



Qualitative and Quantitative Phytochemical Screening of Bitter and Neem Leaves and their Potential as Antimicrobial Growth Promoter in Poultry Feed

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Authors' contributions

This work was carried out in collaboration among all authors. Author RTSO designed the study, wrote the protocol, wrote the first draft managed the literature searches, author EIO performed the statistical analysis, managed the analysis of the study, reviewed the first and second draft. Author EAI conceptualized the study. All authors read and approved the final manuscript.

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ABSTRACT

Gastrointestinal health challenges without in-feed antibiotics are a crucial concern to poultry farmers. Although, quite a few substitutes for antibiotics have emerged, phytochemicals and antibiotic properties of medicinal plants cannot be overlooked with regard to control, good health and minimizing sub-clinical bacteria-induced infections by zoonotic enteropathogenic bacteria in poultry birds. *Vernonia amygdalina* (Bitter leaf) and *Azadirachta indica* (Neem) leaves were collected early in the morning and oven dried to 15% moisture content. The non-essential oil was obtained by cold maceration method using methanol. Appropriate methods were used for the

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qualitative and quantitative analysis of phytochemicals in the respective oils. Qualitative analysis of phytochemicals showed that, saponins and terpenoids were highly detected (+++) in *V. amygdalina*. Saponins and terpenoids were slightly detected (+) in *A. indica*. Phenolic compounds were highly detected (+++) in both plants. Flavonoids were also identified (++) in both plants, but tannins were highly detected (+++) in *A. indica*. Quantitative analysis revealed a rather high concentration of complete phenolic content and overall flavonoid content in *V. amygdalina*. Total phenolic content of 44.76 mg Gallic acid equivalent / g of extract and 57.21 mg Rutin equivalent / g of extract (total flavonoid content) was recorded in *V. amygdalina* and 25.77 mg Gallic acid equivalent / g of extract (total phenolic content) and 24.45 mg Rutin equivalent /g of extract (total flavonoid content) in *A. indica*.

In conclusion, both plant extracts exhibited possibility as suitable substitutes to proprietary antibiotics that can be beneficial to gastrointestinal health and overall performance in animal nutrition.

Keywords: *Azadirachta indica*; *gastrointestinal health*; *phytoGENICS*; *animal nutrition*; *Vernonia amygdalina*.

1. INTRODUCTION

PhytoGENICS or plant extracts are currently being considered as appropriate substitutes to antibiotic growth promoters (AGP) from results of several studies using different plant extracts instead of antibiotics in poultry diets. Although varied results have been reported, however, their potential in enhancing growth performance by influencing gut-related variables such as gut bacteria type and population, gut pH, inducing immune response just to mention a few cannot be overlooked. Medicinal herbs have beneficial properties such as anti-oxidant, anti-microbial and anti-fungal [1] including immune-modulatory and anticoccidial effects. As natural components of plants, herbal preparations assist in digestion and may be regarded as innocuous, cost-efficient and consumer-friendly with little or no knock-on effect on the animals and consumers of livestock products, such as meat and eggs. Earlier report by Vinus et al. [2] advanced the addition of plant extracts in diet of poultry birds to improve performance, make better feed conversion and utilization, sustain health and assuage the harmful impact of environmental stress. Aloe vera, Fenugreek, Ashwagandha, Ginger, *Moringa oleifera*, Cinnamon, Tulsi, Garlic, Pepper, *Ocimum* etc. are examples of medicinal plants that have received particular interest from researchers. Medicinal plants may exert various functions in the bird's body system [3]. Such functions include ameliorating bird performance by improving digestive tract function, eliciting anti-inflammatory action, anti-oxidative and anti-microbial effects. In addition, some have an effect on different physiological functions. The manner of action of medicinal plant extracts has not been completely elucidated. Most of them

have antibacterial, coccidiostatic, anti-helminthic, anti-viral, anti-inflammatory and in particular exhibit antioxidant properties. Herbs and spices can protect feed against oxidative deterioration during storage [2]. As a new class of additives to livestock feeds, a wide variety of medicinal plants exist in nature and will necessitate studying extensively to enable their usage as phytoGENIC feed additives for poultry. The utilization of herbal blends give rise to positive effect on feed digestibility [3] and positively influence gut health parameters [4].

The leaves of *M. oleifera* has been shown to elicit potential prebiotic effects [5]. Phytochemicals with potential antioxidant properties, such as chlorogenic acid and caffeic acid were found in *M. oleifera* [5]. Leaf meal of *M. oleifera* widely available in many tropical countries, is a suitable source of antioxidant compounds – ascorbic acid, flavonoids, phenolics and carotenoids [6]. Garlic and ginger are spices with medicinal potential which can serve as phytoGENIC growth promoters or suitable potential substitutes to antibiotic growth promoters [7]. According to Al-amin et al. [8], ginger extract might control quantity of free radicals and the peroxidation of lipids. An earlier report by Zige and Ofongo [9] highlighted the antibacterial activity of ginger extract. Garlic (*Allium sativum*) has antibacterial, antifungal, antiparasitic, antiviral, antioxidant, and anti-cholesterol properties [10]. Few *in-vivo* nutrition studies carried out with *A. indica* (neem) and *V. amygdalina* (bitter leaf) indicated the possibility of aqueous extract of both plants to modulate gut bacteria [11] and improve growth performance in broiler chickens [12].

Based on outcomes gotten from an earlier *in-vivo* studies, the quantitative and qualitative phytochemical assessment of the methanol extract of these two herbal plants (neem – *A. indica* and bitter leaf – *V. amygdalina* – Fig. 1) was carried out. This is to provide the evidence-based result that will enable further studies which will focus on concentration and standardization of these plant extract.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Leaves

A large quantity of neem (1084.29 g – wet weight) and bitter leaves (3632.28 g – wet weight) were obtained freshly harvested from the University farm early in the morning. They were bagged separately in zip lock bags and taken to the laboratory for phytochemical analysis. The leaves were washed lightly with distilled water separately then oven-dried at a temperature of 40°C for 72 hours until a constant weight was obtained. The dried leaves were milled to powder form with a blending machine. Quantity of *V. amygdalina* and *A. indica* obtained after drying and milling were 702.22 g and 502.51 g; respectively.

2.2 Extraction

The ground powder of *A. indica* and *V. amygdalina* leaf was extracted separately with 80% methanol (Sigma-Aldrich Analytical grade) by the cold maceration method. Each mixture was vigorously stirred intermittently then allowed to stay for 72 hours after which it was sifted through a Whatman No.1 filter paper-lined funnel into a conical flask. The solvent from the filtrate was recovered by means of a rotary evaporator under vacuum at 40°C. The extract was further concentrated and dried using a water bath at 40°C for another 48 hours. The semi-solid crude extracts were kept in a desiccator for 24 hours until constant weights were observed. All extracts obtained were kept in McCartney bottles and stored in the refrigerator until required for use. The extracts of both leaves were evaluated for alkaloids, tannins, glycosides, steroids, flavonoids, saponins, anthraquinone, etc. using standard procedures.

2.3 Phytochemical Screening

Phytochemical screening which implies the extraction and detection of the medicinally active substances obtained in plants, characteristically the *secondary metabolites* was conducted for both plant extracts. Some of the bioactive components of plant extracts are flavonoids, alkaloids, phytosterols, terpenoids, tannin, saponins, reducing sugar, cardiac glycosides, antioxidants, phenolic compounds and triterpenoids. Chemical reagents required for the analysis of each secondary metabolite were utilized for each specific metabolite to be determined. These were: ethanol, Chloroform, Mayer's reagent, Dragendorff's reagent, Hager's reagent, Wagner's reagent, Sodium hydroxide, di hydrogen tetra-oxo-sulphate 6 acid or sulphuric acid (H₂SO₄ Sigma-Aldrich analytical grade), Sodium potassium tartrate, 3,5-dinitrosalicylic acid (Sigma-Aldrich), Lead acetate solution, glacial acetic acid, acetic anhydride, Methanol (Sigma-Aldrich Analytical grade), Hydrochloric acid, Sodium carbonate, Barium hydroxide, Zinc Sulphate, Folin ciocalteu phenol reagent, Potassium ferricyanide, Ascorbic acid, Quercetin, Catechol, Gallic acid and Rutin (Reference standards), Phosphate buffer, Trichloroacetic acid, Iron chloride solution and Tannic acid.

2.3.1 Qualitative analysis

2.3.1.1 Test for reducing sugars (Fehling's Test)

To 2 ml of aqueous extract, 1 ml of Fehling's A solution and 1 ml of Fehling's B solutions were mixed in a test-tube then heated in boiling water in a water bath for 10 min. The appearance of a yellow then brick-red precipitate denoted the occurrence of reducing sugars.

2.3.1.2 Test for Terpenoids

Salkowski's Test was utilized to test for the presence of terpenoids. A tiny quantity (0.5 g) of each extract was weighed into a test-tube, into which 10 ml of methanol was added. This was stirred properly, then filtered to derive 5 ml extract of each plant sample. Thereafter, 2 ml of chloroform was stirred together with each extract of the plant samples. Afterwards, 3 ml of sulphuric acid was put into each sample extract. The development of a reddish-brown colour pointed to the presence of terpenoids in the respective plant extract.



A: *Vernonia amygdalina*

B: *Azadirachta indica*

Fig. 1. Pictures of plants used in the study

2.3.1.3 Test for Steroids

Salkowski's Test was utilized to test for the presence of steroids. A little quantity (0.5 g) of each extract was mixed with 3 ml of chloroform then filtered into a test-tube. The test-tube content was tilted, after that 2 ml of concentrated H_2SO_4 was slowly poured through the side of the test-tube. A brown colour and reddish colour at the chloroform phase denoted the presence of steroids.

2.3.1.4 Test for Phenolic compounds (Lead acetate Test)

Five milliliters (5 ml) of aqueous extracts of neem and bitter leaf were treated with 2-3 drops of lead acetate. Formation of white-yellow colour denoted the occurrence of phenolic compound.

2.3.1.5 Test for flavonoids (Shinoda Test for flavonoids)

A small quantity (0.5 g) of neem and bitter leaf extract was individually dissolved in 95 % ethanol, warmed then filtered to get the ethanolic extract. To the filtrate, four pieces of magnesium turning or filings (ribbon) were added. The filtrate was hydrolyzed by adding 0.5 ml concentrated hydrochloric acid (HCl). The presence of a pink, orange, or red to purple pigmentation point to the occurrence of flavonoids [13].

2.3.1.6 Test for Tannins (Ferric chloride test)

Small amount (0.5 g) of the dried extract was bubbled in 10 ml of distilled water in a test-tube then filtered to obtain a filtrate. To 2 ml of this filtrate, a drop of 1% ferric chloride ($FeCl_3$) solution was added and observed for brownish green-black or a blue-black coloration signified the occurrence of tannin [13].

2.3.1.7 Test for alkaloids

One gram (1 g) of each extract was liquefied in ethanol and dilute hydrochloric acid (50:50) in a test-tube and positioned in a boiling water-bath for 10 minutes. The mixture was filtered.

- Mayer's Test:** This involved treating the filtrates with Mayer's reagent (Potassium Mercuric Iodide) to check for the occurrence of alkaloids. The development of a buff-white to yellow colour precipitate denoted the occurrence of alkaloids.
- Wagner's Test:** Addition of Wagner's reagent (Iodine in Potassium Iodide) to the filtrates and the resultant formation of brown/reddish precipitate showed the occurrence of alkaloids.
- Dragendorff's Test:** Addition of Dragendorff's reagent (solution of Potassium Bismuth Iodide) to each respective filtrate and the development of a

red precipitate further signified the occurrence of alkaloids.

- d) *Hager's Test*: Addition of Hager's reagent (saturated picric acid solution) to each respective filtrate and the formation of a yellow-colored precipitate established the presence of alkaloids.

2.3.1.8 Test for Saponin (Frothing Test)

Each respective extract was diluted with distilled water to 10ml and this was mixed together in a graduated cylinder by shaking for 15 minutes. The formation of 1 cm layer of foam point towards the presence of saponins [14].

2.3.1.9 Test for Anthraquinones (Bontrager's Test)

About 0.5 g of each respective plant extract was boiled with 10% hydrochloric acid (HCl) for few minutes by placing in a water-bath. Each was filtered then allowed to cool. Equal volume of chloroform (CHCl₃) was added to each filtrate after which few drops of 10 % ammonia (NH₃) was added to the mixture then heated. The development of a pink colour in the NH₃ phase showed the occurrence of anthraquinones (Sofowora, 1993).

2.3.1.10 Test for Cardiac glycoside (Keller Kiliani Test)

A small amount (0.5 g) of each extract was treated with 2ml of glacial acetic acid into which a drop of 1% ferric chloride solution was included in a test-tube and the solution filtered. The test-tube was tilted to angle 45 degrees then about 1ml of concentrated H₂SO₄ was added. The formation of a brown ring detected at the interface designated the presence of cardiac glycosides.

2.3.1.11 Triterpenoid (Liebermann-Burchard Test)

A small quantity (0.5 g) was stirred together with 5 ml chloroform, then with 2 ml acetic anhydride followed by concentrated sulphuric acid from the sides of the test-tubes. Appearance of a blue and finally green colour designated the occurrence of triterpenoids.

2.3.2 Quantitative analysis

Quantitative analysis of methanol extract of *A. indica* and *V. amygdylina* was carried out to ascertain the amount of each phytochemical

detected earlier in each respective extract. Values obtained were reported on per gram basis.

2.3.2.1 Estimation of Alkaloids

The alkaline precipitation-gravimetric method as described by Harborne (1998) was used to estimate quantity of alkaloids present. This was achieved by dispersing a measured weight of each extract sample into 10% acetic acid solution in ethanol forming a ratio of 1:10 (10%). This mixture was let to stand for 4 hours at 28°C then sifted afterwards with Whatman No. 42-grade filter paper. The filtrate obtained was condensed to one-quarter of its original volume by evaporation. This condensed filtrate was later treated with drop-wise totting up of concentrated aqueous NH₄OH up until precipitation of alkaloids. Precipitated alkaloid was accumulated into a pre-weighed filter paper, rinsed with 1% ammonia solution then dried in the oven at 80°C. The quantity of alkaloid obtained was estimated and expressed as a percentage of the weight of sample analyzed.

2.3.2.2 Estimation of Phenolic Compounds content

The Folin Ciocalteu reagent method as described by Singleton and Rossi [15] was used to estimate the total phenolic contents of the extracts. In order to plot the calibration curve, 1 ml aliquots of 50, 100, 150, 200, 250, 300, 350, 400 and 450 µg/ml Gallic acid solution was mixed with 5.0 ml of Folin Ciocalteu reagent (diluted tenfold) and 4.0 ml of sodium carbonate solution (75 g/l). The absorbance of this mixture was read at 765 nm after 30 min. One mill (1 ml) of the extracts (1 g/100 ml) was mixed separately with the same reagents, following the same procedure for generating the calibration curve. After one hour, the absorbance was quantified to establish the total phenolic contents in the plant extracts using the formula:

$$T = (C \times V)/M$$

Where,

T = Total phenolic contents in milligrams of Gallic acid equivalent per gram of the extract
C = Concentration of Gallic acid in mg/ml obtained from the calibration curve.

V = Total volume of extract used in assay.

M = Total weight of dry extract (in gram) used in the assay.

2.3.2.3 Determination of Total flavonoid

The method is founded on the formation of a flavonoids - aluminium complex with a maximum absorptivity at 415 nm. A quantity (100 µl) of the sample extracts in methanol (10 mg/ml) was mixed with 100 µl of 20% aluminium trichloride in methanol to which a drop of acetic acid was added. The mixture was then attenuated with methanol to 5 ml. After 40 minutes of incubation, the absorption at 415 nm was read. Preparation of blank samples was carried out using 100 ml of sample extracts and a drop of acetic acid which was later diluted to 5 ml with methanol. The absorption of standard Rutin solution (0.5 mg/ml) in methanol was measured under similar criteria. All determinations were carried out in triplicates.

Total flavonoid concentration was expressed as Rutin equivalents (mg Rutin Equivalents /g extract).

2.3.2.4 Estimation of Reducing Sugars

Fehling's solution (10 mL) was pipetted out into a conical flask (300 mL) by means of a burette, just about the whole of the sample solution required for reduction of all the copper was ran. After this, contents of the flask were boiled gently for 2 min. At the expiration of 2 min of boiling without discontinuing boiling, 1 mL of methylene blue indicator was added. While contents of the flask carry on boiling, a sample cocktail was added in from the burette till the blue coloration of the indicator clearly disappears. The burette reading was then recorded.

2.3.2.5 Quantitative Estimation of Steroids

Gravimetric weight method was used for the quantitative estimation of steroids. Thirty ml (30 ml) of chloroform was used to extract 5 g of the dried sample using Soxhlet apparatus for 15 min. The two filtrates were collected and dried under reduced pressure at 40°C in the laboratory by means of a rotary evaporator to recover the solvent. The residue obtained was placed in an oven at 80°C until a constant weight was attained.

2.3.2.6 Determination of Saponin

Twenty grams (20 g) of each plant sample was weighed into a conical flask (250 ml) into which 100 ml of 20% ethanol was added. This mixture was heated for 4 hours with continuous stirring at about 55°C on a hot water bath. The filtrate was

obtained by sieving through Whatman No.42 filter paper. The residue gotten was re-extracted with another 200 ml of 20% Ethanol. The combined extract was concentrated to 40 ml over a water bath at about 90°C. The concentrated extract was then conveyed into a 250 ml separator funnel and 20 ml of diethyl ether (CH₃CH₂)₂O added to the extract and stirred briskly. The aqueous layer was retrieved and the (CH₃CH₂)₂O layer discarded. This purification procedure was repeated. Sixty mills (60 ml) of n-butanol were then added and the combined n-butanol extract was washed twice with 10 ml of 5% NaCl. The residual solution was afterwards heated on a water-bath in a pre-weighed 250 ml beaker. After evaporation, the residue was dried in an oven at 105°C to a constant weight. The percentage (%) Saponin was estimated by difference.

2.3.2.7 Evaluation of Tannin

The method described by Vetter and Barbosa (1995) was used to determine the quantity of tannins in each extract. To 100 ml of distilled water was added 2 g of the sample and the solution placed in a water bath at 90° C for one hour. This mixture was sieved using Whatman's No. 1 filter paper and the filtrate was re-extracted again. The two filtrates were collected together and set aside to cool down. The filtrate was made up to 500 ml using distilled water. A small amount (100 ml) of this solution was transferred to a beaker, into which 10 ml of 40% formaldehyde and 5 ml of concentrated sulphuric acid was added; respectively. The whole mixture was refluxed for 30 minutes and subsequently allowed to cool down. The mixture was filtered and the filtrate dried, weighed and estimated.

2.3.2.8 Estimation of free anthraquinones

The anthraquinone content was spectrophotometrically quantified in triplicates, after a suitable pre-treatment. A volume of ethyl acetate (0.5 mL) fraction was alkalized with NaHCO₃ (50 mg) and oxidized with an aqueous solution of 10.5% FeCl₃ (20 mL). This mixture was then boiled under reflux for 5 min. Concentrated hydrochloric acid (1 mL) was added, and the reaction medium was kept under a similar condition for 20 more minutes. The mixture was partitioned with diethyl ether three times at room temperature. The ether phase was transferred into a 100 mL volumetric flask, the final volume made up to mark with this organic solvent to obtain a stock solution. Ten mill (10

mL) of the stock solution was evaporated to dryness on a water bath at 60°C. The residual solid obtained was dissolved in 10 mL of 0.5% magnesium acetate as methanol solution. The absorbance was determined at 515 nm with a UV-Vis spectrophotometer. This same analytical procedure was carried out for the hydro-ethanolic fraction of each sample. In order to obtain the analytical curve, a standard solution of 1, 8-dihydroxyanthraquinone (0.005-0.06 mg/mL) in ether was equally evaporated and treated with 0.5% magnesium acetate in methanol. The methanol solution was utilized as equipment blank. The results obtained were expressed as milligrams of hydroxyl-anthracene derivatives per 100 g of the dry sample.

2.3.2.9 Evaluation of Cardiac glycosides

The concentration of cardiac glycosides in each sample was evaluated with Buljet's reagent. One gram of the finely milled powder of each sample was soaked separately in 10 ml of 70% alcohol for 2hrs then filtered. The extract obtained was purified using lead acetate and Na₂HPO₄ solution after which freshly prepared Buljet's reagent (containing 95 ml aqueous picric acid + 5 ml 10% aqueous NaOH) was added. Difference between the intensity of colour of the experimental and blank (distilled water and Buljet's reagent) samples gave the absorbance which is proportional to the concentration of glycosides.

2.4 Statistical Analysis

Statistical analysis was carried out using IBM SPSS version 25 (IBM SPSS Inc, Chicago). Independent t test was carried to compare the phytochemical constituents of both plants and their significant of the difference was assessed both at 95% and 99% probability levels.

3. RESULTS

The results obtained from qualitative and quantitative analysis of both plant extracts is presented in Tables 1 and 2; respectively.

3.1 Qualitative Analysis

Quantitative analysis of the methanol extract of *A. indica* and *V. amygdylina* presented in Table 1 revealed that saponins and terpenoids were heavily detected in *V. amygdylina* (+++) compared to *A. indica* (+). Alkaloids analysis by the different tests revealed similar level of alkaloids (+ i.e., slightly detected) in both plant

extract. This was also the case with reducing sugar and steroids in both plant extracts. Cardiac glycosides were not found in *A. indica* but slightly detected in *V. amygdylina* (+).

3.2 Quantitative Analysis

The results of obtained from quantitative analysis (mg/ g of extract) of methanol extract of *A. indica* and *V. amygdalina* are presented in Table 2. From the results, the lowest concentration (mg/g of phytochemicals analyzed in the methanol extract of *A. indica* and *V. amygdalina* was recorded for Cardiac glycosides (0.17 mg/g – *A. indica* and 0.12 mg/g *V. amygdalina*), Anthraquinones (0.37 mg/g – *A. indica* and 0.13 mg/g *V. amygdalina*) and steroids (0.42 mg/g – *A. indica* and 0.29 mg/g *V. amygdalina*); respectively, which were all different in the two plant extracts. Apart from alkaloids and reducing sugars, which was almost similar, *V. amygdalina* had a higher concentration of Saponins (7.43 mg/g of extract) and tannins (12.33 mg/g of extract).

Total phenolic concentration of *V. amygdalina* (44.76 mg Gallic acid equivalent / g of extract) was over 45% that recorded for *A. indica* (25.77 mg Gallic acid equivalent / g of extract). This was also the case with flavonoids but the concentration recorded in *V. amygdalina* (57.21 mg Rutin equivalent /g of extract) was over 50 % better than the value recorded in *A. indica* (24.45 mg Rutin equivalent /g of extract).

4. DISCUSSION

Several phytochemicals (plant extracts) have been utilized in poultry nutrition especially in broiler feeding. The results from these studies have indicated the advantages of phytochemicals in enhancing weight gain, feed intake, feed conversion ratio [16] and other factors as estimated in these earlier studies. The phytochemical constituent present in medicinal plants have been described to play significant role or involved in the positive response obtained from studies utilizing medicinal plants as probiotics or natural growth promoters.

4.1 Tannins

Tannins are extensively dispersed in the plant kingdom. They are notably profuse in nutritionally valuable forages, shrubs, cereals and medicinal herbs [17,18]. Vulnerable plant parts such as new leaves and flowers of plants are generally abundant in tannins [19,20,21]. Growth

Table 1. Qualitative analysis of phytochemical constituent present in *A. indica* and *V. amygdalina*

Phytochemical constituent	Type of test	<i>A. indica</i> (Neem)	<i>V. amygdalina</i> (Bitter leaf)
Alkaloids	Mayer's test	+	+
	Dragendorff's Test	+	+
	Wagner's Test	+	+
	Hager's Test	+	+
Saponins	Frothing test	+	+++
Reducing sugar	Fehling's test	+	+
Anthraquinones	Borntrager's Test	++	+
Cardiac glycosides	Keller Kalani's Test	-	+
Terpenoids	Lieberman-Burchard	+	+++
Steroids	Salkowski's Test	+	+
Phenolic Compounds	Lead acetate Test	+++	+++
Tannins	Ferric chloride Test	+++	++
Flavonoids	Shinoda's Test	++	++
Triterpenoids	Liebermann-Burchard	++	++

Heavily detected: +++; detected: ++; slightly detected: +; not detected: -

Table 2. Quantitative analysis of methanol extract of *A. indica* and *V. amygdalina* (mg/ g of extract)

Phytonutrient /Phytochemical	Bitter leaf <i>V. amygdalina</i>	Neem <i>A. indica</i>	T value	Mean Difference	Std. Error of Difference
Alkaloids	5.76±0.01	5.09±0.00	67.000**	0.670	0.010
Saponins	7.43±0.02	0.30±0.02	285.400**	7.135	0.025
Steroids	0.30±0.01	0.42±0.02	-7.589*	-0.120	0.016
Tannins	12.33±0.01	9.15±0.02	142.214**	3.180	0.022
Reducing Sugars	3.43±0.01	4.12±0.01	-48.790**	-0.690	0.014
Anthraquinones	0.14±0.02	0.38±0.01	-15.179**	-0.240	0.016
Cardiac Glycosides	0.12±0.01	0.17±0.01	-4.919*	-0.055	0.011
Phenolic compounds	44.76±0.02	25.77±0.03	566.023**	18.985	0.034
Flavonoids	57.21±0.00	24.45±0.02	2184.333**	32.765	0.015

Along the rows, values are presented as Mean ± SE (n=2), the t-value of the difference was presented at 95% (*) and 99% (**) probability levels

conditions and growth stage of a plant is a determining factor on the chemical structure and concentration of tannins which varies significantly among plant species. Earlier reports Frutos et al., [21]; Amsler and Fairhead, [22]; Berard et al. [23]; Li et al. [24]; Huang et al. [25] stated that growth conditions such as temperature, light intensity, nutrient stress and exposure to herbivory play a role in chemical composition and concentration of tannins.

These factors could be the probable reason for the difference in the tannin content detected in *V. amygdalina* in this study. Although tannins were

highly detected in *A. indica* (+++) compared to *V. amygdalina* (++) , however the concentration of tannins /g of methanol extract was higher in *V. amygdalina*. The two plants were gotten from the same farm but planted in different locations on the same farm which further buttresses difference in concentration. Tannins elicit, antimicrobial, anti-parasitic, antioxidant, anti-inflammatory and antiviral properties. Although, initially considered as anti-nutritional components in monogastric diets – pig and poultry – Redondo et al., [26]. However, several recent reports point towards inclusion of low concentrations of tannins for improved health status, nutrition and

animal performance in monogastric farm animals [27-30].

The phytochemical results of *A. indica* and *V. amygdalina* obtained in this study further verifies the prospective of *A. indica* and *V. amygdalina* as possible phytochemical growth promoter for monogastric animals that can impact positively on gastrointestinal health and growth performance. Although the negative effects of tannins on feed intake, nutrient digestibility, performance limits its inclusion in monogastric diets however their capacity to enhance growth performance as reported in recent studies cannot be overlooked.

Several authors have also reported the positive outcomes of tannins in stimulating health status of the intestinal ecosystem by means of their anti-microbial, anti-oxidant and anti-inflammatory activities [31-32]. In addition to these effects therapeutically, other relevant effects such as expectorant, anti-inflammatory and immune-stimulatory effects which are ranked the highest.

4.2 Saponins

Saponins are glycosides found in several plants which elicit surfactant properties. Apart from causing symptoms of intoxication in high concentration, they demonstrate antimicrobial properties particularly against fungi. They also demonstrate antimicrobial properties against bacteria and protozoa. An earlier report by Westendarp [33] elucidated that the addition of saponins in ruminant nutrition suppresses intestinal and ruminal ammonia production. The author stated this effect is largely attributed to an inhibition of proteolytic microorganisms resulting from reduced NH₃-concentrations. A reduction of NH₃ - concentration brings about relief in metabolism which ultimately offers benefits to animal welfare and animal performance. In the light of saponins having capacity to inhibit proteolytic microorganisms, inclusion of phytochemicals containing saponins may influence the growth and proliferation of proteolytic bacteria – *Clostridia* – in poultry gastrointestinal tract. Saponins also exhibit antiseptic properties [34,35]. They play the biological role of membrane-permeabilizing, immune-stimulant and hypo-cholesterolaemic properties. The hypo-cholesterolaemic effect of saponins can be of benefit to meat quality especially when plant extracts containing saponins are incorporated in diets of poultry birds. Saponins significantly have an effect on growth and feed consumption in animals.

Saponin containing extracts or phytochemicals can function in several ways such as anti-coccidial, immune-stimulatory, antibacterial and antifungal. Sustainable poultry production demands continuous supply of feed ingredients and additives that can minimize if not eliminate deleterious effects of the absence of antibacterial growth promoters.

Issues of gut health will be of paramount concern in commercial poultry production and any plant extract or plant-based product that can rescue the situation should be developed to meet the demands. Plants with High-level saponin concentration have been suggested to be grown widely and endorsed by stakeholders for their availability and sustainability [36].

Azadirachta indica and *V. amygdalina* are readily available herbs in Nigeria. So as to serve the purpose of modulating gastrointestinal health in poultry nutrition, further studies must be carried out to tap their potential as phytochemical growth promoters or phytobiotics without inhibiting growth performance. In spite of the benefits stated for saponins, they affect animals in both positive and negative ways [37]. The benefits of saponins to gut health which involves the interface involving nutrition, gut bacteria and immune response cannot be ruled out.

4.3 Flavonoids

The report of Brodowska [38] stated that flavonoids are characterized by antioxidant, pharmacological, anti-inflammatory, anti-allergic, antiviral, anti-carcinogenic, in addition to therapeutic and cytotoxic properties. They also possess a varied scope of usages in diverse fields of industry. Flavonoids have been a focus for immense consideration due to their potential health benefits. According to Kamboh et al. [39], supplementation of poultry diets with flavonoids potentially progresses the nutritional, sensorial and microbiological quality of poultry meat and eggs. Absorption of flavonoids takes place in the ileum which has a pH range of 5 – 6.8. Adequate absorption of flavonoids would imply its benefit expressed in various aspect of the birds' physiology and products obtained from birds that have consumed such flavonoids. Recent studies on flavonoids focused on the advantages of flavonoids to inhibit lipid oxidation, microbial growth, control any pH-dependent deterioration, improve the colour, stability of meat and related products [39]. These studies are further pointers to the prospective of *A. indica* and *V. amygdalina*

as sources of flavonoids with possibility for use as suitable feed additives. Possible use of both plant extract as phyto-genic growth promoters can only be estimated with appropriate *in vivo* feeding trials using poultry birds (broilers and layers). Aside from the possibility to improve gastrointestinal health, the two plant extracts also have potential to improve quality of products of poultry origin. However, in contrast to many studies with model animals and humans, potential anti-oxidative and anti-inflammatory effects of polyphenols have been less investigated in livestock to date [40].

The occurrence and consequences of oxidative stress and inflammation on livestock health and performance can be alleviated with utilization of flavonoids of plant origin. This further emphasizes the need to study the bioavailability and metabolism of polyphenols from *A. indica* and *V. amygdalina* in poultry feeding. Kamboh et al. [39] opined that flavonoid supplementation in chicken diet positively alters fatty acid profile of meat and eggs by reducing the cholesterol and triglyceride content and meat colour, in terms of lightness, which can be improved by up to 5%. Utilization of various plant flavonoids as substitute for synthetic feed additives in commercial poultry production to satisfy consumer demands in terms of quality and safety of animal products is worthy of consideration.

According to Kamboh et al. [39] results from various studies has motivated interest in more research on various flavonoid classes to ascertain the most efficient compounds and their most favorable doses for both broilers and laying hens. These classes of compounds (alkaloids, saponins, tannins, anthraquinones, and flavonoids) from earlier reports have curative activity against numerous pathogens and consequently their use traditionally for the treatment of various illnesses (Hassan et al., 2004; Usman and Osuji, 2007). The mode of action of phyto-genics in poultry feeding is yet to be fully understood or well elucidated. But alteration of gastrointestinal health through their antibacterial action and immune-stimulation are potential means of enhancing gut health. Any positive effect they have on gut health will ultimately lead to improved growth performance which is desirable to poultry farmers besides consumers of poultry products. According to Attia et al. [41], utilization of a blend of plant extract containing oregano, fenugreek, chamomile and fenne exhibited significant capacity to improve total tract apparent nutrient digestibility and

caecal microflora counts. Aqueous extract of both plants in this study were earlier reported to significantly modulate gut bacteria counts without any negative effect on villus height in broiler chickens [11].

5. CONCLUSION

The results of this study indicated the ethnobotanical potential of *A. indica* and *V. amygdalina* as an innovative alternative for enhancing gastrointestinal health. The presence and concentration of antimicrobial, anti-inflammatory, anti-viral and immune-stimulatory phytochemicals in both plants makes them suitable alternatives for further *in-vivo* trials to ascertain potential outcomes if used commercially by farmers as a phyto-genic growth promoter.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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