone of inhibition 0.00.

3.8 Minimum Inhibitory Concentration of Crude Extract

Table 8 shows the minimum inhibitory concentration of crude extracts on test isolates. The MIC of the extract ranged from 5 mg/ml to 50 mg/ml. The highest minimum concentration 50 mg/ml on *Klebsiella pneumoniae* was obtained while the least MIC on *E. coli* and *Pseudomonas mendocina* was obtained using methanol. The n-hexane showed the highest minimum inhibitory concentration of 50 mg/ml on *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, similarly, chloroform showed the least MIC 5 mg/ml on *Staphylococcus epidermidis* and *Micrococcus leteus.*

4. DISCUSSION

Despite the commercialization common antibiotics produced from the pharmaceutical industry, urinary tract infection remains the most health-threatening infectious disease in the human population [7]. It is also the commonest infections seen in hospital settings and the second commonest infections seen in the general population [8]. More than 50% of women will have at least one urinary tract infection (UTI) during their lifetime. UTIs are also a common problem in pregnancy due to the increase in sex hormones and physiological changes during pregnancy [1]. In this present study, the

Table 6. Antibiotic sensitivity pattern of gram-negative bacteria

Antibiotics	% Resistance of the isolates to the antibiotics used								
	EC (90)	PA (27)	KP (50)	PM (24)	PMen (17)	PP (18)	KO (11)	EA (26)	PF (21)
COT	47 (52%)	R	35(70%)	7(29%)	15(82%)	16(89%)	4(36%)	19(73%)	R
CHL	46(51%)	R	34(68%)	9(52%)	R	R	3(27%)	7(27%)	R
CIP	34(37%)	28(85%)	36(72%)	19(79%)	R	R	5(45%)	11(42%)	19(90%)
AMO	56(62%)	R	36(72%)	18(75%)	R	R	2(18%)	9(34%)	R
AUG	33(38%)	R	25(50%)	10(42%)	14(82%)	15(83%)	4(36%)	9(35%)	20(95%)
PER	48(53%)	20(74%)	19(38%)	7(29%)	13(76%)	16(89%)	5(45%)	11(42%)	16(76%)
OFL	50(56%)	25(93%)	20(40%)	11(26%)	15(88%)	R	4(36%)	15(58%)	17(81%)
STR	41(46%)	R	15(30%)	9(38%)	16(94%)	R	5(45%)	10(38%)	13(62%)
NIT	24(27%)	19(70%)	26(52%)	1(4%)	13(76%)	14(77%)	2(18%)	7(27%)	18(86%)

KEY: COT-Cotrimoxazole, CHL- Chloramphenicol, CIP-Ciprofloxacin, AMO-Amoxicillin, AUG-Augmentin, GEN-Gentamycin, PER-Pefloxacin, OFL-Ofloxacin, STR-Streptomycin, NIT-Nitrofurantoin, EC-Escherichia coli, PA- Pseudomonas aeruginosa, KP- Klebsiella pneumoniae, PM- Proteus mirabilis, PMen- Pseudomonas mendocina, PP- Pseudomonas putida, KO- Klebsiella ozaenae, EA- Enterobacter aerogenes, PF- Pseudomonas fluorescens, R- Resistant

19 Enterobacter aerogenes showed the highest resistant (73%) to cotrimoxazole while the least resistant 7(27%) to chloramphenicol and nitrofurantoin respectively

Table 7. Antimicrobial activity of leaf extract of *Chromolaena odorata* on some selected resistant isolates at varied concentration (30mg/ml and 50mg/ml)

Isolate code	Solvent concentrates (50 mg/ml)			Solvent concentrates (30 mg/ml)		
	Methanol	n-Hexane	Chloroform	Methanol	n-Hexane	Chloroform
	Zone of inhibition			Zone of inhibition		
EC2	10.83±0.33[a]	7.86±0.33[b]	8.33±0.33[b]	6.67±0.66[a]	2.67±0.33[b]	3.00±0.57[b]
KP1	10.80±0.46[a]	6.06±0.52[b]	6.33±0.33[b]	9.46±0.24[a]	4.00±0.57[b]	4.67±0.66[b]
SA5	9.50±0.28[a]	7.33±0.33[b]	7.00±0.57[b]	7.67±0.66[a]	4.67±0.66[b]	5.67±1.20[b]
PM1	14.33±0.33[a]	8.33±0.57[b]	9.00±0.57[b]	11.33±0.66[a]	5.00±0.57[b]	7.00±0.57[b]
PP6	1.66±0.33[a]	1.16±0.44[a]	1.66±0.88[a]	0.66±0.33[a]	0.67±0.06[a]	0.46±0.26[a]
SE2	11.00±0.57[a]	6.00±0.57[b]	6.00±0.57[b]	7.33±1.20[a]	2.66±0.33[b]	4.33±0.88[b]
PV1	10.00±0.25[a]	8.00±0.57[b]	9.00±0.00[b]	9.66±1.33[a]	4.00±0.57[a]	5.67±0.88[a]
ML6	12.67±0.33[a]	8.00±0.57[c]	7.33±0.33[c]	11.00±1.15[a]	5.00±0.57[b]	6.67±1.20[b]
KO4	14.00±0.57[a]	7.50±0.57[c]	10.33±0.33[b]	11.33±0.66[a]	5.66±0.66[b]	7.33±0.88[b]
SF1	8.67±0.33[a]	5.67±0.33[c]	8.33±0.44[b]	5.33±0.66[a]	2.66±0.66[b]	3.33±0.66[b]
PF4	1.33±0.67[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
EA10	9.00±0.57[a]	7.00±0.57[b]	7.66±0.33[b]	6.66±0.33[a]	4.00±0.57 [b]	4.66±0.33[b]
PA10	6.46±0.48[a]	3.67±0.33[b]	5.83±0.16[b]	3.86±0.33[a]	2.00±0.57b	2.50±0.28[b]
PMe 3	0.00±0.00[a]	0.00±0.00[a]	1.67±0.33 [a]	0.70±0.15[a]	0.00±0.00[a]	0.33±0.33[a]

Each value is expressed as mean ± standard error (n = 3). Values with different superscript within a row are significantly different at (p ≤ 0.05)

*KEY: **EC –** Escherichia coli, **KP -** Klebsiella pneumonia, **SA -** Staphylococcus aureus,**PM -** Proteus mirabilis,**SE -** Staphylococcus epidermidis, **EA -** Enterobacter aerogenes, **PMe -** Pseudomonas mendocina, **PV -**Proteus vulgaris,**SF –** Streptococcus faecalis, **PF –**Pseudomonas fluorescens, **PA -** Pseudomonas aeruginosa, **PP –** Pseudomonas putida, **ML -** Micrococcus letus, **KO**-Klebsiella ozaenae,**-** No effect*

Table 8. Minimum inhibitory concentration of crude extracts on test Isolates

Isolate	Concentration of extracts (mg/ml)		
	Methanol	n-Hexane	Chloroform
EC2	5	10	10
KP1	50	50	40
SA5	30	40	30
PM1	40	40	40
PP6	30	40	40
SE2	20	10	5
PV1	20	30	10
ML6	10	20	5
KO4	20	30	10
SF1	10	20	20
PF4	10	30	20
EA10	40	40	30
PA10	50	50	50
PMe 3	5	10	10

*KEY: **EC –** Escherichia coli,**KP -** Klebsiella pneumonia, **SA -** Staphylococcus aureus,**PM -** Proteus mirabilis,**SE -** Staphylococcus epidermidis, **EA -** Enterobacter aerogenes, **PMe -** Pseudomonas mendocina, **PV -**Proteus vulgaris,**SF –** Streptococcus faecalis, **PF –**Pseudomonas fluorescens, **PA -** Pseudomonas aeruginosa, **PP –** Pseudomonas putida, **ML -** Micrococcus letus, **KO**- Klebsiella ozaenae.*

microorganisms isolated might be an aetiologic agent of most diseases in man. The high occurrence of *Escherichia coli* in all the samples might be due to its ability to tolerate high pH medium and normal floral in the urinary tract. Isolation of different microorganisms from urine samples of an infected patient in Palestine and two large hospitals in Kuwait has been reported [9,10]. Onifade et al. [3] and Aiyegoro et al. [11] reported *E. coli* as the most commonly isolated pathogen in significant bacteriuria. Isolation of *E. coli* (51.2%), *S. aureus* (27.3%) and *K. pneumonia* (12.8%) from urine samples of infected patients has been reported [12,13]. The

results obtained was similar to the findings of Ebie [4] and Njoku et al. [14] who reported *E. coli* as the commonest pathogenic bacterium in patients with urinary tract infection. The high prevalence of the urinary tract infection from the samples might be due to several factors like promiscuity, peer group influence, pregnancy, a low socio-economic status which are common among Nigerian young men and women living in an urban area [13]. Also, Ebie et al. [4] reported that age contributed to the significant susceptibility of an individual to urinary tract infections. The use of plant extract or chemicals derived from them to treat disease is a therapeutic modality, which has stood the test of time [15]. The antimicrobial efficacy of the leaf extract also depends on the concentration of the plant extract in a diffusing medium due to their high viscosity [16,17]. Sukanya et al. [18] reported that the methanol extracts of plants inhibit the growth of microorganisms more than aqueous extracts of plants. This trend shows that the active ingredients of plant parts may be better extracted with methanol than other solvents. This could be due to the fact that methanol has high polarity, dissolve both the polar and non-polar compounds. The broad spectrum antibacterial activityagainst gram positive and gram negative bacteria of this plant extract has been linked to the presence of secondary metabolites [5]. Vital and Rivera [19] had reported that Gram-positive microorganisms are more susceptible than Gram-negative microorganisms. The result obtained from this study was similar to the findings of Basri and Fan [20] who reported antibacterial activity of the plant extract against Gram-positive bacteria than Gram-negative bacteria which might be due to the permeability barrier provided by the cell wall or the morphological differences between these microorganisms. The resistance of some pathogenic microorganisms to several antibiotics has rendered them ineffective in the treatment of infections caused by these pathogens thereby developed multi-resistant to these therapeutic agents. The high resistant of the bacterial isolates to some of the commercial antibiotics might be due to their misuse or their frequent exposure to these antibiotics [2].

5. CONCLUSION

This study showed that *Escherichia coli* was the most prevalent bacterium in urinary tract infections and this can be treated using antibiotics. *C. odorata* showed the broad spectrum antimicrobial activity on the resistant isolated microorganisms. The methanolic leaf extract exhibited more inhibitory activity against the isolated microorganisms from patients infected with urinary infections than n-hexane and chloroform extracts. Therefore, the use of this plant extract could be promising in research to determine its active components and pharmacological importance in the treatment of infectious diseases in man.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sabahat S, Perween T. Symptomatic and asymptomatic urinary tract infection during pregnancy. Int. J. Microbiol. Res. 2011; 2(2):101-104.
2. Jai PP, Shafique-ur-Rehman M, Zakir HR, Syed AS. Pattern and sensitivity of microorganisms causing urinary tract infection at teaching hospital. J. Liqquat Uni. Med. Health Sci. 2012;11(02):97-100.
3. Onifade AK, Omoya FO, Adegunloye DV. Incidence and control of Urinary Tract Infections among pregnant women attending antennal clinics in government hospitals in Ondo State, Nigeria. J. Food, Agric. Envt. 2005;3:37-38.
4. Ebie MY, Kandakai-Olukemi YT, Ayanbadejo J, Tanyigna KB. Urinary tract infections in a Nigerian military hospital. Nig. J. Microbiol. 2001;15:31-37.
5. Adeshina GO, Onaolapo JA, Ehinmidu JO, Odama LE. Phytochemical and Anti-microbial Studies of the ethylacetate extract of *Alchornea cordifolia* leaf in Abuja, Nigeria. J. Med. Plant Res. 2010;4: 649-658.
6. Trease GE, Evans WC. Pharmacognosy William Charlses Evans as edited in 15th edition. Saunder Publisher. London. 2004; 137–440.
7. Gupta V, Yadab A, Joshi RM. Antibiotic resistance pattern in uropathogens. Ind. J. Med. Microbiol. 2002;20(2):96-98.
8. Valiquette L. Urinary tract infections in women. Can. J. Urol. 2001;8(1):6-12.
9. El-Astal ZY, Ashour AE, Kerrit AA. Antimicrobial activity of some medicinal plant extracts in Palestine. Pak. J. Med. Sci. 2005;21:187-193.
10. Al-Sweih N, Jamal W, Rotimi VO. Spectrum and antibiotic resistance of

uropathogens isolated from Hospital and Community Patients with Urinary Tract Infections in two large hospitals in Kuwait. Med. Principles and Practises. 2008;14: 401-407.
11. Kolawole AS, Kolawole OM, Kandaki-Olukemi, YT, Babatunde SK, Durowade KA, Kolawole CF. Prevalence of Urinary Tract Infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. Int. J. Med. Med. Sci. 2009;1:163-167.
12. Nwanze PI, Nwaru LM, Oranusi S, Dimkpa U, Okwu MU, Babatunde BB, Anake TA, Jatto W, Asagwara CE. Urinary tract infection in Okada village: Prevalence and antimicrobial susceptibility pattern. Scientific Res. Essay. 2007;2:112-116.
13. Aiyegoro OA, Igbinosa OO, Ogunmwonyi IN, Odjadjare EE, Igbinosa OE, Okoh AI. Incidence of Urinary Tract Infections (UTIs) among children and adolescents in Ile-Ife, Nigeria. Afr. J. Microbiol. Res. 2007;1:13-19.
14. Njoku CO, Ezissi NH, Amadi AN. Observations on bacterial infections of Urinary Tract patients. Int. J. Env. Health Human Dev. 2001;2:57-61.
15. Anwannil HG, Atta R. Antibacterial activity of ethanol, crude and water extract of *Chromolaena Odorata* leaves on *Salmonela typhi* and *Escherichia coli. Trends* in Ethnopharmacology J. Ethno-pharmacol. 2006;100:43-49.
16. Esimone CO, Iroha CO, Okpara EM. *In-vitro* evaluation of the interaction between tea extracts and penicillin against *Staphylococcus aureus*. Afr. J. Biotechnol. 2006;5:1082-1086.
17. Adwan G, Mhanna M. Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from clinical specimens. Middle East J. Scientific Res. 2008;3(3):134-139.
18. Sukanya SL, Sudisha J, Hariprasad P, Niranjana SR, Prakash HS. Fathima SK. Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. Afr. J. Biotechnol. 2009;8(23):677-668.
19. Vital PG, Rivera WL. Antimicrobial activity and cytotoxicity of *Chromolaena odorata* and *Uncaria perrottetii* extracts. J. Med. Plant Res. 2009;3(7):511-518.
20. Basri DF, Fan SH. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. Indian J. Pharmacol. 2005;37(1):26-29.

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