http://dx.doi.org/10.22161/ijeab/4.1.34 *ISSN: 2456-1878*

In-vitro Antibiotic Activity of Dry Ginger Root Extract against Potential enteropathogenic Bacteria isolated from two Weeks old Broiler Chickens

Zige, V. D.[1] and Ofongo, R. T. S.[2]

[1]Department of Microbiology, Faculty of Science, Federal University, Otuoke, P.M.B. 126, Yenagoa, Bayelsa State, Nigeria.
Email: zigedouye@gmail.com

[2]Poultry Nutrition and Animal Biotechnology Research Group, Department of Animal Science, Niger Delta University, Wilberforce Island, Bayelsa State Nigeria
Email : ruthofongo@gmail.com ; tariruth@live.de

Abstract**—** *Zoonotic enteropathogenic bacteria of poultry origin is a source of concern to public health. Dependent on various factors, poultry birds may not necessarily fall prey to sub-clinical infections induced by zoonotic enteropathogenic bacteria. A preliminary study was carried out to determine antibacterial activity of dry ginger root (Zingerbain officinalle) extract on Salmonella sp and Escherichia coli isolated from the crop, ileum and caecum of two weeks broiler birds. The in-vitro antibacterial activity was determined by agar disc diffusion method on pre-prepared sensitivity test agar with respective cells inoculated and incubated after 24 hours. Zone of inhibition measured in millimeters after observation gave an inhibition zone of 11.8 ± 2.2, 15.0 ± 2.4, 15.0 ± 2.4 and 14.0 ± 2.9, 13.0 ± 2.3, 8.2 ± 2.6, 15.0 ± 2.2, against Salmonella and E coli respectively, isolated from crop, ileum and caecum. The highest zone of inhibition was observed in the ileum (15.0 ± 2.4) for Salmonella and caecum (15.0 ± 2.2) for E coli. The least was observed in crop (11.8 ± 2.2) and ileum (8.2 ± 2.6) respectively. It is evident that dry ginger root extract has anti-bacterial activity against Salmonella sp and E. coli isolated from the gut of two weeks old broiler birds.*

Keywords**— Antibiotics, Escherichia coli, Ginger root, in-vitro, Salmonella spp.**

I. INTRODUCTION

Enteric infections with pathogenic bacteria can cause mortality in both humans and animals. Such infections are responsible for reduced growth rates and consequent economic losses in animal production. Antibiotics are the main tools for the prevention and treatment of animal infections. Unfortunately, the long term and extensive use of antibiotics for veterinary purpose may eventually result in selection for the survival of resistant bacterial strain [1; 2]. Genes encoding for this resistance can also be transferred to other formerly susceptible bacteria, thereby posing a threat to both animal and human health [3;2]. Consequently, some countries have banned (Sweden) or limited (European Union), total withdrawal (European Union) the general use of in-feed antibiotics as growth promoters in animals [3]. In poultry, the issue of gut health cannot be over emphasized and any attempt to enhance or improve it in the absence of antibiotic growth promoters will be considered an innovation in the right direction. Antibiotics enhance performance of broilers but may not necessarily create an environment that may lead to increased *Lactobacillus* (beneficial bacteria) counts in the gut [4;5]. Although several alternatives to in–feed antibiotics exist, the role of phytobiotic (antibiotic properties of medicinal plants) in the control of certain bacteria in the gut cannot be overemphasized [6]. Many active ingredients from plants are considered as pro–nutrients because of the role they play in enhancing the physiology and microbial load of the animal and as such used in animal feeds [6; 7; 8].

Ginger (*Zingerbain officinale*) roscoe has been exploited for its medicinal value. Some of its pharmacological effect include anti–cancer, antioxidant, potent antibacterial activity, potent anti-fungal activity and anti-helminths' activity [9;10;11]. In–vitro bacteriostatic potential of a commercial ginger paste against *E. coli* has been reported [12]. In view of its potential, a preliminary study was carried out to determine the in –vitro anti-bacterial activity of dry ginger root extract on two potential enteropathogenic bacteria (*Salmonella sp* and *Escherichia coli*) isolated from two weeks old broiler chickens.

II. MATERIALS AND METHODS

http://dx.doi.org/10.22161/ijeab/4.1.34 *ISSN: 2456-1878*

Digesta (content of the gastrointestinal tract - GIT) was collected from different sections (crop, ileum and caecum) of the GIT of two weeks old broiler chicks. Each bacteria type was isolated on their specific agar on triplicate bases. *Salmonella spp* was isolated on *Salmonella Shigella* agar and *Escherichia coli* on Eosine methylene blue agar. Prior to the isolation of pure cultures of each bacterium (*Salmonella sp* and *Escherichia coli)*, dry ginger was milled and the extract obtained by extracting with petroleum ether in a soxhlet apparatus. The extraction was carried out at the Department of Chemical Sciences, Niger-Delta University.

2.1 Antibacterial activity

The disc diffusion method of [13] was used to determine the in-vitro anti-bacterial activity of the ginger extract. To achieve this Whatman No. 1 filter paper was cut into circular disc of 6mm using a pre–sterilized cork borer. Sterilization of the cork borer was done at 121°C for 20minutes to denature and destroy completely the entire chemical used in its preservation as well as making it sterile before imbedding the extract. The dish was then dipped into the ginger extract using sterile forceps for 15sec and transferred aseptically into sterile glass Petri dish and kept in the oven at 55°C until the following day. Each test bacterium was pre – adjusted to the 0.5 McFarlands turbidity standard in a test tube, dipped with sterile swab stick and used to seed on solidified oxoid sensi-test agar in an inoculating chamber. The prepared disc was carefully transferred onto the inoculated culture plates using sterile forceps. The plates were incubated for 24hr at 37°C, after which the zone of inhibition was measured in millimeters and recorded for each bacterium. The same procedure was repeated for each bacterium using ampiclox as a positive control and distilled water as a negative control. The in – vitro antibacterial activity was done on triplicate basis for each bacterium and the test carried out at the Medical Microbiology Unit, Federal Medical Centre Yenagoa, Bayelsa State.

2.2 Statistical analysis

Data collected on the zone of inhibition (ZOI) by *Salmonella spp* and *Escherichia coli* isolated from different sections of the gut were subjected to statistical analysis using SPSS package volume 17 and significant means separated using LSD (least significant difference).

III. RESULTS

Results on in – vitro antibacterial activity of dry ginger extract on *Salmonella spp* and *E. coli* is presented in the Table below. The antibacterial activity of dry ginger extract was more evident and almost similar to that of Ampiclox (a positive control) against *Salmonella spp* and *E. coli* isolated from the caecum. This was also the case with *Salmonella spp* isolated from the ileum. Distilled water (negative control) had no inhibitory effect on *Salmonella spp* isolated from any section of the gut. Ampiclox was more effective against *Salmonella sp* isolated from the crop with a value of 23 mm recorded against 11.8 mm recorded for dried ginger extract. Antibacterial activity of dried ginger extract against *E. coli* indicated in Table 1 showed an almost similar zone of inhibition (15.0 ± 2.2 mm) with Ampiclox (18 mm).

The negative control (distilled water) also had no antibacterial activity against *E. coli.* The extract was least effective (8.2 ± 2.6mm) against *E. coli* isolated from the ileum compared to ampiclox (20 ± 1.3mm). A value of 13.0 ± 2.3 mm was recorded for *E. coli* isolated from the crop against 23 mm for ampiclox the positive control.

Although the extract was not standardized and dosage determined in this study however, the results indicated a positive inhibitory effect of the extract against each bacterium and in certain cases almost similar effect compared to ampiclox a standard drug in use for humans affected with these bacteria. The two bacteria in the current study are zoonotic enteropathogenic bacteria in poultry gastrointestinal tract and are of public health concern to humans, the ultimate consumers of poultry products.

IV. DISCUSSION

The primary constituents of ginger root are gingerol, zingebain, bisaboline, oeloresins, starch, essential oil, mucilage, protein flavonoids and chemical compounds. According to [14] these constituents are active against a wide array of microorganisms from in–vitro studies. The gingerols have antipyretic, antibacterial and gastrointestinal tract mobility activity thereby capable of eliminating harmful bacteria such as *E. coli* [15]. In the light of this and reports of previous in–vitro studies by means of disc diffusion assay as in this study, an inhibition zone of 9mm [16] or more appearing around the disc indicates presence of antibacterial substance in the extracts tested [17]. Results of the current study agree with these observations even though the least value of 8.2 ± 2.6 mm was recorded against *E. coli* isolated from the ileum. The antibacterial potency of ginger is mainly attributed to the presence of oxygenated mono- and sesquiterpenes, phenolic compounds (shagaol, gingerols) – [18]. They are lipid – soluble phenolic compounds isolated from ginger roots [17;19]. These compounds have different mode of action. They can only attack cell walls and cell membranes (although not tested in this study) by affecting the permeability and release of intracellular constituents (e.g. ribose sodium (Na) glutamate) but they also interfere with membrane

functions. Apparently, this could have been one of the likely reasons for the positive zone of inhibition recorded for both bacterium tested. Apparently, electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity might have been involved in the inhibitory effect of the extract against each bacterium. In a further study, [20] these compounds may have several invasive targets which could lead to the inhibition of bacterial pathogens. An earlier report by [12], a commercial ginger paste exhibited antibacterial activity against *E. coli* (0157:H7 in peptone water at 4°C for two weeks. From our study, supplementation of spices and herbs may have many benefits to broiler health and performance. These include having antioxidant properties [21], antimicrobial activity [8] and enhancing digestion by stimulating endogenous enzymes [22] when included in the diet of broiler chickens. Furthermore, It has been reported that the greater the ZOI, the stronger the antimicrobial effect [23]. The antimicrobial effect, in terms of activity and efficacy of plant extracts on gut microbes can however be quite variable as observed in this study and stated in earlier reports [24]; [25]. This variability can be dependent on the plant source, extraction process, quality and consistency of the product as asserted by [23].

V. CONCLUSION

The ginger extract exhibited inhibitory effect on the bacteria tested. However, further studies are suggested to enable standardization and determination of dosage.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Mr Fidelis Dogiye Ifiemi for assisting with sample collection during the experiment.

REFERENCES

[1] Aarestrup, F.M. (1999). Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. *International Jour. Antimicrobial Agents.* Vol. 12:279-285. https://doi.org/10.1016/S0924-8579(99)90059-6

[2] Munk, P., Knudsen, B.E., Lukjancenko, O., Ribeiro Duarte, A.S., Van Gompel, L., Luiken, R.E.C., Smit, L.A.M., Schmitt, H., Garcia, A.D., Hansen, R.B., Petersen, T.N., Bosers A., Ruppe, E., EFFORT GROUP, Lund, O. Hald, T., Pamp, S.J., Virge, H., Heederik, D. Wagenaar, J.A., Mevius, D., Aarestrup, F.M. (2018). Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. *Nature Microbiology* Vol. 3:898–908. https://doi.org/10.1038/s41564-018-0192-9

[3] Montagne, L., Pluske, J.R., Hampson, D.J. (2003). A review of interactions between dietary fibre and the intestinal mucosa and their consequences on digestive health in young non-ruminant animals. *Animal Feed Science and Technology Vol.* 103: 95-117. doi:10.1016/S0377-8401(03)00163-9

[4] Abule, S. T. R.; Obaweki, V. E.; Etebu, E., Ohimian, E. I. (2014). Effect of feed additives on performance and lactobacillus counts in broilers. In Proceedings: Animal Science Association of Nigeria Annual conference (ASAN – NIAS JAM) Ibadan: 2014. Pp.

[5] Ofongo–Abule, R.T.S., Etebu, E. And Ohimain, E.I. (2016). Performance and molecular identification of bacteria isolated from the gut of broiler birds after antibiotic administration and enzyme supplementation. *Journal of microbiology, biotechnology and food sciences.* Vol 6 (3): 924 – 929. DOI: 10.15414/jmbfs.2016/17.6.3.924-929

[6] Dieumou, F.E., Teguia, A., Kuiate, J.R, Tamakou, J.D., Fonge, N.B., Dongmo M.C. (2009). Effect of ginger (*Zingeber officinale*) and garlic (*Allium sativum*) essential oils on growth performance and gut microbial population of broiler chickens. *Livestock Research for Rural Development. Volume 21, Article #131.* Pp. 25 – 34. Retrieved February 20, 2019, from http://www.lrrd.org/lrrd21/8/dieu21131.

[7] Zhang, G. F., Yang, Z. B., Wang, Y., Yang, W. R., Jiang, S. Z., G. S. Gai. (2009). Effects of ginger root (*Zingiber officinale*) processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. *Poultry Science.* Vol. 88 (10): 2159–2166. https://doi.org/10.3382/ps.2009-00165

[8] Doorman, H.J.D., Dean, S.G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of applied microbiology* 2000; 88:308 – 316. https://doi.org/10.1046/j.1365-2672.2000.00969.x

[9] Sarica, S., Cifti, A., Demir, E., Kilinc, K., Yildirim, Y. Use of antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzyme s in wheat-based broiler diets. *South African journal of animal science* 1995; 35: 61 – 72. http://dx.doi.org/10.4314/sajas.v35i1.4050

[10] Akoachere, J. F., Ndip, R.N., Chenwi, E.B. Antibacterial effect of *Zingeber officinale* and Garcinia kola on respiratory tract pathogens. *East African medical journal* 2002; 97 (11):588 – 592. Akoachere, J. F., Ndip, R.N., Chenwi, E.B. Antibacterial effect of *Zingeber officinale* and

Garcinia kola on respiratory tract pathogens. *East African medical journal* 2002; 9 (11):588 – 592. DOI: 10.4314/eamj.v79i11.8804

[11] Great-head, H. Plants and plant extracts for improving animal productivity. Proceedings of the Nutrition Society, 2003; *62*(2): 279-290. https://doi.org/10.1079/PNS2002197

[12] Gupta, S., Ravishankar, S. A comparism of the antibacterial activity of garlic, ginger, carrot and turmeric paste against *Escherichia coli* 0157:H7 in laboratory buffer and ground beef. *Food Borne Pathogen Dis* 2005; 2 (4): 330 – 340. DOI:10.1089/fpd.2005.2.330

[13] Kigigha, L. T., Atuzie, M. N. Assessment of traditional medicinal application of *Alchornea cordifolia African Journal of Biotechnology* 2012;11(8): 2083-2086. http://dx.doi.org/10.5897/AJB09.1615

[14] Ekwenye, U.N., Elegalam, N. N. Antimicrobial agents of ginger (*Zingeber officinale* roscoe) and garlic (*Allium sativumL*) extracts on Escherichia coli and Salmonella typhi. *Inter. Jour. Mol. Med. Adu. Sci.* 2005; 4:411–416. http://medwelljournals.com/abstract/?doi=ijmmas.2005.411.417

[15] Gull, I., Saeed, M., Shaukat, H., Aslam, S.M., Samra, Z.Q., Athar, A.M. (2012). Inhibitory effect of *Allium sativum* and *Zingiber officinale* extracts on clinically important drug resistant pathogenic bacteria. *Ann clin microbiol antimicrob*. Vol 27(11):8. DOI:10.1186/1476-0711-11-8

[16] Güven, K., Yücel E., and Cetintaş F. (2006). Antimicrobial activities of fruits of *Crataegus* and *Pyrus* Species, *Pharmaceutical Biology*, 44:2, 79-83. DOI: 10.1080/13880200600591253

[17] Wang, W., Li, C.Y., Wen, X.D., Li, P., Qi, L.W. Simultaneous determination of 6 – gingerol, 8 – gingerol, 10 – gingerol and 6 – shogaol in rat plasma by liquid chromatography – mass spectrometry. Application to pharmacokinetics, chromatogram B Analytical Technology. *Biomed life science* 2009; 877: 671 – 679. doi: 10.1016/j.jchromb.2009.01.021.

[18] Michielin E. M., Salvador, A. A., RiehI, C.A., Smania, A. J., Smania, E.F. Chemical composition and antibacterial activity of *Cordia verbenacea* extracts obtained by different methods. *Bioresource Technology* 2009;100: 6615 – 6623.

[19] Liu Z. (2011). Diterpene Glycosides as Natural Solubilizers. United States Patent Application Publication. Patent Department Taylor, Porter, Brooks and Philips, 2011.

[20] Bajpai, V.K., Al-Reza, S.M., Choi, U.K., Lee, J.H. and Kang, S.C. (2009). Chemical composition, antibacterial and antioxidant activities of leaf essential oil and extracts of *Metasequioa glyptostroboides* Miki ex Hu. *Food Chemistry Toxicology*. Vol 47: 1876 – 1883. *DOI:10.1016/j.fct.2009.04.043*

[21] Hui, Y.H. (1996). Oleoresins and essential oils. In: Hui, YH, editor. Bailey's industrial oil and fat products. New York, Wiley-Interscience Publication, Cap. 6. 1996, pp: 145-153.

[22] Brugalli, I. (2003). Alternative feeding: the use of Phyto therapeutic or nutraceuticals as modulators of immunity and animal performance. In: SYMPOSIUM ON THE MANAGEMENT AND NUTRITION OF BIRDS AND SWINE, 2003, Campinas, SP.Anais ... Campinas: CBNA, 2003. p.167-182. Sao Paulo Brasil. Campinas: CBNA 2003: pp: 167 – 182.

[23] Ewing, W.N. [2008]. The living gut: An introduction to the identity, location and activity of the gastro – intestinal micro flora and their benefits on health and performance of animals. Nottingham University Press UK. Pp 149 – 157.

[24] Ofongo – Abule, R.T.S. and Ohimain, E.I. (2015). Proximate composition and antimicrobial effect of *Ocimum gratissimum* on broiler gut microflora. Journal of *Advances in Biological and Basic Research*, 1 (1): 24 – 27.

[25] Ohimain, E. I., Ofongo – Abule, R.T.S. and Zige, D.V. (2015). In – vitro antibacterial effect of *Ocimum gratissimum* on broiler gut microflora. *Bulletin of Advance Scientific Research* 1(1): 37 – 41.

Table 1: Antibacterial activity (mm) of dry Zingiber officinale (ginger) extract on bacterial isolates from broiler chicken gut

Gut section/ bacteria specie	Ginger extract (mm)	Positive control (mm)	Negative control (mm)
Salmonella spp			
Crop	11.8 ± 2.2^{b}	23 ± 1.2^{a}	0.00±0.00
Ileum	15.0 ± 2.4^{a}	18 ± 1.8^{a}	0.00±0.00
Caecum	14.0 ± 2.9^{a}	16 ± 1.4^{a}	0.00±0.00
Escherichia coli			
Crop	13.0 ± 2.3^{b}	23 ± 1.4^{a}	0.00±0.00
Ileum	8.2 ± 2.6^{b}	20 ± 1.3^{a}	0.00±0.00
Caecum	15.0 ± 2.2^{a}	18 ± 2.2^{a}	0.00±0.00

[ab]: means along the same row are significantly different ($p<0.05$). N=3