Proximate analysis of nutrients and *in vitro* radical scavenging efficacy in selected medicinal plant powders with potential for use as poultry feed additives

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Abstract

The use of antibiotic feed additives in poultry feed is currently discouraged owing to the development of antibiotic-resistant bacteria and other drawbacks. The benefits and importance of phytogenic feed additives as potential alternatives in poultry nutrition include stimulation of digestion, higher feed conversion rate, reduced incidence of disease and profitability. Evaluation of the mineral content and other properties of plant material generates useful information towards rationalizing the use of plants as alternatives to antibiotic feed additives. Concentrations of macro-and micro-minerals in *Morinda lucida*, *Acalypha wilkesiana*, *Ficus exasperata* and standard broiler feed were determined using ICP-MS, and ICP-OES. Levels of degradation of C and N over specific periods of time were evaluated by dry oxidation (Dumas). Radical scavenging activities were investigated using DPPH and ABTS assays while levels of phytochemical constituents were assessed using standard methods. Macro- and micronutrient concentrations in the plant powders were generally higher than those of standard broiler feed. With few exceptions it was observed that the levels of total C and N as well as the C/N ratio in plant powders and standard feed were proportional to storage time. Macronutrients were higher in *F. exasperata* and *A. wilkesiana* while micronutrients were higher in *A. wilkesiana* and *M. lucida*. The highest total N and C levels were observed in *M. lucida* while *A. wilkesiana* showed the highest C/N ratio. Remarkable radical scavenging activities were displayed by *A. wilkesiana* while the highest total phenolic content (TPC) and total flavonoid content (TFC) were exhibited by aqueous and acetone extracts of *F. exasperata* respectively. High antioxidant activity was correlated with high total C and C/N ratio of the plant powders. In *M. lucida* leaf powder, fructose was the dominant sugar at 8 450 mg/kg DW followed by glucose and sucrose with a good amount of Se at 0.32 mg/kg DW. The contents of malic acid were the highest (20 500

mg/kg DW). Organic acids decrease intestinal *Escherichia coli* and *Salmonella* species in broilers. Cl^- was the most predominant anion. Minerals and organic acids are essential antimicrobial and antioxidant constituents of poultry diets for the maintenance of gut flora health, and their body metabolic, enzymatic and antioxidant defence. This study highlights the nutrients content of the selected plants, providing motivation for further investigation of these species as poultry feed additives.

Keywords: Poultry, medicinal plants, macronutrients, micronutrients, radical scavenging, feed additives.

Abbreviations: ICP – MS, inductively coupled plasma mass spectrometry; ICP – OES, inductively coupled plasma optical emission spectrometer; TPC, total phenolic content; TFC, total flavonoid content; DW, Dry weight; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid; AMR, antimicrobial resistance; AGPs, antibiotic growth promoter; LC- PUFAs, long chain polyunsaturated fatty acids; ARC-ISCW, Agricultural Research Council – Institute for Soil, Climate and Water; SD, Standard Deviation; NA, Not Applicable.

1. Introduction

The poultry industry has been growing persistently over recent years, providing a globally important source of animal protein (Byarugaba, 2007). Poultry are amongst the most intensively reared of all livestock species, with development occurring especially in the areas of nutrition, disease control, genetic improvement, management and organization of dietary requirements along with large scale poultry production and vertical integration (Mirzaei-Aghsaghali, 2012). The ban on the use of antibiotic growth promoters (AGPs) by the European Union (EU) in 2006 due to antimicrobial resistance (AMR) has increased pressure for the introduction of novel alternative additives to optimise bird performance and health (Whitehead, 2002).

The presence of beneficial macro- and micronutrients in various plant species is significant, and when consumed, these substances play major roles in physiological regulation, hormone secretion pathways, immune defence and the body's antioxidant system (Choct et al., 2004). Some of these elements play both curative and preventive roles in combating diseases, thus prompting researchers to explore the preventive medicinal aspects of various plant species (Faizuland and Rahat, 2011). An assessment of beneficial elements in medicinal plants could therefore be helpful in promoting their uses in different fields. For instance, calcium (Ca^{2+}) and phosphorous (P) are the most abundant mineral elements in the body of chickens, and are classified as macrominerals, along with sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}), sulphur (S) and magnesium (Mg^{2+}). These minerals are required in poultry diets at concentrations of more than 100 mg/kg (Ravindran, 2013). Ca^{2+} and P are responsible for formation and maintenance of skeletal structure as well as egg shell quality. The elements Na^{+}, K^{+} and Cl^{-} largely determine the acid-base balance for maintenance of physiological pH (Muir, 2017).

The organically complexed microminerals like copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) are needed for optimal growth and performance of broiler chickens, though in small quantities. The organic trace minerals appear to provide a pathway to minimise the trace mineral levels in the excreta and meet the broiler chicken requirements for optimal growth and health (Leeson, 2003). There is evidence to suggest that organic trace minerals are a lot less toxic than inorganic minerals (Scott et al., 1982). In addition, broiler chickens treated with organic Zn had a higher feed intake compare with birds treated with inorganic salts, suggesting that organic Zn was more easily retained in the body of chickens (Bao and Choct, 2009). Zn is one of the key microminerals, and a deficiency strongly depresses feed intake, and hence growth of broiler chickens. The usage of Zn prevents the symptoms of deficiencies of other microminerals such as Cu, Fe and Mn on the basis of growth response (Bao and Choct, 2009).

Soluble sugars can contribute to taste of feed, potentially affecting aroma or flavour. Additionally, fructose, glucose and sucrose are low-molecular weight carbohydrates and highly digestible, providing readily available energy. Supplying these nutrients at higher levels to meet energy requirements of the birds for improved bird performance is important, especially in starter diets for young broilers. In addition, the approach to include more highly digestible ingredients so as to accommodate deficiencies in digestibility or immaturity of digestive functions has been reported (Brody, 2008). Where the capacity of the digestive system is still not fully developed, there is a general consensus that highly digestible diets should be provided to the birds (Noy and Uni, 2010).

Meat is known to contain a high content of saturated fatty acids which can damage consumer health as well as predisposing them to cardiovascular diseases. Hence, efforts have been made to

incorporate into poultry feed phytogenics or phytobiotics that are rich in long chain polyunsaturated fatty acids (LC-PUFAs) to enhance the levels of LC-PUFAs in the poultry meat and eggs (Sultan et al., 2015). Antioxidants from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids are effective lipid oxidation inhibitors (Laguerre et al., 2007).

Furthermore, loss of flavours and nutrients in fat-containing foods has been attributed to lipid oxidation, which is one of the main factors limiting product quality and acceptability. Herbs and spices can inhibit oxidative rancidity and delay the development of off-flavour in meat products (Sherman and Flaxman, 2001). The use of alpha-tocopherol and other preservatives on poultry products after slaughtering may be reduced with incorporation of LC-PUFAs as sources in feeds (Christaki et al., 2012). In one study the incorporation of LC-PUFA sources in feeds reduced the levels of saturated fatty acids (SFAs) in thigh and breast meat of broilers (Cortinas et al., 2004).

Phytogenic additives have been reported to improve the growth performance of poultry, which has been attributed to interaction of these plant-based additives with intestinal microflora (Lin, 2011). *Morinda lucida* Benth. (Rubiaceae) is a tropical West Africa rainforest tree which is also known as brimstone tree. The stem, bark, root and leaf infusions of *Morinda lucida* are known to be locally used as an antimicrobial, antimalarial, antidiabetic and in the treatment of jaundice (Cowan, 1999). The plant is reputed to be one of the most popular traditional medicines against fever in sub-Saharan Africa (Bello et al., 2009). *Acalypha wilkesiana* Muell.Arg. (copper leaf) is a plant from the family Euphorbiacae. The genus *Acalypha* comprises about 570 species (Riley, 1963), a large proportion of which are weeds while the others are ornamental plants. The plants are found all

over the world, especially in the tropics of Africa, America and Asia. *A. wilkesiana* has antibacterial and antifungal properties (Adeshina et al., 2000, Oladunmoye, 2006). Anokwuru et al. (2012) reported that *A. wilkesiana* leaves had very high antioxidant and anti-denaturing activities for the prevention of the plant protein denaturation. *Ficus exasperata* Vahl (Moraceae) is widespread in tropical Africa and Arabia, and usually inhabits dryer types of forests. *Ficus exasperata* has been ethnobotanically reported to have varied therapeutic uses such as management of infections, hypertension, epilepsy, arthritis, haemorrhoids, cough, intestinal pain and ulcer (Julius et al., 2020; Ahmed et al., 2012).

In commercial practice, macro- and microminerals in plants have been added to poultry diets in high amounts with a large safety margin for the management of both disease and nutrition related conditions (Aksu et al., 2012). The chemical analysis of elemental composition in medicinal plant species and standard broiler feeds are not widely reported. Hence, the present study was undertaken to determine the content of macro- and microminerals, radical scavenging activity, phytochemical constituents of three medicinal plant species. The aim was to select a plant with high levels of macro- and micronutrients for further analysis and possible development as a phytogenic feed additive.

2. Materials and methods

2.1. Preparation of plant and feed powders

Plant collection

Fresh leaves of *Morinda lucida*, *Acalypha wilkesiana* and *Ficus exasperata* were collected from Ibadan Metropolis at Lagelu Local Government Area of Oyo State, Nigeria in June, 2017. The

plants were identified by Mr Esimekhinai, Donatus Ozimede. Voucher specimens were prepared and deposited after identification in the Herbarium of the Department of Botany, University of Ibadan. Nigeria with specimen numbers as follows: *M. lucida* (UIH-2629), *A. wilkesiana* (UIH-22793) and *F. exasperata* (UIH-22626). The thoroughly cleaned and dried plants were ground into powder and kept for subsequent use. Standard broiler starter and grower feed mash (powder) was purchased from Rossgro Feeds, Pretoria North, South Africa and Nova Feeds, Pretoria, South Africa respectively. One hundred grams of each plant and feed powder were submitted for analysis at the Agricultural Research Council-Institute for Soil, Climate and Water (ARC-ISCW), South Africa, while 100 g of *M. lucida* was analysed at the Institute of Plant Nutrition and Soil Sciences, University of Kiel, Germany.

2.2. Determination of elemental composition: macro- and micronutrients

The concentrations of the macronutrients (magnesium, Mg^{2+}; phosphorus, P; potassium, K^{+} and calcium, Ca^{2+}) and micronutrients (iron, Fe; manganese, Mn; Copper, Cu; zinc, Zn and selenium (Se) was achieved using an ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) (Agilent Technologies, US. Version: 725 and 700 Series) at the ARC-ISCW. The ICP-OES is a multi-element and simultaneous instrument where all the elements (and all wavelengths) may be determined simultaneously, except for S. In the testing procedure, 0.5 g of sample was digested with 7 ml HNO_3 (conc. nitric acid) and 3 ml $HClO_4$ (perchloric acid) at temperatures up to 180^0C and made up to 100 ml (Zasoski and Burau, 1977). An aliquot of the digest solution was used for the analysis while several of the elements were determined at more than one wavelength, allowing confirmation of the values, with no increase in analysis time or consumption of digest solution. S was determined separately from other elements by purging the

optics of the instrument with argon (Ar) gas at a very low wavelength (below 190 nm) in order to clear the system of oxygen. Each element was measured at one or two appropriate emission wavelengths, chosen for high sensitivity and lack of spectral interferences. The instrument was set up and operated according to the recommended procedures in the instrument manual and conditions were optimised. Since all the elements were determined simultaneously the determination and optimisation was done for the group of elements. The instrument was calibrated against a series of standard solutions, containing all the elements of interest in the proportions found in typical leaf samples. (Unpublished method developed and optimised at ARC-ISCW, based on the recommended procedures in the instrument manual, Agilent Technologies, 2010). All the analyses were conducted in duplicate.

The dried and finely ground sample of *M. lucida* was used to determine the mineral profile, which was carried out by inductively coupled plasma mass spectrometry (ICP-MS Agilent Technologies 7700 Series, Böblingen, Germany) at the Institute of Plant Nutrition and Soil Sciences, Kiel University, Germany. This further analysis was explored to detect those elements with low concentrations because ICP-MS has been reported to have better detection limits (1-10 part per trillion (ppt) range) compared to ICP-OES, with detection limits of 1-10 part per billion (ppb) range (Tyler and Jobin Yvon, 1995). For elemental determination, dried plant powder (200 mg) was digested with 10 ml of 69% HNO_3 (ROTIPURAN® Supra for ICP, 69%) using a closed-vessel microwave digestion system (MARS 6 Xpress, CEM Corporation) under the following conditions: 2 min at 100°C, 1 min at 120°C, 20 min at 180°C and 20 min cooling time. Consequently, the digested plant samples were diluted with Milli-Q water (18.2 MΩ cm conductivity) to 100 ml and

stored at 4℃ until further analysis. Concentration of selenium was investigated by ICP-MS as described by Abdalla et al. (2020).

2.3. Determination of carbon (C) and nitrogen (N) levels

The analysis of the levels of C and N in plant powders and standard feed was done with the dry oxidation (Dumas) method using a CHNS-O Analyzer (Thermo Scientific, US. Version: Flash 2000 Organic Elemental Analyzer) at the ARC-ISCW. The samples in powdered form were used directly. Approximately 8 to 12 mg of the samples were weighed into tin foil containers for each determination (Matejovic, 1995). The sample and foil container were ignited at high temperature (950°C) in oxygen (on a chrome oxide catalyst) to produce carbon dioxide, nitrogen gas and oxides of nitrogen (plus other oxides). The gases produced pass through silvered cobalt oxide (to remove oxides of S and halogens) and a column of copper (650°C) to reduce the oxides of nitrogen to nitrogen gas and excess free O_2. After removal of water vapour by a trap of anhydrous magnesium perchlorate, the N_2 gas and CO_2 were finally separated from any traces of other gases by gas chromatography using a helium carrier gas and detected by a thermal conductivity detector. The instrument was calibrated against a pure organic compound (Phenylalanine, 8.48% N and 65.4% C) of known composition. After the initial analysis of plant and feed powder, further analysis was conducted every two weeks for 12 weeks and tested in duplicate to monitor the levels of C and N over specific period of time.

2.5. Anions, organic acids and soluble sugar analysis of Morinda lucida

Concentrations of anions including chloride, nitrate, sulfate and phosphate in addition to organic acids and soluble sugars were determined by ion-chromatography (ICS 2500, Dionex, Sunnyvale,

CA, USA) at the Institute of Plant Nutrition and Soil Sciences, Kiel University, Germany. Anions were extracted using de-ionized water in a boiling water bath for 5 min. Additionally, samples were incubated on ice for half an hour, and subsequently centrifuged. Moreover, proteins were excluded from the supernatant using chloroform extraction. The supernatant was filtered through C18-columns (Strata spe. 8BS001-DAK) prior to ion-chromatography.

2.6. In vitro radical scavenging assays

2.6.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The stable DPPH has an absorption maximum at 517 nm, which decreases upon reduction through reaction with a radical scavenger (Olech et al., 2012). For quantification purposes the extracts were screened in microtitre plate format. An aliquot of 40 µl of methanol was added to each well, and 40 µl of the extracts (1 mg/ml) were then added and serially diluted using a multichannel pipette. Trolox and ascorbic acid at 1 mg/ml served as controls for the assay. An amount of 160 µl of DPPH was added to each well and the plates were covered with foil and incubated for 30 min. To blank each sample, methanol was added instead of DPPH. The absorbance values of the plates were read with a spectrophotometer (BioTek Synergy) at 517 nm.

2.6.2. ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

The extracts were quantitatively evaluated by aliquoting 40 µl of methanol in each well, adding 40 µl of the extracts (1 mg/ml) and serially diluting using a multichannel pipette. Trolox and ascorbic acid at 1 mg/ml served as controls for the assay. The ABTS was prepared overnight by adding 76.81 mg of ABTS in 20 ml of methanol and 26.49 mg of potassium persulphate in 20 ml of methanol separately and the combined mixture of 40 ml of methanol was allowed to stand in

the dark for 16 h prior to the experiment. An amount of 160 µl of ABTS was added to each well and the plates were covered with foil and incubated for 5 min. To blank each sample methanol as added instead of ABTS. The absorbance of the mixtures in the plates was read with a microplate reader (BioTek Synergy) at 734 nm.

2.7. Phytochemical evaluation

2.7.1. Total phenolic content

The total phenolic content of the extracts was determined using the 96-well plate method adapted from Zhang et al. (2006). The extracts (20 µl) were aliquoted into the wells of a 96-well microtitre plate to which 100 µl of 20% Folin-Ciocalteu reagent and 80 µl of 7.5% Na_2CO_3 solution were added. The final mixture was shaken and incubated for 60 min in the dark at room temperature, and the absorbance was measured at 760 nm. The total phenolic content was calculated from the linear regression curve of gallic acid, and the results are expressed as mg gallic acid equivalent (GAE) per g of crude extract.

2.7.2. Total flavonoid content

The total flavonoid content of the extracts was determined using the 96-well plate method adapted from Yadav and Agarwala (2011). The extracts (100 µl) were dispensed into the wells of a 96-well plate, and 100 µl of 2% aluminium chloride were added. The final mixture was shaken, incubated for 15 min, and the absorbance was read at 430 nm in a microplate reader. A yellow colour indicated the presence of flavonoids. The total flavonoid content was calculated from the linear regression curve of quercetin, and results are expressed as mg quercetin equivalent (QE) per g of crude extract.

2.8. Statistical analysis

Data obtained from the various experiments were expressed as mean ± standard deviation (SD) and were subjected to descriptive statistics using Student's t-test to compare two sample means where applicable while $P<0.05$ was considered statistically significant. One-way ANOVA was used for the test of significance between groups that were more than two, and Tukey's post-hoc test was used to compare means of all samples using GraphPad Prism, version 5.01, April, 2016 statistical software Chicago, IL, USA.

3. Results

3.1: Elemental composition of the powder of the selected plants and standard feed

The present study (Table 1) shows variations in the contents of macro- and micronutrients of the three selected medicinal plants with antimicrobial and antioxidant potential in the treatment of chicken pathogens. *A. wilkesiana* had the highest level of K^+ and P, while *M. lucida* had the highest level of Mg^{2+}. The highest level of Ca^{2+} and Na^+ was found in *F. exasperata*. Moreover, the highest values of Fe, Al and Zn were observed in *A. wilkesiana*. *M. lucida* had the highest values of S, Mn and Cu of the three plants. The highest level of B was found in *F. exasperata*. The levels of both macro- and micronutrients in the plant powders were relatively higher than those in the standard feed, except for P, Na^+, S, Mn and Cu.

3.2. Determination of N, C and C/N ratios of the three plants

Table 2 shows the values of total N, C and C/N ratios in the plant powders. The highest values for total nitrogen and carbon were recorded in *M. lucida* powder followed by *F. exasperata* and then *A. wilkesiana,* while the inverse trend was observed with the C/N ratio. The C/N ratio was higher

in *A. wilkesiana* than in *M. lucida* and *F. exasperata*. The initial value of total carbon in *M. lucida* was significantly lower than the values at 4, 6, 8, 10 and 12 weeks post-analysis while the same trend was observed with *A. wilkesiana* and *F. exasperata*. The initial value of total nitrogen in *M. lucida*, *A. wilkesiana* and *F. exasperata* was also significantly lower than the values obtained at 2, 4, 6, 8, 10 and 12 weeks post-analysis. The values for the C/N ratio in *M. lucida* and *F. exasperata* were significantly lower than the values two weeks post-analysis, while the C/N ratio in *A. wilkesiana* was significantly lower than the values obtained at two and four weeks post-analysis. The plant powder was stored inside the sample containers in between the analysis.

3. 3. N and C concentrations and C/N ratios in the standard poultry feed

Table 3 shows the levels of total N, C and C/N ratios in the standard feeds. The total C in grower feed was relatively higher than in starter feed while the opposite was true for total nitrogen. The C/N ratio in grower was higher than that of starter feed. There was no significant difference ($P>0.05$) in the values of total carbon in starter feed throughout the study period (12 weeks). However, the initial value of total C in grower feed was significantly lower ($P<0.05$) than the values obtained at 8, 10 and 12 weeks post-analysis. The initial value of total N in starter feed was significantly higher ($P<0.05$) than those values obtained at 2, 4 and 6 weeks post-analysis, and later this became significantly lower ($P<0.05$) than the values obtained at 8, 10 and 12 weeks post-analysis.

The initial value of total nitrogen in grower feed was significantly higher ($P<0.05$) than that obtained at 2, 4, 10 and 12 weeks. The initial value of C/N ratio in starter feed was significantly lower ($P<0.05$) than the values obtained at 2, 4 and 6 weeks while the initial value of C/N ratio in

grower feed was significantly lower ($P<0.05$) than the values obtained at 2, 4 and 10 weeks post-analysis. Generally, in this study with few exceptions it was observed that the levels of total C and N as well as the C/N ratio were proportional to feed storage time.

3.4. Inorganic anions, organic acids and sugar contents of Morinda lucida

Table 4 shows the values of selenium, anions, organic acids and sugar contents of *M. lucida*. From this analysis, K^+ was found to be the highest while Se was the lowest. Fructose was the most abundant sugar (8 450 mg/kg DW) followed by glucose (5 790 mg/kg DW) and sucrose (2 680 mg/kg DW). With regard to organic acids, the contents of malic, citric and oxalic acids of the leaves were 20 500 mg/kg DW, 8 210 mg/kg DW and 1 860 mg/kg DW respectively. The contents of Cl^-, PO_4^{3-}, NO_3^- and SO_4^{2-} were 2 150, 1 110, 490 and 430 mg/kg respectively.

3.5. Phytochemical constituents and antioxidant properties of acetone and aqueous extracts of three plant species

The phytochemical constituents and antioxidant potential of the three selected plants are presented in Table 5. In this study, the best radical scavenging activities were observed with the acetone (13.90 ± 0.00**)** and aqueous (17.32 ± 0.00**)** extracts of *A. wilkesiana* followed by *M. lucida*. The aqueous and acetone extracts of *F. exasperata* displayed the highest total phenolic and flavonoid contents.

4. Discussion

Mineral elements are important constituents of enzymes and play a significant role in human and animal metabolism and are very important with regard to various life processes (Paul et al., 2013).

The mineral analyses for the powders of *M. lucida*, *A. wilkesiana* and *F. exasperata* showed the presence of macro- and microminerals in varying quantities. The levels found were generally greater than those found in the standard broiler feeds with few exceptions.

Generally, K^+ and Ca^{2+} were the most abundant minerals in the three plants, enhancing their value as suitable mineral supplements in the form of additives in broiler diets to maintain physiological pH and bone strength. K^+, P, Fe, Al and Zn levels were highest in *A. wilkesiana*; Mg^{2+}, S, Mn and Cu levels were highest in *M. lucida* while Ca, Na and B levels were highest in *F. exasperata*. However, the findings of Enabulele et al. (2017) showed that K^+ level was higher while Mn and Cu levels were lower in aqueous and ethanolic extracts of *Morinda lucida* compared to *Nauclea latifolia*. The elemental analysis of the aqueous extract of *A. wilkesiana* showed the presence of Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn, Fe, Cu and Mn in moderate quantities (Madziga et al., 2010). The levels of K^+, Ca^{2+}, Mn, Fe and Cu in order of decreasing magnitude have been reported in *F. exasperata* powder (Bello et al., 2014) which supports the results of this study.. Ca^{2+} helps in bone formation and also plays a major role in the release of neurotransmitters (Neher and Sakaba, 2008). Ca^{2+} is essential for conduction of nerve impulses and for neurotransmitter generation (Watts, 1997) as well as in the muscle system and for heart function (Brody, 1994). Moreover, K^+ is the principal cation in intracellular fluid and is essential in acid-base balance, regulation of osmotic pressure, conduction of nerve impulses and muscle contractions, particularly cardiac muscle. In addition, it helps in the functioning of cell membranes and Na^+/K^+-ATPase. Potassium is also required during glycogenesis and the transfer of phosphate from ATP to pyruvic acid, and it probably has a role in many basic cellular enzymatic reactions (Njuguna et al., 2013).

The presence of different microminerals plays a major role in the prevention and management of some infections and nutritional disorders. Cu, Fe, Mn and Zn are required for the growth, development and maintenance of healthy bones (Beattie and Avenell, 1992). The recommended range of minimum and maximum levels (mg/kg) of Cu, Fe, Mn and Zn in broiler diets are 4-250, 35-1000, 45-600 and 40-800 mg/kg respectively (Bao and Choct, 2009). In addition, minerals such as Fe, Mn and Zn are essential because they are important in several enzyme reactions as co-factors (Robert et al., 2000). Vieira et al. (2020) reported that broiler diets containing organic trace minerals were beneficial for broiler performance and reduced the excretion of minerals in the litter.

Zn is a co-factor of several enzymes for bone mineralisation. In broiler chickens, Zn deficiency can cause shortening and thickening of tibia bones (Scott et al., 1982). It has been reported that the bioavailability of minerals is important for optimal growth in broiler chickens (Bao and Choct, 2009). The rate of intestinal absorption of organic macro- and microminerals is higher than that of inorganic minerals in broiler diets (Bao and Choct, 2009). The higher the bioavailability, the higher the bone mineralisation in broilers (Gulz et al., 2019). In the present study, the C/N ratio of the three plant powders was more than 10 with the carbon to nitrogen (C/N) values ranging from 12-18. The highest value of C/N ratio was observed in *A. wilkesiana* powder, which is a good indicator for antioxidant activity, and this can be a possible reason for the excellent radical scavenging potential of the plant.

A positive correlation has been reported between secondary metabolite content, antioxidant activities and C/N ration of medicinal plants (Ibrahim and Jaafar, 2011). It has also been reported that the increase in C/N ratio in plants might be due to low N absorption or fertilization of the

plants (Lindroth et al., 2002). The three plant powders had good amounts of C and N, with the highest observed in *M. lucida*. It is expected that the high levels of C, which is a precursor of carbohydrate, in these plant powders will enhance their potential to provide energy to the broiler chickens. Likewise, since N contributes to protein metabolism and enzymatic activities in the body, the plant powders have the ability to increase the protein content of the broiler feeds. Therefore, in view of the positive correlation between secondary metabolite content, antioxidant activities and C/N ratio (Ibrahim et al., 2011), it is expected that the consumption of these plant powders as additives will promote several antioxidant activities. Generally in this study, with few exceptions such as where degradation of N in starter (2, 4 and 6 weeks post analysis) and grower feed (2, 4, 10 and 12 weeks post analysis) was observed over time, the levels of total C and N as well as the C/N ratio in the standard feeds increased significantly throughout the study period.

On the basis of high levels of macro- and microminerals as well as good C/N ratio, *M. lucida* was subjected to further nutritive analysis which encompassed the evaluation of inorganic anions, organic acids and sugar contents. The anions investigated Cl^-, NO_3^-, SO_4^{2-} and PO_4^{3-} and Cl^- was the most abundant. The major organic acids available in *M. lucida* were malic and citric acids while the concentration of malic acid (20 500 mg/kg) was higher compared to that of citric acid (8 210 mg/kg). Organic acids have been used in commercial feeds for antimicrobial activity and feed preservation (Wang et al., 2009). Furthermore, they are known to increase gastric proteolysis, amino acid digestibility as well as forming complexes with Ca, P, Mg and Zn to enhance mineral digestibility (Li et al., 1998).

Generally, distribution of oxalate content in plant parts varies as it has been reported to be highest in the leaves, followed by the seeds and is lowest in the stems (Osweiler, 1985). Oxalic acid is classified as a major anti-nutrient, binding minerals to form insoluble salts, thereby decreasing their bioavailability or absorption (Muhammad et al., 2011). However, in this study, the levels of the anti-nutrient oxalic acid found in *M. lucida* leaves appeared lower (0.1%) than the minimum toxic level of 3 g per 100 g (Munro and Bassir, 1969). This indicates that the level of oxalate in the *M. lucida* plant leaf sample is within acceptable limits though more work is needed to ascertain this assertion. The presence of essential nutrients and minerals in *M. lucida* leaves implies that they could be utilized to improve growth performance and health status of poultry if used as a feed additive. Other substances such as polyphenols can also be considered as anti-nutrients that can limit food mineral bioavailability (Kaushik et al., 2018).

The soluble sugars found in *M. lucida* were glucose (5 790 mg/kg), fructose (8 450 mg/kg) and sucrose (2 680 mg/kg). The detected sugars of *M. lucida* may be useful as a source of energy. Additionally, carbohydrates and their derivatives play major roles in the working process of the immune system, fertilization, pathogenesis, blood clotting and development (Anupama and Sunilkumar, 2019).

M. lucida contained a reasonable amount of Se (0.32 mg/kg) which enables this plant to be a possible source of selenium species. These function to maintain effective antioxidant defences and prevent detrimental consequences of stress in chickens (Surai et al., 2017). Se is an important component of glutathione peroxidase and in the presence of Vit E was found to prevent exudative diathesis in broilers (Noguchi et al., 1973). Moreover, it is an essential nutrient for the body's

antioxidant system (Choct et al., 2004). A deficiency of Sedepresses growth of broilers by inhibiting hepatic 5^0-deiodinase activity (He et al., 2000). Furthermore, feeding of Se to the poultry may add nutritional values to the eggs and meats because low Se content of human diets has been correlated with higher incidences of cancer (Allan et al., 1999). In view of these observations, Se has been considered to possess immunomodulatory potential, which in turn will enhance the immune response to vaccinations in chickens. The recommended Se requirements for broiler diets is 0.15 mg/kg (Utterback et al., 2005).

There has been an increase in demand for natural antioxidants in food due to their health benefits against oxidative stress and several diseases. Demand is high for plant derived antioxidants in poultry nutrition because their meat has a high content of polyunsaturated fatty acids which are susceptible to lipid oxidation (Christaki, 2012). Many plants have been identified as excellent antioxidants to be incorporated into the poultry diet. Rosemary extracts are some of the most studied natural antioxidants in poultry products and these studies have demonstrated their ability to act as natural antioxidants in various poultry products (Karrel et al., 2013). Much has been reported on the antioxidant activity of flavonoids and other plant phenolic compounds owing to their potential in health promotion and disease prevention (Dillard and German, 2000). The significant antioxidant activity shown by both acetone and aqueous extracts of *A. wilkesiana* may be linked to the higher levels of total phenolic (TPC) and flavonoid (TFC) aqueous extract of *A. wilkesiana*.

The suggested range for moderate antioxidant capacity is 50 µg/ml < IC_{50} < 100 µg/ml (Efferth and Kuete, 2010) which means the other two plant extracts also displayed moderate antioxidant

activities. Mansouri et al. (2005) reported that most of the antioxidant activity of plants is derived from phenols and this is similar to our findings in this study, where the plant extracts with higher TPC displayed the best antioxidant activities. Free radicals may injure cells and tissues directly via oxidative degradation of essential cellular components. The extent of tissue damage is attributed to the result of the balance between the free radicals generated and antioxidant protective defences (Machlin and Bendich, 1987). It is believed that antioxidant activities of medicinal plants must be evaluated by more than one method (at least two methods) in order to take into account different modes of action of a given antioxidant (Parfenov and Zaikov, 2000). Ogbuehi et al. (2014) reported that *A. wilkesiana* enhanced the antioxidant capacity of rats and decreased reactive oxygen species mediated oxidation of lipids. These authors further demonstrated the presence of tannins, flavonoids, saponins, steroids, alkaloids, anthraquinones, cardiac glycosides and carotenoids in the leaves of *A. wilkesiana* which supports the antioxidant effects of the plant methanolic extract. The aqueous extract of *A. wilkesiana* java white, which is a cultivar of *A. wilkesiana* Mull. Arg., showed impressive *in vitro* ABTS and DPPH radical scavenging activities with better activity than the positive control (ascorbic acid) and *A. wilkesiana* Mull. Arg. (Akinloye et al., 2016)

The differences in the composition of minerals from previously published studies, apart from species differences, may be due to the differences in the locality of their growth and the stage at maturity prior to harvesting as well as reflection of elemental composition of their parent soil. This needs further investigation in plants belonging to one species grown in different areas, and at different stages of growth.

5. Conclusions

The current study shows that *M. lucida, A. wilkesiana and F. exasperata* leaves contain appreciable amounts of macro- and microminerals, sugars, organic acids and anions, all of which are nutritional requirements of poultry. It is important to note that there is a demand for antioxidants in poultry meat owing to the presence of highly polyunsaturated fatty acids which are susceptible to lipid oxidation. In light of this, leaves from these plants could be useful as feed supplements in poultry to enhance quality of the meat as well as to improve health and growth performance. It is recommended that *in vivo* trials in chickens using *M. lucida* should be conducted, as this plant had high levels of macro-and micronutrients. Also, further research is needed to evaluate selenium, anions, organic acids and sugar contents of the other two plant powders to determine their potential as poultry feed additives.

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Conflicts of Interest

The authors declare no conflict of interest.

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Table 1. Elemental composition of the powder of the selected plants and standard feed

Elements/Minerals (mg/kg)	*M. lucida* (Mean ± SD)	*A. wilkesiana* (Mean ± SD)	*F. exasperata* (Mean ± SD)	Broiler Starter (Mean ± SD)	Broiler Grower (Mean ± SD)
K	19 821± 78.91	23 674.00 ± 48.00	17 879 ± 628.62	8 509 ± 7.07	7 151 ± 7.78
Ca	21 233 ± 195.87	23 316 ± 59.40	32 855 ± 287.09	7 087 ± 84.85	6 066 ± 299.80
Mg	4 539 ± 62.23	3 036 ± 11.31	3 452 ± 44.55	1 966 ± 0.70	1 384 ± 0.70
S	2 507 ± 35.36	2 196.00 ± 25.46	1 972 ± 11.31	2 463 ± 62.23	2 627 ± 21.92
P	1 968 ± 11.31	4 131 ± 121.16	2 034 ± 17.68	5 960 ± 84.85	5 496 ± 16.26
Na	129.60 ± 1.34	142.90 ± 7.28	154.10 ± 0.42	1 813 ± 0.71	1 408 ±2 8.28
Fe	1 638 ± 10.61	1 892.00 ± 22.63	1 263 ± 205.06	238.6 ± 0.35	197.40 ± 2.83
Al	985 ± 16.97	1 383 ± 19.80	593 ± 4.95	113.30 ± 1.48	93.50 ± 4.95
Mn	223.60 ± 1.41	65.30 ± 1.56	41.20 ± 0.85	91.20 ± 6.15	131.10 ± 9.19
Zn	125.50 ± 5.66	208.50 ± 17.96	141.60 ± 15.63	94.70 ± 0.21	110.30 ± 6.01
B	55.00 ± 2.12	43.20 ± 0.57	88.40 ± 1.91	17.10 ± 1.20	15.00 ± 0.21
Cu	11.60 ± 0.14	10.50 ± 0.49	8.10 ± 0.07	18.30 ± 0.14	18.70 ± 2.83

Table 2. Levels and degradation of total N, C and C/N ratios of the three plants

Period	Total Carbon (%)			Total Nitrogen (%)			C/N ratio		
	M. lucida (Mean ± SD)	*A. wilkesiana* (Mean ± SD)	*F. exasperata* (Mean ± SD)	*M. lucida* (Mean ± SD)	*A. wilkesiana* (Mean ± SD)	*F. exasperata* (Mean ± SD)	*M. lucida* (Mean ± SD)	*A. wilkesiana* (Mean ± SD)	*F. exasperata* (Mean ± SD)
Initial values	42.30 ± 0.00[a]	38.50 ± 0.14[a]	35.70 ± 0.07[a]	3.27 ± 0.00[a]	2.13 ± 0.01[a]	2.79 ± 0.01[a]	12.94 ±0.00[a]	18.07 ± 0.06[a]	12.82 ± 0.04[a]
After 2 weeks	42.60 ± 0.14[a]	38.20 ± 0.07[a]	36.30 ± 0.14[b]	3.62 ± 0.00[b]	2.41 ± 0.01[b]	3.12 ± 0.01[b]	11.77 ± 0.04[b]	15.80 ± 0.08[b]	11.65 ± 0.07[b]
After 4 weeks	44.40 ± 0.07[b]	40.20 ± 0.07[b]	37.00 ± 0.00[b]	3.43 ± 0.01[b]	2.17 ± 0.01[b]	2.84 ± 0.02[a]	12.96 ± 0.07[a]	18.55 ± 0.09[b]	13.05 ± 0.10[a]
After 6 weeks	44.80 ± 0.07[b]	40.20 ± 0.14[b]	37.20 ± 0.00[b]	3.44 ± 0.02[b]	2.19 ± 0.01[b]	2.87 ± 0.02[b]	13.06 ± 0.10[a]	18.40 ± 0.13[a]	12.94 ± 0.09[a]
After 8 weeks	44.70 ± 0.21[b]	40.20 ± 0.21[b]	37.20 ± 0.00[b]	3.46 ± 0.04[b]	2.20 ± 0.01[b]	2.85 ± 0.01[b]	12.92 ± 0.07[a]	18.34 ±0.16[a]	13.06 ± 0.06[a]
After 10 weeks	44.90 ± 0.21[b]	40.80 ± 0.00[b]	37.00 ± 0.00[b]	3.49 ± 0.00[b]	2.25 ± 0.00[b]	2.85 ± 0.02[b]	12.88 ± 0.06[a]	18.13 ± 0.00[a]	12.96 ± 0.10[a]
After 12 weeks	45.40 ± 0.64[b]	40.50 ± 0.00[b]	37.40 ± 0.00[b]	3.48 ± 0.01[b]	2.26 ± 0.01[b]	2.91 ± 0.00[b]	13.05 ± 0.21[a]	17.92 ± 0.11[a]	12.85 ± 0.00[a]

NB: All analyses are means ± standard deviations of means (SD). Similar superscripts denote insignificant differences at $P \leq 0.05$ among the means compared to initial values.

Table 3. The levels of total N, C and C/N ratios of the standard feeds

	Total C (%)		**Total N (%)**		**C/N ratio**	
Period	Broiler Starter (Mean ± SD)	Broiler Grower (Mean ± SD)	Broiler Starter (Mean ± SD)	Broiler Grower (Mean ± SD)	Broiler Starter (Mean ± SD)	Broiler Grower (Mean ± SD)
Initial values	40.70 ± 0.14^{a}	41.60 ± 0.14^{a}	3.33 ± 0.01^{a}	3.00 ± 0.05^{a}	12.23 ± 0.09^{a}	13.85 ± 0.18^{a}
After 2 weeks	41.10 ± 0.21^{a}	42.20 ± 0.35^{a}	3.18 ± 0.00^{b}	2.70 ± 0.03^{b}	12.91 ± 0.07^{b}	15.65 ± 0.30^{b}
After 4 weeks	41.30 ± 0.00^{a}	41.90 ± 0.21^{a}	3.24 ± 0.02^{b}	2.52 ± 0.01^{b}	12.73 ± 0.08^{b}	16.65 ± 0.18^{b}
After 6 weeks	41.11 ± 0.00^{a}	42.20 ± 0.07^{a}	3.22 ± 0.04^{b}	2.91 ± 0.08^{a}	12.77 ± 0.16^{b}	14.52 ± 0.36^{a}
After 8 weeks	41.30 ± 0.07^{a}	42.40 ± 0.07^{b}	3.46 ± 0.03^{b}	3.12 ± 0.04^{a}	11.95 ± 0.07^{a}	13.60 ± 0.18^{a}
After 10 weeks	41.50 ± 0.92^{a}	42.40 ± 0.07^{b}	3.44 ± 0.02^{b}	2.79 ± 0.05^{b}	12.07 ± 0.01^{a}	15.19 ± 0.24^{b}
After 12 weeks	41.80 ± 0.00^{a}	42.80 ± 0.14^{b}	3.46 ± 0.01^{b}	2.86 ± 0.04^{a}	12.07 ± 0.02^{a}	14.10 ± 0.13^{a}

NB: All analyses are means ± standard deviations of means (SD). Similar superscripts denote insignificant differences at $P \leq 0.05$ among the means compared to initial values.

Table 4. The mineral, anion, organic acids and sugar contents of *Morinda lucida* in mg/kg

In organic and organic components	mg/kg of dry weight (DW)
Mineral	
Selenium	0.32
Anions	
Cl^-	**2 150**
NO_3^-	490
SO_4^{2-}	430
PO_4^{3-}	1 110
Organic acids	
Malic acid	**20 500**
Oxalic acid	1 860
Citric acid	8 210
Sugars	
Glucose	5 790
Fructose	**8 450**
Sucrose	2 680

Bold: Highest values

Table 5.Phytochemical constituents and antioxidant activities of acetone and aqueous extracts of three plant species

Plant species	Extracts	TPC (g/mg gallic)	TFC (mg/QE/g)	DPPH IC_{50} (µg/ml)	ABTS IC_{50} (µg/ml)
M. lucida	Acetone	217.62 ± 0.30	169.26 ± 24.44	60.21 ± 3.20	44.56 ± 2.06
	Aqueous	322.19 ± 0.20	40.27 ± 9.54	240.96±49.27	38.59 ± 1.30
A. wilkesiana	Acetone	0.56 ± 0.11	0.40 ± 0.00	**13.90 ± 0.00**	**1.17 ± 0.00**
	Aqueous	228.89 ± 54.66	172.35 ± 2.62	**17.32 ± 0.00**	**1.39 ± 0.00**
F. exasperata	Acetone	212.81 ± 0.10	**466.48 ±52.81**	98.41 ± 18.36	52.36 ± 4.65
	Aqueous	**410.87 ± 0.60**	1.31 ±1.05	210.10 ± 0.00	0.01 ± 0.00
Trolox	-	NA	NA	0.97 ± 0.00	0.03 ± 0.00
Ascorbic acid	-	NA	NA	0.39 ± 0.08	0.02 ± 0.00

Bold: Highest values