Quest Journals
Journal of Research in Pharmaceutical Science
Volume 7 ~ Issue 9 (2021) pp: 07-12
ISSN(Online): 2347-2995
www.questjournals.org

Research Paper

Antimicrobial Assay of Methanolic Extracts of Selected Plants onMultiple Antibiotics-Resistant *Escherichia Coli*

Ohunayo A.S*[1], John-Mese O.J[1],Dauda O.S[1], Oyinlade P.O[1],Afolabi A.T[1].
[1]*Department of Science Laboratory Technology, Ekiti State University, Ado-Ekiti, Nigeria.*

ABSTRACT
Antimicrobial resistance is the ability of a pathogen to develop resistance to antimicrobials that have been previously used to treat infection caused by the microorganism. In this study, 25 presumed E. coli *isolates were collected at Adeoye Teaching Hospital and were subjected to biochemical tests for identification & confirmation. The confirmed isolates were subjected to Antimicrobial Susceptibility Test (AST) using eight different antibiotics. The result showed that Ofloxacin (100%), Gentamicin (92%), Nitrofurantoin (76%), & Ciprofloxacin (72%) had high activity in terms of susceptibility, while the isolates showed resistance to other antibiotics used with Augmentin (4%) having the lowest susceptibility. MAR indexes were measured & three of the isolates (12%) showed resistance to five and above antibiotics (i.e., multidrug-resistance). The three phenotypically confirmed multi-drug resistance isolates were subjected to Minimum Inhibitory Concentration (MIC) at varying concentrations (50, 75, and 100mg/ml) of methanolic extracts of five selected medicinal plants, with gentamicin and 10% methanol serving as positive and negative control respectively. The zones of inhibition increased with a significant increase in the concentration of the methanolic crude extracts (i.e., the zones of inhibition produced by the plant extracts at 100mg/ml were higher than that of 75mg/ml and 50mg/ml). Susceptibility of the three phenotypically confirmed multi-drug resistance isolates to the extracts was in the order Alliumsativum>Vernoniaamygdalina>Zingiberofficinale>Azadirachtaindica>Ocimumgratissium. This study has shown that the selected plant extracts have antimicrobial compounds which makes them effective against multidrug-resistant E. coli isolates and hence can be used to produce drugs with a better mode of action.*

KEYWORDS*: Antimicrobial assay, Methanolic extracts, Escherichia coli*

Received 10 September, 2021; Revised: 22 September, 2021; Accepted 24 September, 2021 © The author(s) 2021. Published with open access at www.questjournals.org

I. INTRODUCTION

Escherichia coli are Gram-negative, facultative anaerobic bacilli which are a profound microbial tenant of the human gastrointestinal tract and other mammals. Being an enteric organism, they belong to the family of Enterobacteriaceae. (Raji *et al.,* 2007). *Escherichia coli* have a circular DNA molecule of 4.6 million base-pairs in length, containing 4288 annotated protein-coding genes (organized into 2584 operons), seven ribosomal RNA (rRNA) operons, 86 transfer RNA (tRNA) genes (Singleton *et al.*, 1999). Most strains of *E. coli* are not harmful but are part of the human gut. However, some types can cause illness in humans which includes diarrhoea, abdominal pain, and fever, and sometimes vomiting (Yvette, 2017). The groupings of diarrheagenic *E. coli* include; enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic (EHEC), enterotoxigenic *E. coli* (ETEC), and diffusely adhering *E. coli* (DAEC). All categories of *E. coli* described may be shed in the faeces of infected humans, creating the potential for spread to other humans, animals, and the environment (Thomas *et al.*, 2012). Their ability to cause diseases while resisting antimicrobials has represented an enormous global challenge. *Escherichia coli* and other enteric microbes have fallen under the WHO priority list because of their pathogenicity and ability to resist antimicrobials. Antimicrobial resistance (AMR or AR) is described as the ability of a pathogenic microbe to develop a resistance to the effects of an antimicrobial medication that was once able to treat that infection caused by the microorganism (Peterson *et al.*, 2018). Antibiotics resistance in *E. coli* is a result of the expression of antibiotics resistance genes (ARGs). The genetic exchange system and the ability of *Escherichia coli* to transfer and propagate genes between humans and animals may make it a significant vector for the spread of rapidly dispersed resistance genes (Wright *et al.*, 2007). ARGs in *E. coli* can be detected using the Polymerase Chain Reaction (PCR) amplification technique some of which includes; Tetracycline *tetA*, Kanamycin *pKD13*,

Ampicillin *ampC*, gentamicin *aac3-1, aac3-III*, Doxycycline *tetB*, Cefotaxime *mphA*, Spectinomycin *aadA14*, Streptomycin *aadA1*, and Ciprofloxacin *ermA, ermB, ermC* (Sheikheldin *et al.*, 2018). Medicinal plants represent dependable reservoirs and natural repositories for antimicrobial agents. A wide variety of natural products are used in the treatment of common infections in traditional medicinein developing countries (Afolayan, 2003). Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. The presence of phytochemical constituents such as alkaloids, flavonoids, tannin, and phenolic compounds has been reported to be important compounds in many other medicinal plants (Jayashree *et al.*, 2014). In this research, methanolic extract of five different plants *Azadirachtaindica* (Neemleaf/Dongorayo), *Vernoniaamygdalina* (Bitterleaf/Ewuro), *Ocimumgratissium* (Scentl eaves), *Zingiberofficinale* (Ginger extracts), and *Allium sativum* (Garlic) were used and tested against resistant strains of *E. coli*.

II. MATERIALS AND METHODS

Collection and Identification

A total of 25 Presumptive *E.coli* isolates (from stool, urine, and wound samples) were obtained and examined. The isolates were collected on a slant from Adeoyo Hospital, Ibadan. And were transported to the Microbiology Department laboratory of Ekiti State University, Ado-Ekiti. The isolates were cultured on Nutrient Agar and subcultured on (EMB agar & MacConkey agar) and incubated accordingly. Gram staining and primary biochemical tests were performed on subcultures obtained from Nutrient Medium for the identification of *E. coli*. The biochemical test includes; Urease test, Citrate test, Oxidase test, Sulphur, Indole, and motility test.

Antibiotic Susceptibility Test:

Antibiotic susceptibility test was performed using the Kirby-Bauer method on Mueller-Hinton agar, an inoculum of 0.5 McFarland, and the plates were inverted and incubated aerobically at 37°C for 18 to 20 hours. The zone of inhibition and resistance was measured, recorded, and interpreted following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). All isolates were subjected to the antibiotics listed in the table below (Table 1).

Table 1. List and of antibiotics and acronyms

N	Acronyms	Antibiotics
1	CAZ	Ceftazidime (30µg)
2	CRX	Cefuroxime (30µg)
3	GEN	Gentamicin (10µg)
4	CMX	Cefixime (5µg)
5	OFL	Ofloxacin (5µg)
6	AUG	Augmentin (30µg)
7	NIT	Nitrofurantoin (300µg)
8	CPR	Ciprofloxacin (5µg)

Minimum Inhibitory Concentration (MIC) determination:

The methanolic plant extracts were prepared in varying concentrations following the protocol of Taura and **Oyeyi (2009),** to obtain 50mg/ml, 75mg/ml, and 100mg/ml concentrations. The antimicrobial activity of the plant extracts was determined using the agar well diffusion method of (Thormberry, 1983; Irobi *et al.*, 1996; Akande and Hayashi, 1983). Briefly, about 0.5ml of the standardized portion of the new microbial culture was aseptically transferred into Petri dishes containing Nutrient Agar (NA) for bacterial isolates (isolate showing resistance to 5 and above antibiotics) and left for about 20 minutes to allow the microorganisms to fix on the media. Wells, where extracts were to be introduced into the plates, were carefully marked using a sterile cork borer (6mm diameter). 1ml of extract at various concentrations (50mg/ml, 75mg/ml, and 100mg/ml) were added into the wells. A well was also made at the central portion of the agar medium and Gentamicin was placed therein to serve as controls. The plates which were prepared in triplicates were incubated at 37OC and the zones of inhibition were measured after 24 hours (Mudi and Ibrahim, 2008).

III. Results & Discussions

A total of 25 clinical isolates were collected. The isolates were collected from three (3) different sources. Out of the 25 isolates, 40% were isolated from stool samples, 40% from urine samples & 20% from wound samples. The isolates were subjected to biochemical tests in which 96% of the isolates were oxidase negative, 100% were catalase-positive, 96% were sulphur negative, 100% were indole positive, 96% were motile, 96% were urease negative, 96% were citrate-negative and 100% were gram-negative (Figure 1.0). Little deviation in the result from the standard biochemical profile of *E. coli* may be because some isolates might have mutated and may also be as a result of differences in some strains of the said organism. In this analysis, 23 out of the 25 biochemically confirmed isolates meet up all the biochemical tests conducted. This result is similar to the study of Parames*het al.*, (2018) where 40 out of the 42 isolates were confirmed *E. coli* after conducting Indole test, citrate test, motility test, and other biochemical tests.

In Figure 2.0, The AST result showed that Ofloxacin had the best activity among all the antimicrobial agents with 100% of the isolate sensitive to it, followed by Gentamicin (92%), Nitrofurantoin (76%), Ciprofloxacin (72%), Cefuroxime (16%), Cefixime (12%), Ceftazidime (12%), and the least activity was obtained against Augmentin (4%). This result is similar to the study of Sumera*et al.*,(2014) where most of the isolates were sensitive to Ciprofloxacin and Gentamicin. Also, in this result, Gentamicin & Nitrofurantoin were 92% & 76% sensitive respectively which is similar to the study of Kibert and Abert (2011) where Gentamicin & Nitrofurantoin were 79.6% and 96.4% sensitive respectively. This result can also be related to the study of Daoud*et al.*, (2020) where Nitrofurantoin, Gentamicin, and Ofloxacin were 97.6%, 93.9%, and 86.9% sensitive respectively.

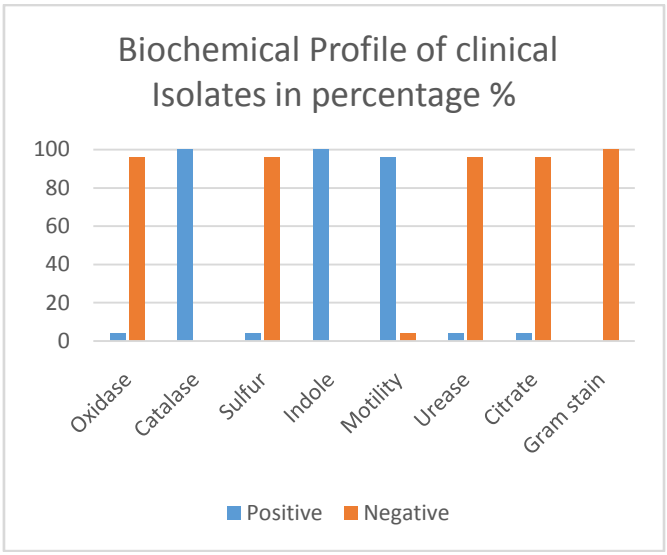

Figure 1.0: **Biochemical profile percentage of the clinical isolates**

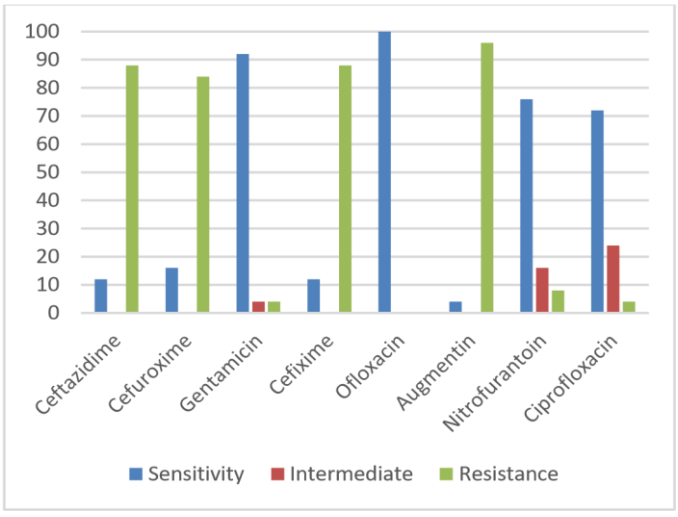

Figure 2.0: **Antibiotics susceptibility profile of *E. coli* isolates**

Isolates that show resistance to more than four antibiotics are considered multidrug-resistant (Adenaike*et al.*, 2016). The M.A.R indexes of the isolates were measured, and it was observed that among the 25 isolates analysed,three (3) isolates which amount to 12% of the total isolates showed resistance to five (5) and above different antibiotics (Table 2.0). The three phenotypically confirmed multi-drug resistance isolates were likely isolated from people that have misused antibiotics which led to the development of the multidrug-resistant strains and may also be due to several other possible reasons such as resistance gene transfer.

Table 2.0.Multiple antibiotic indexes of all the E. coli Isolates

MULTIPLE ANTIBIOTICS INDEX	PERCENTAGE OF ISOLATE
0	0
0.125	0
0.25	8
0.375	28
0.5	52
0.625	8
0.75	4
0.875	0
1	0

Table 3.0. Anti-microbial effects of 50 mg/ml concentration of methanolic leaf extracts of selected plants on multidrug-resistant*E. coli* isolates.

Organisms	Zone of Inhibition (mm) at 50.0 mg/ml conc. (METHANOLIC PLANT EXTRACTS)						
	Gentamicin 10µg (positive control)	*Methanol 10%conc (negative control)*	*Azadirachtaindica (Neem leaf)*	*Vernoniaamygdalina (Bitter leaf)*	*Allium sativum (Garlic)*	*Ocimum gratissium (Scent leaves)*	*Zingiberofficinale (Ginger)*
*E. coli*001	20.00	-	3.40	3.50	3.60	-	2.60
E. coli 002	18.00	-	-	3.20	3.50	2.20	2.40
E. coli 003	24.00	-	2.50	2.90	3.80	1.90	3.30

E. coli* = *Escherichia coli
- = No Zone of Inhibition

Susceptibility of the three phenotypically confirmed multi-drug resistance isolates to 50mg/ml concentration of methanolic plant extract produced zones of inhibition ranging from (2-3mm) (Table 3.0).

At 75mg/ml concentration, the zones of inhibition increased to around (4-5mm) (Table 4.0) and further increased to (5-6mm) at 100mg/ml concentration(Table 5.0). Susceptibility of the three phenotypically confirmed multi-drug resistance isolates to the positive control (Gentamicin- 10µg) produced zones of inhibition ranging between (18-24mm). The susceptibility of all three concentrations of the plant extract is low compared to that of the positive control (Gentamicin- 10µg).

This proves that methanol used for the extraction may not have liberated all the active components of the plants and it is the main reason for the low zones of inhibition that were produced by the plants, this is in accordance to the work by Ohunayo *et al* (2020). It is important to know that Gentamicin which is the positive control had the second-highest activity during Antimicrobial susceptibility testing. The extracts have shown excellent activity when compared to other antibiotics used in this study. Although all the plant extract had activity when tested against the three phenotypically confirmed multi-drug resistance isolates, *Allium sativum, Vernoniaamygdalina* & *Zingiberofficinale* had the best antimicrobial property and showed the highest zone of inhibition at the three different concentrations used. This result is similar to the study of Ugueri *et al.*, (2015) where *Allium sativum* & *Vernoniaamygdalina* had the best antimicrobial property when tested on *E. coli* isolates.

Table 4.0. Anti-microbial effects of 75 mg/ml concentration of methanolic leaf extracts of selected plants on multidrug resistant *E. coli* isolates.

Organisms	Zone of Inhibition (mm) at 75.0 mg/ml conc. (METHANOLIC PLANT EXTRACTS)						
	Gentamicin 10µg (positivecontrol)	*Methanol 10%conc (negative control)*	*Azadirachtaindica(Neem leaf)*	*Vernoniaamygdalina (Bitter leaf)*	*Alliumsativum (Garlic)*	*Ocimumgratissium (Scent leaves)*	*Zingiberofficinale (Ginger)*
E. coli 001	20.00	-	4.60	4.80	5.40	4.40	4.80
E. coli 002	18.00	-	4.30	5.20	5.60	4.20	5.20
E. coli 003	24.00	-	4.20	5.50	5.30	4.60	4.70

***E. coli* = *Escherichia coli* - = No Zone of Inhibition**

Table 5.0. Anti-microbial effects of 100 mg/ml concentration of methanolic leaf extracts of selected plants on multidrug-resistant *E. coli* isolates.

Organisms	Zone of Inhibition (mm) at 100.0 mg/ml conc. (METHANOLIC PLANT EXTRACTS)						
	Gentamicin 10µg (positive control)	*Methanol 10%conc (negative control)*	*Azadirachtaindica (Neem leaf)*	*Vernoniaamygdalina (Bitter leaf)*	*Alliumsativum(Garlic)*	*Ocimumgratissium (Scent leaves)*	*Zingiberofficinale (Ginger)*
E. coli 001	20.00	-	5.70	5.00	6.30	4.30	5.60
E. coli 002	18.00	-	5.50	6.30	5.50	4.50	5.90
E. coli 003	24.00	-	5.20	6.50	6.40	5.70	6.20

***E. coli* = *Escherichia coli* - = No Zone of Inhibition**

Zones of inhibition produced by the plant assay increase as the concentration of the methanolic crude extracts increases. These plants can be further explored by extracting the phytochemical constituents and using them to produce more drugs that can prove effective in the treatment of the multidrug-resistant strains *E. coli*.

IV. CONCLUSION

This study has shown that antimicrobial resistance is a global issue that requires urgent attention. *E. coli* is now becoming resistant to most of these antibiotics and only a few among the most used antibiotics are still effective against the resistant strains. Some of the isolates were resistant to multiple antibiotics which is an indication of multidrug resistance. Prevalence of resistance is mostly a result of drug misuse through self-medication, and other reasons. However, Medicinal plants can provide a strategic tool in combatting antimicrobial resistance and also reducing the incidence of pathogenic *E. coli.*

REFERENCES

[1]. Adenaike O., Olayeni Stephen Olonitola., Ameh J Baba., &Whong C.M.Z, (2016). Multidrug resistance and Multiple Antibiotics Resistance Index of Escherichia coli strains isolated from Retailed Smoked Fish. *Journal of Natural Sciences Research*. Vol. 6, No. 9.

[2]. Afolayan A.J (2003) Extracts from the shoots of Arctotisarctotoides inhibits the growth of Bacteria and Fungi, *Pharmaceutical Biology*, 41:1, 22-25.

[3]. Akande J.A, Hayashi Y., (1983). The potency of extract contents from selected tropical chewing sticks against *Staphylococcus aureus*&*Staphylococcus auricula. World Journal of Microbiology & Biotechnology.* 14: 235-238.

[4]. Daoud N., Hamdoun M., Hannachi H., Gharsallah C., Mallekh W., &Bahri O., (2020). Antimicrobial Susceptibility Patterns of Escherichia coli among Tunisia Outpatients with community-acquired urinary tract infection (2012-2018). Current urology. 14(4): 200-205.

[5]. Irobi O.N, Mo-Young M, Anderson WA, Daramola S, (1996). Antimicrobial activity of bark of *Brideliaferruginea*. *Internal Journal of Pharmacognosy.* 34(2): 87-9.

[6]. Jayashree V Hanchinalmath, Ramesh L. Londonkar, (2014). Comparative phytochemical studies on and antimicrobial potential of fruit extracts of Feronia limonia Linn, *International journal of pharmacy and pharmaceutical sciences* 6(1): 731-734. *Journal of Research Communication*.

[7]. Kibert M., &Abera B., (2011). Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. *African health sciences, 11 Suppl 1* (Suppl 1), S40S45. *Microbiology*. 5(3): 175-186.

[8]. Mudis S.Y, Ibrahim H, (2008). The activity of *Bryophiliumpinnatum* S. KURZ Extraction on respiratory tract pathogenic bacteria. *Bayero Journal of Pure & Applied Sciences.* 1(1): 43-48.

[9]. Ohunayo A.S, Adekeye D.K, Dauda O.S, Odeniyi I.O, PopoolaO.DandAkinwunmi O. (2020). Microbial Profile of the Phyllosphere and the Antimicrobial potency of *Ficusvogelii*extracts. *J. Pharm. Sci. & Res.* 12(1): 191-195.

[10]. Paramesh, B.N., A. Basavaraj, P. Suryakanth, B. Abhilash, and Reveppayya, M, (2018). Isolation and Biochemical Characterization of Escherichia coli from Bovine Mastitis Milk. *Int. J. Curr. Microbiol. App. Sci*. 7(07): 719-722.

[11]. Peterson E, & Kaur P (2018). Antibiotic Resistance Mechanisms in Bacteria: Relationships between Resistance Determinants of Antibiotics Producer, Environmental Bacteria and Clinical Pathogens. *Front. Microbiol.* (9): 2928.

[12]. Raji M., Adekeye J., Kwaga J., Bale J., Henton M., (2007). Serovars and biochemical characterization of Escherichia coli isolated from colibacillosis cases and dead-in-shell embryos in poultry in Zaria Nigeria. *Vet. Archiv*. 77: 495505.

[13]. Sheikheldin A. Abdelgader, Donglin Shin, Mianmian Chen, Lei Zhang, (2018). Antibiotics resistance genes screening and comparative genomics analysis of commensal Escherichia coli isolated from poultry farms between China and Sudan (2): 1-9.

[14]. Singleton, P., (1999). Bacteria in biology. Biotechnology and Medicine. 4thEdn., *John Wiley and Sons Ltd*., Chichester, USA.

[15]. Taura D.W, Arzai A.H &Oyeyi T.I., (2009). Evaluation of antimicrobial activities of Combretummicranthum. L. *Bayero Journal of Pure and Applied Sciences*. 2(1):183-185.

[16]. Thomas J. Montville, Karl R. Matthews, Kalmia E. Kniel, (2012). Food Microbiology: an introduction 3rdEdn, ASM press, Washington, DC.

[17]. Thormberry A.I., (1983). Susceptibility tests and diffusion test animal faecal samples, Human septage, and surface water, procedures. *Journal of clinical pathology*. 19: 492-300. *Applied and Environmental Microbiology* 71(3): 394-404.

[18]. Ugueri U., Omeje F., Uloma I., &Oseiwe F., (2015). Phytochemical analysis of *Verona amygdalina* and *Ocimumgratissimum* extracts and their antibacterial activity on some drug-resistant bacteria.*American Journal of Research Communication*.

[19]. Wright G.D., (2007). The antibiotic resistome: the nexus of chemical and genetic diversity. *Nature reviews. Microbiology*. 5(3): 175-186.

[20]. Yvette J Johnson, Raida S Sayah, John B Kaneene, RoseAnn Miller, (2005). Patterns of antimicrobial resistance observed in *Escherichia coli* Isolates obtained from domestic and wild fecal samples, Human septage, and surface water, *Applied and Environmental Microbiology* 71(3): 394-404.