GSAR Journal of Agriculture and Veterinary Sciences
ISSN: 3048-9075 (Online)
Abbreviated key title: Glob.J. Agri.Vet.Sci.
Frequency: Monthly
Published By GSAR Publishers
Journal Homepage Link- https://gsarpublishers.com/journal-gjavs-home/

ANTIBACTERIAL ACTIVITY OF FERMENTED FRUIT EXTRACT OF *LAGENARIA BREVIFLORA* (ROBERT) AGAINST SOME BACTERIA PATHOGENS ASSOCIATED WITH POULTRY

By

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Article History

Received: 15/02/2026
Accepted: 25/02/2026
Published: 28/02/2026

Vol – 3 Issue –2

PP: -21-28

Abstract

The use of botanical extracts as alternative antibiotics in poultry production is fast gaining acceptance and popularity in Nigeria and other West African countries. This study investigated the antibacterial potentials of fermented Lagenaria breviflora fruit extract (FLBFE) at low concentrations on some bacteria associated with poultry infections with a view to recommending it to farmers for use as alternative antibiotics. Two kilograms of peeled sliced fruits were soaked in 1000 mL of sterile cold water, covered and fermented in dark dried condition for 7 days. The FLBFE (2g /mL) was further diluted to 2, 1 and 0.5 mg/mL respectively. Isolation of bacteria was done following standard microbiological assay from faeces, drinkers and feeders samples. Antibacterial activity of the different concentrations of the FLBFE was tested against bacteria isolates from the samples using Agar well Diffusion Method. Treatments were replicated thrice in a Completely Randomized Design. Data were subjected to two-way analysis of variance via the SPSS statistical package and significant variance among means were separated using Duncan's Multiple Range Test (DMRT).Results revealed the presence of six bacteria genera: Proteus, Salmonella, Klebsiella, Escherichia, Enterobacter, and Pseudomonas. Higher diversity of these organisms were observed in the fecal samples. The isolates from feeders were predominantly Klebsiella species, while those of the drinkers were Enterobacter species and Proteus species. Zone of inhibition of the test bacteria by the fermented extract followed a concentration dependent pattern. 2mg/mL of the extracts was the most effective and had the best inhibition at15.17±4.22, 14.80±2.52 and 14.97±4.58 for feeder, drinker and fecal samples respectively, followed by 1mg/mL and 0.5mg/mL which was the least. Significant variation (P>0.05) of the impact of the FLBFE do not exist largely among isolates from different feeders, drinkers or stool samples. The study has shown that the fermented fruit extract of Lagenaria breviflora has good inhibitory and broad spectrum activities at low concentration against many poultry-associated bacteria, hence can effectively serve as substitute to antibiotics in poultry management.

Keywords: *Poultry, associated, bacteria, fermented extract, Lagenaria breviflora, antibacterial, antibiotics.*

INTRODUCTION

Infection outbreaks have successfully occasioned the liquidation of many poultry establishments while millions of funds are continuously being lost to poultry disease on cost of treatment, prevention, control and mortality of birds. According to [1] to ensure a viable poultry industry that would be responsive to the nation's animal protein demands and nutrition security, prompt recognition and treatment/prevention of disease cannot be over emphasized.

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Poultry rearing, a significant part of the global economy has varying economic significance worldwide and it is growing into organized, specialized and integrated industry in Nigeria. Poultry birds have the efficiency of conversion of grains, other agricultural and industrial products and by-products into quality protein (meat and eggs) for nutritional benefit of man. They have wide use across different cultural background. Their adaptability to intensive management and large-scale production accounts for the growth of the industry worldwide. The poultry sub-sector plays significant role in the Nigerian economy contributing about 6-8% of Nigerian GDP and 25-30% of the Agricultural GDP annually [2].This has a resultant positive impact on poverty and unemployment alleviation rate at 4.42 million suggesting that a significant number of people in Nigeria are employed in the poultry sector [3, 4] while it is estimated that poultry supplies about 19% of the total meat requirement [5].

High risk of mortality of birds linked to infectious disease in most Nigerian poultry farms remains a huge challenge to small and medium scale farmers due to cost of synthetic antibacterial medicine. According to report from Ogun State , Nigeria, 67% of poultry farms experienced disease occurrence while majority (97.67%) were affected by infectious, metabolic/nutritional, parasitic, and behavioral diseases [1]. Antibiotics has a long history of use as preventive measures or for treatment of bacterial infections in farm animals including poultry [6]. They are mostly used at sub-therapeutic level to improve the production performance of poultry birds. Nevertheless, the frequent use and misuse of these antibiotics has led to the development antimicrobial drug resistance in birds [6, 7,8], various health issues and also a major contributor to higher cost of production [9]. Thus, it is imperative to sort for alternatives that could effectively and economically substitute antibiotics.

Antimicrobial resistance (AMR) is a growing public health threat of broad concern to countries and multiple sectors [10, 11]. In 2015, the WHO through its decision making body a global action plant to stem down the progression of AMR which included the use of other antimicrobials. Governments worldwide continue to pay attention to it as a threat to modern medicine. Due to the emergence and spread of drug-resistant pathogens that have acquired new resistance mechanisms, leading to AMR, the ability to treat common infections has drastically declined [12]. Especially alarming is the rapid global spread of multi and pan-resistant bacteria (also known as “superbugs”) that cause infections that are not treatable with commonly existing antimicrobials such as antibiotics. Hence, the need to focus on other natural and safer means of controlling and preventing bacterial infection can never be over emphasized.

Benefits of plants cut across all life forms for supplying of important nutrients, improving health and management of different disease conditions. Extracts of plants imbued with various phytochemical compounds such as alkaloids, tannins, flavonoids and phenols among others, form the basis for all traditional system of medicine [13] and have been the singular most importance source of raw materials for pharmaceutical medicines. As alternative to synthetic antibiotic, phytobiotics which are natural health and growth promoters derived from herbs and species have been devised to manage diseases in poultry [14].

Worthy of mention is *Lagenaria breviflora* (Spotted pumpkin) which has been relevant in Ethno-veterinary medicine and frequently used by rural poultry farmers for prevention and management of some infection in farm animals [15]. *Lagenaria breviflora* Robert commonly known as spotted pumpkin is one of the phytogenic plants used as antibacterial and antiviral herbal remedies and common in West Africa [16, 17]. It is a herbaceous flowering climber which flowers during the rainy season and fruits during the dry season. The leaves are extremely scabrid and sandpapery while the fruit are globose with very hard thick pericarp, dark green with creamy breaches [18]. Different tribes in Nigeria have their indigenous name of this plant as: "Ahuenyi," "Ogbenwa" in Igbo, "Tagiri" in Yoruba, "Luddal" in Hausa. In addition to its medicinal application, so much has been reported on the taxonomy [19]. The fruit of *Lagenaria* has been reported to be a very efficacious herbal treatment for measles, digestive disorder and as wound antiseptic (e.g., umbilical incision wound) in man, while the livestock farmers used it for the treatment of Newcastle disease and coccidiosis in various animal species, especially poultry [20). Its characteristic antibacterial, antifungal and antiviral properties revealed by the frequency of its usage in many local communities in Nigeria have been reported [21, 22, and 23].

Despite its broad-spectrum antimicrobial potentials, its activity in relation to formulations and application for effective control of pathogen in poultry bird has not been fully validated. There is dearth of real scientific studies affirming the said potency and action at reduced concentration via different extraction methods and against some disease-causing bacteria associated with poultry.

Given the need to explore various methods of extraction of this medicinal plant to maximize it potentials in treatment and control of bacteria pathogen in the poultry, the present study was designed to evaluate antimicrobial activity of the fermented extract of *Lagenaria breviflora* against some bacteria pathogens associated with poultry within Umudike environs. The specific objectives were to: isolate and identify bacteria organisms in the feeders, drinkers and faecal matters of poultry chicks in Umudike metropolis; determine the isolates with the highest percentage occurrence and to determine the efficacy of different concentration of fermented extract of *Lagenaria breviflora* against the isolated pathogens.

MATERIALS AND METHODS

Study Area

This study was carried out at the Department of Zoology and Environmental Science Laboratory, Michael Okpara University of Agriculture, Umudike, Abia State Nigeria between February and October 2022.

Collection of Plant Material

*Corresponding Author: **Faustina Njideka Ugwuja**

Fresh fruits of *L. breviflora* were collected from the Michael Okpara University Forest Reserve, South East Nigeria, in the months of April 2023. The Collected plant samples were authenticated at the Department of Plant Science and Biotechnology, College of Natural Science, Michael Okpara University of Agriculture, Umudike,

Preparation of Plant Extracts

The fruits were washed in three changes of sterile distilled water and the hard pericarp peeled off using a sharp kitchen knife while the remaining parts (mesocarp and the seed) were cut into pieces. Two kilogram of the pieces was soaked in 1000mL of distilled water contained in a plastic drinking-water trough. The jar was covered by means a cheese cloth to allow moderate aeration and the lid was secures by rubber bands and the set-up was stored in the dark for 7 days. After fermentation the extract was further diluted to 2, 1 and 0.5 mg/mL respectively.

Collection of Faecal, Drinker and Feeder Samples

A total of six poultry farms within Michael Okpara University premises were visited. Sterile swab tubes containing 10mL sterile distilled water were labelled appropriately for each farm. Sampling was done in the morning hours between 8:am and 10:am and samples were taken separately from drinking trough, feeder and fecal matter by means of a sterile swap stick, stirred into the collecting tube and tightly screwed to secure the content. These sample were used within 2hours after collection.

Isolation, Characterization and Identification of Microorganisms

The samples were subjected to microbiological analysis following the method described by [24]. Tenfold serially diluted bacterial samples from swabs (from poultry feeding troughs, drinkers and poultry droppings) were aseptically inoculated on MacConkey agar (Oxoid, Cambridge, UK) and incubated at 37°C for 24 hours. Pure cultures were obtained by sub-culturing from a typical and well isolated colony on Blood agar. Presumed colonies based on morphological and cultural characteristics from each sample was plated directly onto trypticase soy agar (TSA; HiMedia, Mumbai, India).The morphological characteristics of bacteria include; shape of colonies, colonial outline, colonial evaluation, colour, consistency and size

Gram staining

A drop of sterile distilled water was placed in the middle of a grease free slide with a sterile wire loop. A small portion of the bacteria colony was smeared on it with the mixture evenly spread on the slide to make a thin smear. The smear was flooded with crystal violet for 60 seconds. Then the smear was washed off with water and few drops of Lugol's iodine were dropped on the smear, which was allowed to stand for 60 seconds while the slide was laid across the staining rack. The iodine was rinsed with distilled water. Then few drops of ethanol were placed on the smear and allowed to stand for 30 seconds and washed off immediately with distilled water to avoid excess decolonization. The slide was laid again on the rack and flooded with safranin for 60 seconds and washed off with distilled water. Then the back of the slide was blot dried using filter paper and allowed to air dry [25]. The smear was examined under 100× (oil immersion) objective lens. Gram positive organism appeared purple, while gram negative organism appeared pink or red.

Biochemical Tests

A series of biochemical methods using suspensions of organisms and chemically-defined solutions are described. The tests use the preformed enzymes of the bacterial cells and the results are not complicated by side effects or the multiple reactions that occur in cultures growing in a nutrient medium containing the test substrate [26].

Six bacteria species comprising of only gram-negative organisms namely *Proteus sp., Salmonella sp., Klebsiella sp., Escherichia sp., Enterobacter sp., and Pseudomonas sp.,* were isolated for this study. All the strains were isolated, purified, characterized and identified at the Post Graduate Microbiology Laboratory, Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State.

Susceptibility Test

Susceptibility of the isolates to the FLBFE was determined using the Agar Well Diffusion Technique [27] An aliquot of 0.1ml broth culture of each test bacteria (optical density equivalent to 10^7-10^8 CFU/mL) was aseptically used to seed sterile molten Mueller-Hinton agar mixed with 5% sterile sheep blood maintained at 45^0C. Distilled water was used as negative control while Gentamicin (mg) served as positive control. The seeded plates were allowed to dry in the incubator at 30^0C for 20 minutes. A sterile cork borer with diameter of 5mm was used to make uniform wells on the surface of the agar, into which different concentrations of the test extract were added. The inoculated and seeded medium was allowed to stand 1hour for the extracts to diffuse into the medium and then incubated at 37^0C in an incubator for 48 hours. The diameters of zone of inhibition (in mm) were measured with the transparent metre rule [28].

Statistical Analysis

Two-way ANOVA followed by multiple comparisons test was applied for the comparison of the zone of inhibition of the extract against test bacteria. A two-tailed P-value < 0.05 was considered statistically significant.

RESULTS

Bacteria isolated from samples their percentage occurrence

A total of six bacteria genera including *Proteus* spp., *Salmonella* spp., *Klebsiella* spp., *Escherichia* spp., *Enterobacter* spp., *and Pseudomonas spp* were isolated from the feeders, drinkers and faecal poultry samples. The bacteria species varied in their occurrence from one poultry sample to another, having the highest biodiversity in the faecal sample followed by the drinker while the feeder had the least diversity of the bacteria species **Table1.** Percentage occurrence ranged from 0- 50 with a middle value of 33.33%. *Klebsiella* and *Proteus* species occurred in all the samples

*Corresponding Author: **Faustina Njideka Ugwuja**

with their equivalent percentage occurrence as shown in the table. *Enterobacter* and *Pseudomonas* species did not occur in the feeder and drinker. Escherichia species occurred in all except drinker while *Salmonella* species occurred in all except faeces.

Table 1: percentage occurrence of bacterial isolates in feeders, drinkers and faecal samples

Isolate	Occurrence in feeders (%)	Occurrence in Drinkers (%)	Occurrence in faeces (%)
Escherichia spp.	**(0)**	**+++ (50)**	**+ (16.66)**
Klebsiella spp.	**+++ (50)**	**++ 33.33**	**++ (33.33)**
Enterobacter spp.	**- (0)**	**- (0)**	**++ (33.33)**
Proteus spp.	**+ (16.66)**	**+ (16.66)**	**++ (33.33)**
Pseudomonas spp.	**- (0)**	**- (0)**	**+ (16.66)**
Salmonella spp.	**+ (16.66)**	**++ 33.33**	**- (0)**

The plus sign (+) represents presence of isolate while the negative sign (-) represents absence of isolate. Klebsiella and Proteus species occurred in all the samples. Enterobacter and Pseudomonas species did not occur in the feeder and drinker. Escherichia species occurred in all except drinker while Salmonella species occurred in all except faeces.

Inhibition Zone Effect of FLBFE on Feeder Isolates

Inhibition zone effects of different concentration of FLBFE on isolated bacteria from feeder samples are shown in **Table 2**. The results revealed that 2mg/mL extract concentrations produced inhibition range of 15.17±4.27 mm to 11.60±1.31 mm of feeder isolates. Effect of difference in feeders was not significant (P>0.05). The inhibitory zones at 1mg/mL extract concentrations ranges from 8.43±3.25 mm of feeder 2 to 4.67±0.93 mm of feeder 5. Effect of difference in feeders was also not significant (P>0.05). The inhibitory zone at 0.5 mg/mL extract concentrations ranges from 2.80±1.31 mm of feeder 2 to 0.00±0.00 mm of feeders 1, 3, 4 and 5. Effect of difference in feeders was significant (P≤0.05). The inhibition zone of control treatment ranges from 45.00±3.60 mm of feeder 1 to 15.00±1.02 mm of feeder 5. Effect of differences in feeders was significant (P≤0.05). Bacteria isolates were *Klebsiella* sp. for feeders 1, 2, and 4 while feeders 3 and 5 had *Proteus* and *Salmonella* species respectively. In all the feeders the pattern of inhibitory effect of the extract were in order of 2 mg/mL >1 mg/mL > 0.5mg/mL. Effect of differences in extract concentrations were significant (P≤0.05) in Feeders 1, 2, 3, 4 and 5 as shown in the Table1.

Table 2: Inhibition zone diameter (IZD) of different concentration of FLBFE on bacteria isolated from feeders.

Feeder samples	Inhibition zone (mm) 2 mg/mL	Inhibition zone (mm) 1mg/mL	Inhibition zone (mm) 0.5 mg/mL	Gentamicin	Bacteria isolates
Feeder 1	15.17±4.27^{a,2}	6.53±5.84^{a,1}	0.00±0.00^{a,1}	45.00±3.60^{c,3}	*Klebsiella sp.*
Feeder 2	13.60±0.44^{a,3}	8.43±3.25^{a,2}	2.80±1.31^{b,1}	22.00±1.50^{b,4}	*Klebsiella sp.*
Feeder 3	14.87±3.37^{a,3}	6.23±2.50^{a,2}	0.00±0.00^{a,1}	17.00±2.40^{a,4}	*Proteus sp.*
Feeder 4	12.27±1.97^{a,2}	8.17±4.37^{a,2}	0.00±0.00^{a,1}	16.00±3.10^{a,3}	*Klebsiella sp.*
Feeder 5	11.60±1.31^{a,3}	4.67±0.93^{a,2}	0.00±0.00^{a,1}	15.00±1.02^{a,4}	*Salmonella sp.*

Values are presented as mean ± standard deviation (n = 3) and values with different superscripts are significantly (P<0.05) different from any paired mean in each column. The values with different number superscripts are significantly (P<0.05) different from any paired mean across the row.

Inhibition Zone Effect of FLBFE on Feeder Isolates

The inhibition zone effects of the different concentration of FLBFE on isolated bacteria from drinkers are represented in **Table 3** .At 2 mg/mL, inhibition zone ranged from 14.80±2.52 mm of drinker 5 to 12.03±1.96 mm of drinker 3. Effect of difference in drinkers was not significant (P > 0.05). The inhibition zones at 1mg/ml extract concentrations ranged from 7.50±2.25 mm of drinkers 7 to 3.93±1.44 mm of drinkers 8. Effect of difference in drinkers was also not significant (P > 0.05). The inhibitory zone at 0.5 mg/mL extract concentrations ranges from 4.73±0.56 mm of drinkers 3 to 0.00±0.00 mm of drinkers 1, 2, 7 and 8. Effect of differences in drinkers was significant (P≤0.05). The inhibition zone of control treatment ranges from 41.00±1.00 mm of drinker 8 to 10.00±1.00 mm of drinker 1. Effect of differences in drinkers was significant (P ≤ 0.05). Bacteria isolates were *Proteus* sp. for drinker 1, *Salmonella* for drinker 2 and 3, *Klebsiella* for drinkers 4 and 7 while *Escherichia* species were predominant in drinker 5, 6 and 8 respectively. Except for isolates of drinker 1, all drinkers isolate had pattern of inhibitory effect of the extract in order of control > 2 mg/ml > 1 mg/ml > 0.5 mg/ml. Effect of difference in extract concentrations was significant (P ≤ 0.05) in Drinkers 1, 2, 3, 4, 5, 6, 7, and 8.

*Corresponding Author: **Faustina Njideka Ugwuja**

Table 3: Inhibition zone diameter (IZD) of different concentration of FLBFE on some isolated bacteria from drinkers.

Samples of drinkers	Inhibition zone (mm) (2 mg/mL)	Inhibition zone (mm) (1 mg/mL)	Inhibition zone (mm) (0.5 mg/mL)	control (Gentamicin)	Bacterial isolates
Drinker 1	12.30±1.30^{a,3}	6.06±0.93^{a,2}	0.00±0.00^{a,1}	10.00±1.00^{a,4}	*Proteus sp.*
Drinker 2	14.27±4.56^{a,3}	6.93±3.55^{a,2}	0.00±0.00^{a,1}	20.00±2.00^{c,4}	*Salmonella sp.*
Drinker 3	12.03±1.96^{a,1}	5.00±0.51^{a,1}	4.73±0.56^{b,1}	16.00±1.00^{b,2}	*Salmonella sp.*
Drinker 4	12.17±2.51^{a,2}	7.00±1.20^{a,1,2}	3.90±1.08^{b,1}	20.00±1.00^{c,3}	*Klebsiella sp.*
Drinker 5	14.80±2.52^{a,2}	6.80±3.42^{a,1}	2.83±1.89^{a,b,1}	31.00±3.00^{d,3}	*Escherichia sp.*
Drinker 6	13.73±3.29^{a,2}	6.83±1.12^{a,1}	4.17±1.04^{b,1}	11.00±1.00^{a,3}	*Escherichia sp.*
Drinker 7	12.57±2.04^{a,3}	7.50±2.52^{a,2}	0.00±0.00^{a,1}	30.00±3.00^{d,4}	*Klebsiella sp.*
Drinker 8	13.60±3.06^{a,3}	3.93±1.44^{a,2}	0.00±0.00^{a,1}	41.00±1.00^{e,4}	*Escherichia sp.*

Values are presented as mean ± standard deviation (n = 3) and values with different superscripts are significantly (P<0.05) different from any paired mean in each column. The values with different number superscripts are significantly (P<0.05) different from any paired mean across the row.

Inhibition Zone Effect of FLBFE on Faecal Isolates

Results of the inhibition zone effects of the different concentrations of FLBFE on bacteria isolated from faecal samples revealed a similar trend as that of the feeders and drinkers **Table 4.** The inhibition zones at 2 mg/ml extract concentrations ranges from 14.97±4.58 mm of faecal sample 6 to 9.90±2.52 mm of sample 7. Effect of differences in faecal samples was not significant (P > 0.05). The inhibitory zones at 1 mg/ml extract concentrations ranges from 6.13±2.83 mm of sample 4 to 4.17±3.67 mm of sample 1. Effect of differences in stool was also not significant (P>0.05). The inhibitory zone at 0.5 mg/ml ranges from 3.40±2.46 mm of sample 4 to 0.00±0.00 mm of samples 1, 2, 3, and 7. Effect of differences in samples was not significant (P>0.05). The inhibitory zone of control treatment ranges from 31.00±2.00 mm of sample 2 to 17.00±2.00 mm of sample 4. Bacteria isolates were *Klebsiella* species for faecal sample 3, *Proteus* species for samples 5 and 7, *Enterobacter* species for 1 and 6 while faecal sample 4 and 2 had *Escherichia* and *Pseudomonas* species respectively.

Table 4: Inhibition zone diameter (IZD) of different concentration of FLBFE on bacteria isolated from faecal samples.

Faecal samples	Inhibitory zone (mm) (2 mg/mL)	Inhibitory zone (mm) (1mg/mL)	Inhibitory zone (mm) (0.5 mg/mL)	Control (Gentamicin)	Bacterial isolates
Stool 1	13.20±2.48^{a,2}	4.17±3.67^{a,1}	0.00±0.00^{a,1}	23.00±1.00^{b}	*Enterobacter sp.*
Stool 2	11.60 1.73^{a,2}	7.23±4.25^{a,2}	0.00±0.00^{a,1}	31.00±2.00^{c}	*Pseudomonas sp.*
Stool 3	14.33±3.04^{a,3}	5.67±3.63^{a,2}	0.00±0.00^{a,1}	21.00±2.00^{b}	*Klebsiella sp.*
Stool 4	13.93±1.16^{a,2}	6.13±2.83^{a,1}	3.40±2.46^{a,1}	17.00±1.00^{a}	*Escherichia sp.*
Stool 5	12.77±2.98^{a,2}	5.57±3.69^{a,1}	1.33±0.25^{a,1}	23.00±2.00^{b}	*Proteus sp.*
Stool 6	14.97±4.58^{a,3}	5.20±3.03^{a,1}	0.53±0.92^{a,1}	29.00±2.00^{c}	*Enterobacter sp.*
Stool 7	9.90±2.52^{a,2}	4.83±6.41^{a,1,2}	0.00±0.00^{a,1}	22.00±1.00^{b}	*Proteus sp.*

Values are presented as mean ± standard deviation (n = 3) and values with different superscripts are significantly (P<0.05) different from any paired mean in each column. The values with different number superscripts are significantly (P<0.05) different from any paired mean across the row.

DISCUSSION

The results of the present study have revealed the potency and unique character of fermented fruit extract of *L. breviflora* in its ability to induce significant inhibition of bacterial isolates from poultry samples at very low concentrations. This result is unique as the first report of significant antibacterial activity of *L. breviflora* fruit extract at concentration as low as 2 mg/ml. In addition, the zones of inhibition followed a concentration dependent pattern for the most part, a feature which suggests that increasing the concentration of the extract may result in increased inhibition.

Results revealed that different bacteria species are associated with feeders, drinkers and faecal samples from different

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poultry farms, suggesting that these species which are in close proximity with the birds are most likely to pose potential infection risk to the birds as well as their handlers under certain environmental conditions. Already some of these bacterial species are implicated as common pathogens of birds [29].

The selective occurrence of different bacteria genera in the different poultry samples indicates that diversity of bacteria organisms associated with poultry birds is source dependent. More *Escherichia* species were detected in the drinker samples suggesting their higher presence in the gut environment of the birds. Similarly, the preponderance of *Enterobacter* and *Proteus* species in the faecal matter implies that these species are associated with the digestive system of the bird and could be a major cause of digestive disorder in the bird given the favourable conditions for infection [30].

The different percentage occurrence of bacteria in the various samples suggests that these organisms have their unique niche in the ecosystem of the birds as reflected by their varied existence.

The results revealed that the difference in the inhibitory performance of extracts of *L. breviflora* across various samples was not source dependent. This is because significant differences (P>0.05) did not exist between samples from different drinkers, feeders or stools. This therefore suggests that the extract concentration based inhibitory effect on the bacteria isolates as shown in this study maybe connected to the high metabolite rich nature of *L. breviflora* as revealed by [31] and innate characteristics of the bacterial organisms involved. The authors in their qualitative and quantitative secondary metabolites screening of *Adenopus breviflorus (Benth)* whole fruit ethanol extract revealed the presence of alkaloids, flavonoids, cardiac glycosides, tannins, steroids, saponins, tannins, anthraquin, pyrrolidizine and alkaloid. The authors concluded that these compounds are responsible for the broad antibacterial activity of the extract.

The results showed varied inhibitory effects of the extract on the different species with 2mg/ml showing the highest activity. Except for *Enterobacter* and *Proteus* species which had no inhibition. This results corroborates the findings of [32] who reported that the degree of inhibition by extracts of *L. breviflora* varies from one bacteria colony to the other with higher antimicrobial property against *S. typi, P. fluorescens* and *S. typhi* while *S. dysenteriae* was the least affected. Similarly, the results obtained by [33] in their study on nutritional and medicinal value of raw and fermented *Lagenaria breviflora* root supported the present findings. They reported varied inhibitory effect against *Shigella* (22.00±0.20), *E. coli* (10.00±0.10), *Salmonella typhi* (20.00±0.10), *Klebsiella pneumonia* (5.00±0.10), *Staphylococcus aureus* (7.00±0.20) with *Enterobacter* and *Proteus* showing no zone of inhibition respectively. Hence they inferred that aqueous root extract of *L. breviflora* was good for curative purposes except against *Enterobacter* and *Proteus*.

The extract had moderate activity compared to the standard antibiotics. This could be as a result of the refined nature of the standard drug which has ample amount of active ingredients with little or no impurities as against the crude extract.

Higher, biodiversity of bacterial organisms was observed in the isolates from the stool. This may be associated with nutrient rich nature of the stool and may also be connected to inherent organisms in the internal organs of the birds which may be part of the excreta.

In most of the samples the control treatment performed better than the extracts of *L. breviflora*. Although, the control treatment Gentamicin induced better inhibition than any of the extract concentrations, there is no doubt that the extract at 2 mg/ml showed strong efficacy. This therefore, suggests with 2 mg/ml concentration of FLBFE effective control of certain bacteria including *Proteus, Salmonella, Klebsiella, Escherichia, Enterobacter, and Pseudomonas* in poultry can be achieved.

CONCLUSIONS

A total of six associating bacteria including *Proteus, Salmonella, Klebsiella, Escherichia, Enterobacter, and Pseudomonas* species were isolated from the poultry samples and higher diversity of these bacteria were obtained from the excreta samples.

The isolates from the feeder samples were predominantly *Klebsiella* species while those of the drinkers and excreta were *Escherichia* and *Proteus* species respectively.

The 2 mg/ml concentrations of the fermented extracts of *L. breviflora* was best in the inhibition of growth of the bacteria. Hence, this study proved that fermented extracts of *L. breviflora* has good inhibitory activity against some poultry-associated bacteria and can favorably substitute standard or conventional drugs in poultry disease management.

The antibacterial activity of the fermented extract was concentration dependent implying that higher inhibition of the tested bacteria can be achieved by increasing the concentration of the extract. The study showed that lower concentrations of fermented extracts of *L. breviflora* (1mg/ ml and 0.5 mg/ml was not efficacious in inhibiting growth of most of the bacteria isolates.

Significant variation (P>0.05) does not exist largely on the effect of fermented extracts of *L. breviflora* on bacteria isolates from different feeders, drinkers and stool. Hence, the study showed that efficacy of fermented extracts of *Lagenaria breviflora* were not isolate source dependent.

RECOMMENDATIONS

The findings of the study proves that there is need for poultry farmers to improve on the hygiene practices in the farm in order to control the possible infectious impact of some of the isolated bacteria organisms in the present study.

Farmers are encouraged to adopt the use of fermented extracts of *L. breviflora* at low doses in combination with conventional

*Corresponding Author: **Faustina Njideka Ugwuja**

drugs to prevent bacterial infection on poultry birds pending the determination of the minimum and maximum effective doses as this may be economically cost effective. Further studies, should be carried out *in vivo* to reconfirm the potency of the fermented extract against infectious bacteria in birds as well as determine the active components in that fermented state of the extract.

Phytochemical profiling of the *Lagenaria breviflora* should be carried out in in order to proffer possible correlation link in its metabolite content and antimicrobial effect.

Abbreviations

FLBFE: Fermented *Lagenaria breviflora* fruit extract; SPSS: Statistical package for social sciences; DMRT: Duncan's multiple range test; UNDP: United Nations Development Project; GDP: Gross domestic product; WHO: World Health Organization; AMR: Antimicrobial resistance; TSA: Trypticase soy agar; CFU/mL: Colony forming unit per milliliter; ANOVA: Analysis of Variance; IZD: Inhibition zone diameter.

Acknowledgments

We are immensely grateful to the Directorate of Farms, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria for the support received during the collection of the poultry samples used in this research.

Authors' contributions

UFN: Conceptualized the idea, sourced the *L. breviflora* fruits, formulated the fermented extract and coordinated the write-up. POG: Conducted the antibacterial assay, compiled results and prepared the manuscript draft. OAN: Reviewed and formatted the manuscript and URI: Analyzed all data and proof-read the final manuscript.

Funding

The authors declare that no funding was received for this research.

Availability of data and materials

The datasets used and analyzed during the study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to conduct the research

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicting interests.

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