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ANTIBACTERIAL ACTIVITY OF *ALCHORNEA CORDIFOLIA* LEAF EXTRACTS AGAINST MULTI DRUG RESISTANT EXTENDED SPECTRUM BETA LACTAMASE PRODUCING UROPATHOGENIC *KLEBSIELLA* SPP AND *E. COLI* ISOLATES

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ABSTRACT

Many bacterial species have been reported to develop resistance to antibiotics commonly prescribed for urinary tract infections. Therefore, the need to search for natural alternative for remedy of this problem cannot be overemphasized. The study was aimed to determine the antibacterial activity of *Alchornea cordifolia* leaf extracts against multidrug resistant extended spectrum beta lactamase producing uropathogenic *Klebsiella* spp and *E. coli* isolates. Phytochemical screening of the *Alchornea cordifolia* leaf extracts was carried out using standard methods. Agar well diffusion and agar dilution methods were employed to determine the zone of inhibition, minimum inhibitory concentration, and minimum bactericidal concentration. The phytochemical screening of the leaf extracts revealed the presence of secondary metabolites such as flavonoids, tannins, alkaloids, steroids, glycosides. The ethanol and ethyl acetate extracts showed antibacterial activity against multidrug resistant ESBL producing uropathogenic isolates with ethanol extract having widest zone of inhibition (17 mm) with MICs ranging from 2.5 to 20 mg/ml for both the isolates. The findings of this study suggest that *Alchornea cordifolia* leaves could be employed as potential therapeutic candidate for the treatment of uropathogenic infections.

Keywords: *Alchornea cordifolia*, Multidrug resistance, *E coli*, *Klebsiella* spp

INTRODUCTION

Urinary tract infection (UTI) is the colonization of the urinary tract by pathogenic microorganisms. Infection is caused by fungi, bacteria and viruses. The infection has prolonged admissions in hospital, morbidity in general population and high financial cost implications to the patients (Chegini *et al*., 2021). Majority UTIs are caused by bacteria that are found in the bowel and live as normal flora and often result from faecal and perineal areas. Urinary tract infection may lead to life threatening complications and death (Veeraraghavan *et al*., 2021). Urine culture is the most effective diagnosis of UTI and treatment (Hudson *et al.,* 2022).

Multidrug resistance should be monitored worldwide and surveillance systems should be used to determine the aetiology for UTIs (Veeraraghavan *et al*., 2021). There is a worldwide setback in management of many bacterial infectious diseases due to antibiotic resistance. It is estimated that globally 26 % of deaths are due to infectious diseases such as UTIs of which 98 % occur in low-income countries (Zeynudin *et al*., 2018).

There is an obvious increase in the prevalence of multi drug resistance (MDR) and ESBL producing *E. coli* isolates from human sources which has been observed throughout the globe (Nyirabahizi *et al*., 2020). Infections by enterobacterial isolates resistant to extended spectrum cephalosporin are reportedly becoming a serious problem worldwide (Manges, 2016). Multi drug resistant (MDR) *Enterobacteriaceae* has been frequently reported from different parts of the world as emergence of treatment problem (Adler *et al*., 2016). Antibiotic given empirically without proper antibiotic susceptibility testing are one of the major causes for the development of

MDR. So, to ensure appropriate therapy, knowledge of the organism that causes UTI and their antibiotic susceptibility is mandatory ((Dougnon *et al.,* 2021).

Medicinal plants are plants that have been used in human disease treatment for ages because they contain compounds that possess therapeutic values. Recently, due to pathogens' resistance against the available antibiotics and the recognition of traditional medicine as an alternative form of health care, the research domain for the biological activities of medicinal plants has reopened. Plants have been a source of herbal remedies throughout the history of mankind. Various medicinal plants have been used for years in daily life to treat diseases all over the world (Anand *et al*., 2019).

Search of newer drugs from plants has been increased, since many of the microorganisms are posing serious health related disorders. According to the World Health Organization, 80% of the African population still uses traditional medicinal practices for their primary healthcare needs (Ayéna *et al.,* 2021).

Globally, the application of medicinal plants in the management of urinary tract infections can be considered a common occurrence in most traditional medicine practices. According to the US Agency for International Development, the importance of medicinal plants is growing due to the sharp increase in global demand for medicinal plants and their products in recent decades and the increasing number of users and the diversity of areas in which they are used (Brochot *et al.,* 2017). Despite the growing menace of antibiotic resistance, and the fact that plants are a promising source of antibiotics with possible activity on multidrug-resistant microorganisms, the potential of plant extracts as alternative sources of novel antibiotics against UTIs pathogens have been under-explored, especially in developing countries (Laisan *et al.,* 2014).

Alchornea cordifolia (Schumach. and Thonn.) Müll. Arg (Euphorbiaceae) (referred to as Christmas bush) is native to Senegal, East Kenya, South Tanzania, and throughout Central Africa to Angola. This plant usually grows very close to water bank, moist or marshy places to a significant height while remaining in a shrubby or scrambling habit (Nnamdi *et al.,* 2017). It belongs to the subfamily *Acalypholdeae* and family *Euphorbiaceae* or Spurge family (Nnamdi *et al.,* 2017). *Alchornea cordifolia* is the most studied species in the Alchornea genus (Adeshina *et al*., 2012). It is found in countries like Congo, Nigeria, Ivory Coast, and Ghana (Adeshina *et al*., 2012). *A. cordifolia* is known as buissondenoel in French and Christmas bush in English (Ngene *et al.,* 2022). In Nigeria, it is known as "Bambami" in Hausa, "Ububo" in Igbo, and "Ewe Ipa", "Esinyin" in Yoruba and "mbom" in Efik (Noundou *et al*., 2016; Boniface *et al.,* 2016.

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The increase in prevalence of multiple drug resistance has slowed the development of new

synthetic antimicrobial drugs, and necessitated the search for new antimicrobial from alternative sources such as plant. Screening the active compounds from plants has led to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases (Boakye *et al*., 2018). The aim of this study is to determine the antibacterial activity of *Alchornea cordifolia* leaf extracts against multidrug resistant extended spectrum β-lactamase producing uropathogenic *Klebsiella* spp. and *E coli* isolates.

METHODS

Ethical Approval

Ethical approval with the number AKTH/OFH/1232/25 and MOH/OFF/797/T. I/72 were obtained from the AKTH and Kano State Ministry of Health ethical committee for sample collection respectively**.**

Collection of bacterial isolates

The *Klebsiella* spp. and *E coli* isolates from patients with Urinary tract infections were obtained from the Microbiology Laboratories of each of the three major hospitals in Kano metropolis which are Murtala Muhammad Specialist Hospital (MMSH), Aminu Kano Teaching Hospital (AKTH) and Muhammad Abdullahi Wase Specialists Hospital (MAWSH). The samples were selected using convenience sampling method and only samples which were said to be confirmed *Klebsiella* spp. and *E. coli* isolates were collected within time frame of isolates collection. The isolates were collected within the period of 4 months (May 2018 to September, 2018).

Identification of multidrug resistant (MDR) resistant strains

The number of antibiotics each bacterium was resistant to in the disc diffusion test was noted for identification of multidrug resistant strains. Multidrug resistance (MDR) was identified according to the guidelines recommended by joint initiative of the European Centre for Disease Prevention and Control (ECDC) and the centers for Disease Control and Prevention (CDC) (Magiorakos *et al.,* 2012). According to the guidelines, MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

Detection of ESBLs Producing Bacteria

The double disc synergy test (DDST) was performed. Discs of cefpodoxime and ceftriaxone were placed at a distance of 20 mm to amoxicillin-clavulanic acid on a Mueller Hinton agar plate earlier inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 37 ^{O}C for 18 hrs. Organisms were confirmed ESBLs producers if synergy between cefpodoxime and ceftriaxone and amoxicillin associated with clavulanic acid is detected i.e zones of inhibitions of 5 mm or greater obtained when compared with discs without clavulante (Tawfik *et al.,* 2012).

Collection and authentication of plant material

Healthy, disease-free leaves of *Alchornea cordifolia* were collected from Madalla-Suleja road in Suleja Local Government of Niger State. Authentication of the plant was carried out at the Herbarium, Department of Plant Biology, Faculty of Life Sciences, Bayero University, Kano, by a taxonomist and accession number; *Alchornea cordifolia* – BUKHAN 0404 was given.

Pretreatment of plant parts

The leaves were washed under running tap water and then spread out in the laboratory to air-dry away from sunlight (Lalisan *et al.,* 2014, Ayéna *et al*., 2021). After proper drying has been ensured (Clement *et al*., 2020), the leaves were pulverized by mechanical grinding using mortar and pestle (Lalisan *et al.,* 2014). These were then weighed, packed in nylon bags and labelled.

Preparation of Plant Extracts

The powdered plant materials (500 grams each) were subjected to soxhlet extraction method using two (2) different solvents, ethanol and ethyl acetate as solvents (Dianursanti *et al*., 2020).

Extraction using the Soxhlet method

This was carried out using Soxhlet extraction apparatus described by Dianursanti *et al*. (2020) using ethanol and ethyl acetate as solvents of extraction.

Test for Absence of Micro-organism in the Extract

Each of the extracts obtained was tested to ensure its purity via dropping on a sterile plate containing nutrient agar and well spread on the agar. The plates were incubated at 37 °C based on the method described by Okigbo and Omodamiro (2004). The plates were examined for possible growth of contaminants; the absence of which confirmed the purity of the test extracts.

Qualitative Phytochemical screening of the extracts

The leaves extracts were subjected to phytochemical screening in order to identify the phytochemical constituents of the leaves using the method of Ali *et al.* (2017).

Preparation of different concentrations of the extracts

Stock solution was prepared according to the method of Rahman *et al*. (2011) with slight modifications. Briefly 0.4 g of the extracts was dissolved in 10 ml of DMSO to produce a stock concentration of 40 mg/ml. From the stock solution, various working concentration of 20, 10, 5, 2.5 and 1.25 mg/ml were produced by half fold dilution. Standard drug was used as positive control (ciprofloxacin) (25 mg/ml) while DMSO was used as negative control.

Antibacterial Activity of the Extracts (Bioassay)

The antibacterial activity of ethanolic and ethyl acetate extracts of *Alchornea cordifolia* leaf were tested against Multidrug resistant ESBL producing *Klebsiella* spp and *E. coli* isolates according to agar well diffusion method described by Ghamba *et al*. (2014) and Osei Akoto (2019). Concentrations of 20, 10, 5, 2.5, 1.25 mg/mL were prepared from 40 mg/ml stock solutions of the two extracts using half fold dilutions. A 20 mL of molten Mueller Hinton agar was dispensed into sterile petri dishes and inoculated aseptically with 0.1 mL fresh cultures of the test isolates and the turbidity was compared with that of 0.5 McFarland standards. Holes of 6 mm diameter were made in the agar plates using a sterile metal cork-borer (6 mm). 0.1 ml of the various dilutions of each extract were dispensed in each hole under aseptic condition. 20% DMSO was used as negative control and ciprofloxacin (25 mg/ml) from Pal pharmacy was used as positive control. Then plates were kept at room temperature 30 minutes to allow diffusion of the extracts into the agar and further incubate at 37 °C for 24 hours. The diameters of zones of inhibition were measured to the nearest millimeter (Uddin *et al.,* 2007). The tests were performed in duplicate and the best results were taken. The whole experiments were performed under strict aseptic conditions according to the standard protocol.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by broth dilution technique. Serial dilutions of the plant extracts from 20 mg/ml stock solution were made in test tubes containing sterile normal saline, to obtain concentrations of 10,5,2.5 and 1.25 (mg/ml). The test tubes (containing 2ml of nutrient broth and 2 ml of different concentration of the extract each) were inoculated with 50 µl of suspension of the test bacterium standardized to 0.5 McFarland Standard. The inoculated tubes were incubated aerobically at 37°C for 18-24 h. After incubation, the tubes were examined for turbidity/growth from the brown clear dilution of the extracts. The tube with the lowest concentration of extracts which showed no turbidity was recorded as the MIC value for the tested extract (Ali *et al.,* 2018).

Determination of Minimum Bactericidal Concentration (MBC)

Minimum Bactericidal Concentration was used to reconfirm the results of MIC by determining the number of the surviving organism through observing the growth of the bacteria. MBC represents the concentration at

which 99 % of the bacteria were killed. The minimum bactericidal concentration (MBC) was determined by streaking the contents of the last tubes of MIC with no turbidity on freshly prepared nutrient agar plates. The MBC is the least concentration in the plate from which no growth was observed after 18-24 h of incubation (Adesokan *et al*., 2007).

RESULTS

Phytochemicals constituents of the extracts

The ethanol extract (EE) of *Alchornea cordifolia* leaves was brown in colour, gummy in nature, soluble in water (rapidly in DMSO), Ethanol extraction from 500g powder of the plant yielded 6.2 g of the extract. While ethyl acetate extract was brown, solid, partially soluble in water but rapidly soluble in DMSO and yielded 5.1 g of the extract (Table 1).

Table 1: Physical parameters of the *Alchornea cordifolia* leaf extracts

S/N	Extract	Texture	Solubility in water	Solubility in DMSO	Yield (%)
1	Ethanol	Gummy	Soluble	Rapidly soluble	6.2 (15.5)
2	Ethylacetate	Solid	Partly soluble	Rapidly soluble	5.1 (12.8)

Phytochemical Constituents of *Alchornea cordifolia* leaves Extracts

The qualitative phytochemical screening of the ethanol and ethylacetate extracts of *Alchornea cordifolia* leaves revealed the presence of the following phytochemicals constituents; carbohydrate, cardiac glycosides, resins, flavonoids, tannins and alkaloids, steroids and triterpenes in all the extracts. However, saponins were present in ethanol extract but absence in ethyl acetate extract. Anthraquinones derivatives were absence in both extracts (Table 2).

Table 2: Phytochemical Constituents of the Extracts of *Alchornea cordifolia* Leaves

S/N	Phytochemicals	Test	Ethyl acetate	Ethanol
1	Carbohydrates	Molisch' test	+	+
	Reducing sugar	Fehling's test	+	+
2	Glycosides			
	Cardiac glycosides	Kella-killiani test	+	+
		Kadde test	+	+
3	Anthraquinone derivatives			
	Combined anthracene	Borntrager,s test	-	-
		Modified Borntrager's test	-	-
4	Resin	Acetic anhydride test	+	+
5	Saponin	Frothing test	-	+
6	Flavonoids	Sodium hydroxide test	+	+
7	Tannins	Ferric chloride test	+	+
8	Alkaloids	Mayer's test	+	+
		Wagner's test	+	+
9	Steroids and triterpenes test	Salkowski test	+	+
		Lieberman-Burchard's test	+	+

Key; += Present; - = Absent

Antimicrobial Activities of the Extracts

Table 3 showed the diameter of zones of inhibition of bacterial growth at varying concentrations of *Alchornea cordifolia* ethanolic and ethyl acetate extracts measured in millimeter (mm). The agar well diffusion is carried out to test for the susceptibility of the organisms to the plant extract. The diameter of the zone of inhibition determines the effectiveness of the extract against the microorganism. The larger the diameter, the greater the susceptibility of the microorganism to the extract. The zones of inhibition of *Alchornea cordifolia* extracts on the test isolates were recorded after 24 h and the result indicated that the ethanol extract had the highest zone of inhibition on multi drug resistance ESBL producing *E. coli* and *Klebsiella* spp. The ethanol extract recorded the largest zones of inhibition (17 mm) and also at a concentration of 1. 25 mg/mL, the extract was able to inhibit the growth of some of the multidrug resistant isolates, to some extent the ethanol extract was active against all the *E coli* isolates with the best activity against isolate *E. coli* (2084) and lowest activity against *E. coli* (227). It is moderately active against *Klebsiella* spp with best activity against K16 and lowest against K4. The activity of ethanolic extract was best seen in *E coli* than *Klebsiella* spp even though was active against both. Susceptibility of the ESBLs producing bacteria to the ethanol extracts of *Alchonea cordifolia* varies from one organism to the other as shown in Table 3.

Table 3: Antimicrobial Susceptibility Pattern of the MDR ESBLs producing Isolates to *Alchornea cordifolia* Extracts

		Concentration (mg/ml)/Zone of inhibition (mm)							
S/N	**Isolates code**	**20**	**10**	**5**	**2.5**	**1.25**	**DMSO**	**CIP**	**Extract**
1	2101 E	13	11	10	10	10	0	22	Ethanol
	2101 E	10	7	0	0	0	0	20	E. acetate
2	2084 E	17	13	12	11	8	0	25	Ethanol
	2084 E	12	11	11	0	0	0	25	E. acetate
3	227 E	8	0	0	0	0	0	25	Ethanol
	227 E	10	8	8	0	0	0	25	E. acetate
4	38 E	15	12	10	10	8	0	23	Ethanol
	38 E	13	12	8	8	0	0	24	E. acetate
5	K4	10	0	0	0	0	0	19	Ethanol
	K4	0	0	0	0	0	0	19	E. acetate
6	K16	14	12	10	10	8	0	23	Ethanol
	K16	14	11	10	8	0	0	23	E. acetate
7	K2	12	11	8	8	0	0	24	Ethanol
	K2	12	10	8	0	0	0	23	E. acetate
8	K64	15	11	10	0	0	0	20	Ethanol
	K64	12	10	8	8	0	0	20	E. acetate
9	10E	15	12	10	9	0	0	21	Ethanol
	10E	12	9	0	0	0	0	20	E. acetate

KEY; Size of the cork borer = 6mm, DMSO = Negative control, Ciprofloxacin (25 mg/ml) = Positive control, E= *E. coli*, K= *Klebsiella* spp, E. acetate = Ethyl acetate

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts

Isolates	Ethanol extract (mg/ml)		Ethyl acetate extract (mg/ml)	
	MIC	MBC	MIC	MBC
2101 E	5	20	10	20
227 E	20	NA	5	NA
38 E	2.5	20	5	20
2084 E	2.5	20	5	NA
K2 (*K pneumoniae*)	5	20	10	20
K16 (*K oxytoca*)	10	20	2.5	20
K4 (*K oxytoca*)	20	NA	NA	NA
K64 (*K oxytoca*)	20	NA	5	NA
10 E	2.5	20	5	20

NA= Not available
E= *E coli*

The ethyl acetate extract was also active against all the *E coli* isolates with the best activity observed against *E. coli* isolate (38) and lowest against *E. coli* isolate (2101). The activity of ethyl acetate against *E coli* isolates when compared to ethanol and was less. *Klebsiella oxytoca* (K4) was found to be resistant to ethyl acetate extract at all concentration. (Table 4).

The ethanol extract showed lower values of M.I.C and M.B.C with *E coli* having the lowest values. While *Klebsiella* spp. had the highest values. (Table 4). The Ethyl acetate extract has higher M.I.C and M.B.C values than the ethanolic extract with *E coli* (E38) and (E 2084) having lower M.I.C values. (Table 4).

DISSCUSION

Ethanol and ethyl acetate extract of *Alchornea cordifolia* leaf obtained by soxhlet extraction method yielded 6.2g and 5.1g respectively with variation in color and texture of the extracts. Ethanol extract had higher percentage yield than ethyl acetate extract which agrees with the work of Dhanani *et al.* (2013). In order to extract different phenolic compounds from plants with a high degree of accuracy, various solvents of differing polarities must be used. Ahmad (2014) also stated that there is a need to employ broad range of extractive solvents in the extractions of possible phytochemicals from medicinal plants. The polarity of the solvent used in extracting the ethanol extract which allowed it to draw more constituents than ethyl acetate extract, could have been the reason why the former had more yield than the latter.

Qualitative phytochemical screening of the extracts extracted with ethanol and ethyl acetate revealed the presence of all the following phytochemicals tested, namely alkaloids, flavonoids, reducing sugar, Tannins, resin and carbohydrates which collaborated with the findings of Djimeli *et al.* (2017) and Jia *et al*. (2017). Anthraquinones and saponins were present in the ethanolic leaves extract but were absence in ethyl acetate extract of *Alchornea cordifolia*. Cardiac glycoside and steroids were present in ethyl acetate extract but absent in ethanol extract. Moreover, the active compounds can sometimes be present in very small concentrations, which could be a hinderance when trying to elucidate antimicrobial compound structures (Ekundayo *et al*., 2020). The presence of these phytochemicals has conferred to the leaf's extracts of *Alchornea cordifolia* their medicinal value (Arbonnier, 2004). Akbari *et al*. (2019) indicated that the recoveries of bioactive phytochemical compounds from plants are potentially affected by the conditions of extraction methods and different

solvent formulations. Phenols are generally protoplasmic poisons toxic to all types of cells. Precipitation of proteins occurs with high concentration of phenol, while at low concentrations it denatures proteins without coagulating them. Phenol freely penetrated the tissue because of its denaturing activity (Adeshina *et al.,* 2012). Flavonoids on the other hand have been reported to be synthesized by plants in response to microbial infection; hence they exhibit antibacterial activities (Anyanwu and Okoye 2017). The presence of flavonoids suggested that it can be used as antispasmodic and antioxidant, and confirms the reason for the use of the plant in the treatment of spasmodic bronchitis and other microbial infections.

The ethanolic and ethyl acetate extracts of *Alchornea cordifolia* were subjected to antimicrobial challenge tests against multidrug resistant ESBL strains. The inhibitory results showed that the ethanol extract had more activity than the ethyl acetate extract. The degree of activity varied with the isolates and the extracts. This variation of activity could be due to the differences in the solubility of the secondary metabolite in the different solvents used and also the structural or morphological variability of the tested isolates thus, larger zones of inhibition were produced by the susceptible organisms than the resistant ones. It could also be due to the polarity of the solvents, ethanol been more polar dissolve more of the secondary metabolites. This result is different from the work of Adeshina *et al.* (2012) which showed that the ethyl acetate fraction (non-polar solvent) extract of *A. cordifolia* leaf was relatively more active than the fraction of polar solvent against type isolates of *E. coli, S. aureus, P. aeruginosa* and *Candida albicans*.

The minimum inhibitory concentration (MIC) values of the extracts ranges from 2.5 to 20 mg/ml, while the Minimum Bactericidal concentrations (MBC) was found to be 20 mg/ml. The ethyl acetate extract was observed to have higher MIC values than the corresponding ethanol extract. With the ethanol extract having 20 mg/ml as the highest MIC values in *E. coli* and *K oxytoca* while the lowest MIC values of 2.5 mg/ml was observed in *E. coli* (E 38 and E 2084) as well. These variations could be due to the differences in the chemical composition of the extract as well as in the mechanism of action of its bioactive constituents. Presence of 20 mg/ml as the highest MBC indicated that the extract might have cidal effect against the bacterial isolates. Further studies are needed towards isolation, characterization, identification and determination of active biological compounds present in the leaves of *Alchornea cordifolia*.

CONCLUSION

This investigation has revealed that the leaves of *Alchornea cordifolia* studied have high phytochemical content and have antimicrobial activity on MDR ESBL producing *E coli* and *Klebsiella* spp. isolated from human. This is an indication that they are of high medicinal value. Thus, they could be exploited to be used in the formation of alternative antimicrobial drugs which will be used to cure and control human diseases. Research should be done for the development of safe antimicrobial agents from other plant sources for the treatment of Urinary tract infections caused by *E coli* and *Klebsiella* spp. isolates.

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Conflict of Interest

The authors declare no conflict of interest exist

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