International Letters of Natural Sciences
ISSN: 2300-9675, Vol. 56, pp 82-89
doi:10.18052/www.scipress.com/ILNS.56.82

Submitted: 2016-03-26
Revised: 2016-06-06
Accepted: 2016-06-16
Online: 2016-07-08

Antibacterial Potentials of V*ernonia amygdalina* against Antibiotic-Resistant *Salmonella* Specie Isolated from Nworie River, Imo State, Nigeria

Chikwendu, Chinwe I. [1,a], Egbadon, Emmanuel O. [1,b*], Okuma, Blessing [1,c]

[1]Department of Microbiology, Federal University of Technology, Owerri, Nigeria

[a]chinwechikwendu@yahoo.com, [b]emmanuelegbadon@gmail.com*, [c]okumablessing@yahoo.com

*Corresponding author

Keywords: Antibiotic-resistant *Salmonella* species, *Vernonia amygdalina*, Nworie River

ABSTRACT. *Salmonella* species were isolated from Nworie River and then tested against several antibiotics to include; Gentamycin, Streptomycin, Amoxycillin, Kanamycin, Oxacillin, Ofloxacin, Imipenem, Ciprofloxacin, Aztreonam and Ceftazidime. *Salmonella* isolates obtained from the river were resistant to at least three different antibiotics. All isolates were resistant to Amoxycillin, Oxacillin and Iminipem showing a 100% resistant rate, while showing sensitivity to Ciprofloxacin, Aztreonam, Gentamycin, Ceftazidime, Kanamycin, Streptomycin, and Ofloxacin at sensitivity rates of 71.4%, 7.1%, 77.1%, 11.4%, 44.3%, 48.6% and 78.6% respectively. The antibiotic-resistant *Salmonella* species were further tested against the aqueous, ethanol and acetone extracts of *Vernonia amygdalina* leaf, stem and roots to assess their antibacterial potential at a concentration of 100mg/ml. Aqueous extracts of the leaf, stem and roots showed no activity against antibiotic resistant *Salmonella* isolate, while the ethanol and acetone extracts showed activity rates of 20% and 17% for roots, 14.3% and 12.9% for stem, and, 15.7% and 11.4% for leaf. The results from this study further confirms the antibacterial potentials of *Vernonia amygdalina* against antibiotic-resistant bacterial isolates.

INTRODUCTION

Salmonella species are ubiquitous enteric bacteria [1]. They are one of the most significant pathogens that affect the health of most people [2]. *Salmonella* is a leading cause of many gastroenteritis such as diarrhea in many countries and remains an important public health problem worldwide especially in the developing countries [3]. Such disease like diarrhea caused by *Salmonella* is responsible for high rates of morbidity and mortality in most developing countries [4]. *Salmonella* infections are considered one of the most widely spread zoonotic infections in most developed and developing countries [5]. *Salmonella*e have also been reported to be present in aquatic environments. The presence of *Salmonella* and other enteric microorganisms in aquatic environments can be a source of disease when water is used for drinking, recreational activities or irrigation purposes [6]. Water-borne diseases pose serious health risks which could result in consequences of economic value in many parts of the world. This public health concern is increased if the pathogenic strain of *Salmonella*e in the aquatic environments are resistant to antibiotics. Antibiotic resistant strains of *Salmonella* have been expanding in recent years, showing resistance to tetracyclines, sulfonamides and chloramphenicols [7]. These resistant bacteria can transfer the genes responsible for antibiotic resistance to other bacteria through lateral gene transfer when such resistant genes are carried on mobile genetic elements such as plasmids [8]. The recent trend in the increase in the rate of antibiotic resistance has intensified efforts by researchers to search for alternative sources of antibacterial agents.

Medicinal plants are known to contain certain physiologically active compounds which over the years have found great use in the treatment of a wide variety of ailments [9]. These medicinal plants have also been used as precursors for the synthesis of useful drugs [10]. Various plants have been used in the treatment of various diseases. These plants have little or no side effects in the treatment of diseases because they act as food and also as medicines [11]. The plant *Vernonia amygdalina* is

locally known as bitter leaf due to its characteristic bitter taste and flavor. It is known to contain biologically active compounds that have been shown to exhibit antibacterial, antifungal and anticancer properties [12,13]. Traditionally in Africa, stem and roots of some plants are used in cleaning teeth by chewing them into brush-like ends, while the leaves are generally used for cooking. Research studies have shown that these stems and roots have active ingredients that possess antimicrobial activity against some microorganisms [10]. This study therefore aims to study the antibacterial activity of the stem, root and leaves of *Vernonia amygdalina* against *Salmonella* isolates that are resistant to at least two antibiotics.

MATERIALS AND METHODS

Study Area

The study area was the Naze end of Nworie River, Owerri Local government area of Imo state, Southeastern Nigeria. This river is subjected to intensive human and industrial activities such as dumping of solid and liquid wastes resulting in the discharge of a wide range of pollutants into the water body. This river also serves for various domestic as well as economic activities by the lower segment of the inhabitants of Owerri.

Water Sample Collection

Three sampling points were chosen along the longitudinal stretch of the Nworie River viz: upstream, midstream and downstream. A total of three samples were collected on each sampling day, one sample from each point. The sampling was done during the month of June, 2014, which fell within the rainy season in Nigeria. Sample collection was done aseptically using clean sterile plastic containers. These sample bottles were immersed below the water surface, filled to overflowing, and the cap affixed securely to eliminate contamination. The water samples were transported immediately to the laboratory and analyzed within one hour of collection.

Culturing and Isolation of Bacterial isolates

Water samples from Nworie River were serial diluted and 0.1ml of the sample were transferred into *Salmonella*-Shigella agar (SSA) to adequately isolate *Salmonella* isolates. The plates were incubated in an inverted position at 37^{o}C for 24 hours and colonies were further subjected to biochemical and morphological characterization to confirm *Salmonella* sp. present in the sample. After identification, the bacterial isolates were picked and further sub cultured onto freshly prepared nutrient agar plates for purification. They were further incubated for 24 hours at 37^{o}C after which they were stored on agar slants and stored at a temperature of 4^{o}C until required for use.

Collection of plant materials

Fresh plants of *Vernonia amygdalina* were collected from Ihiagwa in Owerri-West local Government Area, Imo state. The leaves were collected by hand plucking from plant and cleaned of debris, the stems cut from the plant and the root uprooted from the ground and washed.

Sterilization of Plant Materials

All glassware were washed and dried before sterilization in a hot air oven at 170°C for 2 hours. Each of the powdered plant materials were wrapped with aluminum foil before sterilization. The distilled water to be used was also sterilized in the autoclave at 121°C for 15mins.

Preparation of Plant Materials

The collected plants were each processed according to the methods of [14]. The leaves, stem and roots were air dried at room temperature for 14 days. The dried leaves, stem and roots were then blended separately using a manual blender. Powdered samples were then stored in tightly closed reagent bottles for subsequent extraction and assay.

Preparation and Reconstitution of Extracts

Ten gram each of *Vernonia amygdalina* parts (leaves, stem and roots) were soaked in 100 mls of water, ethanol and acetone and allowed to stand for 48 hours at room temperature after thorough

shaking. Each mixture was filtered using Whatman No.1 filter paper. Filtrates from aqueous, ethanol and acetone extracts of the plants were further concentrated in vacuum using rotary evaporator. All the aqueous, ethanol and acetone extracts of the plant extracts were stored in sample bottles at 4^{o}C prior to use. The extracts were further reconstituted with Dimethyl-Sulphoxide (DMSO) to get a stock of 100mg/ml

Sterility Testing of the Plant Extracts
All the plant extracts were tested for sterility. One (1) ml of each extract was added into a test tube containing 5mls of sterile nutrient broth. They were then incubated at 37^{o}C for 24 hours. The tubes were clear after incubation, indicating the absence of microbial contaminants which would have caused turbid appearance in the tubes. Samples were further cultured on solid nutrient agar and incubated for 24 hours. No visible colonies were observed which further indicated the absence of contamination.

Phytochemical Screening of Plant Extracts
The leaf, stem and root extracts of *Vernonia amygdalina* were subjected to various standard phytochemical analysis to identify the chemical constituents present in the plant parts. The plants were screened for Tannins, Saponins, Flavonoids, Cardiac glycosides, Alkaloids and Cyanogenic glycosides according to methods described by [15].

Antibiotic Susceptibility Testing
Antibiotic susceptibility testing was carried out on the isolated *Salmonella* sp. Ten antibiotics were screened using disc diffusion method on Mueller-Hinton Agar. Prepared standardized *Salmonella* inocula that had been adjusted to match a 0.5 MacFarland standard were used as the source of the inoculum. A sterile cotton swab was dipped into the inocula, rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess fluid. The inocula were gently spread on the surface of the prepared Mueller-Hinton Agar. On completion of the inoculation, sterile forceps were used to dispense the single discs onto the Mueller-Hinton agar surface with the disc making complete contact with the agar surface by touching the top of the discs with the forceps. The plates were subsequently incubated at 35^{o}C for 24 hours. After incubation, zones of inhibition were measured using a transparent millimeter (mm) rule. The standard zones of inhibition interpretation chart [16] was used to interpret the sizes of inhibition. The discs used for the susceptibility testing included; Gentamycin, Streptomycin, Amoxycillin, Kanamycin, Oxacillin, Ofloxacin, Imipenem, Ciprofloxacin, Aztreonam and Ceftazidime.

Antibacterial Susceptibility Testing of Plant Extracts
The different extracts were tested against the *Salmonella* isolates that showed resistance to at least two antibiotics using the disc diffusion method by [17]. Each disc of approximately 5mm in diameter were cut from Whatman No. 1 filter paper. The discs were put into a petri dish and then sterilized in a hot air oven at 170^{o}C for 2 hours. The discs were then impregnated with the reconstituted extracts by soaking in the extract for 24 hours. Each of the disc contained approximately 100mg/ml of the aqueous, ethanol and acetone extracts. Standardized culture of *Salmonella* prepared to match a 0.5 MacFarland turdidity standard of up to 1ml were introduced on to the surface of Mueller-Hinton agar plates. They were evenly distributed using a sterile cotton swab stick. Impregnated discs were removed from the reconstituted extracts using a sterile forceps and then applied aseptically to the surface of the prepared Mueller-Hinton agar plates. The plates were then incubated at 35^{o}C for 24 hours. Zones of inhibition were measured using a transparent millimeter (mm) ruler after incubation.

RESULTS

Frequency (%) of Antibiotic-resistance of *Salmonella* specie isolated from Nworie River.
A total of Seventy (70) *Salmonella* specie isolated from Nworie River were tested for antimicrobial sensitivity with Gentamycin, Streptomycin, Amoxycillin, Kanamycin, Oxacillin, Ofloxacin,

Imipenem, Ciprofloxacin, Aztreonam and Ceftazidime. All isolates showed resistance to at least one antibiotic used. Of the total isolates, 70 (100%) were resistant to Oxacillin, Imipenem and Amoxycillin. Similarlry, 65 (92.9%), 62 (88.6%), 40 (55.7%) and 36 (51.4%) were resistant to Aztreonem, Ceftazidime, Kanamycin, and Streptomycin respectively. However, Ciprofloxacin, Gentamycin and Ofloxacin displayed better performing index with 20 (28.6%), 16 (22.9%) and 15 (21.4%) *Salmonella* isolates showing resistance respectively (Table 1)

Table 1: Frequency (%) of Antibiotic-resistance of *Salmonella* species isolated from Nworie River

Antibiotics	Resistance Rates (%)
Amoxicillin	70 (100%)
Imipenem	70 (100%)
Oxacillin	70 (100%)
Aztreonem	65 (92.9%)
Ceftazidine	62 (88.6%)
Kanamycin	40 (55.7%)
Streptomycin	36 (51.4%)
Ciprofloxacin	20 (28.6%)
Gentamycin	16 (22.9%)
Ofloxacin	15 (21.4%)

Table 2: Multiple Antibiotic-resistant patterns of *Salmonella* isolates

S/N	ANTIBIOTICS PATTERN									NO. OF ISOLATES
1.	ATM	OX	CAZ	IMP	K	A				4
2.	ATM	OX	CAZ	IMP	K	S	A			14
3.	CIP	ATM	OX	CAZ	IMP	OFX	K	S	A	1
4.	ATM	OX	CAZ	IMP	S	A				4
5.	CIP	ATM	OX	CAZ	IMP	S	A			3
6.	ATM	GN	OX	CAZ	IMP	S	A			1
7.	ATM	OX	CAZ	IMP	A					1
8.	ATM	GN	OX	CAZ	IMP	K	A			3
9.	ATM	OX	CAZ	IMP	A					5
10.	ATM	GN	OX	CAZ	IMP	K	S	A		6
11.	OX	CAZ	IMP	K	S	A				1
12.	ATM	OX	IMP	K	A					1
13.	CIP	ATM	OX	IMP	OFX	K	S	A		1
14.	CIP	OX	CAZ	IMP	A					2
15.	ATM	OX	IMP	S	A				A	3
16.	ATM	GN	OX	CAZ	IMP	OFX	K	S		1
17.	CIP	ATM	OX	CAZ	S	A				1
18.	CIP	OX	IMP	A						1
19.	CIP	ATM	GN	OX	CAZ	IMP	A	A		2
20.	CIP	ATM	OX	CAZ	IMP	K	S			2
21.	ATM	OX	CAZ	IMP	OFX	S	A	A		1
22.	CIP	ATM	OX	CAZ	IMP	OFX	S	A		1
23.	CIP	ATM	OX	CAZ	IMP	OFX	K			2
24.	ATM	OX	CAZ	IMP	OFX	K	A			1
25.	CIP	ATM	OX	CAZ	IMP	OFX	A			1
26.	ATM	GN	OX	CAZ	IMP	OFX	A			1
27.	CIP	ATM	GN	OX	IMP	OFX	K			1
28.	CIP	ATM	OX	CAZ	IMP	K	A			1
29.	CIP	OX	IMP	OFX	A					1
30.	CIP	OX	IMP	OFX	K	GN	A			1
31.	ATM	OX	IMP	OFX	A					2

KEY: GN-Gentamycin, S-Streptomycin, A-Amoxycillin, K-Kanamycin, OX-Oxacillin, OFX-Ofloxacin, IMP-Imipenem, CIP-Ciprofloxacin, ATM-Aztreonam and CAZ-Ceftazidime

Multiple resistant patterns of *Salmonella* to antibiotics

Thirty-one (31) resistant patterns were displayed by the *Salmonella* isolated from Nworie River with Aztreonem (ATM), Oxacillin (OX), Ceftazidine (CAZ), impenem (IMP), Kanamycin (K), Streptomycin (S) and Amoxycillin (A) pattern having the highest frequency having been exhibited by the 14 isolates as shown in the Table 2 below

Phytochemical contents of Leaf, Stem and Root of *Vernonia amygdalina*

Table 3 shows the results of the phytochemical screening of leaves, stem and roots of *Vernonia amygdalina*. The phytochemical component screened included Tannins, Saponins, Alkaloids, Flavonoids, Cyanogenic glycosides and Cardiac glycosides. The root and leaves possessed all the bioactive compounds tested while the stem possessed all with the exception of Flavonoids.

Table 3: Phytochemical Properties of *Vernonia amygdalina*

Phytochemical components	Leaves	Stem	Root
Tannins	+	+	+
Saponnins	+	+	+
Alkaloids	+	+	+
Flavonoids	+	-	+
Cardiac glycosides	+	+	+
Cyanogenic glycosides	+	+	+

Key: + = Present; - = Absent

Antibacterial Sensitivity of Aqueous, Ethanol and Acetone Extracts of *Vernonia amygdalina*

The *Salmonella* isolates were used to test the efficacy of *Vernonia amygdalina* using the disc diffusion method. Ethanol and Acetone extracts showed zones of inhibition against *Salmonella* isolates, but the aqueous extracts of the plant did not show any activity against the isolates. Of the 70 isolates tested, 14 (20%), 10 (14.3%) and 11(15.7%) were sensitive to ethanol extracts obtained from the roots, stem and leaf while, 12 (17%), 9 (12.9%) and 8 (11.4%) were sensitive to acetone extracts obtained from the roots, stem and leaf respectively.

Table 4: Antibacterial Efficacy of Aqueous, Ethanol and Acetone extracts of *Vernonia amygdalina*

Plant parts	Ethanol extracts (100mg/ml)	Acetone extracts (100mg/ml)	Aqueous extracts (100mg/ml)
Root	14 (20%)	12 (17%)	0 (0)
Stem	10 (14.3%)	9 (12.9%)	0 (0)
Leaf	11 (15.7%)	8 (11.4%)	0 (0)

DISCUSSION

The results from this study confirm the contamination of Nworie River by *Salmonella* sp. This is in agreement with previous studies carried out to show the presence of *Salmonella* sp in Nworie River [18,19]. The presence of *Salmonella* in Nworie River could be due to anthropogenic activities resulting from the rural settlements that are found along the river. Also, most drainages and gutters containing human and animal faeces as well as household wastes from residential areas have been found to discharge into Nworie River and this could have resulted in the contamination of the river by *Salmonella* coming from these settlements. The antibiotic resistance patterns of *Salmonella* sp isolated from the River revealed varying degrees of susceptibility and resistance to the antibiotics used. *Salmonella* isolates from the river were resistant to Amoxycillin, Oxacillin and Iminepem. The isolates were however susceptible to Ciprofloxacin, Aztreonam, Gentamycin, Ceftazidime, Kanamycin, Streptomycin, and Ofloxacin, showing sensitivity rates of 71.4%, 7.1%, 77.1%, 11.4%, 44.3%, 48.6% and 78.6% respectively. It is important to state that *Salmonella* isolates were resistant

to at least three of the antibiotics used at different rates as shown in Table 2. Resistant rates shown in this study to Gentamycin by the *Salmonella* isolates were reported to be 22.9% (16 of 70). This however is quite high when compared to a resistance rate of 10% reported by [20]. This variance could probably have resulted from the different sources the *Salmonella* isolates were obtained from and probably the serotypes. While *Salmonella* isolates for this study were obtained from Nworie River, *Salmonella* isolates for the study conducted by [20] were obtained from poultry farms. The isolates were found to be sensitive to Ciprofloxacin showing resistance and sensitivity rates of 71.4% and 28.4%. This is very high compared to a study carried out by [21] who reported a 1% resistance rate to *Salmonella* isolates obtained from human blood. The 100% resistance to Amoxycillin can be related with a study carried out by [22] who reported an 88.7% resistance rate for *Salmonella* isolates also obtained from human blood. Not much has been reported with respect to *Salmonella* resistance to Iminepem antibiotics. This study also revealed a sensitivity rate of 71.4% and 77.1% for Ciprofloxacin and Gentamycin respectively. Research studies have been carried out to show the sensitivity to Gentamycin and Ciprofloxacin antibiotics by *Salmonella* isolates as carried out by [23] who reported 100% and 81.6% sensitivity rates for *Salmonella* isolates for Gentamycin and Ciprofloxacin respectively. However, the results from this study does not agree with studies carried out by [23] who reported a 57.1% sensitivity to amoxycillin, whereas a 100% resistance rate was recorded in this present study. Resistance to Oxacillin is in agreement with studies carried out by [24,23]. The isolates were resistant to Streptomycin showing a resistance rate of 51.4%. This can be correlated with studies carried out by [23] who reported a 57.1% resistance to Streptomycin.

These antibiotics were first line treatment regimen for *Salmonella* and therefore have been documented to be active against *Salmonella*. However, the high rate of resistance recorded in this current study is a cause for alarm. This high resistance to the broad spectrum antibiotics could have resulted from the indiscriminate use of this antibiotics in poultry feeds as some poultry farms were seen to be sited along the banks of Nworie River. Also, it is also possible that humans shed these antibiotics in their urine and faeces due to the uncontrolled use of these antibiotics as they are easily obtained as over-the counter drugs.

The antimicrobial efficacy of the different parts of *Vernonia amygdalina* in Table 2 shows that ethanol and acetone extract of the root plant showed 14(20%) and 12(17%) activity to the isolates, while ethanol and acetone extracts of the stem showed a 10(14.3%) and 9(12.9%) activity to the isolates. In the same vein, the ethanol and acetone leaf extracts showed 11(15.7%) and 8(11.4%) activity to the isolates. This is in agreement with studies conducted by [25] who reported sensitivity of *Salmonella* isolates to ethanol extracts of *Vernonia amygdalina*. The aqueous extract showed no activity against *Salmonella* isolated. This could have resulted from the presence of impurities in the crude extracts which lowered its potency [26]. The result of the aqueous extract did not agree with studies carried out by [25] who reported sensitivity of *Salmonella* isolates to aqueous extracts of *Vernonia amygdalina* leaf. However, aqueous extracts of *Vernonia amygdalina* has been reported to display activity against *Pseudomonas* sp, *Staphylococcus aureus* and *Escherichia coli* [27,25].

The results from the phytochemical analysis showed that Tannins, Saponins. Alkaloids, Cardiac glycoside and Cyanogenic glycosides were present in the leaves, stems and roots of *Vernonia amygdalina*. Flavonoid was found in both leaves and roots but absent in stem. This is in agreement with studies carried out by [11] who reported the presence of some of the stated bioactive compounds that were also listed in this present study. The leaves, stems and roots of *Vernonia amygdalina* showed no significant difference with respect to the bioactive components.

CONCLUSION

Salmonella species were isolated from Nworie River, an indication of contamination of the river by the pathogen. The results from this study revealed a high prevalence of *Salmonella* from Nworie River and also high prevalence of antibiotic-resistant *Salmonella* specie possibly caused by indiscriminate antibiotic usage. The results also showed sensitivity of *Salmonella* isolates to ethanol and acetone extracts of *Vernonia amygdalina*. This study further confirms that the leaves, stem and

root of *Vernonia amygdalina* have potent antibacterial activity against *Salmonella* species which is due to the bioactive compounds present in *Vernonia amygdalina*. Thus, it can be suggested that the bioactive compounds present in this plant can be used in the treatment of infections resulting from antibiotic resistant *Salmonella* species.

REFERENCES

[1] J. Baudart K. Lemarchand A. Brisabois and P. Lebaron, Diversity of Salmonella strains isolated from the aquatic environment as determined by serotyping and amplification of the ribosomal DNA spacer regons. Applied and Environmental Microbiology, 66, (4): (2000) 1544 – 1552

[2] M.A.N. Medeiros D.C.N. Oliveira D.P. Rodriguez and D.R.C. Freitas, Prevalence and antimicrobial resistance of Salmonella in Chicken carcasses at retail in 15 Brazillian cities. Rev. Panam Salud Publica, 30(6) (2011). 555-560

[3] V. O. Rotimi W. Jamal T. Pal A. Sonnevend T.S. Dimitrov and M.J. Albert, Emergence of Multi drug resistant Salmonella spp. And isolates with reduced susceptibility to ciprofloxacin in Kuwait and the United Arab Emirates. Diagn. Microbiol.Infect. Dis. 60 (2008) 717

[4] M. Gordana A. Bogdanka T. Dragica L. Milena and D. Brankica, Antibiotic Susceptibility of Salmonella Spp.: A Comparison Of Two Surveys With A 5 Years Interval, Journal of IMAB – Annual Proceeding (Scientific Papers) 18, (1) (2012) 216-219.

[5] J. Osterom, Epidemiological studies and proposed preventive measures in the fight against Salmonellosis. Int. J. Food Microbiol. 12 (1999) 41-52

[6] N.R. Atieno O.P. Owuor and O. Omwoyo, Isolation of High Antibiotic resistant faecal bacteria Indicatiors, Salmonella and Vibro Species from raw Abattoir sewage in Peri-Urban Locations of Nairobi, Kenya. Greener Journal of Biological Sciences, 3(5) (2013) 172-178

[7] M. Biendo G. Laurans D. Thomas B. Canarelli F. Hamdad-Daoudi F. Rousseau S. Castelain and F. Eb, Molecular characterization and mechanisms of resistance of multidrug-resistant human Salmonella enterica serotype Typhimurium isolated in Amiens (France). Int. J. Antimicrob Agents, 26 (2005) 219-229

[8] R.S. Seyah J.B. Kaneene Y. Johnson and R.A. Miller, Patterns of antimicrobial resistance observed In *Escherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water, Applied Environment Microbiology, 71 (2005) 1394-1404

[9] A.O. Adebanjo C.O. Adewumi and E.E. Essien, Anti-infection agents from higher plants 5th International Symposium of medicinal plants University of Ife, Nigeria, pp: 152-158 (1983)

[10] A. Sofowora, Medicinal Plants and Traditional Medicines in Africa. Chichester John Wiley & Sons New York,. pp: 97-145 1993.

[11] A. O. Ogundare, Antibacterial properties of the leaf extracts of *Vernonia amygdalina, Ocimum gratissimum*, *Corchorous olitorius* and *Manihot palmate*, Journal of Microbiology and Antimicrobials, 3(4) (2011) 77-86

[12] E.B. Izevbigie, Discovery of water soluble anticancer agents from a vegetable found in Benin City, Nigeria. Exp. Biol. Med., 228 (2003) 293 – 298

[13] A. Tadesse A. Gebre-Hiwot K. Asres M. Djote D. Frommel, The in-vitro activity of *Vernonia amygdalina* on *Leishmania acthiopica*. Ethiop. Med. J., 31 (1993) 183-189.

[14] R.F. Atata A. Sani and S.M. Ajewole, Effect of stem Stem Bark extracts of Enantia chloranta isolates. Nig. Society of Experimental Biology, 15(2) (2003) 84-92

[15] B.A. Amadi E.N. Agomuo and C.O. Ibegbulem, Phytochemical test; Research methods in Biochemistry. Supreme Publishers Owerri, pp. 89-95 (2004)

[16] National Committee for Clinical Laboratory Standard, Method for antimicrobial susceptibility testing. In: Manual of Clinical Microbiology. 5 ed. Washington, D.C. 1105-1125 (1990)

[17] C. Okore O. Mbanefo B. Onyekwere A. Ugenyi A. Ozurumba U. Nwaehiri and C. Akueshi, Comparative analysis of phytochemical and Antimicrobial effects of extracts of some local herbs on selected pathogenic organism. GRF Davos Planet@Risk, 2(4): 241-248 (2014)

[18] C.N. Ohalete I.N.S. Dozie I. O. C. Obiajuru and I. H. Eke, Studies on the ecology of *Salmonella bacilli* in Owerri Metropolis, Imo state Nigeria, Global Research Journal of Science, 1 (2011)109-116
[19] M. Duru and K. Nwanekwu, Physicochemical and Microbial status of Nworie River, Owerri, Imo state, Nigeria. Asian J. Plant Sci. Res. 2(4) (2012) 433-436
[20] S.A. Enabuele P.O. Amune and W.T. Aborisade, Antibiograms of Salmonella isolates from poultry farms in Ovia North East Local Govt Area, Edo State, Nigeria. Agric. Biol. J. N. Am. 1(6) (2010) 1287-1290
[21] A. Mas'ud, and M. Tijani, Prevalent and Multiple antibiotic resistance patterns of *Salmonella* Serovars isolated from Blood samples of Hospitalised patients in Kano, North-west Nigeria. Journal of Pharmacy and Biological Sciences. 10(2) (2015) 125-135
[22] O.G. Adeshina N. Osuagwu C.E. Okeke and J.O. Ehinmidu, Plasmid DNA analysis and conjugative study of antibiotic resistant *Salmonella typhi* and paratyphi to ten selected antibiotics in Zaria, Nigeria. Afr. J. Pharm. Pharmacol. 4(4) (2010) 151-157
[23] G. O. A. Agada I.O. Abdullahi M. Aminu M. Odugbo, S.C. Chollom P.R. Kumbish and A.E. J. Okwori, Prevalence and Antibiotic Resistance Profile of *Salmonella* Isolates from Commercial Poultry and Poultry Farm-handlers in Jos, Plateau State, Nigeria. British Microbiology Research Journal 4(4) (2014) 462-479
[24] T. Suresh A.A. Hatha D. Sreenivasan N. Sangeetha and D. Lashmanaperumalsamy, Prevalence and antimicrobial resistance of *Salmonella enteritidis* and other *Salmonella* in the eggs and egg storing trays South India. Journal of Food Microbiology.23 (2006) 294-299.
[25] O.O. Salami and G.C. Agu, The Assessment of the antimicrobial activities of *Ocimum gratissimum* (wild basil) and *Vernonia amygdalina* (Bitter leaf) on some enteric pathogen causing dysentery or diarrhea in patients. IJES 2(9) (2013) 83-96
[26] A.A. Slayer and D.D. Whiff. Bacteria Pathogens, 1st Edition AMS Press, Washington D.C, pp. 120-240 (1994)
[27] U. Udochukwu F.I. Omeje I.S. Uloma and F.D. Oseiwe, Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. American Journal of Research Communication, 3(5) (2015) 225-235

Volume 56
10.18052/www.scipress.com/ILNS.56

Antibacterial Potentials of V*ernonia amygdalina* against Antibiotic-Resistant *Salmonella* Specie Isolated from Nworie River, Imo State, Nigeria
10.18052/www.scipress.com/ILNS.56.82

DOI References
[1] J. Baudart K. Lemarchand A. Brisabois and P. Lebaron, Diversity of Salmonella strains isolated from the aquatic environment as determined by serotyping and amplification of the ribosomal DNA spacer regons. Applied and Environmental Microbiology, 66, (4): (2000).
10.1128/aem.66.4.1544-1552.2000
[2] M.A.N. Medeiros D.C.N. Oliveira D.P. Rodriguez and D.R.C. Freitas, Prevalence and antimicrobial resistance of Salmonella in Chicken carcasses at retail in 15 Brazillian cities. Rev. Panam Salud Publica, 30(6) (2011). 555-560.
10.1590/s1020-49892011001200010
[3] V. O. Rotimi W. Jamal T. Pal A. Sonnevend T.S. Dimitrov and M.J. Albert, Emergence of Multi drug resistant Salmonella spp. And isolates with reduced susceptibility to ciprofloxacin in Kuwait and the United Arab Emirates. Diagn. Microbiol. Infect. Dis. 60 (2008).
10.1016/j.diagmicrobio.2007.07.007
[4] M. Gordana A. Bogdanka T. Dragica L. Milena and D. Brankica, Antibiotic Susceptibility of Salmonella Spp.: A Comparison Of Two Surveys With A 5 Years Interval, Journal of IMAB - Annual Proceeding (Scientific Papers) 18, (1) (2012) 216-219.
10.5272/jimab.2012181.216
[5] J. Osterom, Epidemiological studies and proposed preventive measures in the fight against Salmonellosis. Int. J. Food Microbiol. 12 (1999) 41-52.
10.1016/0168-1605(91)90046-r
[6] N.R. Atieno O.P. Owuor and O. Omwoyo, Isolation of High Antibiotic resistant faecal bacteria Indicatiors, Salmonella and Vibro Species from raw Abattoir sewage in Peri-Urban Locations of Nairobi, Kenya. Greener Journal of Biological Sciences, 3(5) (2013).
10.15580/gjbs.2013.5.032113539
[7] M. Biendo G. Laurans D. Thomas B. Canarelli F. Hamdad-Daoudi F. Rousseau S. Castelain and F. Eb, Molecular characterization and mechanisms of resistance of multidrug-resistant human Salmonella enterica serotype Typhimurium isolated in Amiens (France). Int. J. Antimicrob Agents, 26 (2005).
10.1016/j.ijantimicag.2005.05.003
[8] R.S. Seyah J.B. Kaneene Y. Johnson and R.A. Miller, Patterns of antimicrobial resistance observed In Escherichia coli isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water, Applied Environment Microbiology, 71 (2005).
10.1128/aem.71.3.1394-1404.2005
[20] S.A. Enabuele P.O. Amune and W.T. Aborisade, Antibiograms of Salmonella isolates from poultry farms in Ovia North East Local Govt Area, Edo State, Nigeria. Agric. Biol. J. N. Am. 1(6) (2010) 1287-1290.
10.5251/abjna.2010.1.6.1287.1290
[23] G. O. A. Agada I.O. Abdullahi M. Aminu M. Odugbo, S.C. Chollom P.R. Kumbish and A.E. J. Okwori, Prevalence and Antibiotic Resistance Profile of Salmonella Isolates from Commercial Poultry and Poultry Farm-handlers in Jos, Plateau State, Nigeria. British Microbiology Research Journal 4(4) (2014).

10.9734/bmrj/2014/5872
[24] T. Suresh A.A. Hatha D. Sreenivasan N. Sangeetha and D. Lashmanaperumalsamy, Prevalence and antimicrobial resistance of Salmonella enteritidis and other Salmonella in the eggs and egg storing trays South India. Journal of Food Microbiology. 23 (2006).
10.1016/j.fm.2005.04.001