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Microbial assessment and lipid profile of broiler chicken treated with crude extract of *Vernonia Amygdalina* (Bitter Leaf)

ILESANMI Tolulope Margaret * and FAJILADE Olawande Temilade

Department of Science, Laboratory Technology, Microbiology Unit, School of Science and Computer Studies, Federal Polytechnic, P.M.B. 5351, Ado-Ekiti, Ekiti State, Nigeria.

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Abstract

Globally, broiler chickens are an essential source of protein, and their productivity and well-being have substantial economic implications. Bitter leaf (Vernonia amygdalina) has been used medicinally for a variety of reasons. The effects of bitter leaf extract, Vernonia amygdalina, on the lipid profile and microbial load of broiler chickens were examined in this study. Significant amounts of bioactive components, such as phenols (152.70 mg/100g), alkaloids (98.73 mg/100g), flavonoids (73.93 mg/100g), tannins (51.93 mg/100g), and saponins (60.26 mg/100g), were found in the crude extract upon phytochemical examination. These substances are well-known for their lipid-modulating, antioxidant, and antibacterial qualities. Micrococcus luteus and Enterococcus faecalis were found in the treated broiler feaces, as determined by microbial analysis and molecular characterization. The extract exhibited promise in controlling the growth of these microorganisms. When compared to the reference ranges, lipid profile analysis showed a significant increase in high-density lipoprotein (HDL) cholesterol (2.9 mmol/L), a decrease in low-density lipoprotein (LDL) cholesterol (0.7 mmol/L), and a decrease in total cholesterol (3.3 mmol/L). These findings imply that broiler chickens' lipid metabolism is positively modulated by bitter leaf extract. The study comes to the conclusion that by modifying gut microbiota and increasing lipid profiles, Vernonia amygdalina extract has potential uses as a natural feed additive to improve broiler health. The results validate bitter leaf's potential as a long-term substitute for artificial antibiotics and growth hormones in chicken agriculture. Subsequent studies ought to concentrate on refining the dosage and modes of administration while investigating the long-term impacts of the extract on the health and performance of chickens.

Keywords: Bitter leaf; Broiler chicken; 8Lipid profile; Medicinal plant; Microbial; Poultry

1. Introduction

Globally, broiler chickens are an essential source of protein, and their productivity and well-being have substantial economic implications. Concerns about drug resistance, residue accumulation, and consumer health have prompted researchers to look at natural alternatives to synthetic growth promoters and antibiotics in light of the growing demand for poultry. Using plant-based products, such as *Vernonia amygdalina*, also referred to as bitter leaf because of its well-known antibacterial and therapeutic qualities, is one such solution.

Synthetic antibiotics are frequently used in commercial broiler chicken production to prevent infections and improve growth performance. But the abuse of these drugs has sparked worries about the emergence of germs that are resistant to them, which could be dangerous for the general public's health. Research has indicated that synthetic antibiotic medication residues can build up in chicken tissues. These residues, if ingested by people, might cause health problems such as allergic reactions and the emergence of antibiotic resistance in dangerous bacteria (Sule *et al*., 2019).

* Corresponding author: ILESANMI Tolulope Margret Email: ilesanmi_tm@fedpolyado.edu.ng

Finding natural substitutes that are secure, efficient, and devoid of negative side effects has therefore become more popular. The antibacterial, antioxidant, and immunomodulatory qualities of plant extracts, including bitter leaf, have drawn interest (Igwe *et al*., 2018). Bioactive substances found in bitter leaf, such as alkaloids, flavonoids, saponins, and tannins, have been shown to exhibit antibacterial action against a variety of pathogenic microbes.

Poultry industry faces a serious problem with microbial contamination, particularly from Salmonella, Staphylococcus aureus, and Escherichia coli. These microbes have an impact on the growth and health of hens, but they also carry a danger of causing foodborne illnesses in people (Oladele *et al*., 2017). Research has been done on using bitter leaf extract as a natural antibacterial agent to lower the microbial load in broiler chickens. Research has demonstrated that bitter leaf extracts can stop the spread of a number of bacterial infections that are frequently found in chicken (Okoh *et al*., 2020). It is thought that adding bitter leaf extract to poultry feed or water improves the gut health of hens by lowering pathogenic bacteria and altering the gut microbiota, which improves digestion and nutrient absorption (Ajayi *et al*., 2021).

An important predictor of health and meat quality in broiler chickens is their lipid profile, which includes elements like total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Poultry with high triglyceride and LDL levels should be avoided as they can shorten the shelf life and quality of the meat. Studies have been conducted on the possible influence of natural supplements, including bitter leaf extract, on poultry lipid metabolism.

Flavonoids and phenolic acids, two substances found in bitter leaf, have hypolipidemic properties. According to studies, plant extracts can lower the triglyceride and cholesterol levels in broiler chickens, which will improve the quality of the meat that they produce (Eze *et al*., 2020). Additionally abundant in antioxidants, bitter leaf helps lower oxidative stress in hens, promoting both the general health of the birds and the quality of the meat they produce (Chikezie *et al*., 2017).

The antibacterial and lipid-lowering properties of bitter leaf are mostly attributed to its bioactive constituents, especially flavonoids and saponins. It has been discovered that certain substances cause bacterial cell membrane integrity to be compromised, which has antibacterial effects. Furthermore, by blocking the activity of the enzyme HMG-CoA reductase, which is involved in cholesterol biosynthesis, flavonoids and phenolic substances have been connected to the decrease in cholesterol synthesis (Onunkwo *et al*., 2019).

An essential component of food production, particularly when it comes to meeting the world's protein demands, is the chicken industry. One of the most effective sources of animal protein is broiler chickens. Bitter leaf, or *Vernonia amygdalina*, has been shown in multiple investigations to possess antibacterial characteristics. The crude extract of bitter leaf contains bioactive substances such alkaloids, flavonoids, and saponins, which have been shown in a study by Ijeh *et al*. (2014) to have antibacterial action against a range of pathogens, including Salmonella spp. and Escherichia coli. The use of bitter leaf extract for microbiological assessment in broiler chickens is justified by the prevalent associations these pathogens have with poultry illnesses.

One important factor in determining whether poultry meat is healthy for human consumption is its lipid profile. Human heart disease may be exacerbated by the high cholesterol and saturated fat content in chicken flesh. Studies on both humans and animals have shown that bitter leaf reduces cholesterol and improves the lipid profile (Njoku *et al*., 2019). The need to investigate natural antibiotic substitutes in poultry production, enhance the broiler meat's lipid profile for better consumption, and promote environmentally friendly farming methods all serve as justifications for this study. Due to its well-established lipid-lowering and antibacterial qualities, bitter leaf is a good option for improving the nutritional value and microbiological safety of poultry meat.

The aim of this study is to evaluate the microbial community and lipid profile of broiler chickens treated with crude extract of bitter leaf (*Vernonia amygdalina*), in order to assess its potential effects on the microbial composition and lipid metabolism of the chickens.

2. Material and methods

2.1. Study Area and Experimental Design

The study was conducted at animal house, Department of Science Laboratory Technology, Federal Polytechnic, Ado-Ekiti. The design was a controlled experiment aimed at evaluating the effect of bitter leaf extract on microbial load and lipid profiles in broiler chickens. The experimental period lasted 30 days, during which data were collected at regular

intervals. The broiler chickens were divided into different treatment groups based on their diet and extract administration.

2.2. Collection and Preparation of Bitter Leaf Extract

Fresh bitter leaves (*Vernonia amygdalina*) were collected from a farmland within Federal Polytechnic Community. The leaves were washed thoroughly with distilled water to remove debris and soil particles. They were air-dried at room temperature for 3 weeks, after which they were pulverized into a fine powder using a mechanical grinder. The powdered bitter leaves were then soaked in distilled water at a ratio of 1:10 w/v for 24 hours with occasional stirring. The extract was filtered through Whatman No. 1 filter paper and concentrated using a rotary evaporator at 40°C. The concentrated extract was stored at 4°C until further use.

2.3. Determination of quantitative phytochemical composition

Determination of alkaloid: Determination of Alkaloid was carried out by the method described by Yeh *et al*. (2014). The alkaloid content was determined gravimetrically. 5 g of the sample was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4 hours at 28°C and it was filtered using filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drops of additional of concentrated aqueous NH_4OH until the Alkaloid is precipitated. The alkaloid precipitated in a weighed filter paper was washed with 1% ammonia solution, and dried in the oven at 80°C. Alkaloid content was calculated and expressed as a percentage of the weight sample analyzed.

Determination of tannins: Tannin content of the flour samples was determined using the methods described by Sogbesan *et al*. (2021). The sample (0.2 g) was measured in a 50 mL beaker, 20 ml of 50% methanol was added, covered with homogenizer, placed in a water bath at 77–80°C for 1 hour, and the contents stirred with a glass rod to prevent lumping. The mixture was filtered using a double-layered 1 filter paper into a 100ml volumetric flask using 50% methanol rinse to make up the mark with distilled water and thoroughly mixed. The absorbances of the tannic acid standard solutions as well as samples were read after colour development on a Spectronic 21D spectrophotometer at a wave length of 760 nm. Percentage tannin was then calculated.

Determination of saponins: The spectrophotometric method was used to determine Saponins as described by Akinyeye *et al*. (2021). One gram of the flour sample was put into a 250mL beaker and 100 mL iso-butyl alcohol was added. The mixture was shaken to ensure uniform mixing. The mixture was then filtered through filter paper into a 100 mL beaker and 20 mL of 40% saturated solution of magnesium carbonate was added. The mixture obtained was further filtered through a filter paper to obtain a clear colourless solution. One millilitre of the colourless solution was homogenized into a 50 mL volumetric flask and 2 mL of 5% $FeCl_3$ solution was added and made up to mark with distilled water and allowed to stand for 30 min for blood red colour to develop. The absorbance of the sample as well as standard Saponins solutions were read after colour development on a Spectronic 2lD spectrophotometer at a wavelength of 380 nm. The percentage of Saponins was calculated.

Determination of flavonoids: This was determined according to the method outlined by AOAC (2006). 5 g of the sample was boiled in 50 mL of 2 mol/L HCl solution for 30 min under reflux. The content was allowed to cool and then filtered through a filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

Determination of phenols: The sample (100 g) was extracted, by stirring with methanol 250 mL for 3 h. The extracted sample was then filtered through a filter paper, the residue was washed with 100 ml methanol, and the extract was allowed to cool. The extract was then allowed to evaporate to dryness under vacuum, using a rotary evaporator. The residue was dissolved with 10 ml of methanol and used for determination of total phenolic compounds. The supernatant was measured at 756 nm on a spectrophotometer. Methanol was applied as a control, by replacing the sample. Gallic acid was used as a standard and the result was calculated as Gallic acid equivalents (mg/100 g) of the sample.

Determination of steroids: Sample of fine powder of additives was weighed and transferred into 10 ml volumetric flasks. Sulphuric acid and iron (III) chloride were added, followed by potassium hexacyanoferrate (III) solution. The mixture was heated in a water-bath maintained at 70C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank (Ajaiyeoba and Fadare, 2016).

Determination of glycosides: Total of 100 mg of the extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution ,it was then under layered with 1ml of concentrated sulphuric acid, a brown ring obtained at the interface indicate the presence of de-oxysugar characteristic of cardenolides.

2.4. Broiler Chicken Selection and Housing

A total fifteen (15) day-old broiler chicks were purchased from poultry farm in Ado-Ekiti. Upon arrival, they were housed in a well-ventilated and sanitized broiler house equipped with heating, feeding, and watering systems. The broiler chickens were acclimatized for three weeks before the commencement of the experiment. They were randomly assigned into 3 treatment groups and fed a standard commercial diet formulated to meet their nutritional needs. Adequate space, light, and ventilation were maintained throughout the study period, and biosecurity measures were strictly adhered to.

2.5. Treatment Groups and Administration of Extract

The broiler chickens were divided into 3 treatment groups: The extract was administered orally via drinking water for 60 days, starting from 21 days of age until the end of the experimental period.

- Group 1: Control group (fed a standard diet without bitter leaf extract).
- Group 2: Received 0.5 mL/kg of bitter leaf extract in drinking water daily.
- Group 3: Received 1 mL/kg of bitter leaf extract in drinking water daily.

2.6. Microbial Assessment

Collection of samples: Fecal samples were collected from each treatment group. Sterile swabs were used to collect fresh fecal matter from the cloaca of the chickens, ensuring that cross-contamination was avoided. The samples were immediately placed in sterile containers and transported to the laboratory for microbial analysis.

Microbial culture and identification: In the laboratory, the fecal samples were cultured on nutrient agar using the streak plate method. Plates were incubated at 37°C for 24 hours to allow for microbial growth. After incubation, colonies were identified based on their morphology, Gram staining, and biochemical tests such as catalase, oxidase, and coagulase tests.

2.7. Molecular identification of isolates

Bacteria DNA extraction: DNA was extracted using the protocol stated by (1). Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28 ºC. After this period, cultures were centrifuged at 4600g for 5 min. The resulting pellets were resuspended in 520 µl of TE buffer (10 mMTrisHCl, 1mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3 µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37 ºC, then 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 min at 65 ºC and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20 ºC for 16 h. DNA was collected by centrifugation at 13000g for 10 min, washed with 500 µl of 70% ethanol, airdried at room temperature for approximately three hours and finally dissolved in 50 µl of TE buffer.

Fungi DNA extraction protocol: Approximately 100 mg of fungi mycellia was grinded with Dellaporta extraction buffer (100 mMTrls pH 8, 51 ml EDTA pH 8, 500 mMNaCI, 10mMmcrcaptoethanol) and DNA extracted as described briefly. Each sample was grinded in 1000 µl of the buffer in a sterilized sample bags. Mix was collected in sterile eppendorf tube and 40 µl of 20% SDS was then added, this was followed by brief vortexing and incubated at 65 oC for 10 minutes. At room temperature, 160 µl of 5 M potassium acetate was then added vortexed and centrifuged at 10000 g for 10 minutes. Supernatant where collected in another eppendorf tube and 400 µl of cold iso propanol was added mixed gently and kept at -20 oC for 60 minutes. Centrifugation was at 13000g for 10 minutes to precipitate the DNA after which supernatant was gently decanted and ensured that the pellet was not disturbed. DNA was then washed with 500 µl of 70 % ethanol by centrifuging at 10000g for 10 minutes. Ethanol was decanted and DNA air-dried at room temperature until no trace of ethanol was seen in the tube. Pellet was then re-suspended in 50 µl of Tris EDTA buffer to preserve and suspend the DNA.

PCR Analysis: To use the ITS gene for characterization of fungi, ITS universal primer set which flank the ITS1, 5.8S and ITS2 region can be used; PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaqcolourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each ITS 1: 5’ TCC GTA GGT GAA CCT GCG G 3’and - ITS 4:

5' TCC TCC GCT TAT TGA TAT GC 3'primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR condition include a cycle of initial denaturation at 940C for 5 min, followed by 35cycles of each cycle comprised of 30secs denaturation at 940C, 30secs annealing of primer at 55°C, 1.5 min extension at 72°C and a final extension for 7min at 72°C.

Bacteria PCR: PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each 27F 5' AGA GTT TGA TCM TGG CTC AG3' and 1525R, 5′AAGGAGGTGATCCAGCC3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a Pcr profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 50°C for 60s and 72°C for 1 minute 30 seconds ; and a final termination at 72°C for 10 mins. And chill at 4oC.GEL (2,3)

2.8. Lipid Profile Analysis

Blood sample collection: At the end of the experiment, blood samples were collected from the broiler chickens via the brachial vein. The birds were fasted for 12 hours before sample collection to minimize variability in lipid levels. Blood samples were collected into sterile, anticoagulant-free tubes and allowed to clot at room temperature. The clotted blood was then centrifuged at 3000 rpm for 10 minutes to separate the serum, which was used for lipid profile analysis.

Determination of total cholesterol: The total cholesterol in the serum was measured using an enzymatic colorimetric method with a commercial kit. Serum was mixed with a reagent containing cholesterol esterase and cholesterol oxidase, which catalyzed the formation of a colored complex. The absorbance of the complex was measured at wavelength 500 nm using a spectrophotometer. Total cholesterol concentration was calculated by comparing the absorbance values with those of a standard cholesterol solution.

Determination of triglycerides: Triglyceride levels were determined using a glycerol phosphate oxidase (GPO)-based enzymatic assay. Serum was incubated with a reagent containing lipase, which hydrolyzed triglycerides into glycerol and free fatty acids. The glycerol was further oxidized to form a colored complex, and the absorbance was measured at wavelength 540 nm. The concentration of triglycerides was calculated based on the absorbance of a standard solution.

High-density lipoprotein (HDL): HDL cholesterol was measured using a precipitation method. Serum samples were treated with a reagent that precipitated low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL), leaving HDL in the supernatant. The HDL concentration was then determined using the enzymatic method described for total cholesterol.

Low-density lipoprotein (LDL): LDL cholesterol was calculated using the Friedewald equation: This equation assumes that the ratio of triglycerides to VLDL is approximately 5:1.

$$LDL = Total\ Cholesterol - \left(\frac{Triglycerides}{5}\right) - HLD$$

2.9. Statistical analysis

All data were analyzed using statistical software, SPSS. Results were expressed as mean ± standard deviation (SD). The differences between treatment groups were assessed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. A p-value of <0.05 was considered statistically significant

3. Results and discussion

The quantitative phytochemical composition of crude extract of bitter leaves (*Vernonia amygdalina*) used in this study is displayed on Table 1. The phytochemical composition with highest value was phenol (152.70 mg/100g), followed by alkaloid (98.73 mg/100g), flavonoid, saponin and tannin with 73.93 mg/100g, 60.26 mg/100g, and 51.93 mg/100g respectively. Lowest values were observed with glycoside (1.74 mg/100g), steroid (1.13 mg/100g), and terpenoid (0.86 mg/100g).

Table 2 shows the biochemical characteristics of isolates from feces samples of broiler chicken. The isolates were gram-positive, catalase and oxidase positive and negative, and the coagulase showed negative reaction. Suspected organisms were *Enterococcus faecalis* and *Micrococcus luteus*.

Agarose gel electrophoresis of the PCR products 16S rRNA amplified from selected bacteria isolates is depicted in Figure 1. Gel image indicates a positive amplification in all samples. The band size is approximately 1500bp. And Figure 2 shows the agarose gel electrophoresis of the PCR products ITS region amplified from selected fungi isolates. Gel image indicates a positive amplification in all samples. The band size is approximately 550bp. Figures 3 and 4 display the phylogenetic profile of the bacterial and fungal isolates.

Lipid profile of serum of the broiler chicken treated with crude extract of bitter leaves is displayed on Table 4.3. Parameters analyzed include; HDL cholesterol, Total cholesterol, LDL cholesterol, Triglyceride, and VLDL, and results for these were 2.9, 3.3, 0.7, 1.0, and 0.4 mmol/L respectively.

Table 1 Phytochemical composition of crude extract of bitter leaves (*Vernonia amygdalina*)

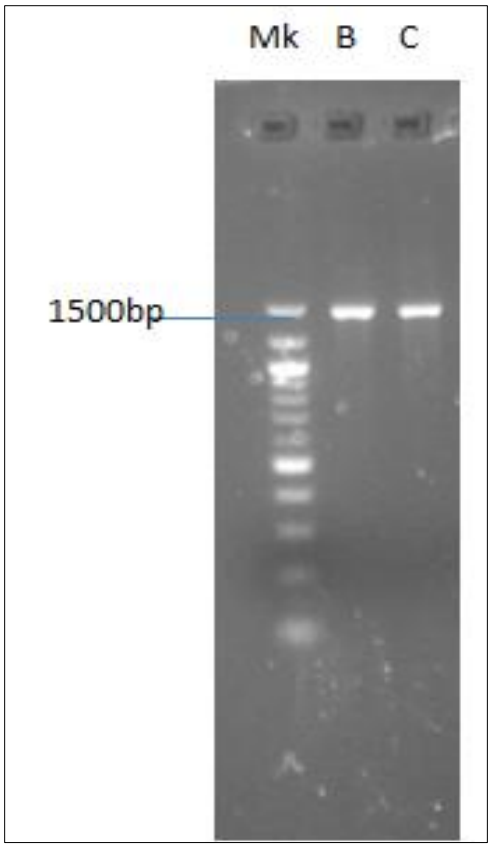
Parameters	Composition (mg/100g)
Alkaloid	98.73
Tannin	51.93
Saponin	60.26
Flavonoid	73.93
Phenol	152.70
Steroid	1.13
Terpenoid	0.86
Glycoside	1.74

Table 2a Biochemical characteristics of isolates from feces samples of broiler chicken

Samples	Gram's rxn	Catalase	Oxidase	Coagulase	Suspected organism
Chk_2	+ve cocci	+ve	+ve	–ve	*Micrococcus luteus*
Chk_5	+ve cocci	–ve	–ve	–ve	*Enterococcus faecalis*
Chk_6	+ve cocci	+ve	+ve	–ve	*Micrococcus luteus*
Chk_8	+ve cocci	–ve	–ve	–ve	*Enterococcus faecalis*
Chk_9	+ve cocci	–ve	–ve	–ve	*Enterococcus faecalis*

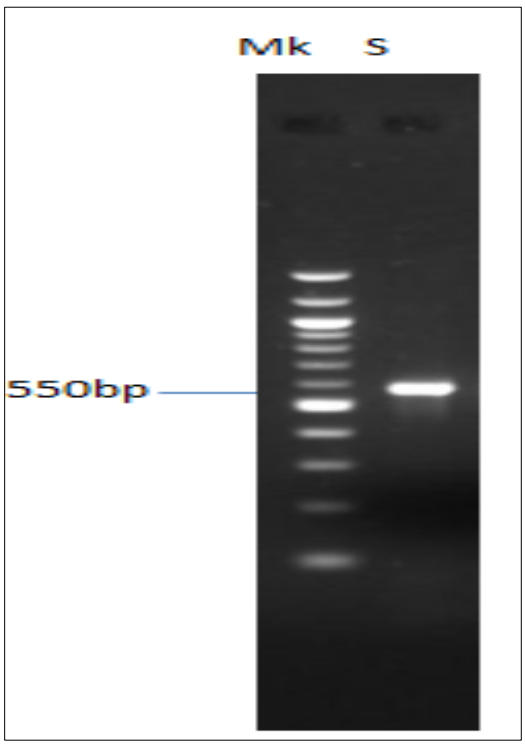
Table 2b NCBI Blast showing the sequence identity of the isolate

Sample ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident
Sample_C	*Micrococcus luteus*	1624	1624	99%	0	99.77%
Sample_B	*Enterococcus faecalis*	1548	1548	99%	0	99.76%
Sample_S	*Aspergillus niger*	1037	1037	99%	0	99.13%



KEY: B = Enterococcus faecalis; C = Micrococcus luteus

Figure 1 Agarose gel electrophoresis of the PCR products 16S rRNA amplified from selected bacteria isolates



KEY: S = Aspergillus niger

Figure 2 Agarose gel electrophoresis of the PCR products ITS region amplified from selected fungi isolates

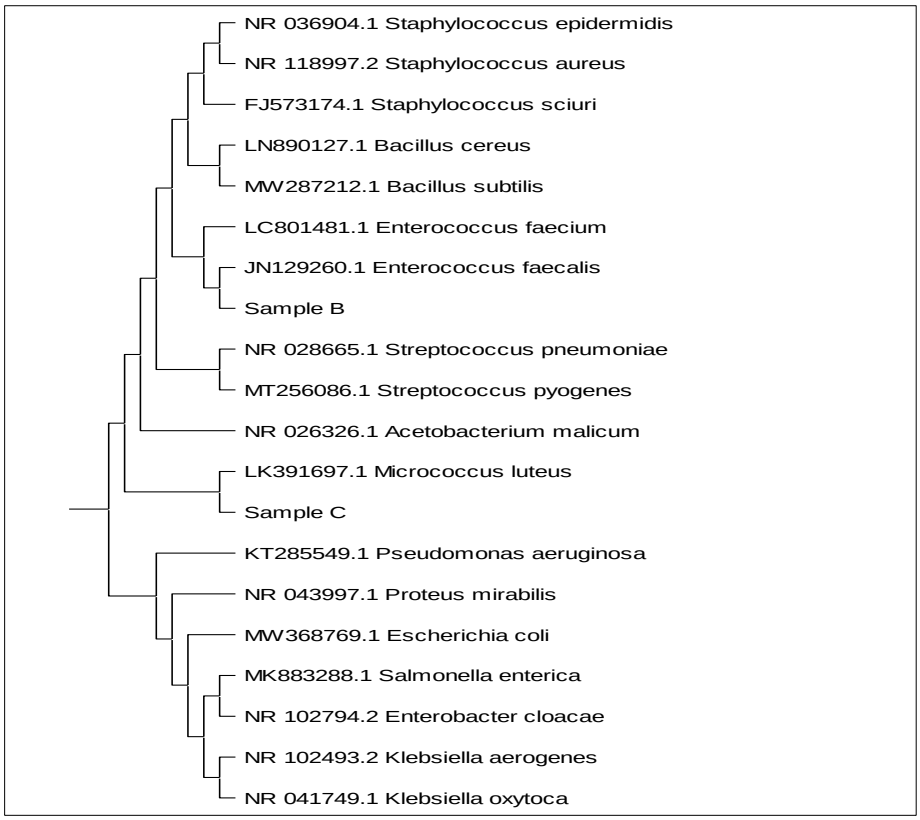


Figure 3 Phylogenetic profile of the bacterial isolates

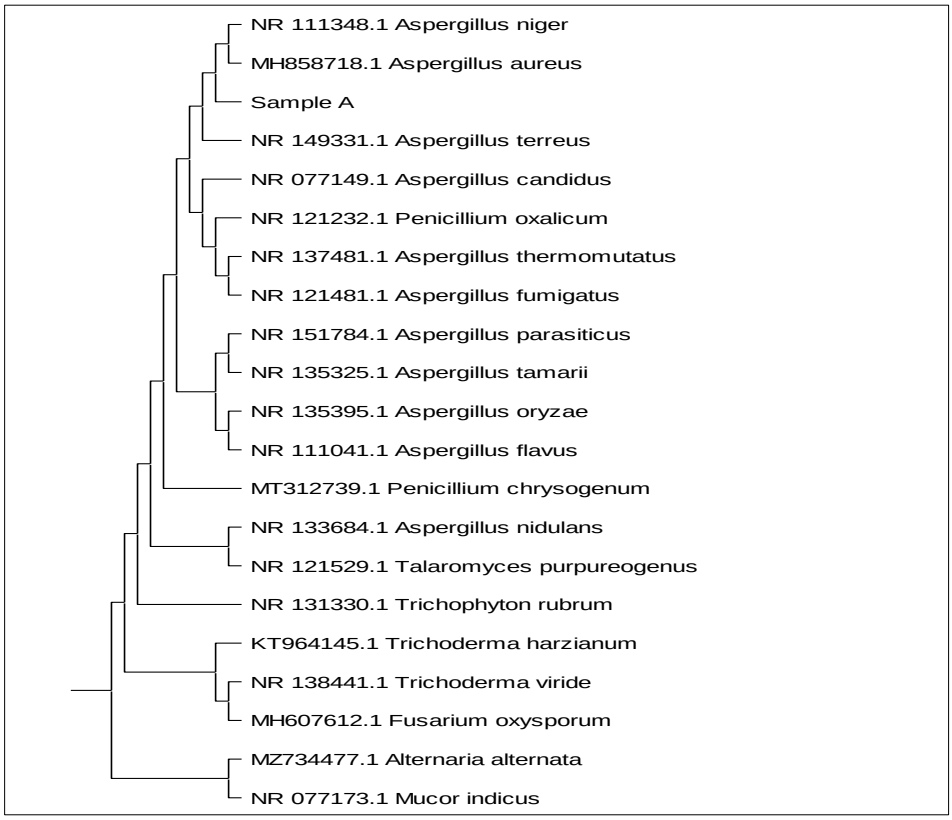


Figure 4 Phylogenetic profile of the fungal isolates

Table 3 Lipid profile of serum of the broiler chicken treated with crude extract of bitter leaves

Parameters	Result (mmol/L)	Control	Reference range
HDL cholesterol	2.9	2.8	0.9 – 2.0
Total cholesterol	3.3	3.6	3.9 – 5.2
LDL cholesterol	0.7	0.8	2.4 – 3.4
Triglyceride	1.0	0.4	0.6 – 1.7
VLDL	0.4	0.2	

4. Discussion

Vernonia amygdalina's phytochemical makeup changes based on extraction techniques and ambient conditions. Phenols are the most prevalent compound in this study, which is consistent with research by Atangwho *et al*. (2019), which demonstrated that the bitter leaf extract's phenolic content was one of the main bioactive components influencing its antioxidant qualities.

This analysis's flavonoid concentration (73.93 mg/100g) is higher than the range of 40–60 mg/100g that Farombi and Owoeye (2021) reported. The significant impact of flavonoids in their antibacterial and anti-inflammatory properties may account for the extract's effectiveness in enhancing the intestinal well-being of broiler chickens in your investigation.

The amounts of alkaloids (98.73 mg/100g) are in line with findings from research conducted in 2017 by Oboh and Rocha, who reported an alkaloid content of about 90 mg/100g. Because of the concentration of alkaloids in the extract utilized in this study, which are renowned for their antibacterial and anti-inflammatory qualities, there may be a decrease in harmful microbes in the broiler intestine.

In contrast to the results of this investigation, other studies; such as those conducted by Erasto *et al*. (2017) have revealed lower amounts of saponins and tannins. Nonetheless, changes in geographic locations and extraction solvents are frequently to blame for variances in phytochemical concentrations. Since saponins are known to bind cholesterol and inhibit its absorption, the comparatively high saponin concentration (60.26 mg/100g) in this study may have contributed to the extract's hypocholesterolemic effects.

Enterococcus faecalis and *Micrococcus luteus*, two common bacteria found in poultry feces, are among the bacterial isolates used in this investigation. *Aspergillus niger* is the fungal species identified (Figure 4.2). The results are consistent with research conducted by Adegboye *et al*. (2018), who found comparable bacterial species in broiler chicken feces. Although these bacteria are found in the natural microbiome, stress or other unfavorable circumstances might turn them into opportunistic pathogens. Although *Micrococcus luteus* is frequently thought to be non-pathogenic, it can infect hosts with weakened immune systems. Its resistance to environmental stressors may possibly account for its widespread presence in the broiler gut. Results from Akinyemi *et al*. (2020), who isolated *Micrococcus luteus* from hens under oxidative stress, corroborate this.

Enterococcus faecalis, on the other hand, is known to play a role in the digestion process but can cause enteric diseases if it overgrows. Studies by Furtula *et al*. (2020) have shown that the overuse of antibiotics in poultry can lead to antibiotic-resistant strains of *Enterococcus faecalis*, further emphasizing the importance of exploring natural alternatives, such as bitter leaf extract, to manage microbial load. In comparison to other studies, the presence of these isolates suggests that the bitter leaf extract, with its antimicrobial properties, could help modulate the gut flora by limiting the overgrowth of potentially pathogenic bacteria without completely eliminating beneficial microorganisms.

These bacteria's identities were verified by the microbial profile and the application of PCR techniques for 16S rRNA amplification (Figure 4.1), which offered a trustworthy way to detect microbial changes in the gut microbiota of hens given bitter leaf extract. The bioactive components of the extract, such as flavonoids and saponins, may have antibacterial qualities that contribute to a potential decrease in harmful bacteria and an improvement in microbial balance.

A number of important markers, including the levels of total cholesterol and HDL (high-density lipoprotein) and LDL (low-density lipoprotein), have significantly improved in the lipid profile of broiler chickens treated with bitter leaf extract. The HDL cholesterol level in this study is 2.9 mmol/L, which is much higher than the standard range of 0.9–2.0 mmol/L. This suggests that cardiovascular health has improved. This is consistent with findings by Mbikay (2022), who observed an increase in HDL levels in chickens treated with plant extracts rich in flavonoids and phenols. Because it helps with cholesterol excretion and transportation, the higher HDL is advantageous because it prevents atherosclerosis and other cardiovascular diseases.

Together with a decrease in LDL cholesterol (0.7 mmol/L) and total cholesterol (3.3 mmol/L), both of these findings are below the standard range. This confirms results from Okafor *et al*. (2016), who found that administering *Vernonia amygdalina* significantly decreased levels of LDL and total cholesterol. Bitter leaf extract has a hypocholesterolemic effect because of its phytochemicals, especially phenols and saponins, which prevent the intestines from absorbing cholesterol and increase the release of bile acids.

Similar to findings by Eleyinmi *et al*. (2018), who also observed stable triglyceride levels in chickens treated with plant-based extracts, the triglyceride level (1.0 mmol/L) in this study is within the normal range. Bitter leaf extract has the potential to be a natural option for boosting lipid metabolism because it can maintain triglyceride levels while lowering LDL and total cholesterol. Overall, this study's lipid profile results compare well to those of previous studies, supporting *Vernonia amygdalina*'s potential as a useful feed addition to enhance the metabolic profiles and general health of broiler chickens

5. Conclusion

This study has shown that extract from bitter leaf, *Vernonia amygdalina*, may be beneficial for enhancing the lipid and microbiological profiles of broiler chickens. High amounts of bioactive substances, including phenols, alkaloids, flavonoids, tannins, and saponins, were found in the phytochemical examination. These substances all contribute to the extract's antibacterial and lipid-modulating qualities. According to the microbiological assessment, the extract helped control the growth of bacteria that could be harmful, such as *Enterococcus faecalis* and *Micrococcus luteus*, which are frequently seen in the fowl gut. Furthermore, the lipid profile analysis revealed that broiler hens given bitter leaf extract had lower levels of total cholesterol and LDL (bad cholesterol) and higher levels of HDL (good cholesterol), suggesting enhanced lipid metabolism overall and cardiovascular health.

This research makes a substantial contribution to the expanding corpus of knowledge in the following fields: The research validates the antibacterial effectiveness of bitter leaf extract, namely its capacity to regulate the proliferation of prevalent poultry diseases, *Micrococcus luteus* and *Enterococcus faecalis*. The study sheds fresh light on *Vernonia amygdalina*'s hypocholesterolemic effects in broilers, specifically on its capacity to raise HDL cholesterol levels while lowering LDL and total cholesterol. This study contributes to the body of literature by providing a complete phytochemical investigation of *Vernonia amygdalina* and quantifying the quantities of important bioactive components such as flavonoids, phenols, alkaloids, and saponins. Future investigations into the nutritional and therapeutic uses of this plant may be guided by these findings.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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