EKETE International Journal of Advanced Research
ISSN 3027-2513 (ONLINE)
ISSN 3027-169X (PRINT)

EKETE: International Journal of Advanced Research
Volume 2 Number 3, May 2024
url: https://journals.journalsplace.org/index.php/EKETE
Email: eketejournal@gmail.com

EVALUATION OF PLASMID PROFILES AND ANTIBACTERIAL ACTIVITY OF SOME PLANT EXTRACTS ON BACTERIA ISOLATES FROM DIARRHEA CHICKS

D. O. Uchendu

Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Afikpo, Ebonyi State, Nigeria.
damimadix@yahoo.com

Abstract

This study aims to evaluate the plasmid profiles and antimicrobial activity of some plant extracts on bacterial isolates from diarrhea chicks. A total fifteen diarrhea stool samples were collected randomly from poultry farm using sterile swab stick. Isolation and characterization were done using standard microbiological procedures. Ethanol, Methanol and Hot water were used as solvent of extraction on coffee seed, Moringa oleifera and scent leaves. Antibiotic resistance profile was determined by standard disk diffusion method and antibacterial activity of the plants extracts were determined using Agar well diffusion technique. While the plasmid isolation and profiling were carried out according to the manufactures instruction and DNA electrophoresis performed with 0.8% agarose gel. The result revealed the presence of Salmonella enteritids, Escherichia coli and Pseudomonas aeroginosa. Methanolic extract of scent leaves had the highest zones of inhibition of 16.655 ± 0.867mm on E. coli, Ethanolic extract of coffee seed had the highest zones of inhibition of 14.876 ± 0.766 mm on E coli whereas Methanolic extract of Moringa oleifera had the highest zones of inhibition of 18.675 ± 0.556 mm on P aeroginosa. The plasmid profiles showed that the two isolates of Escherichia coli had 6.8 kbp and 9.6kbp, three isolates of P. aeroginosa had 5.9kbp, 7.0kbp and 8.9kbp while S. enteritids had 8.9kbp. Antibiotic resistance pattern indicates that all the bacteria isolates had high resistances pattern on the antibiotic used. The results of this research shows that the three plants extracts used exhibited significant in vitro antimicrobial activity against the three isolates from chick diarrhea and can be formulated as an alternative antibiotic for chick diarrhea.

***Keywords**:* Diarrhea, Antibacterial, Plasmid profile, *Moringa oliefera* and Electrophoresis

ISSN: 3027-2513 (ONLINE)
ISSN: 3027-169X (PRINT)
***Ekete** - International Journal of Advanced Research* **Vol. 2 No. 3, May 2024**

INTRODUCTION

Chick diarrhea is a condition characterized by loose of watery stools, often accompanied by increased frequency and volume of defecation. In poultry, can be caused by various microorganisms, dietary imbalance and environmental stressors. There are several diseases of the gastrointestinal tract of farm animals in which diarrhea is major clinical finding. Bacterial agents causes diarrhea in chicks. It is one of the most common disease complexes which the large poultry clinicians have to contend with. It cause a significant economic loss in poultry industries. The utilization of antimicrobial drugs has played important role in poultry industries, as they have been used as prophylaxis, treatment and growth promotion (Abdellah *et al., 2009*). The extensive use of antibiotics in poultry industries especially in developing world, for prophylactic and growth promoting purposes, has generated much debate as for whether this practice contributes significantly to increase frequencies and dissemination of resistance genes into other ecosystem (Aderele, 1991).

Plants have been documented as one of the sources that possesses antimicrobial traits which are chiefly synthesized during secondary metabolism (Kokoska *et al., 2002;* Rusenova & Parvanov, 2009). Plant based antimicrobial compounds have great therapeutic potentials as they can serve the purpose without any side effects associated with synthetic drugs (El-Mahmood & Doughari, 2008). The inherent utility and practical application of plants extracts have been explored for improving poultry health as well as production with fruitful results (Miles *et al.,* 2006).

There have been several approaches to combat diarrhea in livestock among these are the resurgence of interest in the use of medicinal plants and this subject has received extensive coverage in many publication (El-Mahmood & Doughari, 2008).

Plasmids are extremely valuable tools in the field of molecular biology and genetics, specifically in the area of genetic engineering and the study is important to medical microbiology because plasmia can code genes for antibiotic resistance or virulence factors (Pitout *et al.,* 2009). Resistance have been carried out on plasmia or the chromosomes of resistant bacteria (Tanxe, 1999) plasmids have been a major factor in the spread of antibiotic resistance between bacteria (Davis, 1994).

MATERIALS AND METHODS

Sample Collection

Fifteen diarrhea stool samples from chicks were collected with a sterile swab stick moistened with sterile normal saline and were aseptically transported to laboratory for specific bacterial contents. The samples were aseptically streak on already prepared nutrient agar, MacConkey agar and SS agar. Plate were incubated at 37^{o}C for 24 hours. Distinct colonies were randomly selected and were streaked onto new plates until pure

cultures were obtained. The bacterial isolates were identified base on their cultural, morphological and biochemical characteristics.

Plants Extraction Preparations

The plant materials used are coffee seed *Moringa oleifera* leaf and scent leaf. The were collected from local farmers in Afikpo and authenticated by a botanist in the department. The samples were washed with xi a tap water, disinfected, rinsed with distilled water and finally dried in shade. The dried plant materials of each plant species was grounded into fine powder to pass 100 mm sieve. Five hundred grams (500g) of the fine powder each was inacerated with Ethanol, methanol and Hot water for 3 x 24 hours. The solvent extract was concentrated using a rotary evaporator to produce ten grams of different plant extract with ethanol, methanol and Hot water. The extract yields were weighed, stored in a storage bottle and kept in a fridge at 5^{o}C for further use.

Antibiotics Resistance Profile of the Isolates

Antibacterial activities for the isolate were done using standard dish diffusion method in Mueller Hilton Agar (MHA) as specifically by the National Committee for Clinical Laboratory Standards (NCCLS, 2001). All the isolates were tested for resistance to the following antibiotics (Oxoih England), Ampicillin (AMP) 10g, Ofloxacin (OFL) 5$^{\mu g}$, Cetlazidim (CAZ) (30$^{\mu g}$) Ciprofloxacine (CIP) 5$^{\mu g}$, Erythromycin. (ERY) (10$^{\mu g}$) Cephoperazone (CFP) 30$^{\mu g}$. Gentamycin (GEN) (10$^{\mu g}$), Cloxacine (CXC) 5$^{\mu g}$. Chloramphenical (C) 30$^{\mu g}$. Streptomycine (S) 10$^{\mu g}$, Amoxicillin (AUG) 30$^{\mu g}$, Ceftizotimc (ZOX) 30$^{\mu g}$, Tetracycline (TE) 10$^{\mu g}$, Cephalotin (KF) 30$^{\mu g}$, Canamycin (K) 30$^{\mu g}$. A turbility suspension of the isolates was made in nutrient broth using 0.5 Mcfarland standard as a comparator. A sterile wire loop was dipped into bacteria suspension and then used to evenly streak the entire surface of the already prepared media. Sterile forceps were then used to place the multiple antibiotic disk in a circular pattern on the media. A clear zones or rings were present around the antibiotic disc after incubation. (The zones of the inhibition were measured in (mm) and recorded. Interpretation of the zones of inhibitions was done using interpretative chart according to (CISI, 2014). The interpretation result, classify bacterial isolated either as resistant or susceptible.

Antibacterial Activity of the Plant Extracts

The plants extract was dissolved in DMO (Dimethyl Sulfoxide, Sigma-Aldrich to obtain extraction solution. A 24 hours old bacterial isolates were standardized against 0.5 McFarlanh Standard. Pour plate method was used and the media was perforated using a perforator, and 50 $^{\mu l}$ of each of the extract solution was put into different holes.

The plates were incubated at 37^{o}C for 24 hours. Zones of inhibition were measured and recorded, which was the result of inhibition of the bacterial growth by the plant

EKETE International Journal of Advanced Research
ISSN: 3027-2513 (ONLINE)
ISSN: 3027-169X (PRINT)
Ekete - International Journal of Advanced Research **Vol. 2 No. 3, May 2024**

extracts. The sensitivity was done three times and their mean were calculated. DMSO was used as a negative control while Amoxicillin was used as a positive control.

Plasmid Isolation and Profiling

Plasmid isolation was carried out according to the manufacturer instruction of plasmid miniprep kit, Zymogen Co. Ltd. UK.

Gel Electrophoresis; Electrophoresis of the DNA was carried out in a 0.8% agarose gel according to Bikandi (2004) procedure.

Result

Table 1: Characteristics of the bacterial isolated from chick diarrhoeal samples

Gram r x n, Cell shape			
Characters	***Salmonela enteritids***	***E.coli***	***P. aeroeginosa***
Pigmatation	Creamy, dull, transparent, entire, circular and raised	Opaque colones with deeper fellow colour	Green colonies with rough and spreed surface
Catalase	+	+	+
Oxidase	+	+	+
Citrate	+	-	+
Motility	+	+	+
Hydrogen sulphite production	-	-	-
Indole production	-	-	-
Gas production	+	+	_
Carbohydrate utilization			
Glucose	+	+	-
Lactose	+	+	-
Sucrose	+	+	-

ISSN: 3027-2513 (ONLINE)
ISSN: 3027-169X (PRINT)

Ekete *- International Journal of Advanced Research* **Vol. 2 No. 3, May 2024**

Table 2: Scent Leaf Extracts showing antibacterial activity on the bacteria isolates.

	Diameter of zones of inhibition in mm		
Extract Control	*Salmonella enteritids*	*E.coli*	*P. aeroginosa*
Ethanol	12.655±1.585	13.685±1.562	12.556±1.760
Methanol	15.657±1.576	16.655±0.867	14.462±1.574
Hot water	10.677±0.655	11.577±0.577	10.865±0.756
DSMo –ve control	Nil	Nil	Nil
Amoxicillin +v control	18.682±1.166	16.876±0.672	17.857±1.576

Table 3: Coffee seed extracts showing antibacterial activity on the bacterial isolates.

	Diameter of zones of inhibition in mm		
Extract Control	*Salmonella enteritids*	*E.coli*	*P. aeroginosa*
Ethanol	13.775±1.538	14.876±0.766	12.765±0.559
Methanol	13.668±0.588	12.587±1.752	12.556±1.675
Hot water	10.655±1.566	11.555±0.675	9.876±0.655
DSMo –ve control	Nil	Nil	Nil
Amoxicillin +v control	18.588±0.556	17.867±1.587	18.565±1.557

Table 4: *Moringa oleifera* leaf extracts showing antibacterial activity on the bacteria isolates.

	Diameter of zones of inhibition in mm		
Extract Control	*Salmonella enteritids*	*E.coli*	*P. aeroginosa*
Ethanol	13.577±1.555	14.867±1.676	14.567±1.685
Methanol	16.768±1.686	16.776±0.584	18.675±0.556
Hot water	12.755±0.583	10.558±1.675	12.586±1.674
DSMo –ve control	Nil	Nil	Nil
Amoxicillin +v control	20.857±1.677	20.776±1.682	19.876±0.5787

ISSN: 3027-2513 (ONLINE)
ISSN: 3027-169X (PRINT)
Ekete - International Journal of Advanced Research **Vol. 2 No. 3, May 2024**

Table 5: Plasmid profiles and resistance pattern of isolates form chick diarrhea samples

Isolates	Plasmid size	(cbp)	Resistance pattern
E-coli	2	6.8,9.6	OFL, AUG, CRX, CTR, CXC & ERY, I, AM
P. aeruginosa	3	5.7,7.0, 8.9	OFL, AUG, CRX, CTR, GEN, I, AM
Salmonella enteritids	1	8.9	CTR, CXC, GEN, AUG, ERY, I, AM

DISCUSSION

Poultry farming is one of the world's growing sources of meat. Chick diarrhea is a condition characterized by increased frequency and volume of defecation. in poultry can be caused by various microorganisms. This study evaluate the plasmid diarrhea profiles and antimicrobial activity of plant extracts on bacterial isolates from diarrhea stool samples from chicks. The morphological, biochemical and cultural characteristics carried out indicate the presence of *Escherichia coli, Salmonella enteritis* and *Pseudomonas aeruginosa* as shown in table 1. The antibiotic resistance pattern carried out using multiple antibiotic resistant drugs showed that the level of resistance exhibited by the isolated bacteria from diarrhea chick samples is alarming, *E-coli* is resistance to OFl, AUG, CRX, CTR, CXC, ERY, I, AM, and AM, *Salmonella enteritis* is resistance to OFL, AUG, CRX, CTR, GEN, AM, I and *P.aeruginosa* is also resistance to CTR, CXC, GEN, AUG, ERY, I, AM. This high level of resistance is an indication that indiscriminate use of conventional antibiotics has led to a steady increase in the antibiotic resistance and low income countries are mostly affected by this development (Radyowiacti & Haak, 2003). This resistance may be due to production of beta-lactamase enzymes, and the most common mechanism for resistance to cotrimozazoic is acquisition of plasmid 2 – encoded, variants diamino-pyrimidine folate reductose enzemes (WHO, 2001).The emergence of multidrug resistant strains and its varation over the years have been increased (Taneja *et al.,* 2004). The results of the crude extracts of the plants (coffee seed scent leaves and *Moringa olerifera* leaves) against *E-coli, S.enteritids* and *P. aeruginosa* isolates from chick diarrhoeal samples showed high leave of zones of inhibitions as shown in (tables 2, 3,and 4,).Methanolic extract of scent leaves had the highest zone diameter of 16.655 ± 0.867 mm against *E. coli* and the least was hot water extraction of scent leaves with 10.677 ± 0.655 mm against *S. enteritids.* According to Ogundara & Onifade (2009), the inhibition of establishment of *E. coli* by methanol extract of *M. lucida* in vitro and in vivo using agar well diffusion method and albino rats respectively, showed good antibacterial activity with 25mg/ml of the extract inhibited *E. coli* with a zone of inhibition measuring 5 mm. In a similar manner, Ndukwe *et al.*, (2005) reported appreciable activity of the aqueous root extract of *M. lucida* against *S. aureus, E.coli* and *P.aeruginosa*. All the solvents used for coffee seed extraction shows

appreciable diameter of zones of inhibition on the isolates and the highest was methanolic extract with 14.876±0.766 mm against *E. coli*. According to several authors, organic solvents like methanol and ethanol remains better extractants in antimicrobial substance compared to other solvent like water (Eloff, 1998). Methanolic extract of *Moringa oliefera* leaves had the highest zone diameter of inhibition of 18.675±0.556 mm against *P. aeroginosa*. Methanol was noted to be the best extractant for screening and isolation of antibacterial compounds from plants (Eloff, 2019). This is because methanol has high capacity to extract compounds with a wide range of polarity. This does not imply that other solvents are not equally useful, as results obtained from extracts made with ethanol, methanol and hot water were similarly promising.

The inherent utility and practical application of plant extracts such as coffee seed, scent leaves and *Moringa olifera* have been explored for improving the poultry health as well as production, with fruitful results, (Miles *et al., 2006*). The plasmid size of *E-coli* isolated are 6.8 kbp and 9.6 kbp, *P.aeruginosa* are 5.7 kbp, 7.0 kbp and 8.9 kbp while. S. e*nteritids* is 8.9 kbp. Plasmid profile analysis has been widley used in epidemiological investigations (Aderele, 1991). The findings of this research work suggest that the three plants extracts can be formulated to develop more efficacious drugs that can be therapeutic agent for the treatment of diarrhea in chicks caused by the isolated bacteria. Plants have many bio-active compounds which are potential medicinal agents, but of recent, there has been an increased, in the use of herbal medicines in the developing world (Mogal *et al.*, 2018). There are plenty of plants in traditional medicine which have protective and therapeutic properties (Alzohairy, 2016). Bacteria resistance of antibiotic is a major problem to poultry farmer and great efforts are been made to screen wide varieties of medicinal plants from the traditional system of medicine with the hope of getting some newer, safer and more effective agents that can be used to fight diarrhea in chick. (Paula *et al.*, 2019).

CONCLUSION/RECOMMENDATION

The selected plant extracts had varying antibacterial activity against *E.coli ,P. aeroginosa* and *S.enteritids* isolated from diarrhea chick samples. This indicates that the potency of these plant extracts as a candidates for formulation of antibacterial medication for the treatment of diarrhea in chicks that is associated with the bacterial isolates.

References

Abdellah, C., Fouzia, R. F., Abdelkader, C. Rachida, S. B., & Mouloud, Z. (2009). *African Journal of Microbiology Research*; *3*,5: 215-219.

Aderele, O. (1991). Medicinal plants; Policies and priorities. *Conservation of medicinal plants*. Great Britain Cambridge University Press, Cambridge.Pp 200-252.

Alzohairy, M. A. (2016). Therapentic role of *Azadirachta indica* (Neem) and their active constituents in diseases preventive and treatment Evid Based Complement Alternate Med.

Bikandi, J. R., Sanmilliian, A., Rementeria & Garaizar, J. (2004). In Silico analysis of complete bacterial genomes. PCR AFLP-PCR and endonuclease restriction. *Bioinformatics* 20, 798-799.

Clinical and Laboratory Standards Institute (CISI) (2014). Performance Standards for antimicrobial susceptibility Testing, Twenty-four informational supplement. CLSI document M100-S54, 34, 47-135.

Davis, J. (1994). Inactivation of Antibiotics and Dissemination of Resistance Genes. *Science* 26, 375-382.

El-Mahmood, A. M. & Doughari, J. H. (2008). Phytochemical screening and antibacterial evaluation of leaf and root extracts of *Cassia alata* Linn. *African Journal of Pharm. Phamacol* 2, 124-9.

Eloff, J.N.(2019). Avoiding pitfall in determining antimicrobial activity of plant extracts and publishing review*: BMC Complement Altem Med* : 19: 1-8

Eloff, J.N.(1998). Which extranctant should be used for the screening and isolation of antimicrobial components from plants. *J. Ethanopharmacol*, 60:1-8

Kokoska, L. Poleny, Z., Rada, V., Nepovim, A., Vanek, T. (2002). Screening of some Siberian Medicinal plants for antimicrobial activity. *Journal of Ethno-Pharmacol 82*, 51-53.

Miles, T. D., Mclaughlin, W., & Brown, P. D. (2006). Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and Human BMC *Vet Res* 2006: 21-19.

Mogal, M. A., Balam, A., & Anastasia, C. V. (2018). An overview of the anticancer activity of *Azadirachta indica* (Neem) in gynaecological cancers. *Int Mol. Sci 19* ,12, 3898.

National Committee for Clinical Laboratory Standard (2001). Eleventh informational Supplement M100, Vol. 21. Washington D. C. NCCLS, 2001. Performance standards for antimicrobial susceptibility testing; P. 123.

Ndukwe, K.C., Okeke, I.N., Lamikanra, A., Adesina,S.K. & Aboderin,O.(2005): Antibacterial activity of aqueous extracts of selected chewing stick. *J. Contemp Dent Pract*, 6: 86-94

Ogundara, A.O. & Onifade, A.I.(2009): The antimicrobial activity of *Morinda lucida* leaf extract on *E.coli. J. Med Plant Res*; 3: 319-323.

Paula, A. R., Riberio, A., Lemos, F. J. A., Silva, C. P. & Salmuels, R. I. (2019). Neem oil increases the persistence of the Enthromopathogenic Fungus *Metarhizium anisopliea* for the control of Aedes aegyptic (*Diptera culicidea*) Larvae. *Parasite Vetors 12*,1, 163.

Pitout, J. D. D., Daniel, B. G., Lorraine, C. & Kevin, B. L. (2009). Molecular characheristics of extended-spectrum-B-Lactamse-producing *Escherichia coli* isolates causing bacterium in the Calgary Health region from 2000 to 2007.

Emergence of done ST131 as a cause of community-acquired infection. *Antimicrobial Agents Chemotherupeutic 53*,7, 2846-2851.

Radyowiacti, A., & Haak, H. (2003). Improving antibiotic use in low-income countires. An overview of evidence on determinants. *Soc Sci Med* 57, 733-44.

Rusenova, N., Parvanov, P. (2009). Antimicrobial activities of twelve essential oils against microorganisms of veterinary importance. *Traka Journal of Science 7*, 37-43.4

Taneja, N., Mohan, B., & Khurana, S. (2004). Antimicrobial resistance in selected bacterial enteropathogens in North India. *Indian Journal Med. Res.* 120, 39-43.

Tanxe, R. (1999). *Salmonella enteritids* and *Salmonella typhimurium* DT 104. Successful subtypes in the modern world. In: Scheld, W. M. Craig, W. A. Hughes, J. M. Eds; Emerging Infections ASM Press, *Washington* 37-52.

Who Health Organisation (2001). Antimicrobials resistance in *Shigellosis*, *Cholera* and *Campylobacteriosis*. Geneva. 2001. WHO/CDS/ESR/DRS.