

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

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%). *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 terpenoids 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 5.0mg/ml to 50.0mg/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 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 collect, 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 high level of poverty, ignorance and poor hygienic practices, there is also 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 alternative source of biological treatment against resistance microorganisms in herbal medicine.

52 **2. Materials and Methods**

53 **2.1 Collection of Samples**

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

59 **2.2 Isolation of Microorganisms**

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

64 **2.3 Collection of Plant Material**

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

69 **2.4 Preparation of Extracts from Leaves of *C. Odorata***

70 This was carried out according to the method of Adeshina *et al.* [5]. The leaves were grinded
71 to powder, then transferred into a grease free closed airtight container. Four hundred grams
72 (400g) of the sample was weighed into three (3) separate clean containers containing 750ml
73 of methanol, chloroform and n-hexane. The mixture was allowed to stand for 72 hours with
74 frequent stirring, sieved using three folds sterile muslin cloth and then filtered using Whatman
75 filter No. 1 paper. The filtrates was collected into a sterile beaker and concentrated in –
76 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 (0.2µm).

$$\text{Percentage recovery} = \frac{\text{Weight of extract recovered after extraction}}{\text{Initial weight of plant sample}} \times 100\%$$

2.5 Qualitative Analysis of Phytochemicals 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 (ANOVA) 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 State Hospital/Mother and Child

100 Hospital Ondo 7.00% while General Hospital Ikare had the lowest total positive prevalence (11)
101 with 1.83%.

102 **3.2 Prevalence of uropathogens isolated from male and female patients and their** 103 **different age group**

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

117 **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

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

128 **Table 2: Prevalence of uropathogens isolated from male and female patients and their**
129 **different age groups**

IC	NI	%I	Male		Female		Age Group							
			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	-
PM _e	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	-	-

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

137 3.3 Percentage yield of the Leaf Extract after Solvent Extraction

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

141 3.4 Qualitative Analysis of Phytochemical Content of the Plant Extract

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

146 3.5 Antibiotic sensitivity of Gram Positive Bacteria

147 Table 5 shows the antibiotic sensitivity patterns of gram positive bacteria. Out of 39
148 *Staphylococcus aureus* isolated, 36 shows high resistance (92%) to Cefuroxime likewise
149 *Staphylococcus aureus* out of 48, 41 (85%) shows resistant while *Staphylococcus epidermidis*
150 showed the least resistant to erythromycin with 3(6%). *Micrococcus luteus* shows the least

151 resistant 2(8%) to cotrimoxazole. *Proteus vulgaris* and *Streptococcus faecalis* showed high
152 resistant 18(90%) and 17 (65%) to ofloxacin out of 20 and 26 respectively.

153 3.6 Antibiotic Sensitivity Pattern of Gram Negative Bacteria

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

162 **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

163 Each value is expressed as mean $\pm$ standard error (n = 3). Values with different
164 superscript within a row are significantly different at ($p \leq 0.05$).

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169 **Table 4: Qualitative Analysis of Phytochemical Content of the Plant Extract**

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

170 **KEY:** + = Present, - = Absent

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185 **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%)

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

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201 **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%)

202 **KEY:** COT-Cotrimoxazole, CHL- Chloramphenicol, CIP-Ciprofloxacin, AMO-
203 Amoxicillin, AUG-Augmentin, GEN-Gentamycin, PER-Perfloxacin, OFL-Ofloxacin,
204 STR-Streptomycin, NIT-Nitrofurantoin, EC-*Escherichia coli*, PA- *Pseudomonas*
205 *aeruginosa*, KP- *Klebsiella pneumoniae*, PM- *Proteus mirabilis*, PMen-
206 *Pseudomonas mendocina*, PP- *Pseudomonas putida*, KO- *Klebsiella ozaenae*, EA-
207 *Enterobacter aerogenes*, PF- *Pseudomonas fluorescens*, R- Resistant.
208 19 *Enterobacter aerogenes* showed the highest resistant (73%) to cotrimoxazole while the least
209 resistant 7(27%) to chloramphenicol and nitrofurantoin respectively.

210 **3.7 Antimicrobial Activity of Leaf Extract of *Chromolaena odorata* on Some** 211 **Selected Resistant Isolates**

212 Table 7 shows the antimicrobial activity of leaf extract of *Chromolaena odorata* on
213 some selected resistant isolates at varied concentration (30mg/ml and 50mg/ml). At
214 50mg/ml concentrates, methanolic extract had the highest zone of inhibition of
215 14.33mm on *Klebsiella ozaenae*, *Pseudomonas mendocina* show no zone of
216 inhibition, there was significant difference in the zone of inhibition of the leaf
217 extracted with n-Hexane and chloroform on the isolates while at 30mg/ml concentrate,
218 the methanolic extract had the highest zone of inhibition 11.33mm on *Proteus*
219 *mirabilis* and *Proteus mirabilis*. The highest zone of inhibition using n-hexane and

chloroform extract of *Chlomolaena odorata* were 5.66mm and 7.33mm 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 5mg/ml to 50mg/ml. The highest minimum concentration 50mg/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 50mg/ml on *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, similarly, chloroform showed the least MIC 5mg/ml on *Staphylococcus epidermidis* and *Micrococcus leteus*.

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 (50mg/ml)			Solvent concentrates (30mg/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±0.57 ^b	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).

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

240 **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

241 KEY: **EC** – *Escherichia coli*, **KP** - *Klebsiella pneumonia*, **SA** - *Staphylococcus aureus*, **PM** -
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 243 *Pseudomonas mendocina*, **PV** -*Proteus vulgaris*, **SF** – *Streptococcus faecalis*, **PF** –
 244 *Pseudomonas fluorescens*, **PA** - *Pseudomonas aeruginosa*, **PP** – *Pseudomonas putida*, **ML** -
 245 *Micrococcus letus*, **KO**- *Klebsiella ozaenae*.

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5. DISCUSSION

Despite the commercialization common antibiotics produced from pharmaceutical industry, urinary tract infection remains the most health threatening infectious disease in 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 six hormones and physiological changes during pregnancy [1]. In this present study, the 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 flora 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. pneumoniae* (12.8%) from urine samples of infected patients has been reported [12, 13]. The results obtained were similar to the findings of Ebie [4] and Njoku *et al.* [14] who reported *E. coli* as the most 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, low socio-economic status which are common among Nigerian young men and women living in urban areas [13]. Ebie *et al.* [4] reported that age contributing 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 the 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 are 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 activity against 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 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 mis-use or their frequent exposure to these antibiotics [2]. However, this study showed that *Escherichia coli* was 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 treatment of infectious diseases in man.

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