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Toxicological Study and Antibacterial Activities of Effectively Validated Medicinal Plants against Enteric Bacteria Isolated from Chicken Feeds

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Abstract
This study focused on the toxicological study and antibacterial activities of effectively validated medicinal plants against enteric bacteria isolated from chicken feeds sold in Anambra State. A total of 1,536 different chicken feed samples (starter, growers, finisher and layers) were collected from open markets and shops and screened for the presence of enteric bacteria using pour plate technique. The isolates were characterized and identified using their colony descriptions, biochemical and molecular characteristics. The toxicological study of the plant extracts was investigated by exposing the chicks to various concentrations of the extracts for a period of 4 weeks. The isolates were screened for their *in vitro* susceptibility to medicinal plant extracts (*Gongronema latifolium, Piper guineense, Xylopia aethiopica* and *Zingiber officinale*) at 500mg/ml using agar well-diffusion method. The result of this study revealed that *Escherichia coli* O157:H7 SS52 (EC), *Salmonella* serovar Typhimurium U288 (ST), *Escherichia coli* SEC470 (ES), *Salmonella* serovar Enteritidis YU39 (SY) and *Salmonella* serovar Enteritidis FM366 (SE) were isolated from the feed samples. The toxicological study revealed that 0.5 ml of the plant extracts increased the white blood cell indices, and there was no toxic effect on the haematological indices and body organs. The *in vitro* activity showed that the test extracts significantly ($P<0.05$) inhibited the test organisms and *Zingiber officinale* (ZO) extract proved to be most significant ($P<0.05$) against the tested organisms. This study has revealed that EC, ST, ES, SY and SE were the enteric bacteria detected from the studied feed samples. The tested plant extracts have proved to be safe and effective against the isolates, of which *Zingiber officinale* (ZO) extract showed the most pronounced activity.
Keywords: Toxicological study, Medicinal plants, Enteric bacteria, Chicken Feeds.

INTRODUCTION

Chicken diseases have contributed significantly to increase in mortality rate and economic losses in the chicken industry. As a result, antibiotics, sometimes at sub-therapeutic concentrations, are often included in chicken feeds to prevent disease, enhance feed conversion efficiency and improve growth rates (Oguttu *et al.,* 2008). However, the use of antibiotics in chicken feeds is not totally safe. One of the main concerns is the development of antibiotic resistant bacteria (Oguttu *et al.,* 2008). The rampant use of antibiotics in chicken production has resulted in the development and maintenance of populations of antibiotic-resistant enteric bacteria in the intestinal tracts of these chickens and their products (Oguttu *et al.,* 2008). The clinical significance of this phenomenon is that selective pressure for resistance caused by using antibiotics may result in multiple antibiotic resistance and these antibiotic resistant bacteria are known to be transmissible from chicken to man (Oguttu *et al.,* 2008). The use of naturally produced antimicrobial agents without any adverse effects on human health to inhibit the proliferation of pathogenic bacteria in chicken feed is a more congenial option to overcome the problems associated with feed contamination (Tharmanaj and Shah, 2009).

Use of medicinal plants as a source of relief and cure from variety of illness is as old as humankind itself. Even today, medicinal plants provide a cheap source of drugs for the greater number of the world's population. Plants have provided and will continue to provide not only directly usable drugs, but also various chemical compounds that can be used as starting points for the synthesis of new drugs with improved pharmacology properties .Many modern medicines have their origins in plants (Yang *et al*., 2012).

Although Africa contains about 10% of the world's plant diversity, there is still much work that needs to be done on the medicinal plants from this continent (Yang *et al*., 2012). Recently ethnopharmacology is progressing in Africa but in general, there is limited research and investigation regarding the therapeutic potential of medicinal plants (Yang *et al*., 2012).

Several studies have indicated the possibility that the use of plant extracts in high doses could lead to toxic injury to the kidney which interfere with renal tubular functions and induce acute renal failure. Patients are often unaware of the similarities and differences between medicinal plants and approved medications. Some mistakenly think of herbs as natural alternative to chemicals, failing to recognize that herbs are composed of bioactive chemicals some of which may be toxic. For example, *Allium* vegetables, including garlic are used

throughout the world for their sensory characteristics as well as their apparent health benefits. However, high consumption of crushed raw garlic was reported to produce many undesirable clinical effects such as anaemia, weight loss, growth retardation and decrease of caecal microflora and serum protein (Soetan and Aiyelaagbe, 2009). The adoption of crude levels of plants, such as infusions, for self medications by the general public has risen in the possibility that the impact of several diseases may be either ameliorated or prevented by improving the natural constituents of natural nutrients (Soetan and Aiyelaagbe, 2009). Although few of these medicinal plants have been validated scientifically. These calls for a need to further investigate safer preparations of ethnomedinal preparations in view of reducing undesired side effect associated with them. Therefore this study focused on the toxicological study and antibacterial activity of effectively validated medicinal plant against enteric bacteria isolated from chicken feeds.

MATERIALS AND METHODS

Collection of Samples: A total of 1536 commercially produced chicken feed samples (starter, growesr, finisher and layers) were aseptically collected from the wholesalers, retailers and consumers. The feed types which included X (756 samples), Y (756 samples) and Z (756 samples) were aseptically collected from twenty-one (21) major towns located within Anambra State. One cup of the feed sample was aseptically collected from each feed type by randomly collecting one Table spoon of the feed sample from each bag containing the feed type. The feed samples were mixed and homogenized to generate a representative sample for each feed type. The feed samples were collected from Broiler starter (128 samples), Grower mash (128 samples), Broiler finisher (128 samples) and Layer mash (128 samples) for each feed type (X, Y and Z) using aluminum foil. The samples were carefully labeled and classified based on the sources of collection. The feed samples were transported in cooler containing ice block for laboratory for analysis.

Culture and Isolation of Enteric Bacteria: This was carried out using the modified method of Cheesbrough (2000). One gram (1.0g) of each sample was dissolved in 5.0 ml of sterile distilled water, then make up the volume to 10.0 ml prior to serial dilution. One milliliter aliquot was aseptically transferred into a sterile test tube containing 9.0 ml of the diluent (distilled water) and from this; ten-fold serial dilutions were made up to 10^{-3}. One milliliter of the sample was plated on *Salmonella-Shigella* agar (SSA/Biotech) for *Salmonella* and *Shigella* species and MacConkey agar (MA/Biotech) for *E. coli*. All the plates in triplicates were incubated inverted at 44.5˚C for 24 h for *E. coli* and 37˚C for 24 h for other enteric bacteria.

Characterization and Identification of the Isolates: The isolates were subcultured on nutrient agar (Biotech), incubated invertedly at 37˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions (Cheesbrough, 2000).), biochemical reactions (Cheesbrough, 2000) and molecular characteristics (Habtamu *et al.,* 2011; Gabriela *et al.,* 2014).

Preparation of plant materials: The fresh leaves of *Xylopia aethiopica, Piper guineense* and *Gongronema latifolium* and rhizomes of *Zingiber officinale* were collected from cultivated land at Uli in Ihiala L.G.A of Anambra State, Nigeria. The sample was authenticated by Ukpaka C. J, a botanist in Biological Science Department, Faculty of Sciences, Ana nmbra State University, Uli. The fresh leaves of *Xylopia aethiopica, Piper guineense* and *Gongronema latifolium* and rhizomes of *Zingiber officinale* were dried under shade at room temperature for 14 days. The dried leaves were ground to powdered form using sterile electric grinder. Twenty gram of the ground leaves each was macerated with distilled water and ethanol respectively for 72 h. The mixture was filtered using Whatman No 1 filter paper. The extracts were concentrated by evaporating to dryness at room temperature in a steady air current (Nwobu *et al.,* 2010)

Phytochemical analysis of the plant extracts: The phytochemical components (alkaloids, glycosides, flavonoids, phenolics, tannins, steroids and saponins) of the plant extracts were determined quantitatively using the methods described by Iheukwumere and Umedum (2013)

Alkaloids: Five milliliters of the sample was mixed with 96% ethanol and 20% tetraoxosulphate (VI) acid (1:1). One milliliter of the filtrate from the mixture was added to 5 ml of 60% tertraoxosulphate (VI) acid and allowed to stand for 5 minutes. Then 5 ml of 0.5% formaldehyde was added and allowed to stand for 3 h. The reading was taken at absorbance of 550nm.

Glycosides: This was carried out using Buljet's reagent. One gram of the fine powder of the sample was soaked in 10 ml of 70% alcohol for 2 h and then filtered with Whatman No. 1 filter paper. The extract was then purified using lead acetate solution and disodium hydrogen tetraoxosulphate (VI) solution before the addition of freshly prepared Buljet's reagent. The absorbance was taken at of 550nm.

Flavonoids: Five milliliters of the extract was mixed with 5 ml of dilute hydrochloric acid and boiled for 30 minutes. The boiled extract was allowed to cool and then filtered with Whatman No. 1 filter paper. One milliliter of the filtrate was added to 5 ml of ethyl acetate and 5 ml of 1% ammonia solution. The absorbance was taken at 420nm.

Phenolics: Ten milliliters of the sample was boiled with 50 ml acetone for 15 minutes. Five milliliters of the solution was pipetted into 50 ml flask. The 10 ml of distilled water was added. This was followed by addition of

2 M ammonium hydroxide solution and 5 ml of concentrated amyl alcohol solution. The mixture was left for 30 minutes and absorbance was taken at 550nm.

Tannins: Ten milliliters was pipetted into 50 ml plastic containing 50 ml of distilled water. This was mixed for 1 h on a sterile mechanical shaker. The solution was filtered with Whatman No. 1 filter paper, and 5 ml of the filtrate was mixed with 2 ml of iron (III) chloride solution in 0.1 N hydrochloric acid. The absorbance was taken at 550nm.

Steroids: The extract was eluted with normal ammonium hydroxide solution. Two milliliters of eluate was mixed with 2 ml of chloroform in a test tube. Three milliliters of ice cold acetic anhydride was added to the mixture and allowed to cool. The absorbance was taken at 420nm.

Saponins: Five milliliters of the sample was dissolved in aqueous methanol. The 0.25 ml of aliquot was taken for spectrophotometric determination for total saponins at 544nm.

Toxicological studies of the plant extracts: A total of fifteen (15) chicks (two-week old) were used for this study. The chicks were fed on diet specially prepared from chick growers mash (Pfizer Company, Nigeria) and were giving water ad libitum throughout the study period. The toxicity study was carried out using the modified method of Bulus *et al.* (2011). The chicks were divided into five groups and three chicks in each. The first group was giving 0.5 ml aqueous extract of *Zingiber officinale,* second group was giving 0.5 ml aqueous extract of *Piper guineense,* third group was giving 0.5 ml aqueous extract of *Gongronema latifolium,* fourth group was giving 0.5 ml aqueous extract of *Xylopia eathiopica and* the fifth group was giving 0.5 ml of normal saline (control group). The chicks were fed with chick growers' mash and maintained in their cage for 4 weeks. The chicks were monitored daily for 24 h, 48 h and 72 h for lethal toxicity. Recovery and weight gain were seen as indication of having survived the lethal toxicity. The hematological parameters of the chicks were determined after the period of 4 weeks. After 4 weeks, all the surviving chicks were sacrificed and autopsied at the Department of Anatomy, Anambra State University Uli. The internal organs were examined macroscopically for pathological changes compared to the control group.

Determination of extract value of the plant materials: The concentration of the extract was determined by evaporating 1.0 g of the extract in an evaporating dish of known weight in an oven to dryness and weighed. The dish containing the residue was allowed to cool and weighed. The weight of the residue was obtained by subtracting the weight of the empty dish from the weight of the dish and residue. The above method was done in duplicate (Iheukwumere *et al*., 2012)

Preparation of the test samples of the plant extracts for *in vitro* antibacterial susceptibility tests: In this study the concentration of 500 mg/ml of the extract was used to screen for the antimicrobial activity. This was done by using the modified method of Iheukwumere *et al*. (2012). Here, 2.5 g of the extract was dissolved in 5.0 ml of peptone water.

***In vitro* antibacterial susceptibility testing of the plant extracts using agar well diffusion method:** This was carried out by the modified method of Iheukwumere and Umedum (2013). Each labeled plate was uniformly inoculated with the test organism using pour plate method in Muller Hinton Agar (MHA). A sterile cork borer of 5mm diameter was used to make the wells on the medium. One tenth milliliter of various concentrations of the extracts were dropped into each labeled wells and then incubated at 37˚C for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation. Diameter less than 5.50 mm was considered resistant while diameter 5.50 mm and above was considered sensitive.

Statistical Analysis: The results of the data generated were expressed as mean ± standard deviation (SD). The statistical analysis of data generated from protective study was carried out using chi-square at 95% confidence limit (Wafaa *et al.,* 2012). The data generated from this study were examined using SPSS package program version 20.0. Data were analyzed by one-way Analysis of Variance (ANOVA) to determine the significant difference of the mean values at 95% confidence limit. Pair wise comparison of mean was done by Least Significant Difference (LSD) (Wafaa *et al.,* 2012, Dashe *et al.,* 2013)

RESULTS

The morphological characteristics of the isolates are shown in Table 1. Isolates 5, 7 and 11 were isolated from *Salmonella-Shigella* agar (SSA) and they exhibited similar morphological characteristics on SSA plates. In addition, isolates E and G exhibited similar morphological characteristics on MacConkey agar (MA) plates. The biochemical characteristics and identities of the enteric bacterial isolates are shown in Table 2. The results of the present study reveal that isolates 5, 7 and 11 exhibited similar biochemical characteristics; they showed positive results to hydrogen sulphide production, catalase, and methyl red, utilize citrate as carbon source and able to ferment glucose, dulcitol, arabinose and maltose. Isolate 5 fermented inositol, showed slight positive reaction to xylose and was negative to mucate unlike isolates 7 and 11 that fermented xylose but negative to inositol. Isolates E and G exhibited similar biochemical properties; they showed positive results to Indole reaction, methyl red, catalase and able to ferment glucose, maltose, arabinose and lactose.

The results of the sequencing of 16s rRNA using universal primer (16s) revealed the presence of

Escherichia coli 0157:H7 strain SS52 (isolate E), *Escherichia coli* strain SEC 470 (isolate G), *Salmonella enterica* subspecies *enterica* serovar Typhimurium strain U288 (isolate 5), *Salmonella enterica* subspecies *enterica* serovar Enteritidis strain FM366 (isolate7) and *Salmonella enterica* subspecies *enterica* serovar Enteritidis strain YU39 (isolate11) (Table 3).

The quantitative phytochemical constituents of *Gongronema latifolium, Piper guineense, Xylopia aethiopica* and *Zingiber officinale* extracts are shown in Table 4. The results showed that *G. latifolium* extract contained significantly ($P<0.05$) higher alkaloids, tannins and saponins than other extracts. *Piper guineense* extract contained significantly ($P<0.05$) higher cardiac glycosides and non-significantly higher phenolics than other extracts. *Xylopia aethiopica* extract contained non-significantly higher steroids than other extracts. *Zingiber officinale* extract contained significantly ($P<0.05$) higher flavonoids than other extracts. The presence of these phytochemical constituents in the extracts could be responsible for the antibacterial activities of the extracts.

The toxicity of the plant extracts was studied and the results are shown in Tables 5, 6, and 7. The result of the acute lethal effect of aqueous plant extracts administered orally to the chickens (Table 5) showed that no death was recorded after exposing the chickens to the extracts for period of 72 h. The haematological parameter values obtained after administering 0.5ml aqueous plant extracts (Table 6) showed that there was no significant difference among the values of pack cell volume (PCV), Haemoglobin (Hb), neutrophil, eosinophil, basophil and red blood cell counts (RBCs) obtained after administering 0.5ml aqueous plant extracts and normal samples. The lymphocytes and white blood cells counts (WBCs) values obtained after administering 0.5ml aqueous extracts of *Z. officinale* and *G. latifolium* were slightly higher than the values obtained from the normal samples. Also, the red blood cell counts (RBCs) and Haemoglobin (Hb) values obtained after administering 0.5 ml aqueous extract of *Z. officinale* were slightly higher than the values obtained from the normal samples. The post mortem result for the toxicity of aqueous plant extracts (Table 7) showed that the lungs, hearts, kidneys and spleens of the test chickens were normal, with congestion in their livers, except those chickens administered aqueous extract of *Z. officinale* which showed slight congestion in their livers.

The diameter zones of inhibition of the extracts against the tested organisms using 5mm cork borer is shown in Table 8. The ethanolic extracts of *Z. officinale, G. latifolium, P. guineense,* and *X. aethiopica* significantly ($P<0.05$) inhibited the test organisms more than the aqueous extracts. The extracts significantly ($P<0.05$) inhibited *E. coli* O157:H7 SS52 most and *S.* ser. Typhimurium U288 least. Also, the extracts of *Z. officinale* significantly ($P<0.05$) inhibited *E. coli* O157:H7 SS52 and S. ser. Enteritidis FM366 and non-significantly inhibited *S.* ser. Typhimurium U288 more than other extracts.

Table 1: Morphological characteristics of the isolates from chicken feed samples

Parameter \ Isolate	E	G	5	7	11
Appearance on agar plate	Red colony on MA	Red colony on MA	Colourless with black center on SSA	Colourless and dark at the center on SSA	Colourless and dark at the center on SSA
Edge	Entire	Entire	Entire	Entire	Entire
Size (mm)	1.00	1.20	2.20	1.40	1.60
Consistency	Soft	Soft	Soft	Soft	Soft
Optical property	Opaque	Opaque	Opaque	Opaque	Opaque
Elevation	Slightly raised	Convex	Slightly raised	Slightly raised	Slightly raised
Pigmentation	–	–	–	–	–
Gram Reaction	–	–	–	–	–
Shape	Rod	Rod	Rod	Rod	Rod
Motility	+	+	+	+	+

SSA = *Salmonella-Shigella* Agar, MA = MacConkey Agar, + = Positive, – = Negative

Table 2: Characteristics and identities of the enteric isolates from the chicken feed samples

Parameter \ Isolate	E	G	5	7	11
Indole production	+	+	–	–	–
Hydrogen Sulphide	–	–	+	+	+
Ornithine decarboxylase	–	–	–	–	–
Methyl Red	+	+	+	+	+
Voges-Proskauer	–	–	–	–	–
Citrate Utilization	–	–	+	+	+
Catalase	+	+	+	+	+
Urease	–	–	–	–	–
Glucose	+	+	+	+	+
Maltose	+	+	+	+	+
Dulcitol	–	–	+	+	+
Lactose	+	+	–	–	–
Xylose	+	+/–	+/–	+	+
Arabinose	+	+	+	+	–
Inositol	–	–	+	–	–
Mucate	–	–	–	+	+

E – *Escherichia coli*, G – *Escherichia coli*, 5 – *Salmonella* species, 7 –*Salmonella* species
11 – *Salmonella* species, + = Positive, – = Negative

Table 3: Molecular identities of the isolates

Isolate	Max score	Total score	Query Cover	Gap	Identity	Accession Number	Description
E	2856	2967	100%	0%	100%	CO010304.1	*Escherichia coli* strain 0157:H7 str SS52 Complete genome
G	1297	1297	100%	0%	96%	CP007594.1	*Escherichia coli* strain SEC470 Complete genome
5	2193	4386	100%	0%	98%	CP003836.1	*Salmonella enterica* subsp. enterica serovar Typhimurium str U288 Complete genome
7	660	660	100%	0%	96%	NG03836.1	*Salmonella enterica* subsp. enterica serovar Enteritidis str FM366 Complete genome
11	2844	2844	100%	0%	100%	CP011428.1	*Salmonella enterica* subsp. enterica serovar Enteritidis str YU39 Complete genome

Table 4: Phytochemical constituents of various extracts studied

Phytochemical constituent	*G. latifolium* (g/100g)	*P. guineese* (g/100g)	*X. aethiopica* (g/100g)	*Z. officinale* (g/100g)
Alkaloids	10.19	1.86	1.92	10.12
Tannins	7.62	2.81	0.62	4.38
Saponins	3.14	0.18	0.22	0.81
Flavonoids	1.06	0.10	0.44	5.62
Phenolics	1.25	1.81	1.51	1.32
Steroids	0.04	0.06	0.11	0.02
Cardiac glycosides	0.32	1.64	0.35	1.08

Table 5: Acute lethal effect of aqueous plant extracts administered orally to the chickens

Substances Used	No of Chickens			No of Death After		No of Chickens Survived	Administered
	24 h	48 h	72 h				
AEZ	3		0	0	0	3	
AEP	3		0	0	0	3	
AEG	3		0	0	0	3	
AEX	3		0	0	0	3	
Distilled	3		0	0	0	3	water

AEZ - Aqueous Extract of *Zingiber officinale*, AEP- Aqueous Extract of *Piper guineense*
AEG- Aqueous Extract of *Gongronema latifolium*, AEX- Aqueous Extract of *Xylopia aethiopica*

Table 6: Haematological effect of the aqueous plant extracts administered orally to the Chickens

Parameter	AEZ	AEP	AEG	AEX	Control
PCV (%)	28.50±1.32	27.00±1.00	28.00±1.00	26.50±1.41	28.00±0.00
Hb (g/l)	8.80±0.10	8.40±0.30	8.70±0.20	8.20±0.30	8.70±0.10
Neutrophils	25.00±2.00	28.00±1.00	23.50±2.10	27.50±2.00	25.00±1.00
Lymphocytes	68.00±3.00	66.50±1.00	68.00±2.00	66.00±3.00	67.00±0.00
Eosinophils	5.00±0.00	5.50±0.15	6.50±0.10	4.50±0.10	6.00±0.10
Basophils	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
Monocytes	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
RBC (x 10^9)	2.53±0.03	2.48±0.02	2.51±0.02	2.38±0.03	2.51±0.01
WBC (x 10^9)	8.80±0.15	8.20±0.10	8.70±0.10	8.15±0.20	8.50±0.10

PCV – Pack Cell Volume Hb – Haemoglobin RBC – Red Blood Cell Counts
WBC – White Blood Cell Counts AEZ – Aqueous Extract of *Zingiber officinale*
AEP – Aqueous Extract of *Piper guineense* AEG – Aqueous Extract of *Gongronema latifolium*
AEX – Aqueous Extract of *Xylopia aethiopica*

Table 7: Post mortem result for toxicity of aqueous plant extracts administered orally to the chickens

Organ	AEZ	AEP	AEG	AEX	Control
Liver	Slight congestion	Congestion	Congestion	Congestion	Normal
Lungs	Normal	Normal	Normal	Normal	Normal
Heart	Normal	Normal	Normal	Normal	Normal
Spleen	Normal	Normal	Normal	Normal	Normal
Kidney	Normal	Normal	Normal	Normal	Normal

AEZ – Aqueous Extract of *Zingiber officinale*, AEP – Aqueous Extract of *Piper guineense*
AEG – Aqueous Extract of *Gongronema latifolium*, AEX – Aqueous Extract of *Xylopia aethiopica*

Table 8: Diameter (mm) zones of inhibition of the plant extracts against the test organisms using 5mm cork borer

Extract (500mg/ml)	*E. coli*	*S.* ser. Typhimurium U288	*S.* ser. Enteritidis FM366
EEZ	14.10 ± 0.26	10.90 ± 0.10	11.60 ± 0.10
EEP	12.20 ± 0.26	10.80 ± 0.21	10.90 ± 0.20
EEG	11.30 ± 0.44	10.80 ± 0.10	10.40 ± 0.17
EEX	9.50 ± 0.20	7.40 ± 0.17	8.90 ± 0.17
AEZ	6.60 ± 0.17	5.90 ± 0.30	6.20 ± 0.16
AEP	—	—	—
AEG	—	—	—
AEX	—	—	—

EEZ — Ethanolic Extract of *Zingiber officinale,* EEP — Ethanolic Extract of *Piper guineese*
EEG — Ethanolic Extract of *Gongronema latifolium,* EEX — Ethanolic Extract of *Xylopia aethiopica*
AEZ — Aqueous Extract of *Zingiber officinale,* AEP — Aqueous Extract of *Piper guineese*
AEG — Aqueous Extract of *Gongronema latifolium* AEX — Aqueous Extract of *Xylopia aethiopica*

DISCUSSION

The presence of *Escherichia coli* O157:H7 SS52, *Escherichia coli* SEC470, *Salmonella enterica* subspecies *enterica* serovar Typhimurium U288, *Salmonella enterica* subspecies *enterica* serovar Enteritidis FM366 and *Salmonella enterica* subspecies *enterica* serovar Enteritidis YU39 from studied feed samples supported the occurrence enteric bacteria in the samples (Davies and Wales, 2010; Chowdhuri *et al.,* 2011; Fredrick and Huda, 2011).

The absence of death after 72 h exposure of the chickens to the tested plant extracts suggested that the extract could be saved for *in vivo* study (Bulus *et al.,* 2011).The increase in lymphocytes and WBCs observed from the study could be attributed to some phytochemicals of the plant capable of boosting the immune system. Flavonoid acts as an antioxidant which is known to protect the lymphocytes and reduce their destructive abilities (Duthie *et al.,* 1996). The decrease in red blood cells counts (RBCs) and haemoglobin (Hb) values associated with *P. guineense* and *X. aethiopica* extracts, point to the fact that there is an indication of microcystic anaemia (Adebiyi, 2007; Post *et al.,* 2007). Kar (2007) reported that variation in RBCs could be due to quantitative values of saponins in the plant. Saponins are known to be poisonous as they cause haemolysis of erythrocyte.

The liver congestion, which was the major pathology accompanying the *in vivo* administration of the plant extracts could be attributed to its detoxification action of the liver (Bulus *et al.,* 2007; Bulus *et al.,* 2011). From

the results of the toxicological study of plant extracts, the aqueous plant extracts are safe for usage in both traditional medicine and *in vivo* study. And aqueous extract of Z. officinale proved to be potent and safe for *in vivo* study.

The phytochemical constituents present in the plant extracts could be responsible for the antibacterial activity of the various sample extracts. Similar findings were made by different researchers (Parekh *et al.,* 2005; Iheukwumere *et al.,* 2012).The higher activity of ethanolic extracts than the aqueous extracts shows that the active components of the plants dissolved in ethanol than water. Similar conclusion was drawn by different researchers (Iheukwumere *et al.,* 2012). The study further highlighted that ethanol was able to extract most of the phytochemical constituents because it is an organic and polar solvent, and most of the phytochemicals are organic in nature. This finding suggested that the organic solvent extraction is suitable to verify the antibacterial properties of the medicinal plants (Ali *et al.,* 2001; Parekh *et al.,* 2005). *Zingiber officinale* extracts proved to be highest among the tested extracts. This could be due to quantitative variations and potency of the phytochemical constituents of the plants (Ghoshal *et al.,* 2006).

CONCLUSION

This study has revealed the presence of *Escherichia coli* O157:H7 SS52*, E. coli* SEC470 *Salmonella* serovar Typhimurium U288, *Salmonella* serovar Enteritidis FM366 and *Salmonella* Enteritidis YU39 in the chicken feed samples randomly collected from major towns in Anambra State, of which the occurrences of E. coli SEC470 and S. serovar Enteritidis YU39 were negligible due to very low counts of the isolates from the studied samples. The *in vitro* study of the susceptibility patterns of these organisms to leaves extracts of *Xylopia aethiopica, Piper guineense* and *Gongronema latifolium* and rhizomes extracts of *Zingiber officinale* showed the safety and pronounced activities of the extracts, of which *Zingiber officinale* extracts proved to be most effective.

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