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Research Article

Studies on Inhibitory Effects of Extract of *Vernonia amygdalina* Used in Traditional Poultry Farming against Some Bacteria Isolated from Poultry Droppings

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Abstract: Recent findings have incriminated the use of antibiotics in poultry feeds to be associated with emergence of resistance among bacteria. The use of traditional plants remedies has not been found to be associated with resistance. This work determined the inhibitory effects of extracts of *Vernonia amygdalina* used in traditional poultry farming against *Salmonella* sp, *Escherichia coli*, *Proteus* sp, *Pseudomonas* sp and *Staphylococcus aureus* isolated from poultry droppings. The method of maceration was adopted to extract the plant materials with methanol (70%) and sterile water as solvents for the extraction. About one gram of each sample (poultry dropping) was diluted in selenite F broth and another one gram each was inoculated directly onto *Salmonella-shigella* agar, blue green agar, McConkey agar, manitol salt agar and eosin methylene blue agar (EMB). After an overnight incubation at 37^{o}C for 24hrs plates were read. Typical colonies were grams Stained using Standard procedures, consequent upon that, the isolated organisms were subjected to biochemical test. The only Gram positive organism isolated was further confirmed to be *S. aureus* by catalase and coagulase tests. Bioassay of the isolates was conducted and further Subjected to the plant extract, using agar well diffusion method and conventional antibiotics using disc diffusion (Kirby bouer). Findings shows significant effect of the plant extract on the isolated bacteria and also indicated that it can serve as an alternative to conventional antibiotics as used in traditional poultry production in Nigeria. In addition, small scale farmers can utilize this plant extract for sustainable poultry production. This practice can also go a long way in minimizing Emergence of resistance strains of bacteria in human diseases since drugs derived from plants are safe and dependable, compared to synthetic drugs.
Keywords: *Vernonia amygdalina*, Extract, Poultry droppings, Isolated organisms, McConkey agar

INTRODUCTION

Diseases are the main constraints of indigenous poultry production in northern Nigeria where Indigenous poultry farming is an integral part of mixed farming [1]. The birds are raised mostly on low input extensive free-range system of production. Farmers usually have to start with new flock after heavy losses incurred due to disease outbreaks. The existing conventional disease control programs favor the high investment intensive systems of production with birds in confinement and not small-scale farmers with less than 100 birds [2]. Most of the developing countries including Nigeria rely wholly or partly on traditional herbal medicine for treatment and control of animal and human diseases [3]. In Nigeria, traditional veterinary practices play important roles in many areas [4]. Rural poultry farmers are aware of the need to keep birds in good health and when they are sick to source for prescription and procure medicaments for treating rural poultry diseases. They often do so through various means and the use of traditional (indigenous) method of Medicare seems to be the main method of treatment. The use of traditional medicine may be due to its low cost, availability and ease of application compared to modern veterinary medicine [5]. The unavailability and cost of veterinary drugs in most pharmaceutical shops and local market will have contributed to low patronage by farmers in sourcing drugs for treatment of sick chickens. A study by Mapiye and Sibanda [6] has shown that large flock sizes were obtained by farmers that gave traditional medicine to their chickens and indicates that traditional medicines in some instances have potential to improve the health status of rural household flocks. Hence, there is a need for researchers to take inventory of common medicaments used by local farmers in treating rural household chickens and determine their chemical properties and routes of application. There has been very little research

conducted on medicinal plants used for control although their use is wide spread in the treatment of other livestock diseases. Due to high cost of conventional medicines and vaccines coupled with the lack of knowledge on their use, these drugs are usually out of reach of the small-scale farmers. There is therefore need for cheap easy to use and sustainable local poultry disease control programs [1]. Since ancient times, plants and plant parts have an indispensable source of medicine for indigenous poultry production systems. Although modern medical science has developed to a great extent, many farmers in Northern Nigeria depend on plant parts and herbal remedies for indigenous poultry health management. Unfortunately, local medical traditions are being lost because they are communicated orally from generation to generation and are largely un-documented. Very little has been done to verify and validate information gathered [7]. A major concern in poultry production today is the need for reduction in use of antibiotics and other allopathic medicinal products, largely due to concerns over bacterial resistance [8]. Alongside this trend, however, is an ever-growing demand for low-priced, high quality food, improved feed hygiene, greater poultry health and welfare, and reduced environmental impact. Much research has focused on the development of alternative strategies to maintain poultry health and enhance performance within intensive systems, and numerous substances, commonly known as natural growth promoters (NGPs) have been identified as effective alternatives to antibiotics. Phytobiotics are NGPs that have been growing in popularity as feed additives, due to their beneficial effect on gut health and immunity and growth performance [9].

Phytobiotics can be defined as plant derived products added to feed in order to improve performance. They originate from leaves, roots, tubers or fruits of herbs, spices and other plants. They may be available in solid, dried, and ground forms, or as extracts (essential oils). In simple terms, Phytobiotics are products of plant origin, and preparations such as thyme, oregano specie, turmeric specie and garlic are gaining interest among researchers and poultry producers [10]. The beneficial effects of Phytobiotics in poultry may arise from the activation of feed intake and the secretion of digestive enzymes, immune stimulation, antibacterial, coccidiostatic, anthelmintic, antiviral or anti-inflammatory activities, or from antioxidant properties. Many plant secondary metabolites, such as isoprene derivatives, flavonoids and glucosinolates, may act as antibiotics or as antioxidants in vivo. As a result of these beneficial effects, they lead to an increase in performance. Many plants have beneficial multifunctional aspects which are derived from their specific bio-active components [11]. Biologically active constituents of plants are mostly secondary metabolites, such as terpenoids (mono-and sesquiterpenes, steroids, phenolics (tannins), glycosides and alkaloids (present as alcohols, aldehydes, ketones, esters, ethers, and lactones). There is a lot of variation in composition due to biological factors such as kind of plant, growing location, harvest conditions, manufacturing factors such as extraction/distillation, stabilization and storage conditions like light, temperature, oxygen tension and time [12].

The challenge is to identify and quantify the multitude of actions and claims, improving feed utilization, animal physiology and health status.

MATERIALS AND METHODS

Collection and identification of the plant materials

Leaves of *Vernonia amygdalina* (bitter leaf) was collected at three different locations in Makarfi local government. Identification of the plant was aided by local people in the area and confirmed by Botanist in the Department of Biological sciences, Ahmadu Bello University, Zaria. The voucher numbers of 675 for *Vernonia amygdalina* was deposited at the reference catalogue of herbarium plants section. The plant material was dried under shade and ground to powder and dispensed into container and labeled appropriately ready for extraction.

Extraction and preparation of plants materials

Maceration method was employed for the extraction of plant active constituents, and methanol (70%) and water as solvents for the extraction [13].

Phytochemical screening of the plant extracts

Phytochemical screening using simple chemical tests as described by Sofowora [3] were performed to detect the presence of carbohydrates, flavonoids, Glycosides, saponins, tannins, alkaloids and steroids in the plant extracts. The tests were carried out as follows:

Test for carbohydrates (Molisch test)

A few drops of molisch reagent were added to two cubic centimeters of test extract in a test tube. One milliliter of concentrated tetraoxosulphate VI acid was allowed to run down the side of the inclined test tube so that the acid forms a layer beneath the aqueous solution without mixing it. The appearance of a reddish brown colour indicates a positive test [14].

Test for Flavonoids

To the alcoholic solution of the extracts, magnesium powder and few drops of concentrated HCL were added. Formation of orange or pink colours indicated positive while absence indicated negative results [14].

Test for Glycosides

To 50mg of the methanolic extracts, 2mls of chloroform was added and few drops of conc. H_2SO4 were also gradually added by the side of the test-tube. A brown ring at the inter phase indicated positive while absence indicated negative results [14].

Test for saponins

To about 0.5g of the powder in a test tube, 5mi of distilled water was added and shaken vigorously. A persistent froth that lasted for at least 15minutes indicated the presence of saponins [14].

Test for tannins

A few drops of $FeCl_3$ (5%w/v) solution was added to the extract in a test tube and shaken. A dark-blue color indicated a positive result [14].

Test for alkaloids

A small portion of the acidified extract solution was subjected to reactions with Wagner's reagent. A reddish brown precipitate indicated the presence of alkaloids [14].

Test for steroids

One cubic centimeter of concentrated H_2SO_4 was added to one cubic centimeter of test extract. A red colour indicates the presence of a steroidal glycoside [14].

Isolation of bacteria from poultry droppings

Poultry droppings were systematically sampled with chickens kept in separate cages numbered one to twenty (1-20) for 100samples.

Isolation and Identification of Staphylococcus *aureus*

Enrichment of the bacteria was done by adding one gram (1g) of the sample into peptone water in sterile McCartney bottle and incubated at 35^{o}C for 18hrs.Isolation of the *Staphylococcus aureus* was achieved by streaking the pre-enriched culture from the peptone water on to a selective differential agar plate of Manitol Salt Agar. The plates were then incubated at 37^{o}C for 24hrs under aerobic conditions. Colonies showing golden yellow with yellow zones in the media were considered to be presumptive *Staphylococcus aureus* and further subjected to biochemical tests such as catalase and coagulase [15].

Isolation and Identification of *Escherichia coli*

Enrichment of the bacteria was achieved by adding one gram(1g) of the sample into a sterile McCartney bottle containing nutrient broth and incubated for 18hrs at 35^{o}C The pre-enriched culture was then streaked on to surfaces of an Eosin Methylene Blue(EMB)agar and incubated for 24hrs at 37^{o}C.Dark colonies with metallic sheen indicated the presence of lactose fermenters and were to be considered as presumptive *E. coli.* The culture was streaked on nutrient agar slant and kept for further biochemical [15].

Isolation and Identification of *Salmonella* sp

Enrichment was done by transferring 1g of the sample into test tube containing selenite F broth and incubated for 18hrs at 37^{o}C.The enriched culture after incubation was then streaked on to the surfaces of *Salmonella-Shigella* Agar (SSA) and incubated at 37^{o}C for 18hrs.After incubation; typical black colonies were regarded as presumptive *Salmonella.* The colonies were then inoculated on nutrient agar slants and kept for biochemical identifications [15].

Isolation and Identification of *Pseudomonas* sp

Enrichment of the bacteria was achieved by adding one gram (1g) of the sample into a sterile McCartney bottle containing nutrient broth and incubated for 18hrs at 35^{o}C.The pre-enriched culture was then streaked on to surfaces of blood agar and incubated for 24hrs at 37^{o}C Colonies that appear large, flat, spreading which are heamolytic and usually pigment producing (dark greenish-blue color) which is an indication of *Pseudomonas* characteristic. The colonies were inoculated on nutrient agar slants and kept for further biochemical identifications [15].

Isolation and Identification of *Proteus* sp

Enrichment of the bacteria was achieved by adding one gram (1g) of the sample into a sterile McCartney bottle containing nutrient broth and incubated for 18hrs at 35^{o}C.The pre-enriched culture was then streaked on to surfaces of MacConkey agar and Blue Green Agar (BGA) and incubated for 24hrs at 37^{o}C.On BGA a pinkish coloration indicate non-lactose fermenting organism which could be either *Proteus* or *Salmonella*, but on MacConkey agar, colonies appeared as colorless with swarming characteristic. The colonies were inoculated on nutrient agar slants as presumptive *Proteus* sp and kept for further biochemical identifications [15].

Gram staining: the isolates were gram stained using standard procedure and recorded accordingly.

Biochemical characterization of the isolates

Suspected colonies were subjected to biochemical tests which are motility, Indole, Urease, citrate, methyl red, voges-proskauer triple sugar iron (TSI) agar, citrate utilization test, for *Escherichia coli*, *Salmonella species*, *Proteus species*, *Pseudomonas species,* while catalase and coagulase tests for *Staphylococcus aureus* [15].

Standardization of the isolates

McFarland standard (0.5) was prepared by combining 0.05ml of 1% barium chloride dehydrate($Bacl_2.2H_2O$) with 9.95ml of 1% Sulfuric acid (H_2SO_4) to yield 1.0%w/v barium sulphate suspension. The turbid solution (McFarland standard scale No 1) formed was transferred into a test tube for comparison with bacterial inoculums suspension [16].

Preparation of different concentrations of plant extracts

A concentration of 200mg/ml was prepared for each of the plant extracts; by weighing approximately 2g of the plant extract in 10ml of distilled water as stock solution. A doubling serial dilution of the plant extracts

was carried out from their stock solutions to obtain varying concentrations. The varying concentrations obtained were 100, 50 and 25 mg/ml respectively.

Susceptibility test of the plant extracts

Agar well diffusion technique was used as described by Cheesbrough [16], to determine the antibacterial activity of plant extracts. Mueller Hinton agar plates were prepared following the manufacturer's instructions. One ml aliquot of each test organism suspension (standardized) was transferred onto the well-dried Mueller Hinton agar plates in duplicate and was spread evenly following slow rotation of the plates and excess was decanted into disinfectant container. The plates were allowed to dry, a standard sterile cork borer of 4mm diameter was used to cut uniform wells on the Mueller Hinton agar plates and the centre well served as the control. Each well was appropriately labeled on the reverse side of the plates. A sterile syringe (2mls) was used to transfer 0.5 ml of the plant extracts into respective wells in the agar plates. Water was used as control in centre wells. The plates were allowed to stand for 30 min at room temperature to allow proper diffusion of the extract to occur. All the plates were incubated at 37°C for 24, after which, the inhibition zone were measured using transparent meter ruler to the nearest millimeters.

Determination of susceptibility to conventional antibiotics

Clinical laboratory institute standard [17] disk diffusion method was adopted: About five (5) colonies of the test bacteria from culture medium were inoculated into Mueller Hinton broth and incubated at 37oC for 24hrs. Using sterile syringe and needle 0.5ml of the culture was transferred on to the surfaces of Mueller Hinton agar and spread evenly by gently rotating the plates. Using sterile forceps the antibiotic discs were placed appropriately and evenly on the inoculated plates. The plates were incubated at 37°C overnight. The following commercially prepared antibiotic discs were used for susceptibility testing.

Septrin (SXT) 30µg, Amoxicillin (AM) 30µg, Chloranphenicol (CH) 30µg, Gentamycin (CN) 10µg, Ciprofloxacin (CPX), Pefloxacin (PEF) 30µg, Augmentin (AU) 30µg, Tarivid (OFX), Streptomycin (S) 30µg and Sparfloxacin (SP) 10µg [16].

Determination of minimum inhibitory concentration (MIC) of plant extracts

The broth dilution method was used. A stock solution of 400 mg /10ml was prepared for each plant extract separately. One ml of nutrient broth was dispensed into four test tubes and sterilized by autoclaving at 121°C after 15 min. The different extracts were serially diluted from the solutions of 25mg/ml of the stock solution to obtain varying concentrations. The concentrations were; 12.5, 6.25and3.125mg/ml. Zero point one (0.1 ml) of each test isolate was inoculated into the various test tubes containing varying concentrations and then, a set of test tubes containing only nutrient broth were used as negative control, another set of test tubes containing nutrient broth and test organisms were used as positive control. All the test tubes and control were then incubated at 37°C for 24 h. After incubation, the presence or absence of growth on each tube was rated using the following scale: _ = no growth, + = scanty growth, ++ = moderate growth, +++ = heavy growth. The least concentration of plant extract in the test tube with no turbidity was considered as the minimum inhibitory concentration (MIC) [18].

Determination of minimum bactericidal concentration (MBC) of the plant extracts

Subsequently, those tubes that showed no turbidity were plated out on sterile nutrient agar plates and incubated at 37°C for 24 h absence of growth after incubation period of 48hrs was considered the MBC [18].

Statistical Analysis

The data obtained in this study were statistically analyzed using Analysis of variance (ANOVA), with the help of Duncan Multiple Range Test (DMRT) to separate the means.

RESULTS

Five different isolates were identified from the cultured samples of poultry droppings. These isolates are; *Staphylococcus aureus*, *E.coli*, *Proteus spp, Pseudomonas spp, and Salmonella spp*. Table 1 showed the result of phytochemicals constituents of the plant extracts, where the constituents screened for *Vernonia amygdalina* showed presence of all the tests carried out.

Table.1 Phytochemical constituents of the *Vernonia amygdalina*

Test	VAE
Carbohydrate	
Molish's test	+
Glycosides	
Fehlings test	+
Tannins	
Fe3Cl2	+
Alkaloid	
Wagner's test	+
Dragendoff's test	+
Saponins	
Frothing test	+
Flavonoids	
Shinoda test	+
Steroids5k	
Salkwoski test	+

Key: + =presence of the phytoconstituents, VAE=*Vernonia amygdalina* Extract

Table 2 showed zones of inhibition against the bacterial isolates to *Vernonia amygdalina* extract, at 25mg/ml with *Salmonella sp* been the most susceptible, followed by *S .aureus* and *Pseudomonas sp*. At 50mg/ml also *Salmonella sp* had the highest susceptibility, followed by *S. aureus*, *E. coli*, *Pseudomonas sp*. Similarly at 100mg/ml *salmonella sp*, had the highest zone, while *E. coli*, and *S. aureus* having similar activity with significance difference at $p \leq 0.001$. Table 3, below showed the result of zones of inhibition against the conventional antibiotics against the test isolates, the result showed that Ciprofloxacin (CPX) had the highest zones of inhibition across the isolates (most effective), followed by argumentin (AU), *Pseudomonas sp* showed higher resistance, but with little activity by Argumentin and Gentamycin. The Table 4 showed comparison of zones of inhibition of the bacterial isolates to the plant extract and standard antibiotics CPX, CN and OFX at 25mg/ml, *Salmonella* sp appear to have higher activity compared to the other isolates, with significance difference at $p \leq 0.05$. Table 5 showed comparison of susceptibility pattern of bacterial isolates to plant extracts and standard antibiotics CPX, CN and OFX at 50mg/ml, *Salmonella* was more susceptible to *V. amygdalina*. *E. coli* was more sensitive to *V. amygdalina* than *Proteus and Pseudomonas* which had similar activity on the plant extract. The antibiotic Ciprofloxacin was effective across the test isolates but 100% resisted by *Pseudomonas*, also Ofloxacin was effective against the test bacteria but 100% resisted by two organisms (*Pseudomonas and Salmonella*). Similarly, Table 6 showed comparison of zones of inhibition of bacterial isolates to plant extracts and standard antibiotics CPX, CN and OFX at 100mg/ml, *V. amygdalina* also was more effective against *Salmonella* sp. The antibiotic was also effective against the test isolates, but 100% resisted by *Pseudomonas* sp. There exist significance differences at $p \leq 0.05$. Table 7 shows the results of Minimum Inhibitory concentration (MIC) and Minimum Bactericidal concentrations (MBC) of the test isolates in the presence of *V. amygdalina. V. amygdalina* Showed MIC against *Salmonella* sp at 6.25mg/ml and 12.5mg/ml for the other test isolates. While as for the MBC *V. amygdalina* showed MBC against the test isolates at 12.5mg/ml, but the MBC for *Salmonella* sp appeared at 6.25mg/ml.

Table 2: Inhibitory activity of *Vernonia amygdalina* extract against the bacterial isolates

Bacteria	Mean ± SEM (mm)		
	25 mg/ml	50 mg/ml	100 mg/ml
S. aureus	14.75 ± 0.75^{ab}	17.00 ± 0.00^{b}	18.00 ± 0.00^{b}
E. coli	14.00 ± 0.00^{b}	16.00 ± 0.00^{c}	18.00 ± 0.00^{b}
Proteus sp	13.00 ± 0.00^{b}	14.25 ± 0.25^{d}	15.00 ± 0.00^{d}
Pseudomonas sp	14.75 ± 0.75^{ab}	15.50 ± 0.50^{c}	16.50 ± 0.50^{c}
Salmonella sp	16.25 ± 0.25^{a}	18.00 ± 0.00^{a}	19.00 ± 0.00^{a}

Note: Values are expressed as Mean ± SEM (standard error of means).Means having different superscripted alphabets along the columns are different at $p<0.05$.

Table 3: Sensitivity of the test bacterial isolates to commercially available antibiotic discs

Isolate	Mean ± SEM								
	SXT	CH	SP	CPX	AU	CN	S	OFX	PEF
S. aureus	20.25 ± 0.25^{b}	20.25 ± 0.25^{a}	18.00 ± 0.00^{b}	40.25 ± 0.25^{a}	30.25 ± 0.25^{a}	20.25 ± 0.25^{a}	20.00 ± 0.00^{b}	24.00 ± 0.00^{c}	25.00 ± 1.00^{a}
E. coli	14.00 ± 0.00^{c}	16.25 ± 0.25^{c}	0.00 ± 0.00^{d}	20.00 ± 0.00^{d}	18.00 ± 0.00^{b}	20.00 ± 0.00^{a}	14.50 ± 0.50^{c}	28.00 ± 0.00^{b}	15.00 ± 1.00^{c}
Proteus sp	30.25 ± 0.25^{a}	18.25 ± 0.50^{b}	20.25 ± 0.25^{a}	37.50 ± 0.50^{b}	18.00 ± 0.00^{b}	20.25 ± 0.25^{a}	30.50 ± 0.00^{a}	30.25 ± 0.25^{a}	20.50 ± 0.50^{b}
Pseudomonas sp	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	18.25 ± 0.25^{b}	14.50 ± 0.50^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
Salmonella sp	14.25 ± 0.25^{c}	10.50 ± 0.50^{d}	14.25 ± 0.25^{c}	24.50 ± 0.50^{c}	10.50 ± 0.50^{c}	20.00 ± 0.00^{a}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	14.00 ± 0.00^{c}

Statistics F = 3225.833, <0.001**, F=537.150, F= 3858.750, F=2305.667, F = 671.250, F = 84.583, F=3391.000, F = 18441.000, F= 197.889

Note: Values are expressed as Mean ± SEM (standard error of means).Means having different superscripted alphabets along the columns are different at $p<0.05$.

Key: Septrin (SXT) 30µg, Amoxicillin (AM) 30µg, Chloranphenicol (CH)30µg, Gentamycin(CN)10µg, Ciprofloxacin (CPX)10µg, Pefloxacin (PEF) 30µg, Augmentin (AU) 30µg ,Ofloxacin (OFX) 10µg, Streptomycin (S) 30µg and Sparfloxacin(SP)10µg.

Table 4: Comparison of sensitivity of bacterial isolates to plant extracts and standard antibiotic CPX, CN and OFX at 25 mg/ml

Bacteria	Mean inhibition Zone Diameter(mm)			
	VAE	CPX(10µg)	CN(10µg)	OFX(10µg)
S. aureus	14.75 ± 0.75^{d}	40.25 ± 0.25^{a}	20.25±0.25^{c}	24.00±0.00^{b}
E. coli	14.00 ± 0.00^{d}	20.00 ± 0.00^{b}	20.00±0.00^{b}	28.00±0.00^{a}
Proteus	13.00 ± 0.00^{e}	37.50 ± 0.50^{a}	20.25±0.25^{c}	30.25±0.25^{b}
Pseudomonas	14.75 ± 0.75^{a}	0.00 ± 0.00^{b}	14.50±0.50^{a}	0.00±0.00^{b}
Salmonella	16.25 ± 0.25^{c}	24.50 ± 0.50^{a}	20.00±0.00^{b}	0.00±0.00^{e}

Note: Values are expressed as Mean ± SEM (standard error of means).Means having different superscripted alphabets across the columns are different at p<0.05.

Key: VAE= *Vernonia amygdalina* extract, CPX= Ciprofloxacin, CN=Gentamycin, OFX= Ofloxacin

Table 5: Comparison of sensitivity of bacterial isolates to plant extract and standard antibiotics (CPX, CN and OFX) at 50mg/ml

Bacteria	Mean inhibition Zone Diameter(mm)			
	VAE	CPX(10µg)	CN(10µg)	OFX(10µg)
S. aureus	17.00±0.00^{e}	40.25 ± 0.25^{a}	20.25±0.25^{c}	24.00±0.00^{b}
E. coli	16.00±0.00^{d}	20.00 ± 0.00^{b}	20.00±0.00^{b}	28.00±0.00a
Proteus	14.25±0.25^{d}	37.50 ± 0.50^{a}	20.25±0.25^{c}	30.25±0.25^{b}
Pseudomonas	15.50±0.50^{a}	0.00 ± 0.00^{b}	14.50±0.50^{a}	0.00±0.00^{b}
Salmonella	18.00±0.00^{c}	24.50 ± 0.50^{a}	20.00±0.00^{b}	0.00±0.00^{e}

Note: Values are expressed as Mean ± SEM (standard error of means).Means having different superscripted alphabets across the columns are different at p<0.05.

Key: VAE= *Vernonia amygdalina* extract, CPX= Ciprofloxacin, CN=Gentamycin, OFX= Ofloxacin

Table 6: Comparison of Zones of sensitivity of bacterial isolates to plant extracts and standard antibiotics (CPX, CN and OFX) at 100 mg/ml

Bacteria	Mean inhibition Zone Diameter(mm)			
	VAE	CPX(10µg)	CN(10µg)	OFX(10µg)
S. aureus	18.00 ± 0.00^{d}	40.25 ± 0.25^{a}	20.25±0.25^{c}	24.00±0.00^{b}
E. coli	18.00 ± 0.00^{c}	20.00 ± 0.00^{b}	20.00±0.00^{b}	28.00±0.00a
Proteus	15.00 ± 0.00^{e}	37.50 ± 0.50^{a}	20.25±0.25^{c}	30.25±0.25^{b}
Pseudomonas	16.50 ± 0.50^{a}	0.00 ± 0.00^{b}	14.50±0.50^{a}	0.00±0.00^{b}
Salmonella	19.00 ± 0.00^{c}	24.50 ± 0.50^{a}	20.00±0.00^{b}	0.00±0.00^{e}

Note: Values are expressed as Mean ± SEM (standard error of means).Means having different superscripted alphabets across the columns are different at p<0.05.

Key: VAE= *Vernonia amygdalina* extract, CPX= Ciprofloxacin, CN=Gentamycin, OFX= Ofloxacin

Table 7: MIC and MBC of *Vernonia amygdalina* against the test isolates at various concentrations

Isolates	MIC (mg/ml) V AE	MBC (mg/ml) VAE
S. aureus	12.50	12.50
E. coli	12.50	12.50
Proteus sp	12.50	12.50
Pseudomonas sp	12.50	12.50
Salmonella sp	6.25	6.25

Key: VAE= *Vernonia amygdalina Extract*, MBC= Minimum Bactericidal concentrations, MIC= Minimum Inhibitory concentration

DISCUSSION

The results of phytochemical screening of *Vernonia amygdalina* showed the presence of carbohydrates, flavonoids, saponins, tannins, alkaloids, steroids and glycosides (Table 1). The presence of these organic substances may have been responsible for antimicrobial activity of these plant extract. Tannins are polyphenols obtained from various parts of different plants [19]. In addition to use in leather processing industries, tannins have shown potential antiviral activity [20], and antibacterial activity [21, 22]. In the past few years, tannins have also been studied for their effects against cancer through different mechanisms and have been found to form proline-rich-protein which leads to inhibition of cell wall synthesis, a property that may explain the mode of action of these extracts. Alkaloid is a member of a large group of chemicals that are made by plants and have nitrogen in them. Many alkaloids possess potent pharmacological effects. The alkaloids include cocaine, nicotine, strychnine, caffeine, morphine, pilocarpine, atropine, methamphetamine, mescaline, ephedrine and tryptamine [23]. According to Enyi-Idoh *et al*. [24], bioactive components present in *V. amygdalina* extract may be responsible for the

observed antibacterial activities. This corroborates the findings in the present study. According to Clinical and laboratory standard institute-CLSI [17] , any plant material should be considered an effective therapeutic agent if it extract produces zones of inhibition of between 15-22mm on the target pathogenic organism. Activity of plant extracts to test bacteria is normally expressed *in-vitro* as zones of inhibition in millimeter (greater than or equals to 7mm) around the organism on test [25].

The zones of inhibition of *V. amygdalina* extract at 25mg/ml was more effective against *Salmonella* sp, *S. aureus* and *Pseudomonas* sp than *Proteus* sp. Statistical analysis revealed significant difference ($p \leq 0.05$) in the zones of inhibition at all the concentrations tested. This indicated that the antibacterial efficacy of the extract is dependent on the concentration [26, 27]. The antibacterial activities observed in this study might be due to the presence of phytoconstituents such as flavonoids and tannins in these plants. This further buttresses the well established fact that antibacterial substances are present in the higher plants. This is in agreement with the findings of [23].

The comparisons between the antibacterial activities of methanolic extracts of *V. amygdalina* and that of standard antibiotics (CPX, CN and OFX) showed that the extracts had broader spectrum than the antibiotic even though the antibiotic had better activity against some organisms. The differences were statistically significant ($p \leq 0.05$). The better activity of the standard antibiotic over the extracts could be due to the fact that organic extracts are in crude and not in pure form as the synthetic antibiotics; hence the secondary active metabolites could be present in low concentration or even be masked in the extracts [28].

The test bacteria showed different levels of susceptibility to the different antibiotics used in the study as shown by the zones of inhibition. The highest activity (40.25mm) was recorded for ciprofloxacin against *S. aureus*, followed by *Proteus* sp, *Salmonella* sp, and *E coli; Pseudomonas* sp was however found to be resistant to ciprofloxacin. Gram positive bacteria are often more susceptible to antibiotics than the Gram negative bacteria due to the fact that Gram negative organisms have an additional outer phospholipids membrane and porines that makes the cell wall impermeable to lipophilic and hydrophilic solutes respectively [29, 30]. Augmentin and gentamycin were also active against *S. aureus, E.coli, Pseudomonas* sp and *Proteus* sp with the least activity on *Pseudomonas* sp. This might be possible because among the aminoglycosides, gentamycin have an extended spectrum of activity on most of the Enterobacteriaceae family, particularly *Pseudomonas* sp [31].

As for Minimum Inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), the general trend is that lower concentrations inhibited the organisms while higher concentrations had cidal effects on the organisms. The MIC and MBC values for *V. amygdalina* were 6.25mg/ml against *Salmonella* sp, while for *S. aureus*, *E. coli* and *Pseudomonas* sp, the MIC and MBC were observed at 12.50mg/ml. The order of ease of susceptibility to the *V. amygdalina*, it was; *Salmonella sp, S. aureus, E. coli, Pseudomonas sp and Proteus* sp. Antimicrobial agents with low activity against an organism had a high MIC while a highly active Antimicrobial agent gave a low MIC. The antibacterial effectiveness with the increasing concentration of phytoconstituents observed in the present study is in agreement with the earlier investigations [32, 33].

This has also shown that the concentration required to achieve bactericidal activity as observed in this study varied. This is in line with the finding of Abalaka *et al*. [34].

CONCLUSION

The study provides an impetus in the use of natural products which can be used both as a diet and antibiotic in animal feds. The study presents a plant with stable and biologically active antibacterial components with low drug resistance against common bacterial strains infecting both human and livestock. Hence the work contributes to the exploration of an alternative source of antibiotics with view to reducing overdependence on commercial antibiotics associated with the emergence of drug resistant bacteria strain.

REFERENCES

1. Gibson GR, Fulle R; Symposium: probiotic bacteria: Implications for human health. Aspects of in vitro and in vivo research approaches directed toward identifying probiotics and prebiotics for human use. Journal of Nutrition, 2002; 130:391S-395.
2. Fernandez F, Hinton M, Van Gils B; Dietary mannan-oligosacccharides and their effect on chicken caeca microflora in relation to *Salmonella* enteritidis colonisation. Avian journal of Pathology, 2002; 31:49-58.
3. Sofowora A; Medicinal plants and traditional Medicine in Africa. John Wiley and Sons, NewYork, 1993: 100-117.
4. Kudi AC, Myint SH; Antiviral activity of some Nigerian medicinal plant extracts. Journal of Ethnophamarcolog., 1999; 68: 289-294.
5. Alders R, Spradbrow PB; Controlling Newcastle in village chicken. A field Manual Australian Center for International Agriculture, 2001.
6. Mapiye C, Sibanda S; Constraints and opportunities of village chicken production systems in the smallholder sector of Rushinga district of Zimbabwe. Livestock Research for Rural Development, 2005; 17 (10): 1-7.
7. Okitoi LO, Ondwasy HO, Siamba DN, Nkurumah D; Traditional herbal preparations for indigenous poultry health management in Western Kenya.

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8. Cavazonni V, Adami A, Castrovilli C; Performance of broiler chickens supplemented with *Bacillus coagulans* as probiotic. Broilers and Poultry Science, 1998; 39:526-529.
9. Allen PC, Danforth HD, Augustine PC; Dietary modulation of avian coccidiosis. Int. J. Parasitolog., 1998; 28:1131-1140.
10. Abdu PA, Faya JN; The efficacy of some Nigerian plants on helminths found in local chickens. Ethnoveterinary practices research and development. Proceedings of an International Workshop on Ethnoveterinary Practices held 14-18 August 2000, Kaduna, Nigeria. 2000: 65-71.
11. Kamel C; A novel look at a classic approach of plant extracts. Feed Mix, 2000; 8:16-17.
12. Atawodi SE, Usman M, Bulus ST, Atawodi JC, Wakawa L, Ameh D; Herbal treatment of some protozoan and parasitic diseases of poultry in the middle belt region of Nigeria. Proceedings of an International Workshop on Ethnoveterinary Practices held 14-18 August 2000, Kaduna, Nigeria. 2000: 78-84.
13. Salisu L, Garba S; Phytochemical screening and insecticidal activity of *Hyptis sauveolens and Striga her monthica.* BEST Journal, 1997; 5(1): 160-163.
14. Silva GL, Lee I, Kinghorn AD; In Cannell RJP editor; Special problems with the extraction of plants Natural products isolation. Methods in bactechnology: Human press inc., Totowa, NJ, 2004: 343-363.
15. Cheesbrough M; District laboratory practice in tropical countries. Part 2, UK; Cambridge University Press, 2004.
16. Cheesbrough M; District laboratory practice in tropical countries. Part 2, UK; Cambridge University Press, 2006.
17. Clinical Laboratory institute standards (CLIS); Performance standards for antimicrobial susceptibility 15th informational supplement (CLIS document M100-S15). Volume 25, 2005.
18. Andrews MJ; Determinative of MIC.Department of Microbiology, City Hospital NHS Trust, Birmingham B 187 QH, 2004.
19. Gajendiran N, Mahadevan A; Utilization of catechin by Rhizobium species. Plant Soil. 1990; 108: 263-266.
20. Lin LU, Shu-wen L, Shi-bo J, Shu-guang W; Tannin inhibits HIV-1 entry by targeting gp41. Acta Pharmacol Sin., 2004; 25(2): 213-218.
21. Akiyama H, Kazuyasu F, Yamsaki O, Oono T, Iwatsuki K; Antibacterial action of several Tannins against *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy, *2001;* 48(48): 487-491.
22. Funatogawa K, Hayashi S, Shimomura H, YoshidaT, Hatano T, Ito H *et al.*; Antibacterial activity of hydrolysable tannins derived from medicinal plants against *Helicobacter pylori*. Microbiol Immunol., 2004; 48(4), 251-261.
23. Sani AA, Emmanuel TA, Abdulrahim RO, Abdulkareem SS, Ramat AB, Ilyas M; A study review of documented phytochemistry of *Vernonia amygdalina* as the basis for pharmacological activity of plant extracts. Journal of Natural Sciences Research, 2012; 2(7): 25-57.
24. Enyi-Idoh KH, Etim SE, Ofor UA, Mandor BI, Ogar MJ; Evaluation of the antibacterial potential of *Vernonia amygdalina* on food borne pathogens isolated from Kunu sold in Calabar, Nigeria. Microbiol Biotech Res., 2012; 2 (5): 778-782.
25. Gislene GF, Locatelli J, Freitas PC, Silva GL; Antimicrobial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian Journal of Microbiology. 2000; 31(04): 20-33.
26. Abdu PA, Sa'idu L, George BDJ; Diseases of local poultry in Nigeria. Discovery and Innovation, 2000; 14(1/2): 107-118.
27. Cos P, Hermans N, De Bruyne T, Apers S, Sindambiwe JB, Vanden Berghe D *et al.*; Further evaluation of Rwandan medicinal plant extracts for their antimicrobial and antiviral activities. Ethnopharmacology, 2002; 79(2): 155-163.
28. Ahmed I, Olonitola OS, Yakubu SE, Ado SA; Characterization and susceptibility of *Escherichia coli* isolated from infantile diarrhea to *Momordica charantia* and *Aeschynomene uniflora* extracts in kano state, Nigeria. A Ph.D thesis, Ahmadu Bello University, Zaria, Nigeria, 2014.
29. Akortha EE, Aluyi HAS; Plasmid mediated antibiotic resistance among some members of the Enterobacteraceae from diarrhoeal patients. Trop J Environ Sci Health, 2002; 5(1): 13-16.
30. Isyaku I, Sule M, Akujobi CO; Antidiarrhoeal activity of leaf extract of *Chrozophora senegalensis* (Euphorbiacea). Techno Science Africana Journal, 2001; 2(1): 115-118.
31. Sherris JC, Kenneth JR, George CR; Sherris medical microbiology, an introduction to infectious diseases.4th Edition and revised, Mc GRAW Hill Medical Publishing Division, USA, 2003.
32. Kurosoki F, Nishi A; Isolation and antimicrobial activity of the phytoalexin–6 methoxymellein from cultured carrot cells. Phytochemistry, 1983; 22(3): 669-672
33. Banso A, Adeyemo SO; Evaluation of antibacterial properties of tannins isolated from *Dichrostachys cinerea*. African Journal of Biotechnology, 2007; 6 (15): 1785-1787.
34. Abalaka ME; Studies on the antimicrobial activity of bark extracts of *Khaya senegalensis*. An M.Sc. Thesis, University of Ilorin, Nigeria, 2003.