IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 16, Issue 6 Ser. IV (Nov. –Dec. 2021), PP 25-29
www.Iosrjournals.Org

Antimicrobial Assay of Methanolic Extract of Selected Plants on Antibiotic-Resistant Salmonella Spp.

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Abstract
Background: *In this study, presumed* Salmonella spp *isolates were collected from Adeoye Hospital sourced from stool, urine, wound, high virginal swab, and semen samples.*
Materials and Methods: *Biochemical tests were performed for identification & confirmation. The confirmed isolates were subjected to Antimicrobial Susceptibility Test (AST) using eight antibiotics and their sensitivity was recorded*
Results*: Nitrofurantoin (92%), Ofloxacin (80%), Ciprofloxacin (88%), Augmentin (72%), Gentamicin (56%), Cefuroxime (60%), and Cefixime (44%). Multiple antibiotic indexes were measured and 20% of the isolates showed resistance to five and above different antibiotics. The multidrug-resistant isolates were subjected to 50mg/ml, 75mg/ml & 100mg/ml concentrations of methanolic extracts of five selected medicinal plants to determine the minimum inhibitory concentration (MIC). Susceptibility of the multidrug-resistance isolates to the extracts was in the order Allium sativum> Vernonia amygdalina> Zingiber officinale> Azadirachta indica> Ocimum gratissimum.*
Conclusion: *This study has shown that the selected plant extracts have antimicrobial compounds which makes them effective against phenotypically confirmed multidrug-resistant* salmonella *isolates and hence can be used to produce drugs with a better mode of action.*
Keywords: *Salmonella spp., Antimicrobial Resistance, Multi-drug Resistance, Antimicrobial Susceptibility Testing.*

Date of Submission: 20-12-2021 Date of Acceptance: 31-12-2021

I. Introduction

Antimicrobial Resistance (AMR) occurs when bacteria, viruses, fungi, and parasites change over time and no longer respond to drugs. Resistance to antibiotic tends to make infections difficult to treat, increases the risk of disease spread, and most importantly, add to the severity of illness which could then lead to death. As a result of drug resistance, antibiotics and many other antimicrobial compounds are becoming less effective and infections caused by superbugs become increasingly difficult or impossible to treat (Quetin-Leclercq *et al*., 2007). Currently, the whole world is not only battling with the emergence and spread of drug-resistant microbes but also pathogens that have evolved by acquiring various resistance genes, leading to antimicrobial resistance. Pathogens having the ability to resist several antimicrobials continue to threaten our public health (Ohunayo *et al*., 2021). Salmonellosis is an infection of the digestive tract caused by a gram-negative bacterium- *Salmonella enterica*. The organism is taught to be widespread, found in many dairy products and on many species of animals, including mammals, birds, insects, reptiles, and humans (Oliveira *et al*., 2006). This bacterium usually infects animals, with a suppressed immune system or when it is exposed to high doses of the organism. The resistance genes found in *Salmonella* are closely related or even indistinguishable from those found in other bacteria. Problems caused by the increasing resistance include not only apparent difficulties in antimicrobial therapy but also the tendency of the organism to cause serious diseases (Burt *et al*., 2007). An investigation of the molecular evolution of multi-resistance in nontyphoid salmonellae has shown that progressive acquisition and accumulation of plasmid-mediated resistance determinants arose as a result of the exchange of plasmids and other mobile elements between *Salmonella* and other members of the family *Enterobacteriaceae* (Delhalle *et al*., 2009). The term "***medicinal plant***" includes various types of plants used in *orthodox medicine;* the use of plants for medicinal purposes, and the study of such uses. Plants have been useful to man for food and medicinal purposes long before the prehistoric period (Karns *et al*., 2005). Various studies have shown that some of these

plants which include; Ginger, Garlic, Scent leaves, Neem tree parts, and so on, are rich sources of antioxidants (phenolics, carotenoids, anthocyanins, and tocopherols). These antioxidants control and reduce the oxidative damage in foods by delaying or inhibiting oxidation caused by reactive oxygen species (ROS), ultimately increasing the shelf-life and quality of these foods. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables. Beta carotene, ascorbic acid, and many phenolics play dynamic roles in delaying ageing, reducing inflammation, preventing certain cancers and also capable of inhibiting the growth of microorganisms (Quetin-Leclercq *et al*., 2007). This study aims to investigate the antimicrobial assay of methanolic extract of scent leaf, ginger, neem and garlic on antibiotics resistant *salmonella species.*

II. Materials And Method

Collection and identification of samples

The samples used for this research are clinical isolates collected randomly from patients between March-April, 2021 at Ade Oyo hospital, Ibadan, Nigeria. The isolates were collected in an enrichment media-Selenite F broth and transported to the Microbiology laboratory, Ekiti State University. The cultures from the transport media were inoculated into Brilliant green agar (BGA) while typical colonies of *Salmonella* spp. were subcultured on MacConkey agar to obtain a pure culture, all cultures and subcultures were done at 37^{o} C for 24 hours. Identification of colonies were done based on morphological, cultural, gram staining and a series of biochemical tests.

Antimicrobial Susceptibility Testing.

Susceptibility testing was done using the Kirby-Bauer procedure. Briefly, an inoculum of 0.5 MacFaland standards was spread aseptically on Mueller-Hinton agar and antibiotics motor disc containing eight different antibiotics (CAZ- Ceftazidime 30ug, CRS- Cefuroxime 30ug, GEN- Gentamicin 10ug, CMX-Cefixime 5ug, OFL- Ofloxacin 5ug, AUG- Augmentin 30ug, NIT- Nitrofurantoin 300ug, CPR- Ciprofloxacin 5ug) were cultured at 370C for 18-20 hours. Measurement and interpretation were done following the guidelines of the Clinical and Laboratory Standard Institute (CLSI)

Minimum Inhibitory Concentration (MIC) determination.

Plant extraction procedures and Minimum inhibitory concentration was done following the methods of Taura and Oyeyi, 2009, and Akande and Hayashi, 1998 respectively. Briefly, the extracts were diluted to obtain 50mg/ml, 75mg/ml, and 100mg/ml. The antimicrobial activity of the plant extracts was determined using the agar well diffusion method.

III. Results

All the 25 biochemically confirmed *Salmonella spp*. were subject to 8 different types of antibiotics, the result of the AST are presented in figure1. In this study, nitrofurantoin, ciprofloxacin and ofloxacin were the most active of all the 8 antibiotics used with 92%, 88% and 80% sensitivity respectively. Only 5 out of 25 showed resistance to 5 or more different antibiotics. In Tables 1 to 3, the activities of methanolic extracts of five selected plants (*Allium sativum, Vernonia amygdalina, Zingiber officinale, Azadirachta indica, Ocimum gratissimum*) were examined on the 5 phenotypically confirmed multidrug-resistant isolates. In table 1, the assay of methanolic extracts of these plants was examined at 50mg/ml, while table 2 and table 3 shows the assay results at 75mg/ml and 100mg/ml respectively. In all of those experiments, Gentamicin was used as a control.

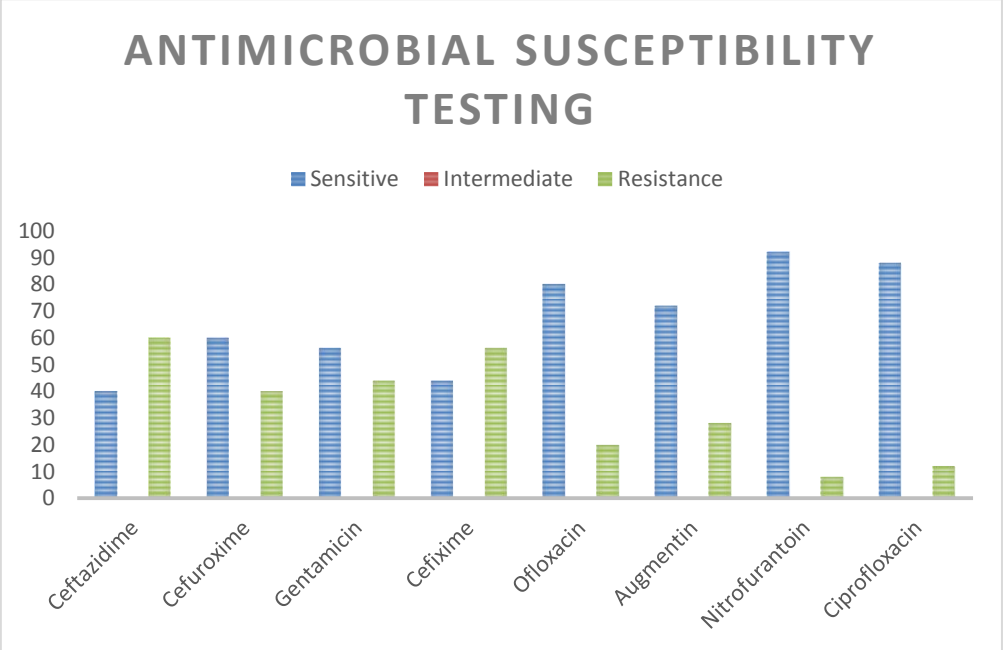

Figure 1: Antibiogram of Salmonella spp. isolates

TABLE 1: Antimicrobial assay of the selected plants at 50mg/ml

		Zone of Inhibition (mm) at 50.0 mg/ml conc. (METHANOLIC PLANT EXTRACTS)						
Multidrug resistant isolates	Source	*Gentamicin 10µg (positive control)*	*Methanol 10% conc (negative control)*	*Allium sativum (Garlic)*	*Ocimum gratissium (Scent leaves)*	*Zingiber officinale (Ginger*	*Azadirachta indica (Neem leaf)*	*Vernonia amygdalina (Bitter leaf)*
SM 001	Stool	16.00	-	4.00	3.20	3.21	3.20	3.50
SM 002	Stool	20.00	-	4.45	3.10	3.25	4.00	3.55
SM 003	Urine	17.00	-	4.25	3.00	3.41	3.31	3.50
SM 004	Stool	14.00	-	4.01	3.01	3.45	3.25	4.00
SM 005	Stool	18.00	-	4.20	3.05	3.43	4.10	4.12

TABLE 2: Antimicrobial assay of the selected plants at 75mg/ml

		Zone of Inhibition (mm) at 75mg/ml conc. (METHANOLIC PLANT EXTRACTS)						
Multidrug resistant isolates	source	*Gentamicin 10µg (positive control)*	*Methanol 10% conc (negative control)*	*Allium sativum (Garlic)*	*Ocimum gratissium (Scent leaves)*	*Zingiber officinale (Ginger*	*Azadirachta indica (Neem leaf)*	*Vernonia amygdalina (Bitter leaf)*
SM 001	Stool	16.00	-	5.00	4.15	4.02	4.00	5.50
SM 002	Stool	20.00	-	5.25	4.12	4.22	4.01	4.41
SM 003	Urine	17.	-	5.42	4.10	4.25	4.05	5.02
SM 004	Stool	14	-	5.31	4.09	4.01	4.07	4.50
SM 005	Stool	18	-	5.05	4.06	4.00	4.10	4.52

Table 3: Antimicrobial assay of the selected plants at 100mg/ml

		Zone of Inhibition (mm) at 100 mg/ml conc. (METHANOLIC PLANT EXTRACTS)						
Multidrug resistant isolates	Source	*Gentamicin 10µg (positive control)*	*Methanol 10% conc (negative control)*	*Allium sativum (Garlic)*	*Ocimum gratissium (Scent leaves)*	*Zingiber officinale (Ginger*	*Azadirachta indica (Neem leaf)*	*Vernonia amygdalina (Bitter leaf)*
SM 001	Stool	16	-	7.00	6.12	5.01	5.50	7.20
SM 002	Stool	20	-	6.69	6.10	5.50	5.00	7.01
SM 003	Urine	17	-	6.50	6.09	5.06	5.05	5.60
SM 004	Stool	14	-	7.20	6.04	5.80	5.01	5.45

SM 005	Stool	18	-	7.25	6.51	5.42	5.02	6.01

IV. Discussion

The incidence of salmonellosis is often accomplished with greenish stool, this infection is mostly caused by the consumption of food or drinking of water contaminated by faeces. Urinary tract infection can be caused by nontyphoidal salmonella is uncommon but carries a high risk of relapse and mortality (Chen *et al*., 2012). Studies on the 25 biochemically confirmed isolates showed 85% of the isolates were recovered from stool samples. This is because *salmonella spp* are predominant in stool but the incidence of *Salmonella spp* have also been associated with blood, urine and in more rare cases, the wound has also been implicated.

In this study, Nitrofurantoin had the highest activity with 92% of the isolates being sensitive, closely followed by Ciprofloxacin (88%), Ofloxacin (80%), Augmentin (72%), Gentamicin (56%), Cefuroxime (60%), Cefixime (44%), Ceftazidime (40%) had the lowest activity. This result is similar to the study of Mijovic *et.al*., (2012) where most isolates were sensitive to Nitrofurantoin but contrary to Maharjan *et al.* (2021*) in the study conducted in* 2021 The highest rate of antibiotic susceptibility was observed towards cefixime (100%) followed by azithromycin (97.5%) and ceftriaxone (95%), whereas the lowest rate of susceptibility was observed towards nalidixic acid (12.5%), followed by ofloxacin (15%) and levofloxacin (20%). However, this result is similar to the study of Adabara *et al.* (2012), which shows that ofloxacin and ciprofloxacin are strongly sensitive even though ofloxacin and ciprofloxacin antibiotics are sometimes ineffective against resistant strains of *Salmonella typhi*.

As cases of antimicrobial resistance threaten our public health, shreds of evidence are also shown in this study where 32% of the isolates were resistant to one antibiotic, 4% were also resistant to two different antibiotics, 4% were resistant to three different antibiotics, 16% were resistant to four different antibiotics, another 16% were resistance to five different antibiotics, 4% were resistant to six different antibiotics while none were resistant to seven and eight antibiotics. From the aforementioned results, it can be deduced that only 5 isolates showed resistance to 5 and above different antibiotics. Among the five phenotypically confirmed multi-drug resistance isolates, four were isolated from stool samples, and one was isolated from urine samples. The results are consistent with the fact that Salmonellosis is a food and water-borne infection, this disease cannot be under-emphasized due to poor hygiene status and misuse of antibiotics.

The five phenotypically confirmed multidrug resistance was subjected to varying concentrations (50, 75 and 100mg/ml) of five different methanolic extract plants which were; *Allium sativum, Vernonia amygdalina, Zingiber officinale, Azadirachta indica, Ocimum gratissimum.* The results showed that the multidrug-resistant isolates were susceptible to all the plant extracts although, the activity of individual plant extracts on the test organism increases as concentration is being increased. It is important to note that across all plant extracts at a concentration of 50mg/ml, produced zones of inhibition ranging between 3-4mm. At 75mg/ml concentration, the zones of inhibition increased, ranging between 4-5mm and further increased to (5-7mm) at 100mg/ml concentration. However, the activity of the five plants is in the following order; *Allium sativum> Vernonia amygdalina> Zingiber officinale> Azadirachta indica> Ocimum gratissimum.*

Plants and other natural products have abundant phytochemicals known to inhibit pathogen. Over the years, plants have been greatly explored to treat many human ailments and to also manage some infectious diseases (Ohunayo *et al.*, 2020).

V. Conclusion

Antimicrobial resistance is a public health concern, hence sourcing for compounds or chemicals with high potency and little or no toxicity cannot be overemphasized. This study has shown how reliable medicinal plants and other natural products can be if explored.

References

[1]. Adabara NU., Ezugwu BU, Momojimoh A, Madzu A, Hashiimu Z, Damisa D. The Prevalence and Antibiotic Susceptibility Pattern of Salmonella typhi among Patients Attending a Military Hospital in Minna, Nigeria", Advances in Preventive Medicine, 2012, (4, Article 875419)

[2]. Akande JA and Hayashi Y. Potency of extract contents from selected tropical chewingsticks against Staphylococcus aureus and Staphylococcus auricularis. World Journal of Microbiology and Biotechnology. 1998; 14: 235-238.

[3]. Burt SA, Fledderman MJ, Haagsman HP, van Knapen F, Veldhuizen EJ. Inhibition of Salmonella enterica serotype Enteritidis on agar and raw chicken by carvacrol vapour. Int J Food Microbiol. 2007 ;119(3):346-50. doi: 10.1016/j.ijfoodmicro.2007.05.002.

[4]. Chen CP, Shi ZY, Chen CH, Chen WM, Lin YH, Tsai CA, Lin SP, Huang SR, Liu PY. Nontyphoidal salmonella urinary tract infection in a case of hyperparathyroidism and nephrocalcinosis. West Indian Med J. 2014 ;63(1):94-7. doi: 10.7727/wimj.2012.241.

[5]. de Oliveira FA, Brandelli A, Tondo EC. Antimicrobial resistance in Salmonella enteritidis from foods involved in human salmonellosis outbreaks in southern Brazil. New Microbiol. 2006 ;29(1):49-54. PMID: 16608125.

[6]. Delhalle L, Saegerman C, Farnir F, Korsak N, Maes D, Messens W, De Sadeleer L, De Zutter L, Daube G. Salmonella surveillance and control at post-harvest in the Belgian pork meat chain. Food Microbiol. 2009;26(3):265-71. doi: 10.1016/j.fm.2008.12.009. Epub 2009 Jan 7. PMID: 19269567.

[7]. Karns JS, Van Kessel JS, McCluskey BJ, Perdue ML. Prevalence of Salmonella enterica in Bulk Tank Milk from US Dairies as Determined by Polymerase Chain Reaction, Journal of Dairy Science, Vol 88, Issue 10, 2005, Pages 3475-3479, ISSN 0022-0302, https://doi.org/10.3168/jds.S0022-0302(05)73031-9.
[8]. Maharjan A, Dhungel B, Bastola A, Thapa Shrestha U, Adhikari N, Banjara MR, Lekhak B, Ghimire P, and Rijal KR. Antimicrobial Susceptibility Pattern of Salmonella spp. Isolated from Enteric Fever Patients in Nepal. Infectious disease reports. 2021. 13(2), 388–400. https://doi.org/10.3390/idr13020037
[9]. Mijović G, Andrić B, Terzić D, Lopičić M, Dupanović B. Antibiotic susceptibility of *Salmonella spp*.: a comparison of two surveys with a 5 years interval. J of IMAB. 2012; 18(1):216-219. doi: 10.5272/jimab.2012181.216
[10]. Ohunayo AS, Adekeye D, Dauda O, Odeniyi I , Popoola O, and Akinwunmi O. Microbial Profile of the Phyllosphere and the Antimicrobial potency of Ficus vogelii extracts. J. Pharm. Sci. & Res. Vol. 2020; 12(1):191-195
[11]. Ohunayo AS, **John-Mese OJ**, Dauda OS, Oyinlade PO, and Afolabi AT. Antimicrobial assay of methanolic extracts of selected plants on multiple antibiotics resistance Escherichia coli. Journal of research in pharmaceutical science. 2021; 7(9): 7-12
[12]. Quetin-Leclercq J, Gurib-Fakim A, and Subratty AH. Screening for Anti-infective Properties of Several Medicinal Plants of the Mauritians flora. Journal of Ethnopharmacology. 2007; (109): 331–337
[13]. Taura DW and Oyeyi T I. Prevalence of Malarial parasites in pregnant women attending Sir Muhammad Sunusi Specialist Hospital, Kano, Nigeria. Bayero Journal of Pure and Applied Sciences. 2009; 2(1):186 – 188.

Adeniyi Ohunayo, et. al. "Antimicrobial Assay of Methanolic Extract of Selected Plants on Antibiotic-Resistant Salmonella Spp.*" IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 16(6), (2021): pp. 25-29.