EFFECT OF STEM BARK AND ROOT BARK EXTRACTS OF *Adansonia digitata* ON EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL)- PRODUCING Escherichia coli ISOLATED FROM POULTRY

***ANUEYIAGU K. N., TIMOTHY P., OLANREWAJU O. D.**

Federal College of Animal Health and Production Technology Vom, Plateau State

*Corresponding author: anueyiagunnamdi@yahoo.com; Phone number: +2348035841582; https://orcid.org/0000-0002-2566-7982

ABSTRACT

The aim of this study is to determine the antibacterial effect of Adansonia digitata extracts on ESBL-producing E. coli. One hundred cloaca samples were collected from chickens in poultry houses in Jos South Local Government of Plateau state. By standard methods, Ethanol extraction method was used to extract the secondary metabolite of Adansonia digitata. The fecal samples collected were inoculated into 9ml of sterile peptone water and incubated overnight at 37°C. A loop full of peptone water broth was streaked on EMB using the quadrant streak method. The plate is incubated aerobically at 37°C for 24 hours and Escherichia coli colonies identified. Preserved E. coli isolates were subjected to DDST method to identify ESBL producers. The antibacterial activity of the extracts was determined using the agar well diffusion technique. The phytochemical composition of A. digitata plant was found to contain saponins, Tannins and Terpenoids in both bark and root, while flavonoids and steroids absent in both bark and root. Cardiac Glycoside was present in bark while absent in root. Out of the 75 E. coli isolates, 9 were ESBL producers. Statistically, there is no significance difference between the isolation of ESBL E. coli in all the districts with p-value less than 0.05. The positive control had the highest zone of inhibitions followed by 500mg/ml compared to other dilution such as 31.25mg/ml which has lowest zone of inhibition for root, bark, and 1:1 combination of the root and bark extracts. The threat of antimicrobial resistance (AMR) of pathogenic bacteria such ESBL producing E coli has becoming a public health problem of global dimension and is growing at an alarming rate, a situation perhaps worsened in third world countries by gross abuse and misuse of antibiotics.

***Key words**: Adansonia digitata, ESBL, E. coli, AMR, root and bark extract*

INTRODUCTION

A good number of trees and shrubs have been claimed by ethno-veterinary practitioners, ethno-medical practitioners and other local people to have medicinal benefits against infectious and non-infectious animal and human diseases. *Adansonia digitata* has among many plant species, been reported to have ethno-medicinal uses and has been widely used in the traditional systems of medicine. According to the UN (2005), the fruit pulp of *A. Digitata* is traditionally used for the treatment of fever, diarrhea, dysentery, haemoptysis and small pox in humans. Leaf infusions are used as treatment for diarrhea, fever, inflammation, kidney and bladder diseases, blood clearing and asthma in humans. The bark is used for treatment of fever, especially that caused by malaria. As far as ethno-veterinary medicine is concerned, reports indicate that bark of *A. Digitata* is used for treatment of diarrhoea in poultry (Guèye, 1999) and fruits are used for treatment of Newcastle disease in poultry (Guèye, 1999; Wynn and Fougère, 2006).

Some work has been done to validate the efficacy of *A. Digitata* and identify its bioactive constituents. For example, experiments performed on rats showed that administration of fruit pulp extract at a dose between 400 and 800 mg/ml had a marked anti-inflammatory effect and reduce formalin-induced oedema in the animals (UN, 2005). These effects are comparable with those produced by 15 mg/ml of phenylbutazone, a common anti-inflammatory drug used as an internal standard (UN, 2005). Equally, extracts from *A. Digitata* fruit pulp have analgesic (pain killing) and antipyretic (temperature reducing) activities (UN, 2005). This activity could be due to be presence of sterols, saponins and triterpenes in the fruit pulp (UN, 2005). Results of antibiotic studies show that methanolic extract of *A. Digitata* root bark or leaves has antibacterial activity against *Staphylococcus aureus, Streptococcus faecalis*, *Bacillus subtilis, Escherichia coli* and *Mycobacterium phlei* (Ananil *et al*., 2000). Methanolic extract from root of *A. Digitata*has also been reported to have anti-trypanosome activity against *Trypanosoma congolense* and *T. brucei brucei* (Atawodi *et al*., 2003).

Antibiotic resistance of bacteria is commonly seen in daily medical practice with multi- drug resistant Gram negative bacteria posing the greatest threat to human health (Gayathri *et.al,*. 2012). According to the WHO (2017), increased threat of antibiotic resistance is a direct result of overuse and misuse of antibiotics in animals and humans. The "Path of Resistance" begins by administering antimicrobials to food-animals such as chickens to keep them health. These antimicrobials protect the chickens against known bacterial infections. The acquired resistance of the bacteria, however, resists the antimicrobials making them ineffective. This increasing resistance has received considerable national and international attention. There is an alarming increase of antibiotic resistance in bacteria that causes infection, of particular interest are the multi-drug resistant pathogens, for example, *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), Penicillin-Resistant *Streptococcus pneumonia*, Vancomycin-Resistant Enterococus and extensively drug- resistant *Mycobacterium tuberculosis* (Alkshun and Levy, 2007)

Beta lactam antibiotics are the most predominantly prescribed antibiotics to treat bacterial infections, especially in Nigeria hospitals. (Gayathri *et al*., 2012; Yusuf and Arzai., 2011). Extended Spectrum Beta Lactamase (ESBL) are enzymes that confer resistance to most beta-lactam antibiotics such as Penicillins, Cephalosporins, Monobactam and

Aztreonam. Infections. ESBL-producing Gram negative bacteria are associated with increased morbidity and mortality which is linked to inappropriate or delayed antimicrobial treatment (Knudsen and Anderson, 2014).

It has been documented that drug resistance among *E. coli* has increased globally mainly as a result of high prevalence of ESBL producing bacterial (Veenemans *et al*., 2014 and Overdevest *et al.,* 2011). This high prevalence of ESBL producing *E. coli* has resulted from growing reservoirs in food animals such as chickens and the antimicrobials (Veenemans *et al*., 2014). Animals food sources such as chickens have been reported as potential reservoir for the spread of ESBL producing *E. coli* to humans in close proximity or via the food chain (Overdevest *et al*.,2011, Nguyen *et al*., 2019 and Falgenhauer *et al*., 2019). The aim of this study is to determine the antibacterial effect of *Adansonia digitata* extracts on ESBL-producing *E. coli*.

METHODOLOGY

Study Location

The research area is Jos South Local Government area of Plateau State It is located between latitude 08o 24N and Longitude 008o32', the postal code of the area is 930. Temperature ranges from 21-25oC (70-77oF), and from mid-November to late January, night temperature drops as low as 11oC (52oF). Jos receives about 1,400 millimeters (55 inches) of rainfall annually, the precipitation arising from both conventional and geographic sources. The bench work was conducted at the Microbiology Laboratory of the Central Diagnostic Laboratory of National Research Institute Vom.

Sample Collection

One hundred cloaca samples were collected from chickens in poultry houses in Jos South Local Government of Plateau state. The root, and stem bark of *A. digitata* is collected from Jos South Local Government area of Plateau State. They are cut into smaller chips and dried in hot air oven for two to three hours and ground into powder using sterile Pestle and Mortar under laboratory condition at Biochemistry Lab of the Federal College of Animal Health and Production Technology, Vom. Thereafter they are kept in air tight container for future use.

Plant Extraction

Ethanol extraction is used for the extraction of secondary metabolite of the plant. 70g of Root bark was soaked in 750ml of ethanol. The water is boiled to 100^{o}C then allowed to cool at room temperature to 70^{o}C gradually in sterile conical flask (Renuka and Berla, 2010). The filter is filtered using Whatman No. 1 filter paper and the filtrate is evaporated to dryness until solid residue is obtained. The solid filtrate is stored in universal bottles in the refrigerator at 4o before use.

Phytochemical screening

Sample of *A. Digitata* is screened phytochemically for the presence of secondary metabolites using the standard methods of Victor and Chidi, 2009. The secondary metabolites will be screened for the Saponins, Terpenoids Tannins, Cardiac Glycosides, flavonoids and steroids.

Media preparation

a) **Eosin Metheny blue**

35.96g of the powdered was weighed and dispensed into 1 liter of distilled water, it was then properly mixed after which it was sterilized by using autoclave for about 15 minutes of 121c, cooled to about 40c and finally poured into the plates.

b) **Muller Hinton agar**

38g of the powder was weighed and dispensed into 1 liter of distilled water, it was then properly mixed after which it was boiled to 100c then allowed to cool to about 45c and finally poured into the plates.

c) **Nutrient broth**

18g of the powder was weighed and dispense into 1 liter of distilled water it was properly mixed, after which it was sterilized by using autoclave for about 15minute to about 121c, cooled to 47c and 9ml was poured each into sterile bottle.

Bacterial Examination of the Fecal Sample

The fecal samples collected were inoculated into 9ml of sterile peptone water and incubated overnight at 37^{o}C. A loop full of peptone water broth is streaked on EMB using the quadrant streak method. The plate is incubated aerobically at 37^{o}C for 24 hours. The plates are examined for gross colony morphology, pigmentation and haemolytic characteristics on blood agar and green metallic sheen on EMB at 24 to 48 hours. Presumptive colonies of *E.coli* are selected and purified on Nutrient agar. Purified isolate is preserved in agar slants for future use such as gram reaction, morphology and catalase test.

Double Disk Synergy Test

Preserved *E. coli* isolates were subjected to DDST method to identify ESBL producers. The MHA plate is inoculated with bacterial suspension matched to 0.5 McFarland. Ceftriaxone (30µg), and Cefepime disks is placed 15 mm distant Centre to Centre from amoxicillin-clavulanic disk (20 µg amoxicillin and 10 µg of clavulanic acid) which is placed at middle. The extension of inhibition zone of antimicrobial disks (one or more) towards amoxicillin-clavulanic disk is confirm the presence of ESBL. This is according to Jabeen *et al.,* (2003).

Determination of antibacterial activity of extracts

The antibacterial activity of the extracts is determined using the agar well diffusion as described by Adeniyi *et al.,* (2006). A ml of a standardized overnight broth culture of each bacterial (ESBL) isolate (equivalent to 107 – 108 CFU/ ml-1) is placed and mixed in a molten MHA at about 50oC and poured into a petri dish. The seeded plates are allowed to solidify and sterile cork-borer of 8mm diameter is used to cut equivalent wells on the surface of the agar. The wells are filled with 0.1 ml solution of each extract reconstituted with ethanol at a concentration of 500mg/ml. Thereafter, doubling dilution was conducted to have 250mg/ml, 125mg/ml, 62.5mg/ml, and 31.25mg/ml concentrations. Gentamycin at 5mg/ml is used as positive control and sterile distilled water used as a negative control. The plate is incubated at 37oc for 24 hours after which the diameter of zones of inhibition is measured

RESULTS

Table 1 shows the phytochemical composition of *A. digitata* plant contains saponins, Tannins and Terpenoids in both bark and root, while flavonoids and steroids absent in both bark and root. Cardiac Glycoside was present in bark while absent in root. Table 2 shows that out of the 75 *E. coli* isolates, 9 were ESBL producers. While all three districts had 3 ESBL *E. coli* each, Kuru had none. Statistically, there is no significance difference between the isolation of ESBL *E. coli* in all the districts with p-value less than 0.05.

Tables 3 and 4 shows that the positive control has the highest zone of inhibitions followed by 500mg/ml compared to other dilution such as 31.25mg/ml which has lowest zone of inhibition. Similar trend could be seen in Table 5.

Table 1. Phytochemical properties of *Adasonia digitata*

S/N	Phytochemicals	Observation	
		Bark	Root
1.	Saponins	++	++
2.	Tannins	++	++
3.	Cardiac Glycoside	+	-
4.	Flavonoids	-	-
5.	Steroids	-	-
6.	Terpenoids	++	++

Table 2. Prevalence of ESBL producing *E. coli* in Jos South Local Government Area

District	No of *E. coli* (%)	No of ESBL *E. coli* (%)	χ^2	p value
Du	23 (30.67)	3 (13.04)	2.6988	0.4404
Gyel	19 (25.33)	3 (15.79)		
Kuru	15 (20.00)	0		
Vwang	18 (24.00)	3 (16.67)		
Total	75	9 (12.00)		

Table 3. Antibacterial activity of ethanol root bark of the extract at various concentrations on ESBL-producing *E. coli*

ESBL *E. coli* Isolates	Diameter of zones of inhibition (mm)						
	Pos	500mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	Neg
A1	35	13	8	7	7	6	-
A2	35	6	6	5	5	4	-
A3	30	12	9	7	6	6	-
B1	35	11	6	6	6	5	-
B2	25	8	8	6	6	5	-
B3	35	8	9	8	7	6	-
C1	27	10	7	5	4	5	-
C2	19	8	8	6	7	6	-
C3	24	8	7	7	5	5	-

Key: Pos – positive control; Neg – negative control; A1, A2, A3 – isolates from Du; B1, B2, B3 – isolates from Gyel; C1, C2, C3 – isolates from Vwang

Table 4. Antibacterial activity of ethanol stem bark of the extract at various concentrations on ESBL-producing *E. coli*

ESBL *E. coli* Isolates	Diameter of zones of inhibition (mm)						
	Pos	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	Neg
A1	28	14	8	8	6	6	-
A2	21	12	10	8	6	6	-
A3	35	15	10	7	7	5	-
B1	30	11	9	6	5	4	-
B2	30	15	12	8	6	6	-
B3	28	13	10	8	6	6	-
C1	30	12	10	6	6	5	-
C2	30	15	13	11	7	7	-
C3	30	15	8	9	6	5	-

Key: Pos – positive control; Neg – negative control; A2, A3, A9 – isolates from Du; B1, B2, B3 – isolates from Gyel; C1, C2, C3 – isolates from Vwang

Table 5. The synergistic (ratio1:1) effect of aqueous stem bark and ethanol root bark extract on bacterial isolates on ESBL-producing *E. coli*

ESBL *E. coli* Isolates	Diameter of zones of inhibition (mm)						
	Pos	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml	Neg
A1	28	15	7	6	5	4	-
A2	38	9	7	5	5	5	-
A3	30	19	10	8	6	6	-
B1	30	10	7	6	6	6	-
B2	20	7	9	5	4	3	-
B3	35	11	8	7	6	6	-
C1	27	1	8	9	9	6	-
C2	30	13	13	8	8	5	-
C3	34	9	10	7	5	5	-

Key: Pos – positive control; Neg – negative control A1, A2, A3 – isolates from Du; B1, B2, B3 – isolates from Gyel; C1, C2, C3 – isolates from Vwang

DISCUSSION

In 2014, WHO reported that over 50% population of pathogens like *E. coli, K. pneumoniae, S. aureus*, and *P. aeruginosa* were evolved as MDR and β-Lactamase producer (Atef *et al,* 2019). The results obtained from the research work showed the antibacterial effect of aqueous stem bark and ethanol root bark extracts of *A. digitata*

on ESBL-producing *E. coli* isolated and also synergistic effect on them. Out of 100 samples isolated 75 was found to be positive to *E. coli* and 9 positive to ESBL-*producing E. coli*. The present study showed that the plant extract used were effective against ESBL-producing *E. coli*. This in corroboration with Afoussatou *et al.,* (2022) which showed a broad zone of inhibition on strains of ethanolic extract one ESBL-resistant strain of *E. coli*.

The phytochemical screening of the plant in this study showed the presence of terpenoids, saponins and tannins which correlates with that of Abdallah *et al.,* (2020).
The ethanol extract of the plant showed higher antibacterial activity against the isolates at the 500mg/ml compare to other dilutions this in agreement with *Abdallah et al.,* (2020). The synergistic (ratio 1:1) effect of aqueous stem bark and ethanol root bark extract has same antibacterial activity on the isolate as to individual extracts The antimicrobial activity of ethanol root extracts against bacterial could be a result of the present of tannins, terpenoids, saponins, in the stem and root bark extracts.

The antimicrobial activity of *A. digitata* ethanol extracts could be due to the presence of tannins as these have been previously reported to cause antimicrobial activities (Datsugwai and Yusuf, 2017). Tannins are known antimicrobial agents that could inhibit the growth of microorganisms by precipitating the microbial protein and thus depriving them of nutritional proteins needed for their growth and development (Jame, 2019). These properties gave this extract an antimicrobial activity exploitable in the context of antimicrobial resistance.

Samatha *et al.,* (2017), reported that antibacterial activity of methanol extract of different part of *A. digitata* found to have activity against some bacterial like *E. coli, Enterobacter, Proteus and Staphylococcus*. According to Al-bakri *et al.,* (2006), stated that the leaves *of A. digitata* has antimicrobial activity against *E. coli* and Salmonella. Researchers have propounded that ethanol is the most effective solvent for extracting compounds from plant that has broad spectrum activities against bacterial. It also stated that ethanol extract of baobab plant shows the presence of tannins, terpenoids and saponins (Cowan, 1999), thereby supporting this present study. Terpenoids have also been reported to be effective in the prevention and therapy of most diseases such as cancer. It also found to have antimicrobial, antifungal, antiparasitic, antiviral and anti-inflammation properties (Rabi and Bishayee. 2009).

CONCLUSION AND RECOMMENDATION

The threat of antimicrobial resistance (AMR) of pathogenic bacteria such ESBL-producing *E coli* has become a public health problem of global dimension and is growing at an alarming rate, a situation perhaps worsened in third world countries by gross abuse and misuse of antibiotics. AMR can lead to high mortality and morbidity cases since available antimicrobials are no longer effective for the treatment of common infections in humans and animals.

It is therefore, recommended that pharmaceutical organizations, research-funding organizations, governments, Non-governmental organizations (NGOs) and other well-meaning individuals should boost research in this field in other to curb the increase in the spread of antimicrobial resistant pathogens.

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