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ANTIBACTERIAL ACTIVITY OF *Colocasia esculenta* LEAF EXTRACTS AGAINST MULTIDRUG RESISTANT EXTENDED SPECTRUM B-LACTAMASE PRODUCING *Escherichia coli* AND *Klebsiella pneumoniae*

Rabiu, I.,[1] Yusha'u, M.[2] and Abdullahi, A.M.[2]
[1]Department of Microbiology, School of Science and Information Technology, Skyline University Nigeria. No. 2, Zaria Road, Kano, Nigeria
[2]Department of Microbiology, Faculty of Life Science, College of Natural and Pharmaceutical Sciences Bayero University, PMB: 3011, Kano, Nigeria.
*rtismail24@gmail.com ismail.rabiu@sun.edu.ng +2347037515320

ABSTRACT
The motivation to test the activities of Colocasia esculenta plant was as a result of its efficacy in treating a wound infected patient. This propels the urge to analyse it scientifically, against MDR-ESBL isolates. Clinical wound isolates of K. pneumoniae and E. coli were collected from Aminu Kano Teaching Hospital (AKTH) and Murtala Muhammad Specialist Hospital (MMSH) and screened for MDR and ESBL. Methanol and Aqueous leaf extracts of Colocasia esculenta were subjected for phytochemical analysis, and Bioassay using disc diffusion method at a concentration of disc potency of 480, 240, 120, 60 and 30µg/disc. The Minimum Inhibitory concentration (MIC) and Minimum bactericidal concentrations (MBC) of the extracts were determined. Phytochemical analysis revealed the presence of different phytoconstituents. Out of the 220 isolates collected, 85(38.6%) of all the isolates were confirmed MDR, with 45(40.9%) E. coli and 40(36.4%) K. pneumoniae. Prevalence of MDR-ESBL isolates was 24(10.9%), with 15(13.6%) and 9(8.2%) for E. coli and K. pneumoniae respectively. The Highest Multiple Antibiotic Resistance (MAR) index was 0.88. The extracts were observed to have varying activity against the isolates, with an increase in activity as the concentration increases. The MIC and MBC values were within the ranges of 120 to 960µg/ml and 480 to 960µg/ml respectively. Among the two extracts, higher activity was recorded by the methanol extracts, showcasing the possibility of better chemotherapeutic outcome in the treatment of MDR-ESBL infections. This study has shown the increase in the occurrence of MDR-ESBL pathogens in Kano with resistance to some commonly used antibiotics.
Keyword: Colocasia esculenta, Extended Spectrum β- Lactamase, Wound infection and Multidrug Resistance

INTRODUCTION

The use of *Colocasia esculenta* in traditional medicine has a long history, yet lacks adequate scientific knowledge. It has been estimated that 25% of the modern medicines are made from plants (Adamu *et al.,* 2021; Bale *et al.,* 2021). *Colocasia esculenta* Linn. (Family: Araceae) is an herbaceous plant, known as Taro (English), and 'Gwaza' in Hausa (Krishnapriya and Suganthi, 2017). The plant possesses various biological activities and has been widely used as therapeutics. There are reports of its activity against, *Klebsiella pneumoniae*, *E. coli* and other Enterobacteriaceae (Arman *et al.,* 2015; Agyare *et al.,* 2016).

Extended Spectrum β-Lactamase (ESBLs) are enzymes that evolved from a narrow spectrum parent ESBL enzyme but with an additional capability to inactivate broad spectrum cephalosporins, penicillins, and aztreonam, but not the cephamycins (cefoxitin) or carbapenems by hydrolytic activity and are inhibited by β-lactamase inhibitors, that is, clavulanic acid (Sadeeq *et al.,* 2018). ESBLs have been detected among several bacteria, from many countries and can be said to be spread to almost all the continents of this planet. Members of Enterobacteriaceae remain their chief hosts (Rabiu *et al.,* 2022a). This research is therefore, aimed at determination of the antibacterial activity of *Colocasia esculenta* leaf extracts against Multidrug Resistant Extended Spectrum β-Lactamase Producing *Escherichia coli* and *Klebsiella pneumoniae.*

MATERIALS AND METHODS

Study Area

Sample Size determination

Using the prevalence rate of (5.9%) reported by Iseghohi *et al*. (2020), sample size (n) of 85.3 (rounded up to 220) was calculated using the formula (n) = $\frac{Z1-\frac{\alpha^2}{2}P\,(1-P)}{d^2}$.

Specimen Collection and Processing

Wound specimens were collected from Aminu Kano Teaching Hospital (AKTH) Kano and Murtala Muhammed Specialist hospital (MMSH) Kano State Nigeria. Clinical isolates of *K. pneumoniae* (55) and *Escherichia coli* (55) were isolated from each of the hospital, to give a count of 110 isolates per hospital and a total of 220 isolates.

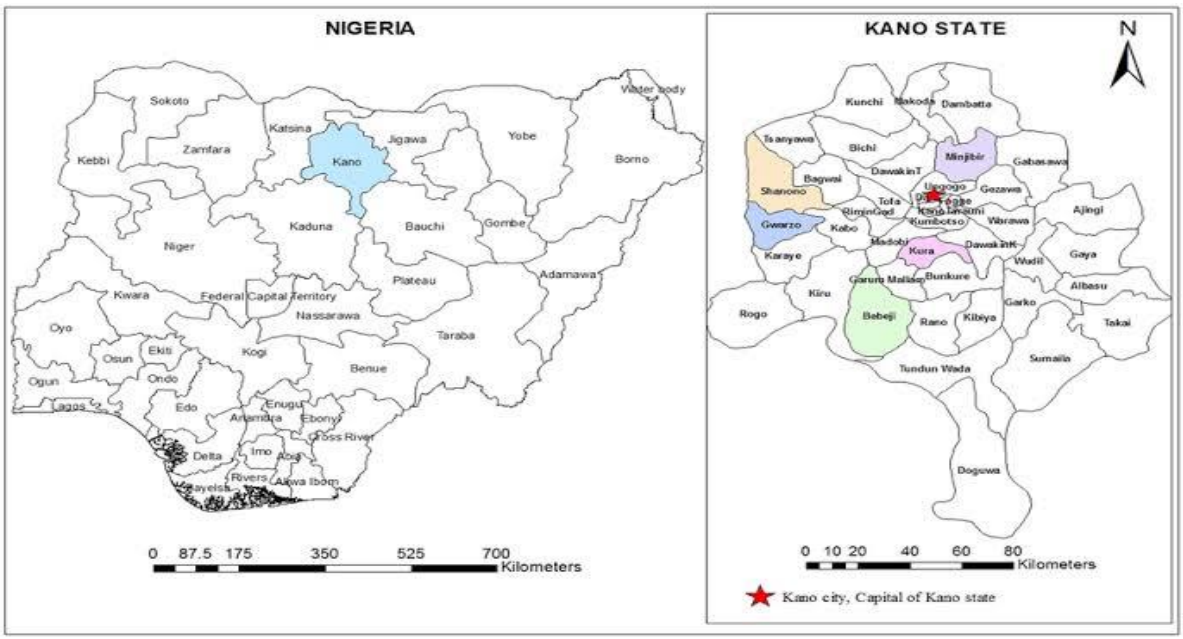

Figure 1: Map of Nigeria and that of Kano State, displaying all the Local Gov't and state to which the state shares boundaries with (National population Commission, 2006).

Morphological and Biochemical Identification of Isolates

The bacterial isolates were reconfirmed by sub culture, Gram staining, Morphology and other biochemical test as described by Cheesbrough (2018).

Screening for multi-drug resistance

All confirmed isolates were subjected for multiple drug resistance test. The standardised inocula were inoculated on a Mueller Hinton agar plate, and different classes of antibiotics discs obtained from (Bio-Rad, Turkey) and (Oxoid UK) were used. These includes; Streptomycin (10µg), Ciprofloxacin (5µg), Gentamycin (10µg), Nalidixic acid (30µg), Ampicillin (10µg), Chloramphenicol (30µg), Ceftazidime (30µg) and Ceftriaxone (30µg). Only organisms which are resistant to more than three different classes of drugs were employed as multidrug resistant isolate.

Calculation of Multiple Antibiotic Resistance (MAR) Index

This was calculated as the ratio of the number of antibiotics to which an organism is resistant to the total number of the antibiotics to which the organism is exposed to.

$$\textbf{MAR Index} = \frac{\text{Number of antibiotics isolate was resistant to}}{\text{The total number of antibiotic used}}$$

Bacteria having MAR index ≥0.2 originate from a high-risk source of contamination where several antibiotics are used (Sofowora, 1986; Afunwa *et al.,* 2020).

ESBL Screening test

This was carried out as described by CLS (2014). The isolates were treated for sensitivity to 3rd generation cephalosporin discs using disc diffusion method, according to the CLSI guidelines. Isolates showing inhibition zone size of ≤ 22 mm with Ceftazidime (30 µg) (Bio-Rad, Turkey) and ≤ 25 mm with Ceftriaxone (30 µg) (Bio-Rad, Turkey) were identified as potential ESBL producers and employed for confirmation of ESBL production.

Confirmatory Tests for ESBLs (The Double disc synergy test)

The Double disc synergy test (DDST) method was employed for the confirmation of all ESBL screened isolates. This was carried out as described by CLSI (2014). This test requires the use of two discs of third generation cephalosporin, either Cefotaxime or Ceftriaxone or Ceftazidime or Cefpodoxime. A Ceftriaxone and Ceftazidime 30µg disc each (Bio-Rad, Turkey), were placed beside amoxicillin+ clavulanic acid 20+10µg disc (Bio-Rad, Turkey) at distances of 25 – 30 mm apart, centre-to-centre on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate and Incubated overnight at 37°C. ESBL production was inferred when the zone of inhibition around the ceftazidime or Ceftriaxone disc was expanded by the clavulanate in a clover leaf fashion.

Plant Sample Collection, Authentication and Preparation

Fresh *Colocasia esculenta* leaves were collected from Kaduna, Kano and Bauchi States. The plant was authenticated by the chief Herbarium, at the Plant Biology Department BUK and a Voucher specimen was deposited. The leaves were processed as described by Bale *et al.* (2021) and Krishnapriya and Suganthi (2017). The leaves were rinsed in clean running water, air dried at room temperature and grounded to a fine powder using a clean Mortar and pistil, and sieved through 250µm mesh sieves to ensure homogeneity.

Aqueous and Methanol Extraction

These were carried out using the method as described by Sofowora (1986). The powdered plants leaves were extracted by percolation using sterile distilled water. Hundred (100) gram of the powdered leaves was dispensed in 500ml of distilled water in a labelled conical flask, and was kept for two weeks in a mechanical shaker. The methanol extracts were extracted by Soxhlet extraction set at a working temperature of 35-40^{o}C for 12 hours. The extracted plant part was filtered and evaporated using rotary evaporator at a working temperature of 60^{0}C and 45^{0}C for the aqueous and methanol extracts respectively.

Qualitative Phytochemical Analysis

Qualitative phytochemical analysis was carried out for the Methanolic and Aqueous extracts using standard phytochemical methods as described by Sofowora (1986). Dragendoff's test was employed for Alkaloids, Fehling's test for Glycosides, Ferric chloride test for Tannins, Hydrogen chloride test for Flavonoids, Frothing test for Saponins, Phenol test for Phenols, Liebermann-Burchardt test for steroids and Born Trager's test for Anthraquinones.

Preparation of Sensitivity Discs

This was done as described by CLSI (2014). Whatman No. 1 filter paper discs (185mm, Whatman International Ltd Maidstone England) of 6.0 mm was punched, using a puncher and 50 discs each, was dispensed into 20 clean Bijou bottles and sterilized by autoclaving at 121°C for 15 min.

Preparation of Different Extracts Concentrations

This was carried out as reported by Yusha'u *et al.* (2011). Exactly 96mg of the plant extract was weighed, transferred and dissolved aseptically into 1ml of Dimethyl sulfoxide (DMSO) to give a stock concentration of 96mg/ml. Five varying concentrations were attained by dissolving serially, 0.5ml of each preceding plant extract into one ml of Di-methyl sulfoxide (DMSO) into 20 different bijou bottles. The subsequent concentrations achieved were: 48, 24, 12, 6mg/ml and 3mg/ml. This was followed by the addition of 50 discs into 0.5ml of each of the 5 varying concentrations of each plant extracts such that each disc absorbed 0.01ml of the solution to arrive at concentration of disc potencies of 480, 240, 120, 60 and 30µg/disc respectively.

Bioassay

The method described by CLSI (2014) was adopted. The standardised test isolate was separately swabbed uniformly across the culture plate of Mueller Hinton agar (Hi-media, UK) and the paper discs impregnated with the various plant extracts concentrations (480, 240, 120, 60 and 30µg/disc) were placed on the surface of the agar and allowed to stand for 30mins in an inverted position to allow for pre-diffusion of the extract into the agar. The plates were incubated at 37°C for 18 to 24hrs after which the diameter zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (MIC)

The method described by CLSI (2014) was adopted. Various extracts concentrations were prepared by serial doubling dilution using nutrient broth (Titan Biotech Ltd, Bhiwadi-301-091, Rajasthan, India) to obtain concentrations of 960, 480, 240, 120, 60 and 30µg/ml. Two (2) ml each of extract and that of Mueller Hinton broth were mixed, and 0.1ml of standardized inoculum (1.5 x 10^{6} CFU/ml) was added to each of the test tubes above. The tubes were incubated at 37^{0}C for 24 hours. Tube containing broth and leaf extracts alone serve as positive control while tubes containing broth and inocula were used as negative control. The tubes were observed after 24 hours of incubation to determine the MIC, which is the lowest concentration that showed no evidence of visible growth (Kawo *et al.,* 2011).

Determination of Minimum Bactericidal Concentration (MBC)

Sterile Mueller Hinton agar (Hi-media, UK) plates were separately inoculated with culture from each of the MIC tubes that showed no evidence of turbidity. The plates were incubated at 37^0C for 24 hours. The MBC was determined as the highest dilution that yielded no single bacterial colony on the solid medium (CLSI, 2010).

Statistical Analysis

The results obtained were subjected to two-way ANOVA, Chi-square test, and Charts using SPSS software version 20.0.

RESULTS AND DISCUSSION

Following the subculture, Biochemical test and their Gram stain reaction (Table 1), both *K. pneumoniae* and *E. coli* are H_2S and Oxidase negative and test positive for catalase, Glucose, Lactose and Gas production. *K. pneumoniae* is non-motile and Indole negative, and test positive for Citrate and Voges-Proskauer, while *E. coli* is motile and Indole positive and test negative for Citrate and Voges-Proskauer. These results are the same with that of Cheesbrough (2018).

Table 1: Biochemical, Gram Staining and Microscopic Characteristics of Test Isolates

Category of test	Type of test	*Klebsiella pneumoniae*	*E. coli*
Biochemical test	Indole	-	+
	Gas production	+	+
	H_2S production	-	-
	motility	-	+
	Citrate	+	-
	Oxidase	-	-
	Glucose	+	+
	Lactose	+	+
	Voges-Proskauer	+	-
	catalase	+	+
Microscopy	Morphology	Rod	Rod
	Gram reaction	Gram negative	Gram negative

Keys: + = Positive, - = Negative, H_2S = Hydrogen sulphide

The isolates were further analysed according to the patient demographic profile (Table 2), and the highest number of isolates were obtained among the (31 and above) age group, followed by (21-30) age group. Based on gender, the males were found to have the highest occurrence of these pathogens. The higher occurrence of these wound pathogens isolated in male might be related with the fact that the male gender is more exposed to a number of occupational hazards which make them prone to a number of different infectious agent.

Table 2: Distribution of MDR-ESBL producing *E. coli* and *K. pneumoniae* isolates according to patients Demographic characteristics

Patient Demographic profile		No (%) MDR Isolates (n=85)		No (%) MDR-ESBL producers (n=24)	
		***E. coli* (%)**	***K. pneumoniae* (%)**	***E. coli* (%)**	***K. pneumoniae* (%)**
Age	<10	1(1.2)	0	0	0
	10-20	8(9.4)	6(7.1)	1(4.2%)	3(12.5)
	21-30	15(17.6)	19(22.4)	9(37.5)	4(16.7)
	31 above	21(24.7)	15(17.6)	5(20.8)	2(8.3)
Gender	Male	37(43.5)	34(40)	15(62.5)	7(29.2)
	Female	8(9.4)	6(7.1)	0	2(8.3)

Keys: MDR= Multidrug resistant, ESBL= Extended Spectrum β-lactamase

A total prevalence of multidrug resistant (MDR) isolates was observed to be 85(38.6%), with a higher occurrence in *E. coli* having 45 (40.9%) as compared with *K. pneumoniae* having 40(36.4%) (Table 3). This varies with the findings of Ye *et al.,* (2018) who reported a prevalence of 6.7% in the Ha'erbin city of China and Parvez *et al.,* (2017) who reported a prevalence of 29% in Bangladesh. This study further varies with the findings of Iseghohi *et al.* (2020), who recorded a prevalence of 5.9% among MDR-ESBL producing *E. coli* in Minna, Niger state (North central), Nigeria.

However, this study recorded a higher prevalence of MDR isolates when compared with the findings of Ejikeugwu *et al.* (2013) who reported 27.7% in Enugu and 26.1% in Ile-Ife, south western Nigeria by Olufunke *et al.* (2014). The prevalence of MDR along with MDR-ESBL isolate is geographically dependent. This is linked to the variability in the use of antimicrobials and measures put in place to control infections in these locations (Iseghohi *et al.,* 2020). The presence of ESBL isolates as observed in this study may have a serious implication in the public health as these bacteria may harbour different genetic determinants which may be transferred to other bacteria including pathogens (Korzeniewska and Harnisz, 2013). ESBL isolates possess more threat when they exhibit resistance to different antibiotics that are commonly prescribed (Parvez *et al.,* 2017).

Table 3: Occurrence of Multi-drug resistant *E. coli* and *K. pneumoniae* isolates

Isolate	Total No. of Isolates	Occurrence (%)
K. pneumoniae	110	40(36.4)
E. coli	110	45 (40.9)
Total	**220**	**85 (38.6**)

A total occurrence of MDR-ESBL isolates were observed to be 24 (10.9%) in this study. Among the two isolates, *E. coli* 9 (8.2%) recorded the highest MDR-ESBL isolates followed by *K. pneumoniae* 15 (13.6 %) (Table 4). This is lower than the result of Nepal *et al.* (2017) who reported 96 (54.2%) in Nepal. This is in conformity with the report of Afunwa *et al.* (2020), Ye *et al.* (2018) and Manenzhe (2015) who reported that the prevalence of MDR-ESBLs in clinical Settings varies depending on geographical location. The result of this study slightly varies with that of Denisuik (2013), who reported an ESBL- prevalence in *E. coli* and *K. pneumoniae* with 78.8% and 66.7%, respectively, demonstrating an MDR phenotype.
Ciprofloxacin (5µg) and Ampicillin (5µg) have the highest number 18(75%) of MDR-ESBL isolates (n=24) (Table 5). This agrees with the results of Iseghohi *et al.* (2020) who reported *K. pneumoniae* and *E. coli* been resistant to Aminoglycosides, Cephalosporins and Penicillins. However, the result of the present study differs with the report of Ye *et al.* (2018) and Iseghohi *et al.* (2020) who recorded a lower resistance profile against most of the antibiotics by the MDR-ESBL isolates. The Highest (MAR) index of (0.88) was recorded in this study, while (0.13) was the lowest. Bacteria having MAR index ≥ 0.2 originate from a high-risk source of contamination where several antibiotics are used. This suggests that most of the isolates have lost sensitivity to the conventional antibiotics used in this study (Tanko *et al.,* 2020. Afunwa *et al.* (2020) in the Eastern parts of the country identified high resistant pattern to antibiotics used, with *E. coli* showing the highest resistance. The MAR index of 0.88, which is an indication of the organisms been resistant to most of the antibiotics tested against the 2 isolates confirm the presence of multidrug resistant genes originating from the environment where there is an abuse of these drugs. The study conducted by Yasir *et al*. (2018) also established that most ESBL isolates are multidrug resistant (MDR), especially to 3rd and 4th generation cephalosporins. Moreso, 24(10.9%) of all the isolates were observed to be confirmed MDR-ESBL producers (Table 4), with higher occurrence in MMSH Hospital. This agrees with the findings of Akujobi *et al*. (2010) in a study conducted in the south-Eastern Nigeria. In this study, *E. coli* 2(3.6%) recorded the least, followed by *K. pneumoniae* 6(10.9%) in AKTH. This indicates that there exist differences in ESBLs among the different bacterial species and Hospitals. This is in agreement with the findings of Yusha'u *et al.* (2010) who recorded a higher occurrence of ESBL in *K. pneumoniae* and Garba and Yusha'u (2012) who recorded a higher occurrence in *E. coli*.

Table 4: Occurrence of confirmed MDR-ESBL Bacteria

Bacterial isolate	Total No. of isolates collected	No. of MDR-ESBL Producers	Occurrence (%)
K. pneumoniae	110	9	(8.2)
E. coli	110	15	(13.6)
Total	**220**	**24**	**(10.9**)

Table 5: Susceptibility profile of MDR-ESBL isolates to different antibiotics

	Inhibition zones of Antibiotics conc. in (µg/disc)									
Isolate (n=24)	**[a]Str(10)**	**[b]Cip(5)**	**[a]Gen(10)**	**[b]N.A (30)**	**[c]Amp(10)**	**[d]Chl(30)**	**[e]Cef(30)**	**[e]Ceft(30)**	**No of Antib. resistance**	**MAR Index**
K1	16±0.5	24±0.5	16±0.3	16±0.3	13±0.5	27±0.5	14±0.7	13±0.2	+5	0.63
K2	16±0.2	32±0.7	13±0.3	19±0.7	17±0.3	17±0.2	21±0.3	24±0.3	-2	0.25
K3	12±0.5	28±0.2	12±0.5	13±0.2	15±0.2	28±0.2	12±0.5	15±0.2	+7	0.88
K4	13±0.7	22±0.3	15±0.5	13±0.2	13±0.3	21±0.5	14±0.3	13±0.7	+6	0.75
K5	12±0.2	27±0.7	11±0.5	12±0.5	15±0.2	27±0.2	16±0.7	23±0.5	+6	0.75
K6	10±0.5	33±0.2	16±0.2	21±0.5	15±0.3	21±0.5	14±0.3	15±0.7	+4	0.5
K7	11±0.2	18±0.2	11±0.7	11±0.2	12±0.7	19±0.2	12±0.2	12±0.7	+7	0.88
K8	16±0.5	31±0.7	16±0.5	19±0.2	19±0.2	20±0.7	21±0.7	15±0.7	-1	0.13
K9	14±0.7	29±0.2	16±0.7	15±0.2	13±0.7	21±0.7	14±0.2	13±0.7	+6	0.75
E1	16±0.5	26±0.2	15±0.2	13±0.5	15±0.2	17±0.2	15±0.5	12±0.7	+6	0.75
E2	11±0.5	24±0.5	13±0.2	15±0.2	13±0.7	25±0.5	16±0.2	24±0.7	+6	0.75
E3	11±0.2	27±0.7	17±0.7	15±0.2	11±0.5	32±0.5	13±0.2	13±0.7	+6	0.75
E4	14±0.7	28±0.5	9±0.3	12±0.5	13±0.5	29±0.3	12±0.5	15±0.5	+7	0.88
E5	16±0.5	26±0.3	11±0.3	11±0.7	12±0.5	20±0.7	12±0.5	13±0.3	+6	0.75
E6	15±0.3	33±0.7	9±0.3	21±0.5	18±0.7	27±0.5	21±0.3	23±0.7	-1	0.13
E7	13±0.7	24±0.5	14±0.5	12±0.7	14±0.5	27±0.7	21±0.5	13±0.5	+6	0.75
E8	17±0.7	31±0.5	16±0.3	20±0.5	17±0.3	23±0.3	21±0.3	13±0.7	-1	0.13
E9	9±0.3	25±0.3	11±0.3	15±0.7	12±0.3	22±0.5	10±0.7	23±0.5	+6	0.75
E10	11±0.5	26±0.7	11±0.3	11±0.5	14±0.7	21±0.7	21±0.5	15±0.5	+6	0.75
E11	9±0.5	24±0.7	14±0.5	11±0.3	13±0.3	17±0.5	13±0.3	23±0.5	+7	0.88
E12	14±0.5	27±0.5	9±0.5	16±0.5	18±0.7	28±0.7	20±0.5	15±0.3	+6	0.75
E13	12±0.7	28±0.5	16±0.7	15±0.7	13±0.5	22±0.5	16±0.5	15±0.7	+6	0.75
E14	16±0.5	31±0.5	13±0.3	19±0.3	19±0.5	25±0.5	21±0.3	24±0.7	-1	0.13
E15	16±0.5	19±0.7	12±0.7	19±0.5	13±0.7	16±0.3	15±0.3	13±0.7	+6	0.75
R-I (%)	**15(62.5)**	**18(75)**	**15(62.5)**	**17(70.8)**	**18(75)**	**4(16.7)**	**17(70.8)**	**17(70.8)**	**19 MDR-ESBL**	
S-I (%)	**9(37.5)**	**6(25)**	**9(37.5)**	**7(29.2)**	**6(25)**	**20(83.3)**	**7(29.2)**	**7(29.2)**	**5 Non-MDR- ESBL**	

Values are mean inhibition zone (mm) ±SD of two replicates

Keys: **Str**: Streptomycin, **Cip**: Ciprofloxacin, **Gen**: Gentamycin, **N.A**: Nlidixc acid, **Amp**: Ampicillin, **Chl**: Chloramphenicol, **Cef**: Ceftazidime, **Ceft**: Ceftriaxone**.** **a**: Aminoglycosides, **b:** Quinolones, **c**: Penicillins, **d**: phenicols, **e:** Cephalosporins. **+**: MDR, **-** Non MDR**.** **R-I**: Resistant Isolates, S-I: Susceptible isolates.

All the extracts were observed to have a gummy texture and chocking smell, with the methanol extract having a greenish black colour while the aqueous was having a reddish brown colour (Table 6). Higher yield was observed in the Aqueous extracts (16.0%) than the Methanol extracts (8.2%), having observed a significant difference in the yield of the two extracts ($P<0.05$). This results differs with the findings of Al-Kaf *et al*. (2019) who reported a higher yield in the methanol extracts (29%) compared with the aqueous extracts (26%). The variation might be due to the varying quantity of Phytoconstituents present in these plants as reported by Yusha'u *et al*. (2011).

Table 6: Physical Properties of *C. esculenta* leaf extracts

Plant	Plant extracted	Solvent used	% Yield	Odour	Colour	Texture
C. esculenta	100g	Methanol	8.2	Chocking smell	Greenish black	Gummy
	100g	Aqueous	16.0	Chocking smell	Reddish brown	Gummy

Phytoconstituents such as flavonoids, Saponins, tannins, steroids and phenols were identified to be present in all the extracts (Table 7). These metabolites have been reported to possess a number of multiple biological effects including antibacterial and antioxidant activity. In particular, the flavonoids were reported to be responsible for antimicrobial activity associated with some ethno-medicinal plants (Bale *et al.,* 2021). Abdulfatai *et al*. (2018) reported that the presence of tannins implies that the extract can be pharmacologically used as astringents and the astringent activity of tannins is by precipitating proteins, thereby protecting the underlying tissue leading to improvement of wound healing. Alkaloids are known to play some metabolic roles and control development in living system. It interferes with cell division, hence the presence of alkaloids in the extracts could account for their use as antimicrobial agents (Yusha'u *et al.,* 2011). The variations in some phytochemicals among plants could be due to the geographical conditions of the plant or solvent used in extraction of the phytoconstutuents (Kawo *et al*., 2011).

Table 7: Phytochemical Constituents of *C. esculenta* Leaf extracts

Phytoconstituents	Test	Methanol extracts	Aqueous extracts
Flavonoids	Shinoda test	+	+
Saponins	Frothing/Foam test	+	+
Tannins	Braemer's test	+	+
Glycosides	Fehling's test	+	-
Alkaloids	Dragendoff's test	+	+
Steroids	Liebermann-Burchardt test	+	+
Anthraquinones	Born Trager's test	-	-
Phenols	Phenol test	+	+

Keys: (+) = Present, (-) = Absent

The activity of the extracts (Table 8) show that the extracts exerts varying activity against the isolates, with an increase in activity as the concentration of the extracts increases. This agrees with the findings of Seong *et al.* (2008) and Nakade *et al.* (2013) who also reported the two extracts as having activities against *K. pneumoniae* and *E. coli.* Higher activity was observed in the methanol extracts. At 100% extracts concentrations, the isolates (K4, E1 and E9), recorded the highest ZOI (19mm) in the methanol extracts while only the isolate (E5) recorded (19mm±0.5) in the aqueous extracts. This is in conformity with previous studies conducted by Al-Kaf *et al.* (2019). Similar result was observed when compared with results of Dutta and Aich (2017) who recorded varying activity among *E. coli* and *K. pneumoniae* at the same concentration with the methanol extracts exerting higher inhibitory activity against *E. coli*. The result of the present research also corresponds with the findings of Nakade *et al.* (2013) who also reported a higher activity in the methanol extracts when tested against *E. coli.* Following the comparisons of means of both the extracts and antibiotics activities against the isolates, no significant difference ($P>0.05$) was observed. The variation in the extracts activity may not be unconnected with the fact that plant extracts contain both active and inactive compound in their unrefined form (Yusha'u *et al.,* 2011)

Table 8: Antibacterial Sensitivity (mm) of MDR ESBL isolates to Aqueous and Methanol extracts of *C. esculenta* using disc diffusion method

Isolate code	CeA Extracts conc. in (µg/disc)					CeM Extracts conc. in (µg/disc)					Antibiotics conc. in (µg/disc)				
	480	**240**	**120**	**60**	**30**	**480**	**240**	**120**	**60**	**30**	**Chl. (30)**	**Amp. (30)**	**Cip. (5)**	**Caz. (30)**	**Gen (10)**
K1	11±0.3	11±0.3	10±0.7	8±0.5	8±0.5	18±0.5	18±0.7	13±0.5	8±0.5	7±0.3	25±0.5	13±0.2	29±0.2	13±0.5	15±0.5
K3	13±0.5	11±0.2	9±0.7	8±0.2	7±0.2	12±0.7	11±0.7	10±0.7	10±0.7	9±0.7	25±0.7	15±0.5	30±0.5	12±0.7	12±0.7
K4	12±0.2	10±0.2	9±0.7	7±0.7	6±0.2	19±0.5	18±0.2	15±0.5	10±0.2	6±0.2	22±0.3	14±0.7	22±0.5	14±0.7	15±0.3
K5	11±0.7	7±0.2	6±0.7	6±0.7	6±0.2	15±0.2	13±0.5	10±0.2	7±0.2	6±0.2	28±0.3	16±0.5	30±0.3	17±0.3	13±0.5
K6	12±0.7	11±0.2	10±0.5	8±0.7	7±0.5	16±0.2	10±0.7	8±0.7	7±0.5	6±0.2	21±0.3	18±0.7	29±0.7	12±0.5	14±0.3
K7	14±0.5	12±0.7	9±0.2	8±0.7	6±0.2	13±0.5	11±0.5	10±0.5	8±0.2	4±0.5	20±0.5	13±0.5	18±0.3	15±0.3	11±0.7
K9	18±0.2	12±0.5	10±0.5	9±0.7	6±0.2	17±0.2	15±0.5	13±0.5	9±0.5	9±0.5	21±0.3	13±0.3	29±0.5	14±0.5	14±0.3
E1	12±0.7	11±0.7	10±0.5	9±0.2	7±0.5	19±0.5	8±0.5	7±0.2	7±0.5	7±0.5	15±0.5	15±0.5	30±0.3	14±0.3	15±0.7
E2	18±0.7	14±0.5	12±0.2	10±0.5	8±0.7	18±0.7	15±0.5	10±0.5	9±0.5	8±0.2	23±0.3	12±0.7	30±0.5	16±0.7	13±0.3
E3	12±0.5	12±0.2	12±0.5	10±0.5	9±0.7	18±0.5	13±0.7	9±0.7	8±0.7	7±0.7	28±0.7	13±0.5	29±0.3	13±0.3	15±0.5
E4	12±0.2	9±0.5	10±0.7	6±0.7	6±0.5	16±0.5	16±0.5	15±0.5	14±0.5	12±0.5	29±0.5	15±0.3	27±0.3	13±0.5	12±0.3
E5	19±0.5	16±0.7	12±0.7	9±0.3	7±0.5	11±0.7	11±0.3	8±0.3	7±0.7	6±0.7	19±0.3	12±0.7	22±0.3	12±0.7	11±0.3
E7	17±0.7	15±0.3	14±0.3	12±0.7	11±0.5	18±0.3	14±0.7	10±0.7	9±0.3	7±0.3	28±0.3	14±0.5	26±0.3	21±0.3	14±0.3
E9	16±0.3	13±0.5	9±0.7	7±0.5	6±0.3	19±0.7	15±0.3	8±0.7	7±0.3	6±0.3	22±0.7	12±0.7	29±0.3	10±0.5	11±0.3
E10	14±0.5	12±0.7	9±0.5	7±0.3	6±0.7	12±0.5	11±0.7	10±0.3	8±0.3	7±0.7	21±0.3	15±0.5	30±0.5	22±0.3	11±0.5
E11	16±0.3	14±0.3	11±0.3	9±0.7	7±0.3	14±0.7	13±0.3	10±0.3	8±0.7	8±0.7	16±0.5	13±0.7	24±0.3	14±0.3	14±0.3
E12	13±0.7	11±0.3	10±0.7	8±0.5	8±0.7	18±0.3	12±0.7	10±0.3	9±0.7	6±0.3	23±0.3	15±0.5	27±0.3	12±0.5	11±0.3
E13	18±0.5	12±0.7	11±0.5	9±0.3	6±0.7	16±0.3	14±0.3	12±0.7	9±0.3	6±0.3	21±0.3	12±0.7	28±0.5	16±0.7	14±0.3
E15	17±0.7	15±0.3	13±0.5	9±0.7	9±0.3	13±0.3	12±0.7	9±0.3	8±0.3	8±0.3	15±0.7	13±0.3	30±0.5	15±0.3	11±0.7
ANOVA (Row)			(p>0.05)*					(p≤0.05)**					(p>0.05)*		
ANOVA (Column)			(p>0.05)*					(p>0.05)*					(p>0.05)*		

Values are mean inhibition zone (mm) ±SD of two replicates.

Keys: CeA= *C. esculenta* Aqueous extracts. CeM= *C. esculenta* methanol extracts. Chl. = Chloramphenicol; Amp. = Ampicillin; Cip. = Ciprofloxacin; Caz. = Ceftazidime. Gen = Gentamycin. E= *E. coli,* K= *K. pneumoniae*

***Chi-square test**: *= No significant difference (p>0.05). a=Comparison of Aqueous and Methanol extracts of C. esculenta (p>0.05)**

b= Comparison of Aqueous extracts of *C. esculenta with* commonly used antibiotics (p>0.05)* c= Comparison of Methanol extracts of *C. esculenta* with commonly used antibiotics (p>0.05)* ANOVA (two-way analysis): *= No significant difference (p>0.05). ****=** Significant difference (p≤0.05).

The minimum inhibitory concentrations (MIC) values of the extracts (Table 9) ranges from 120 to 960µg/ml, while the Minimum Bactericidal concentrations (MBC) were within the ranges of 480µg/ml to 960µg/ml. The aqueous extracts were observed to have higher MIC values than the corresponding Methanol extracts. With the Aqueous extracts having 960µg /ml as the highest MIC values in K1, K5, E4 and E12 isolates while the lowest MIC values of 120µg /ml was observed in E2 and E5. These variations could be due to the differences in their chemical composition of the extracts as well as in the mechanism of action of their bioactive constituents. According to Kuete (2010) and Kuete and Efferth (2010), the antibacterial activity of a plant extract is considered significant when MIC values are below 100µg/ml, moderate when the MIC is ≥100µg/ml but ≤625µg/ml and weak when MIC > 625µg/ml. Comparing the MIC results obtained in this study with these scale reveals that none of the extracts MIC is within the "significant range". Many of the MIC of the methanol extracts were within the "moderate range" while many of the MIC of the aqueous extracts are within the range of "weak". These indicate that the methanol extracts is effective at lower concentrations when compared with the aqueous extracts. The MIC results (146.9µg/m) reported by Agyare *et al.* (2016) is in agreement with the MIC values reported in this study. However, the obtained MIC values are very important when considering that extracts are from edible plant parts and also when considering the medicinal importance of the extracts against the tested MDR bacteria (Dzotam *et al.,* 2016). However, some isolates were observed to have MBC values greater than 960µg/ml, and this was observed in all the extracts. The low MBC recorded in some of the extracts is an indication of the effectiveness of the extracts even at lower concentrations. However, the MBC values exceeding 960µg/ml could be due to high resistance rate of the test isolates. More so, seasonal variations can affect the chemical composition of the plants and thus biological activity (Kawo *et al.,* 2011; Rabiu *et al.,* 2022b).

Table 9: Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) for aqueous and methanol extracts of *C. esculenta.*

Isolate code	CE Aqueous Extracts (µg /ml)		**CE Methanol Extracts (µg/ml)**	
	MIC	**MBC**	**MIC**	MBC
K1	960*	-	120**	480
K3	240**	960	240**	960
K4	240**	960	120**	480
K5	960*	-	120**	480
K6	480**	-	960*	-
K7	240**	960	120**	480
K9	240**	480	240**	960
E1	480**	960	480**	960
E2	120**	480	480**	480
E3	240**	960	480**	960
E4	960*	-	120**	480
E5	120**	480	960*	-
E7	240**	960	240**	480
E9	480**	-	480**	960
E10	480**	-	960*	-
E11	480**	960	480**	960
E12	960*	-	480**	960
E13	480**	960	120**	960
E15	240**	960	480**	**-**

Keys: K= *K. pneumoniae*; E= *E. coli*; CE= *C. esculenta,* ME= *C. esculenta,* *=weak, **=Moderate, ***= Significant; - = Value exceeding 960µg/ml.

CONCLUSION

The result of this study indicated that, out of all the samples collected, higher levels of MDR-ESBL producing isolates were recorded with *E. coli* having higher occurrence compared to *K. pneumoniae*. Higher MAR-index recorded among the isolates which is an indication of the higher resistance status. The activity recorded by the leaf extracts of *C. esculenta* indicates their pharmacological importance and could justify the use of these plants in the treatment of wound infections as claimed by traditional herbalists. This showcases the possibility of better chemotherapeutic outcome of MDR-ESBL producing *K. pneumoniae* and *E. coli* even under the presence of multidrug resistance.

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