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Original Article

In-vitro antibacterial effect of *Ocimum gratissimum* on Broiler gut microflora

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Abstract

Ocimum gratissimum Linn is a medicine herb that is used in tropical world particularly in Asia, Africa and South America. Because of the restrictions in the use of antibiotics in animal health, alternative drugs are being considered. This study was designed to assess the antibiotic activity of O. gratissimum for the control of gut microorganisms. Proximate and phytochemical analysis of the herb was carried. To 100 day old chicks, aqueous extract of the herb was administered to a set of 50 chicks (Ocimum treatment) but was not given to the second set (control). Antibiotic sensitivity testing of gut microbes (*Salmonella* and E. coli) isolated from the chicks was tested using 0.1 g/l dried *O. gratissimum* extract. In the control , the zone of inhibition (ZOI) of Salmonella was 13.2, 11.8 and 14.8mm at the crop, ileum and caecum respectively whereas in the Ocimum treatment, the ZOI were significantly higher ($P<0.05$) being 15.0, 15.6 and 15.6 mm respectively. The pattern of sensitivity of Salmonella and *E.coli* was similar. The zone of inhibition was significantly wider in the chicks administered with *Ocimum gratissimum* extract compared to the control. Antibiotic activity of the herb may be due to the presence of phytochemicals particularly sapronins, alkaloids, flavonoids and tannins. We conclude by recommending the use of the plant for the control of pathogenic microbes of broilers.

1. Introduction

Ocimum gratissimum Linn, which belong to the family Lamiaceae[1-3] or Labiate[4-7], is a medicinal herb/spice used in the tropical world particularly in Asia, Africa and South America. The plant is variously called wild basil, cove basil, sweet basil, scent leaf, fever plant, teabush[8]. The plant is a wonder herb used for the treatment several ailments such as headache and stomach ache, constipation, insomnia, asthma and bronchitis, wounds and ulcers, epilepsy, rheumatism and paralysis[5,8-11]. *O. gratissimum* has analgistic activity, anti inflammatory and antimutagenic activity, antihypertensive and immunostimulatory effects, antidiabetic and hepatoprotective effects, antioxidant, prevent hair loss, and protects the central nervous system, Cardiovascular and renal function properties, anti-carcinogenic, free-radical scavenging strike[5.8,10-13]. *O. gratissimum* also possess broad spectrum antimicrobial and anti parasitic activities[7], specifically antibacterial Labiate[4,5,8], antifungal[2,4,5,8], anti helminthic[5.8], insect repellant[8,14] and nematicidal activity[5]. Due to its broad antimicrobial activities, *O. gratissimum* extracts have been used for the treatment of upper respiratory tract infections and cough, various types of fever including typhoid fever, urinary tract infection, skin diseases (eczema, scabies and dermatitis), sexually transmitted diseases (gonorrhea and other urogenital infections), enteric diseases (diarrhea, dysentery, shigellosis, salmonellosis), measles, ear and eye infections (conjunctivitis, red eye)[5,8].

The antimicrobial and therapeutic properties of *O. gratissimum* has been variously reported to be due to the presence of phytochemicals including alkaloids, tannis, glycosides, phytate, saponins, phenolics, flavonoids, resins, steroids, terpenes, aromatic and volatile oil, linolenic acid, oleic acid and other essential oils (eugenol, methyl eugenol, ocimene, germaerne, cineole and selinene[1,3,7,9,12-16]. *O. gratissimum* also contain vitamins and minerals[10,11]. Because of these properties, *O. gratissimum* is used for the production of soup[4,14,17], spice meat[14] and for the protection of grains[8] and as insect repellant[14]. Aluko et al[17]; Nweze and Eze[6] reported several culinary uses of *O. gratissimum* including soup, pastas, salads, vinegar and jellies).

Globally, the poultry industry is challenged with the problem of microbial infection, which has resulted in mortality of birds, productivity and at great economic losses to the industrial. And because of increasing resistance and possible carry over to human, the use of antibiotic growth promoters (AGP) have been restricted in many countries. Hence, a need for alternative intervention to control bacteria induced poultry infections and enhanced performance of birds. Probiotics and prebiotics have been promoted to control infections in poultry[18-21]. Mushrooms have been used to control infections particularly coccidiosis, salmonella and *E. coli*, while enhancing beneficial lactobacillus[22-29]. The use of traditional veterinary medicine (ethno-veterinary medicine) is gaining prominence especially in Africa and Asia[30-34]. Ethno veterinary medical application have been demonstrated for the control of animal diseases of small ruminants[35]; cattle[36], poultry[37] and pig[38]. The utilization of underutilized medicinal plants in animals health have been found to be effective, cheap and practicable[39]. Hence, in this study, we aim to demonstrate the effectiveness of *O. gratissimum* in the control of enteric infections in broilers.

2. Materials and Method

2.1 Source of Experimental Birds and preparation of brooder house

One hundred day old (ANAK 2000) commercial broilers were purchased at CHI farm, Ibadan, Nigeria and transported to Niger

Delta University Teaching and Research Farm where the experiment was carried out. Vittalyte was administered to the birds due to stress resulting from transportation.

The brooder house and it environment was cleaned with detergent and disinfectant (Z-germicide) two weeks prior to the arrival of the birds. Electric bulb (200Watts) was used at the brooding stage as a source of heat and light. The feeders and drinkers were properly washed prior to brooding. The birds were brooded for seven days before random distribution to their respective treatments and replicates.

2.2 Source of Ocimum gratissimum and its preparation methods

Ocimum gratissimum used in this experiment were purchased from Tombia junction market in Yenagoa Local Government Area of Bayelsa State, Nigeria. The *Ocimum gratissimum* leaves inclusive of the stem was dried in the laboratory using an oven at a temperature of 55^{o}C for four days respectively. The dried samples were milled to semi powder form. Thereafter, 25ml of petroleum spirit was utilized for extraction using soxhlet method. The extract was later placed on a Rotatory evaporator to allow the petroleum spirit to evaporate and subsequently followed by placing in water bath. This was to ensure that extract obtained was from *Ocimum gratissimum*.

2.3 Phytochemcial screening of *Ocimum gratissimum*

Phytochemical screening of alkaloids, flavonoids, saponins, tanins and glycosides was carried out on the obtained extract using the method previously described by AOAC (1977)[40]

Alkaloids

About 0.2g of the sample was boiled with 5ml of (2%) hydrochloric acid on a steam bath for 5min, the mixture was allowed to cool and filtered. The filtrate was shared in equal proportion into three test tubes labelled A, B, and C. One mile (1ml) of the filtrate from each individual test tube was treated with two drops of Mayer's reagent and a creamy white precipitate was observed. Also to confirm this first result, 1ml portion of the filtrate was treated with Dragendroffs reagents which gave a red precipitate to indicate the presence of alkaloids.

Flavonoids

To 0.5g of the sample, 10ml of ethyl acetate was added and heated in boiling water for 1 minute. The mixture was filtered and 4mls of the filtrate was shaken with 1ml of (1%) aluminum chloride solution and left to stand for some time. Formation of yellow coloration in the presence of 1ml dilute ammonia solution indicated the presence of flavonoids.

Saponins

About 0.1g of the sample was boiled with 5ml of distilled water for 5min and the mixture filtered while still hot. To 1ml of the filtrate, 2 drops of olive oil was added, the mixture was shaken and observed for the formation of emulsion. One mille (1ml) of the filtrate was diluted with 4ml of distilled water. The mixture was shaken then observed for the formation of a stable froth up standing.

Tannins

To 2g of the sample, 5ml of (45%) ethanol was added and boiled for 5minute the mixture was cooled and filtered, and to 1ml of the filtrate, 3 drops of lead sub acetate solution was added. The formation of a gelatinous precipitate indicates the presence of tannins. For the confirmation test for the presence of tannins, 1ml of the filtrate was collected and 0.5ml of bromine water was added and the formation of a pale brown precipitate indicated the presence of tannins.

Glycosides

About 2g of the sample was mixed with 30ml of distilled water and heated for 5minute on a water bath and filtered. To 5ml of the filtrate, 0.2ml Fehling's solution A and B was added and heated in a water bath for 2minute. A light blue coloration was observed instead of the brick red coloration which indicates the absence of glycoside.

2.4 Proximate composition of *Ocimum gratissimum*

The proximate parameters determined for the water hyacinth include, protein, lipid, ash, fiber, moisture etc. the guide provided by AOAC (1977) was used for the analysis[40].

2.5 Experimental design and Digesta Collection

The experiment was design as a complete design. The birds were distributed to two treatment having five replicates of ten birds per replicate. Birds in treatment A (control) were not administered aqueous *Ocimum gratissimum, W*hile birds on treatment B (ocimum treatment) were administered aqueous *Ocimum gratissimum.* Aqueous *Ocimum gratissimum* was administered on day eight after brooding. Digesta was collected seven days after the administration of aqueous *Ocimum gratissimum* from one bird per replicate of each treatment after slaughtering to obtain digesta from the crop, ileum and caceum. Digesta collected was used to isolate for *Salmonella spp* and *Escherichia coli* for in-vitro antibacterial effect of the *Ocimum gratissimum* extract previously obtained to isolate for the aforementioned bacteria and for further in-vitro study stated earlier. All digesta collected from the gastrointestinal tracts i.e. - ileum, caecum and crop were collected into sterile container for the microbial analysis.

2.6 Identification of the test Isolates

The test organism used for the antimicrobial studies was isolated clinically from the gastrointestinal tracks (i.e. crop, ileum and ceacum) of the experimental birds using MacConkey agar for *Escherichia coli* and *Salmonella-Shigella* Agar for *Salmonella* species respectively. The presence of pinkish red growth with a metallic sheen or reflection on MacConkey Agar indicated *Escherichia coli*[41] after aerobic incubation at 44º C for 24 hours. The presence of black colonies indicated *Salmonella* species on *Salmonella-Shigella* Agar after 37°C for 24 hours. The organisms were placed in pure culture.

2.7 Antibacterial Activity

Antibacterial sensitivity of the pure culture of bacteria isolates were determined using agar well diffusion method as reported by Kigigha and Atuzie (2012)[42] with slight modification. Whatman No. 1 filter paper was cut into circular disc using a perforator giving a diameter of 6mm. The disc was autoclaved at 121^{0}C for 20 minutes so as to denature the chemical used in its preservation as well as making it sterile before imbedding the extracts.

Pure cultures of test isolates were transferred into sterile nutrient broth and incubated at 37^{0}C for 18h. The test organisms were pre- adjusted to the 0.5 McFarland's turbidity standards. About 0.1ml of the test organism was introduced on the surface of sterile Mueller Hinton agar plates and evenly distributed using spreading rod. The disc was dipped into the plant extract using a sterile forceps for 15 seconds and transferred aseptically into the surface of the plates containing the test organism. The zone of inhibition (ZOI) was measured using transparent meter rule.

2.8 Statistical analysis

Data collected were subjected to general linear model (GLM) using SPSS version 17, while Duncan multiple Range test was used to separate the means at P=0.05.

3. Results and Discussion

Phytochemical analysis indicated that the *O. gratissimum* used in the study is high in sapronins, moderate in glycosides but low in tannins, alkaloids and flavonoids (Table 1). Several authors have reported the presence of these phtochemicals in *O. gratissimum*. Many authors have reported the presence of alkaloid, tannin, sapronin, flavonoid and glycoside in *O. gratissimum*[3,6,11,43]. Efiong[12] reported the concentration of phytochemical in the leaves s follows; 0.56% tannin, 1.93% flavonoids, 0.41% alkaloids, 1.0% sapronins.

Emeka and Chimaobi [10] reported 0.167 – 0.267 mg/g alkaloid, 0.307 – 0.437 mg/g flavonoids, 0.286 – 0.370 mg/g tannins and 0.349 – 0.406mg/g phytate. Adewole [14] reported 11.43% alkaloid, 7.5% phenol, 10.90% tannin, 8.2% flavonoid and 12.87% sapronin. Ladipo et al[7] reported 0.17% phenol, 0.82% alkaloids, 1.95% tannins, 0.68% flavonoid and 1.92% sapronin.

Table1: Phytochemical Analysis of *Ocimum gratissimum*

Phytochemicals	Concentration
Saponins	+++
Tannins	+
Glycoside	++
Flavonoids	+
Alkaloids	+

+ = Low; ++ = Moderate and +++ = High concentration

The proximate composition of fresh *O. gratissimum* used in the study had a proximate composition of 4.21 crude fibre, 2.24% crude protein, 1.06% ash, 87.60% moisture and 4.14% carbohydrate (Table 2). Other authors have reported the proximate composition of *O. gratissimum* leaves. Adewole [14] reported 10.30% moisture, 2.45% ash, 2.78% fat, 16.51% protein, 9.07% fibre and 58.89% carbohydrate. Idris et al[4] reported 82% moisture, 13.67% ash, 3.33% protein, 8.5% fat, 9.52% fibre and 64.98% carbohydrate. Aluko et al[17] reported 63.96% carbohydrate, 0.04% protein, 12% ash, 17% fibre and 7%crude fat. Efiong [12] reported 4.43% protein, 2.7% fat, 4.2% crude fibre and 1.15% ash. The differences in the proximate composition of *O. gratissimum* reported in literature may be due to the preparation of the samples which makes the moisture content to vary and the type of solvent used in extraction.

Table 2: Proximate Composition of fresh *Ocimum gratissimum*

Parameters	Proximate composition, %
Crude fibre	4.21
Crude protein	2.24
Ash	1.06
Moisture content	87.60
Carbohydrate	4.14
Crude Lipid	0.98

Table 3 presents antibiotic activity of *O. gratissimum* extract against Salmonella obtained from the gut of broiler chicken. Results show that Salmonella species obtained from the gut of chicks administered with Ocimum (Treatment B) was more sensitive than those obtained from the chicks that were not given *O. gratissimum* extract (Treatment A) ($P<0.05$). The reason for these differences in sensitivity pattern is not known, but it could be due to the prior exposure of the organisms to the herb in treatment A. In treatment A, the zone of inhibition (ZOI) was 13.2, 11.8 and 14.8mm at the crop, ileum and caecum respectively whereas in Treatment B, the ZOI were significantly higher ($P<0.05$) 15.0, 15.6 and 15.6 mm respectively. At a concentration range of 25 – 75% Ocimum water extract, Bankole et al[44] reported 7 – 15mm, which is comparable to our findings. At 20 – 30mg/ml, water extract of Ocimum had a lower ZOI of 10mm against *Salmonella*[7]. Other authors reported higher ZOI values than ours. Adebolu and Oladimeji[45] reported ZOI of 26.0, 18.0 and 8.0mm against *Salmonella typhi* at a concentration of 0.1%, 0.01% and 0.001%. At a concentration of 6.25 – 100g/ml, Olamide and Agu[46] reported a ZOI of 10 – 30mm against Salmonella species. At a concentration of 1.5 – 7.5 mg/g, Matasyoh et al[47] reported a ZOI of 7.5 – 20.5 mm against *S. typionurium*.

Table 3: Antibiotic activity *Ocimum gratissimum* against *Salmonella* species on gastrointestinal track of chickens

Gut section	Sample	Zone of inhibition (mm)	Positive Control (mm)	Negative Control (mm)
Crop	Treatment A	13.2 ± 2.9b	23.0	0.0
	Treatment B	15.0 ± 2.3d	16.0	0.0
Ileum	Treatment A	11.8 ± 3.3a	20.0	0.0
	Treatment B	15.6 ± 2.1e	19.0	0.0
Caecum	Treatment A	14.8 ± 2.3c	21.0	0.0
	Treatment B	15.6 ± 2.1e	22.0	0.0

Treatment A was not administered with *Ocimum gratissimum*; Treatment B was administered with aqueous extract of *Ocimum gratissimum*; Negative Control = Distilled water; Positive Control = Ampiclox; n= 3

The sensitivity analysis of *E. coli* was similar to the pattern observed with Salmonella. The ZOI was significantly wider in treatment B than treatment A. in treatment A, the ZOI of aqueous Ocimum extract against *E. coli* was 12.4, 11.4 and 15.8mm at the crop, ileum and caecum respectively, whereas for Treatment B they were 14.6, 13.6 and 16.2 mm respectively. Nakamura et al[48] reported a ZOI of 16-17mm at a concentration of 24 – 48mg/100ml. Ocimum extract in demethyl sulfoxide. Nwinyi et al[49] reported a ZOI of 3mm *against E. coli* at an aqueous concentration of 10mg/ml Ocimum extract. Nweze and Eze[6] reported lack of inhibition of *E. coli* at 6.25 – 100mg/ml extract of *O. gratissimum*. Mabekoje et al[3] reported a ZOI of 8mm for water extract and 6.0 mm for ethanol extract of Ocimum against *E. coli*. Adebolu and Oladimeji[45] reported a ZOI of 29mm at 0.1% and 11 mm at 0.01% extract of ocimum against *E.coli.* Olamide and Agu[46] reported a ZOI of 12 – 23 mm at 6.25 – 100g/ml ocimum extract against *E. coli*.

Table 4: Antibacterial activity of *Ocimum gratissimum* against *Escherichia coli* isolated from the gut of chickens

Gut section	Sample	Zone of inhibition (mm)	Positive Control (mm)	Negative Control (mm)
Crop	Treatment A	12.4 ± 1.8 b	23.0	0.0
	Treatment B	14.6 ± 2.9d	21.0	0.0
Ileum	Treatment A	11.4 ± 3.8a	24.0	0.0
	Treatment B	13.6 ± 2.1c	22.0	0.0
Caecum	Treatment A	15.8 ± 1.9 e	19.0	0.0
	Treatment B	16.2 ± 1.3 f	22.0	0.0

Treatment A was not administered with *Ocimum gratissimum*; Treatment B was administered with aqueous extract of *Ocimum gratissimum*; Negative Control = Distilled water; Positive Control = Ampiclox; n= 3

4. Conclusion

Ocimum gratissimum has many medicinal uses that have been well documented. This study was designed to assess its possible use for the control of salmonella and E. coli infections in the gut of broilers. *Salmonella* and *E. coil* that were obtained from chickens that were previously administered with aqueous extract of the herb in their drinking water were tested for antibiotic sensitivity using the disc diffusion method. OUR Results showed that the herb exhibited antibiotic activity that was sensitive to the two gut microorganism tested.

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