The Antibacterial Effect of *Aloe Vera* Gel Extract against *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* Isolated from Gastrointestinal Tract of Poultry Birds.

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ABSTRACT

The Antibacterial effect of Aloe vera gel extract against Escherichia coli, Salmonella typhi and Staphylococcus aureus isolated from the gastrointestinal tract of poultry birds was investigated. Standard microbiological methods were used for the isolation of Escherichia coli, Salmonella typhi and Staphylococcus aureus. The prevalence of Escherichia coli, Salmonella typhi and Staphylococcus aureus in the droppings of the poultry birds was 89%. The organisms were identified following various biochemical tests, like Gram Staining, catalase test, Coagulase test, Citrate test and Indole test. The antibacterial activity of Aloe vera gel extracts were standardized by plating out 1.05×10^5 cfu/ml, while serial dilutions of 10^{-1}-10^{-10} were used to evaluate efficacy of the inhibitory effect using the Agar Gel diffusion method on different concentration on the test organisms. The result shows that Aloe vera gel extract at different concentrations inhibited the growth of the microorganisms. Though there was a higher inhibitory effect on Staphylococcus aureus at 10^{-1}, 13.5 mm and 14.5 mm for Ist and 2nd agar well respectively and a lower inhibitory growth of 8 mm and 8.3 mm at 10^{-8}. There was no inhibition on 10^{-9} and 10^{-10}, followed by Escherichia coli at 10^{-1}, 11.5 mm and 12.5 mm for Ist and 2nd agar well respectively and a lower inhibitory growth of 10 mm at 10^{-6}. There was no inhibition on 10^{-7} to 10^{-10} and lastly Salmonella typhi at 10^{-1}, 10.5 mm and 11.5 mm for Ist and 2nd agar well respectively and a lower inhibitory growth of 9 mm at 10^{-7}. There was no inhibition on 10^{-8} to 10^{-10}. The study revealed that Aloe vera gel extract possessed antibacterial properties. Therefore, it can be added to the feed of poultry birds as a prophylactic to reduce bacterial infections and would be used to test the natural efficacy of Herbal medicine.

Key Words: Antibacterial; Aloe vera; Escherichia coli; Salmonella typhi; Staphylococcus aureus; Gastrointestinal tract; Poultry Birds

INTRODUCTION

Plants have evolved the synthesis of some chemical compounds that aids in the eradication of many microorganisms, most especially the enteric microorganisms. One of the most effective plants that possess this feature is the Aloe vera (Aloe barbadensis miller). This study will examine the effect of this plant on some selected enteric microorganisms. The synthesis of some natural compounds from the plant plays a part

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in antibacterial effect [1]. There are a growing number of antibiotics resistant strains of bacteria now threatening our health, which mostly are the enteric bacteria. Aloe vera extracts has been known to have healing powers for centuries over some of these enteric bacteria. Though, practically everybody is aware of Aloe vera gel which is used for treating burns, skin infections, acne, diabetes, leg ulcer and 'gastro intestinal ulcers. Whole leaf of Aloe Vera can eliminate dozens of harmful bacteria. The diseases associated with these bacteria are some of the most common and fatal of all in our time [2]. Enteric microorganisms are those that dwell in digestive tracts of an animal which is the largest reservoir of human flora. Bacteria like *Bacillus subtilis, Esherichia coli, Streptococcus mutans, Serratia marcescenes, Salmonella typhi, Pseudomonas aeruginosa* and *Lactobacillus spp*, and Staphylococcus aureus, are some of most disease causing microbial agents in medical research. Amongst these listed bacteria, research has shown that Escherichia coli, Salmonella typhi and Staphylococcus aureus are the most severe of all. Aloe vera extract will be used against to know their susceptibility and resistance effects. These microorganisms have done more harm than good in the gastro intestinal tract of animals especially poultry birds, as they cause various infectious diseases. Escherichia coli is a gram-negative rod shaped bacterium that is commonly found in the lower intestine of warm-blood organism. Staphylococcus aureus is facultative anaerobic, Gram-positive coccus, which causes its enteric pathogenicity through food poisoning and most common cause staphylococcus infections. *Staphylococcus aureus* is commonly a body micro flora found on part of the skin, the nose and in the mouth. About 20% of the human populations are long term carriers of *staphylococcus aureus* [3]. The corotenoid pigment staphyloxaqthin is responsible for its characteristic golden color, which may be seen in the colonies of the microorganism. S. aureus can cause a range of illness from minor skin infections, such as Impetigo, Boils (furuncles), Cellutis, and also cause intestinal tract infection mostly through food poisoning [4]. Typhoid fever, also known as typhoid, is a common world wild illness, transmitted by the ingestion of food or water contamination with the feces of an infected person, which contain Salmonella typhi. The bacterium perforates through the intestine wall and phagocytosed by macrophages. It is a gram- negative, short bacillus that is motile due to its peritrichous flagella.

Poultry farming is a type of animal husbandry in which domestic birds like chicken, ducks, turkeys and geese are raised and cared for to produce meat or eggs for food and income. Poor handling and management of poultry birds make them very susceptible to diseases and an outbreak can easily spread throughout the entire farm [5]. This can result in significant financial losses and affect the reputation of the business. More so, due to high cost of conventional medicines and vaccines coupled with lack of knowledge of herbal medicine by farmers, these drugs are out of reach of some small scale farmers. There is therefore need for cheaper and easier to use medicinal plants for local poultry diseases control and sustainable healthy living of the poultry birds; hence the study of the potentials of aloe vera gel extract on enteric organisms.

The aim of this research is to determine the antibacterial effect of Aloe vera gel extract on the above discussed enteric microorganisms, compare the effect of Aloe vera plant on each of the three studied enteric microorganisms and also determine the organism amongst the three that is most sensitive to Aloe vera plants. Therefore, this study offers an improvement in the medicinal and natural activities of the plants which will in a long way promote the medical world and deepening the interest for microbiology as a discipline.

MATERIALS AND METHOD

- **Study area, sample collection and analysis**

This study was conducted at the Microbiology Laboratory (Caritas University, Amorji-Nike, Enugu State-Nigeria). Aloe vera plants were harvested from backyard garden and the gastrointestinal tract (collected from slaughter slab of Ogbete market, Enugu) of 20 poultry birds (Gallus gallus domesticus). The gastrointestinal tracts were analyzed immediately upon reaching the laboratory for the presence of

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Escherichia coli, Salmonella typhi and Staphylococcus aureus following the procedures in the bacteriological analytical manual of the FDA- USA [6].

- **Processing, Extraction of the plant sample and preparation of different concentration of the extract.**

A full expanded leaf of Aloe vera was selected from the whole plant, washed with distilled water and was subjected to surface sterilization with 70% alcohol. After the process, the parenchymatous covering of the leaf was peeled out and briefly, the Aloe vera gel was extracted under aseptic condition. Slurry was formed with the help of pestle and mortal which are sterile. 10g of the slurry Aloe vera gel was suspended in 50ml of 80% ethanol and kept on a shaker for 24 h for proper dissolution. One in 10 serial dilutions was made, in which the extract was diluted in a serial dilution from 10^{-1} to 10^{-10} dilutions; each test tube was added with 9ml of distilled water. A millimeter of the extract was added to the first test tube, and 1ml was pipetted from the same test tube down to 'the last test tube serially [7].

- **Isolation and Identification of the Test Organisms**

For the isolation of *Escherichia coli,* one g of gastrointestinal tract content was pre-enriched in 10 ml of Buffered Peptone Water (BPW) and incubated at 37^{o}C for 18-24 h. After which, the aliquots were plated on Levine Eosin-Methylene Blue Agar and incubated again at 37^{o}C for 24 h. For *Salmonella typhi*, aliquots from BPW were further enriched in Rappaport Vassiliadis (RV) and Selenite (SN) Broths. Samples in RV broth were incubated at 42^{o}C for 24 h while those in SN broth were incubated at 37^{o}C for 24-48 h. After incubation, the aliquots from RV and SN broths were plated on xylose lysine deoxycholate and brilliant green agar, and incubated at 37^{o}C for 24 h. For *staphylococcus aureus*, the GIT content was inoculated onto 5% sheep blood agar plates and plate were incubated for 24h at 37^{o}C. For presumptive, *Escherichia coli* and *Salmonella typhi* were purified on trypticase soy agar (incubated at 37^{o}C for 24 h) and confirmed using the appropriate biochemical tests as stated by [5]. Incubations done under aerobic conditions and all media used were purchased from Oxiod, Basingstoke, UK.

- **Standardization of Isolates and Screening for antibacterial Action**

The test organisms were standardized by plating about 1.05×10^{5}cfu/ml for each of the test organisms. Agar well diffusion method was used for the screening. The test organisms were inoculated in different Petri dishes and a sterile cup borer was used to bore hole (5mm) in each of them. Three holes were bore, in which one of them is a control for the test. Then the Aloe vera gel which has been diluted in a serial dilution from 10^{-1} to 10^{-10} dilution, in which each test tube was added with 9ml of sterile diluted water. 1ml the Aloe vera gel was added to the first test tube and, one drop was pipetted from there and diluted down to the last test tube. After which the Aloe gel was pipetted again from the prepared dilution, 0.1ml of the gel was dropped in each of the hole in the Petri dishes except the control which has only the Aloe gel. They were incubated for 34h^{rs} under 37°C. Clear zones of incubation were observed after incubation showing positive effects of the Aloe vera gel on the test microorganisms

RESULTS AND DISCUSSION

Table 1: Microscopic and Biochemical Characteristics of the Selected Isolates.

Colony Colour	**Texture**	**Form**	**G.rxn**	**I**	**C**	**CI**	**CO**	**Growth on agar(mm)**	**Probable Organism**
Whitish-Gold	creamy	Cocci	+	–	+	+	+	13.5	***Stapylococcus aureus***
Dark	Smooth	bacillus	–	–	+	–	–	10.5	***Salmonela typhi.***

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Greenmetallic sheen	Rough dry texture	Rods	–	+	+	–		11.5	***Escherichia coli.***

Key: G. rxn : Gram reaction I : Indole; C : Catalase; CI :Citrate; CO : Coagulase + = Positive; – = Negative;

Table 2: Antibacterial activity of Aloe vera gel extracted using different dilution from 10^{-1} and 10^{-10} serial dilution on *Staphylococcus aureus*

Agar Well (mm)	**Test Tube Dilution (mm)**									
	10^{-1}	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**10-6**	**10^{-7}**	**10^{-8}**	**10^{-9}**	**10^{-10}**
Ist Well	13.5	12.5	12	11.5	10.4	10.1	8.7	8.0	–	–
2nd Well	14.5	13	12.5	12	10.5	10	8.5	8.3	–	–
Average	14	12.7	12.3	11.8	10.5	10.05	8.6	8.2	–	–

Key: – Negative (No inhibition)

Table 3: Antibacterial activity of Aloe vera gel extracted using different dilution from 10^{-1} and 10^{-10} serial dilution on Escherichia coli

Agar Well (mm)	**Test Tube Dilution (mm)**									
	10^{-1}	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**10-6**	**10^{-7}**	**10^{-8}**	**10^{-9}**	**10^{-10}**
Ist Well	11.5	11.5	11.3	11	10.5	10	–	–	–	–
2nd Well	12.5	11.7	10.7	10.5	10.4	10.1	–	–	–	–
Average	12	11.6	11	10.8	10.5	10.05	–	–	–	–

Key: – Negative (No inhibition)

Table 4: Antibacterial activity of Aloe vera gel extracted using different dilution from 10^{-1} and 10^{-10} serial dilution on Salmonella typhi

Agar Well (mm)	**Test Tube Dilution (mm)**									
	10^{-1}	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**10-6**	**10^{-7}**	**10^{-8}**	**10^{-9}**	**10^{-10}**
Ist Well	10.5	10.5	10.3	10	9.8	9.5	9.0	–	–	–
2nd Well	11.5	11	10.5	10.5	10.4	9.6	9.0	–	–	–
Average	11	10.8	10.4	10.3	10.1	9.6	9.0	–	–	–

Key: – Negative (No inhibition)

Table 5: The Comparison of antibacterial activity of Aloe vera gel extracted using different dilution from 10^{-1} and 10^{-10} serial dilution on Escherichia coli, Salmonella typhi and Staphylococcus aureus

Sample Bacteria	**Ist Agar Well(mm)**	**2nd Agar Well (mm)**	**Least Agar well (mm)**	**No inhibition**
Staphylococcus aureus	13.5(10^{-1})	14.5(10^{-1})	8.0(10^{-8})	10^{-9} – 10^{-10}
Escherichia coli	11.5(10^{-1})	12.5(10^{-1})	10.0(10^{-6})	10^{-7} -10^{-10}
Salmonella typhi	10.5(10^{-1})	11.5(10^{-1})	9.0(10^{-7})	10^{-8} -10^{-10}

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This report revealed that the prevalence of Escherichia coli, Salmonella typhi and Staphylococcus aureus in gastrointestinal tract of poultry birds was 89% due to poor feeds, handling and management techniques used by our local farmers.

Aloe vera that was used in this study is a greenish succulent plant, spreading offsets; the leaves are thick and fleshy, similar to the work as described by [8]. The microorganisms that were used in this study showed the same characteristics as shown in their identification, which is so similar to the work done by [9]. Table 1; shows the result of the microorganisms after microscopic and biochemical reactions. Gram staining reaction shows that Staphylococcus aureus was Gram positive and Salmonella typhi was gram negative and Esherichia coli was Gram negative as shown by [10]. Gram-positive bacteria have a thick mesh like cell wall comprising of 50-90% peptidoglycan, while Gram-negative bacteria on the other hand have a thinner cell wall and an additional outer membrane composed of lipids. Gram-positive bacterium remains purple while the Gram negative ones pick the positively charged Safranin counter stain to stain pink. These characteristics agree with the bacterial characteristics guidelines published by [11].

Table 2, shows that Staphylococcus aureus was observed to be the most inhibited organism, thus showed at 10^{-1}, 13.5 mm and 14.5 mm for Ist and 2nd agar well respectively and a lower inhibitory growth of 8 mm and 8.3 mm at 10^{-8}. There was no inhibition on 10^{-9} and 10^{-10}, followed by Escherichia coli at 10^{-1}, 11.5 mm and 12.5 mm for Ist and 2nd agar well respectively and a lower inhibitory growth of 10 mm at 10^{-6}. There was no inhibition on 10^{-7} to $10^{-10}$and lastly Salmonella typhi at 10^{-1}, 10.5 mm and 11.5 mm for Ist and 2nd agar well respectively in Table 3 and a lower inhibitory growth of 9 mm at 10^{-7}. There was no inhibition on 10^{-8} to 10^{-10} seen in table 4. The three microorganisms have a great effect on the Aloe vera extract used. Staphylococcus aureus showed the highest laboratory actions compared to Esherichia coli and Salmonella typhi. Salmonella typhi had the second inhibitory action followed by Esherichia coli. Irshad [12] reported that Aloe vera extract produced an average inhibition zone of 2 mm against *Escherichia coli.* [13]. Irshad [12] again reported that Aloe vera extracted using acetone exhibited stronger activity against *Escherichia coli* as compared to aqueous or ethanol extracts. Ferro et al [13] reported that sap water extract was more effective than leaf extract against *Escherichia coli.*

The result showed that there is a variation in the degree of antibacterial activities of the extracts. The serial dilution that was made really showed the inhibitory effects of the extract against the test organisms as also shown in Table 2. The data gotten from this experiment agrees with the work done by [14]. There was no effect of distilled water, which serves as the negative control while the 100% extract serves as the positive control have a great effect on the test organisms. There was no inhibition in the Petridishes containing 10^{-7} dilution to 10^{-10}, thus shows that there was absolutely low antibacterial activity in the dishes. This is because the concentration of the aloe vera gel extract has been reduced due to the dilution from the most concentrated one from 10^{-1} to 10^{-10}·. This observation was also very similar to the work done by [15]. Another work done by [16] examined the antimicrobial activity of Aloe vera gel by using disc diffusion method and reported that, the gel was effective against Salmonella *enteric,* thus was in line with the findings of this study [17] indicated that, the Aloe vera gel is rich in variety of secondary metabolites, such as anthraquinones glycosides, glycoproteins, gamma-lanoline acid, prostaglandins and mucopolysaccharides, which are mainly responsible for its antimicrobial activity. In this study, there were differences in the antimicrobial activities of the Aloe vera gel extract. Azwanida [18] revealed that differences in antibacterial activity of Aloe vera plant extracts can be attributed to the age of the plant, physical factors such as temperature, light, water, time of harvesting of plant method used before the extraction process. This agrees with the work done by [19] on the factors that are suitable for the cultivation of Aloe vera. Coleby [20] suggest that some species of *Aloe vera* have been reportedly intolerant of very heavy frost or snow. These species re relatively resistance to most birds, insect, pest, which could cause a decline in the plant health.

From the result in Table 5, The comparism of the results gotten from the effects of Aloe vera gel against

Staphylococcus aureus, Escherichia coli and Salmonella typhi. It was clearly stated that Aloe vera gel work in a higher degree of inhibition on *Staphylococcus aureus* with 13.5 mm and 14.5 mm for Ist and 2nd well respectively; followed by Escherichia coli with 11.5 mm 12.5 mm for Ist and 2nd well respectively. The activity of the Aloe gel extract was least observed on Salmonella typhi with inhibition diameter of 10.5 and 11.5 for Ist and 2nd well respectively. Thus, this suggests that Aloe vera gel extract could be very effective in assisting healing incase of gastrointestinal disease, chronic wound [21]. Choi et al [22] again highlighted that Aloe vera gel extract may be able to assist in the cure of ulcerative colitis in birds, diverculitis and bowel irritable syndrome.

CONCLUSIONS

From the research, it could be seen that the probable organisms that were inhibited by Aloe vera gel extract used in the study were *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. The action of Aloe vera gel extract rendered the organisms unfit for bacterial virulent abilities. The presence of these bacteria in the gastrointestinal tract of poultry birds has for long being potential pathogens for poultry birds. Numerous antibiotics have showed incessant resistance to these organisms. This study obviously agreed on the fact that Aloe vera gel extract has a potential as an antibacterial agent in agriculture particularly in poultry farming. It is therefore recommended that the use of natural herbal medicine should be encouraged and pharmaceutical industries for the production of prophylactic antibiotic to reduce the overuse of many conventional antibiotics, which in most cases lead to development of resistant strains. Further study on toxicity evaluation should be done on Aloe vera gel to determine the safety indicators of the extract.

Competing Interest

The authors hereby declare that no competing interests exist regarding the publication of this research.

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