Evaluation of *Chrysophyllum albidum* seed cotyledon as a feed additive in broiler diet and possible antimicrobial

Ezeala Freeman, Gabriel Adeyemi Francis, Moses Olabiyi ABIODUN

University of Abuja, Nigeria

Abstract: **The increasing cost and short supply ofpoultryfeeds have created the need to search for alternatives as feed additives to enhance feed quality and quantity. This research work was undertaken to evaluate the nutritive and non-nutritive potentials of *Chrysophyllum albidum* seed cotyledon (CASC) as a feed additive in broiler diet, possible antimicrobial activity and FTIR study of the functional groups. Results of nutritive mineral, vitamin and proximate composition revealed that CASC contained the highest content of copper (2.44 mg/kg), ascorbic acid (0.554 mg/kg) and carbohydrate (81.64mg/kg) and lowest contents of chromium (0.16 mg/kg), Niacin (0.0046mg/kg) and Nitrogen free extract (0.51%) respectively. Also, the non-nutritive contents of CASC revealed alkaloids (1.28mg/kg) as highest and the lowest is phenolics (0.48mg/kg) not excluding the cyanide content of 1.4798mg/kg. In addition, the result of the antimicrobial analysis shows that *E. coli* was highly susceptible to the aqueous extract (p<0.05) whereas *Bacillus* spp. and *Candida albicans*had the least susceptibility to the test extracts. The toxicity studies show no significant difference in the blood parameters of both the control and the experimental groups and no visible lesions were observed in the vital organs of both the control and experimental groups. The Fourier Transform Infrared (FTIR) study on the oilidentified some functional groups such –OH, -CH, C=O and C-O.**

Keywords: ***Chrysophyllum albidum*, feed additive, antimicrobial**

I. INTRODUCTION

The global increase in the demand for poultry products has seriously led to the surge in the cost of feeds [1].Plants have been used as animal feed and medicine since time immemorial and their parts such as roots, leaves, fruits and seeds often provide food and medicine for humans and feed for animals [2,3].

African star apple (*Chrysophyllum albidum*), belongs to the family *Sapoteacea*. It is a tropical fruit mainly distributed in the low land rain forest zones and frequently found in good quantities in rural areas. It is popularly called *Udara* in Igbo, *Agbalumo* in Yoruba, *Agwaluma* in Hausa and *Otien* in Edo [4]. It is common in both urban and rural centres in Nigeria especially during the months of December through April [4].

Chrysophyllumalbidum has been adjudged as one of the most auspicious plants with diverse ethnobotanical uses [5]. The leaves are used as palliatives for the treatment of dermatological problems, stomachache and diarrhea [6]. The cotyledons are used as unguents for the treatment of vaginal infections [7] and as hypoglycemic and hypolipidemic agent [8]. Even with the numerous health benefits attributed to the plant, *Chrysophyllum albidum*seed cotyledons remains traditional and underutilized but discarded or threaded as anklets in dancing or to play outdoor games [9] after which they are thrown away.

In recent times, the application of medicinal plants in animals transcend therapy and is on the increase due to the great existent concern at world level for the crossed possible resistance to the antibiotics for many microorganisms as a response to indiscriminate sub-therapeutic use in animals [10].

Studies have demonstrated that phytobiotics in the diets of farm animals improved gut integrity and nutrient absorption resulting in general bodily development, antioxidant activity, and immunity [11]; these natural products have been considered as an effective alternative to feed additives and antibiotics, mainly to diminish or decrease the residual effects of synthetic antibiotics in meat and eggs [11]. The present study seeks to evaluate the potentials of African star apple seed cotyledon as a potential feed fortifier and useful drug as well.

II. MATERIALS AND METHODS

Chemicals and reagents

The chemicals and reagents used were all from Sigma Aldrich. Media used were from hi-media laboratories limited, India. Pathogenic clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Candida albicans* were collected from Ritchez medical laboratory, Maitama, Abuja, Nigeria.

Collection and Preparation of Plant Materials

Ripe and fresh *Chrysophyllum albidum* fruits were collected locally within Gwagwalada Area Council of the F.C.T. The plant material was prepared according to the methods described by Anibijuwon and Udeze [12]. The *C. albidum*fruits were washed and the seeds removed from the fruits and shade-dried to constant weight. The dried seeds were deshelled and the cotyledon pounded into smaller granules using laboratory Mortar and pestle and the granules were pulverized using laboratory blender. The pulverized cotyledon sample was kept in an airtight container.

Preparation of the plant extract

The method described by Arekemase*et al*. [13] and Akin-Osanaiye*et al*. [14] were used. Pulverized plant samples of known quantity (500g) were extracted successively with different solvents of varying polarity ranging from hexane, ethyl acetate, ethanol and distilled water usingsoxhlet extraction method. The crude extracts were filtered and concentrated in a rotary evaporator at 40^{o}C. The extracts were dried to completion in a hot air-oven at 40^{o}C, kept in Mc-Carthny bottles and refrigerated at 2-4 ^{o}C for further use. The crude extracts were weighed and yielded 7.44g, 1.37g, 16.06g and 65.44g for hexane, ethylacetate, ethanol and water extracts respectively.

Isolation and purification of crude hexane extract

The procedures that were used for isolation and purification of the components were column chromatographyand thin-layer chromatography as described by Pavia *et al*. [15]. A total of 22 eluents of 20 mL each were obtained. Thin-layer chromatography was performed to ascertain the purity of the fractions. The fractions 1-2, 3-8, 9, 10-11, 12-15, 16, 17-22 were separately pulled together based on their similar R_f values. These groups were lettered a, b, c, d, e, f and g respectively.

Feed Formulation

The pulverized cotyledon samples were included in broilers diet mixtures. Five diets were formulated to comprise graded levels of the pulverized sample at inclusion rates of 0, 5, 10, 15 and 20% in replacing the conventional vital feed (starter) in the diets of the birds. The analytical nutritional composition of the vital starter feed contains; crude protein (18 %), fat (10 %), crude fibre (12 %), calcium (1.2 %), phosphorus (0.45 %) and metabolized energy (3000 kcal/kg).

Animal Care and Approval

Twenty (n = 20) one month old broilers weighing 0.5 ± 0.1 kg were purchased from Agrobetveterinary Nyanya, Abuja Nigeria. According to the procedure used by Annongu*et al*. [16] The broilers were housed in cages under a controlled light cycle (12 h light / 12 h dark) and randomly divided into five groups including the control group and were acclimatized for one week, placed on commercial starter feed and water administered during the acclimatization period.

Experimental diets and drinking water were given to the chicks for a period of one month and treated in accordance with recommendations of National Institute of Health [17] Guidelines for the care and use of laboratory animals. The physical appearance of both the control and experimental broilers were monitored while the bodyweight of each broiler recorded weekly.

Experimental Protocol

There were five groups each consisting of four broilers:

Group 1: Fed daily with a feed mixture of 0% of the pulverized sample of *Chrysophyllum albidum* seed cotyledon and 100% of starter feed for one month and served as normal control group. This group is cage one.

Group 2: Fed daily with a feed mixture of 5% of the pulverized sample of *Chrysophyllum albidum* and 95% of starter feed for one month. This group is cage two.

Group 3: Fed daily with a feed mixture of 10% of the pulverized sample of *Chrysophyllum albidum* cotyledon and 90% of starter feed for one month. This group is cage three.

Group 4: Fed daily with a feed mixture of 15% of the pulverized sample of *Chrysophyllum albidum* cotyledon and 85% of starter feed for one month. This group is cage four.

Group 5: Fed daily with a feed mixture of 20% of the pulverized sample of *Chrysophyllum albidum* cotyledon and 80% of starter feed for one month. This group is cage five.

Analyses of the Nutritive and non-Nutritive Components

Proximate Analysis

The proximate analysis was carried out in the Department of Chemistry SHEDA Science and Technology Complex Gwagwalada Abuja. Each sample (pulverized seed cotyledon of *Chrysophyllum albidum* fruits) was analyzed in triplicate. This was carried out according to the method of AOAC (1990) [18] to determine moisture content, ash content, crude protein, crude fibre, fat content, carbohydrate content and energy content.

Quantitative Analysis of Mineral / Heavy Metals

This was carried out according to the method of AOAC [20], the samples were ashed at 550 ^{O}C and the obtained ash was boiled with 10 ml of 20 % hydrochloric acid in a beaker and then filtered into a 100 ml volumetric flask. The filtrate was made up to the mark with deionized water. The minerals were determined from the resulting solution. Flame emission photometer was used for sodium (Na) and potassium determination while Atomic Absorption Spectrophotometer (AAS) was used for calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), chromium (Cr), Nickel (Ni), cadmium (Cd) and copper (Cu) at SHEDA Science and Technology Complex Gwagwalada Abuja. All values were expressed in mg/kg.

Quantitative Analysis of Phytochemical Constituents

The quantitative phytochemical analysis was carried out in the Department of chemistry SHEDA Science and Technology Complex Gwagwalada Abuja. Each sample (pulverized seed cotyledon of *Chrysophyllumalbidum*) was analyzed in triplicate. This was carried out according to the method of AOAC [18] to determine; alkaloid (Obadoni and Ochuko,

[21], saponin AOAC, [20], tannin [22], flavonoid [23], phenols[20], phytate [24] oxalates [24] vitamins B1, B2, B3, C [18][25], and HCN content.

Antimicrobial Analysis

Preparation and sterilization of media

Media employed in this study were weighed appropriately and prepared according to the manufacturers' instruction and sterilized in the autoclave at 121°C for 15 min. After sterilization, the media were allowed to cool before aseptically dispensing into petri-dishes and then allowed to solidify [12].

Preparation of Test Organisms

The clinical isolates were maintained on agar slant in bijou bottles and then sub-cultured for 24 hours before use. Pure isolates of each test organisms were emulsified into 5 ml of sterile normal saline using a sterile bacteriological loop to make a suspension of the test organism. The suspension was standardized to match 0.5 McFarland standards, shaken vigorously to ensure thorough mixing and stored overnight [12]

Reconstitution of extracts

Reconstitutions of the extracts were done according to the method described by Arekemase*et al*. [12]. This was done by diluting the each seed cotyledon extract (160 mg, 80 mg, 40 mg and 20 mg) in 1 ml of different solvent soluble to the extracts to give different concentrations of 160 mg/ml, 80 mg/ml, 40 mg/ml and 20 mg/ml, respectively.

Determination of the Antimicrobial Activity of the Seed Extract

The antimicrobial activity of the seed extracts was determined using the agar well diffusion method described by NCCLS [27] using the prepared concentrations of 20mg/ml, 40mg/ml, 80mg/ml, and 160mg/ml. The control experiments were done using 5mg/ml of Chloramphenicol. The inhibition zone was measured with a transparent meter rule and the sensitivity test of the seed extracts was done in duplicates.

Haematological Studies

Blood Collection from broilers

After the 1 months of the experiment, the blood samples were collected from the wing web using syringes and sterilized needles in accordance with the ethical requirements for using animals in experiments into heparinized tubes for the studies. The tube contains EDTA with calcium serving as anti-coagulant for hematological analysis.

Haematological studies were carried out at haematology unit of Saint Maris Hospital Gwagwalada, Ilishan–Remo using automated analyzer (Swelab Alfa haematology analyzer by Boule Medicals). These were analyzed in accordance with the methods described by Baker *et al*. [28].

Histological Examination

Tissues Preparation

After the 2 months of the experiment, the broilers were sacrificed under mild anesthetics with chloroform after overnight fast. The liver, heart, kidney, lungs, and spleen were promptly excised soon after decapitation, weighed and stored in 10% formalin for 24 h to allow proper fixation.

Histological Examination

Histopathological examination was carried out at Histology unit, Asokoro General Hospital, Abuja. The sections were embedded in paraffin, cut into 5 µm sections, prepared routinely and stained with haematoxylin and eosin (H and E) for histopathological changes and photomicrography using a light microscope, fitted with a digital camera for histological examination.

FTIR Study

The surface functional groups of the column fractions (a–g) of the hexane extracts of *Chrysophyllumalbidum*seed cotyledon were studied by Fourier Transform Infrared (FTIR) Spectrophotometer (Shimadzu Corporation, IR Prestige 21 model).

Statistical Analysis

The differences among experimental and control groups were determined using standard Statistical Software Package of Social Science (SPSS) for Window XP Software Program (version 13.0). Group comparisons were done using one-way analysis of variance (ANOVA) test. Antimicrobial analysis was done using one-way analysis of variance (ANOVA) test. Significant differences between control groups and experimental groups were assessed by Least Significant Difference (LSD) to test the significance at $p< 0.05$. All data were expressed as mean ± SEM.

III. RESULTS AND DISCUSSION

Table 1: Weight of Broiler Birds

Treatment		Groups			
Period	Group 1	Group 2	Group 3	Group 4	Group 5
Adaptation	0.74 ± 0.12^{a}	0.72 ± 0.01^{a}	0.72 ± 0.00^{a}	0.69 ± 0.02^{b}	0.68 ± 0.12^{b}
Week 1	0.88 ± 0.01^{a}	0.83 ± 0.01^{a}	0.83 ± 0.01^{a}	0.83 ± 0.01^{a}	0.82 ± 0.00^{a}
Week 2	1.27 ± 0.00^{b}	1.18 ± 0.04^{b}	1.37 ± 0.06^{a}	1.25 ± 0.01^{b}	1.26 ± 0.02^{b}
Week 3	1.72 ± 0.26^{a}	1.55 ± 0.02^{b}	1.57 ± 0.01^{b}	1.58 ± 0.01^{b}	1.62 ± 0.02^{b}
Week 4	2.10 ± 0.01^{a}	1.83 ± 0.04^{b}	1.94 ± 0.01^{b}	1.99 ± 0.01^{b}	2.05 ± 0.13^{a}

Values are expressed as mean ± SEM. Values in the same row with different superscripts are significantly different at $P< 0.05$.

Table 2: Proximate composition and phytochemical composition of CASC

Parameter	Values (%)	Phytochemicals	Values(mg/kg)
Moisture	11.47 ± 0.10	Phenolics	0.48 ± 0.02
Ash	1.74 ± 0.02	Oxalates	1.18 ± 0.15
Crude fibre	2.42 ± 0.02	Saponins	0.55 ± 0.02
Crude fat	3.28 ± 0.04	Flavonoids	1.15 ± 0.06
Nitrogen free extract	0.51 ± 0.00	Terpenoids	1.24 ± 0.03
Energy gross	1518 ± 2.00	Phylates	0.20 ± 0.02
Crude protein	1.86 ± 0.01	Alkaloids	1.28 ± 0.13
Carbohydrates	81.64 ± 0.06	Tannins	0.84 ± 0.01

Values are expressed as mean ± SEM.

Table 3: Vitamin and Minerals composition of CASC

Minerals	Values(mg/kg)	Vitamins	Values(mg/kg)
Iron (Fe)	2.24 ± 0.03	Niacin (B_1)	0.0046 ±0.001
Chromium (Cr)	0.16 ± 0.01	Riboflavin (B_2)	0.0035 ± 0.007
Nickel (Ni)	0.51 ± 0.02	Thiamine (B_3)	0.0064 ± 0.005
Lead (Pb)	0.06 ± 0.02	Ascorbic acid (C)	0.5542 ± 0.172
Cadmium (Cd)	0.03 ± 0.00	Hydrogen cyanide	1.4798± 0.025
Cupper (Cu)	2.44 ± 0.00		
Manganese (Mn)	0.62 ± 0.02		
Sodium (Na)	0.26 ± 0.00		
Magnesium (Mg)	1.26 ± 0.00		
Calcium (Ca)	0.94 ± 0.00		
Potassium (K)	1.32 ± 0.00		

Values are expressed as mean ± SEM.

Table 4: Antimicrobial Analysis of the classified crude extracts

Zone Diameter of Inhibition at 160 mg/ml					
Organisms	Hexane Extract	Aqueous Extract	Ethanol Extract	Ethyl acetate Extract	Control
C. albicans	NZ	13.0 ± 0.71	9.0 ± 1.41	NZ	21.0 ± 1.40
Bacillus spp.	NZ	8.0 ± 2.83	10.0 ± 1.40	5.0 ± 0.00	18.0 ± 0.00
Staphylococcus spp.	19.0 ± 0.70	NZ	18.0 ± 0.60	NZ	25.0 ± 0.20
E. coli	14.0 ± 0.71	20.0 ± 2.12	15.0 ± 0.14	13.0± 0.40	35.0 ± 0.56
P. aeruginosa	NZ	NZ	13.0 ± 0.50	14.0 ± 0.40	24.0 ± 0.30

NZ = No zone of inhibition. Values are Zone of Inhibition ± SEM (Standard Errors of Means). Control= chloramphenicol

Table 5: Hematological Analysis

Hemoglobin characteristics	Treatment Groups				
	Group 1	Group 2	Group 3	Group 4	Group 5
WBC (10^9/L)	197.1±38.73^b	209.8 ± 3.15^b	128.5 ± 3.50^c	123.1 ± 1.33^c	253.3 ± 1.67^a
RBC (10^{12}/L)	1.73 ± 0.37	1.97 ± 0.34	1.45 ± 0.15	1.50 ± 0.12	2.37 ± 0.15
Hb (g/dL)	15.50 ± 2.83^b	13.60 ± 3.20^b	19.85 ± 0.25^a	20.30 ± 0.15^a	10.63 ± 0.09^b
Hct (%)	26.73 ± 5.15^b	28.60 ± 4.11^b	21.90 ± 3.70^b	25.30 ± 0.40^b	36.93 ± 0.29^a
MCHC (g/dL)	45.55 ± 16.7^b	55.03 ± 21.38^b	51.15 ± 27.15^b	80.50 ± 0.59^a	29.0 ± 0.40^c
MCV (fl)	160.63± 1.95^a	147.2 ± 4.34^a	157.0 ± 3.00^a	158.73 ± 1.39^a	158.2 ± 8.30^a
Platelets (10^9/L)	70.00 ± 9.25^b	36.33 ± 9.35^c	67.00 ± 14.0^b	72.7 ± 1.48^b	116.0 ± 30.53^a

Values are expressed as mean ± SEM. Values in the same row with different superscripts are significantly different at P< 0.05. RBC = Red Blood Cell Counts, WBC = White Blood cell count, MCV = Mean Corpuscular Volume, MCHC = Mean Corpuscular Hemoglobin Concentration, Hb = Hemoglobin, Hct = Hematocrit.

Table 6: Organ Weight of Experimental Broilers

Organs	Groups				
	Group 1	Group 2	Group 3	Group 4	Group 5
Liver	44.33 ± 1.45^b	51.00 ± 0.58^a	52.33 ± 0.88^a	53.33 ± 0.33^a	54.00 ± 0.58^a
Spleen	2.00 ± 0.00^a	2.33 ± 0.33^a	2.33 ± 0.33^a	2.67 ± 0.58^a	2.67 ± 0.33^a
Heart	19.00 ± 0.58^a	19.00 ± 0.57^a	20.00 ± 0.57^a	19.33 ± 0.67^a	20.00 ± 1.00^a
Lungs	17.30 ± 0.88^a	17.33 ± 0.57^a	17.00 ± 0.58^a	18.00 ± 1.00^a	18.33 ± 0.33^a
Kidney	20.00 ± 1.15^a	19.33 ± 0.33^a	19.67 ± 0.33^a	20.00 ± 0.58^a	19.67 ± 0.88^a

Values are expressed as mean ± SEM. Values in the same row with different superscripts are significantly different at P< 0.05.

Table 7: Histopathological Examination of Broiler organs

Organs	Groups	
	Control groups	Experimental
Liver	No visible lesion	No visible lesion
Spleen	No visible lesion	No visible lesion
Heart	No visible lesion	No visible lesion
Lungs	No visible lesion	No visible lesion
Kidney	No visible lesion	No visible lesion

Table 8: Table of FTIR results summary of the bonds present in the spectra

Bonds	Wave number (cm^{-1})	Suspected functional group	Spectrum present
OH Stretch	3200 – 3600cm^{-1}	Primary Alcohol Carboxylic Acid	a, b, c, d, e, f, g
Sp^2 CH Stretch	3000 – 3100cm^{-1}	Olefins (General)	a, b, c, d and g
Sp^2 CH Stretch	2850 – 2970cm-1	Aliphatic hydrocarbon	a, b, c, d, e and g
C=O Stretch	1700 – 1780cm^{-1}	Carboxylic acid Aliphatic acetate	a, b, c, d, e, f,

		ester	
C=C Stretch	1600 – 1630cm^{-1}	Olefins	a, b, c, d
C-X Stretch	1400 – 1420cm^{-1}	Aliphatic acid halide	a, b, c, d, e
Sp^2CH bending vibrations	1490cm^{-1}	Olefins	a, b, c, d, e, f, g
Sp^3CH symmetric vibrations	1390 – 1378cm^{-1}	Aliphatic hydrocarbons	a, b, c, d, e, f, g

Sp^2 C-O Stretch	1200 – 1260cm^{-1}	Aliphatic Alcohol Aliphatic Esters	F
Sp^3 C-O Stretch	1050 – 1080cm^{-1}	ester or Furan	f, g
Sp^2 CH rocking vibrations	710 – 720cm^{-1}	Aliphatic hydrocarbons	a, b, c, d, e, f, g

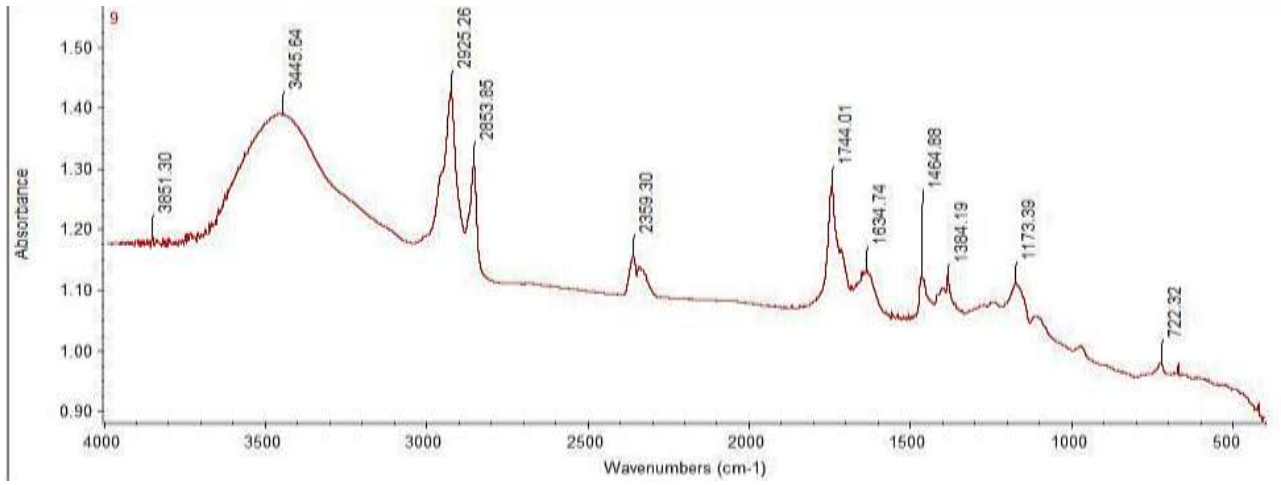

Figure 1: FT-IR spectrum of the hexane extract of *Chrysophyllum albidum* seed

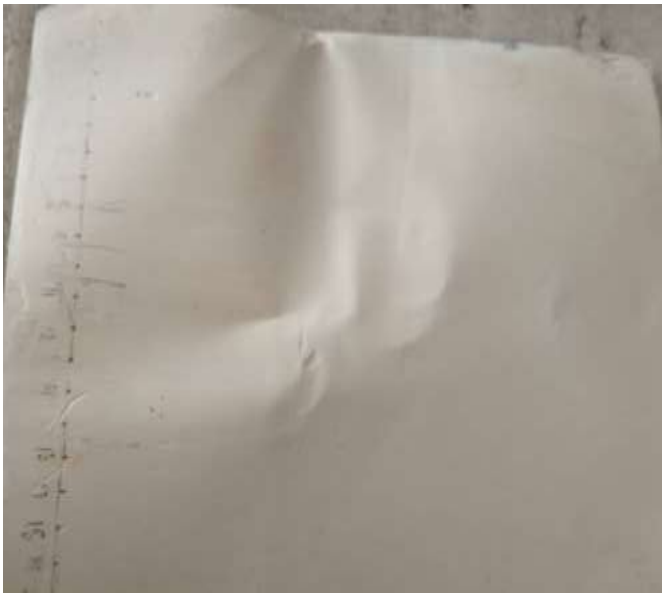

Figure 2: TLC plate of the hexane extract of *Chrysophyllum albidum* seed

IV. DISCUSSIONS OF FINDINGS

The weight of broiler (table 1) in the adaptation week was significantly higher in group 1, group 2 and group 3 than group 4 and group 5 ($p<0.05$). The result of this analysis indicates that there is a gradual increase in the weight of broiler birds fed with different percentage of *Chrysophyllum albidum* cotyledon as feed additive compared to the control group. This increase in weight of treatment animals is an indication that *Chrysophyllum albidum* did not exert any deteriorative effect on the growth of the animals. This increase in weight of the animals suggests that they increasingly accumulated calories by consuming more of the experimental diet. This result is in agreement with findings of Ajayi and Ifedi [29].

The result of the proximate analysis indicated that the moisture content of the plant material was 11.47 ± 0.10%, the ash content was 1.74 ± 0.02%, the crude fibre and crude fat were 2.42 ± 0.02% and 3.28 ± 0.04% respectively while the Nitrogen free extract was 0.51 ± 0.00%, crude protein was 1.86 ± 0.01% while carbohydrate was 81.64 ± 0.06% and gross energy of 1.52 kcal. The gross energy observed in this study is in agreement with the report of Ibrahim *et al*. [30]. The authors reported gross energy of 1.53 kcal from the seed shell pericarp of *Chrysophyllum albidum*. However, the moisture content, crude protein, fat, crude fibre, ash content, and carbohydrate reported by Ibrahim *et al*. [30] was different from values observed in this study.The difference could be attributed to the different parts of *Chrysophyllum albidum* studied.

The assessment of the result of the minerals indicated that cupper had concentration of 2.44 ± 0.00 mg/kg, iron (2.24 ± 0.03 mg/kg), potassium (1.32 ± 0.00 mg/kg), Magnesium (1.26 ± 0.00 mg/kg), calcium (0.94 ± 0.00 mg/kg), nickel (0.51 ± 0.02 mg/kg), chromium (0.16 ± 0.01 mg/kg), cadmium (0.03 ± 0.00 mg/kg). The result of the heavy metal analysis was lower than heavy metal reported by Ajayi and Ifedi [29]. The authors reported 5100.00 mg/kg of potassium, 2100.00 mg/kg of magnesium, 1960.00 mg/kg of calcium, 210.00 mg/Kg of sodium, 47.20 mg/kg of iron, 24.20 mg/kg of manganese, 12.90 mg/kg of copper and 6.70 mg/kg of zinc. The authors also reported absence of nickel, chromium and lead which were present in this study.

However, Minerals are critical in the regulation of a number of cell membrane, permeability, muscles contraction, heart function, blood clothing, prkotein and red blood synthesis as reported by Aremu*et al*. [31]. These essential minerals are important components of the daily diet. The high contents of potassium, magnesium, iron, calcium, and copper content in the extract is an indication that *C. albidum* can supply some essential minerals needed for healthy life.

The sample has high quantity of oxalates, flavonoids and terpenoids. Ajayi and Ifedi [29] reported the presence of saponin, flavonoids and alkakloids in *Chrysophyllumalbidum* seed powder. The phytochemical quantities observed in this study were lower than those reported by Ibrahim *et al*. [30] who thoroughly studied the quantitative phytochemical constituents of *Chrysophyllum albidum* seed pericarp, fruit pulp and fruit skin. The authors reported that the seed pericarp had a significantly higher alkaloids, tannins flavonoids and vitamin C compared to the fruit pericarp and fruit skin.

The result of analysis of vitamins indicated that ascorbic acid content was high compared to Niacin, riboflavin and thiamine. This is in agreement with findings of Ibrahim *et al*. [30] who reported high ascorbic acid content of the seed pericarp of *Chrysophllumalbidum.*

The sample had much higher HCN value of 1.4798mg/100g. The values recorded for the sample were high and exceeded the range allowed in monogastric animal diets as reported by Udedibie*et al*. [32]. HCN levels above 50-100 ppm (0.5mg/kg) have been shown to be detrimental to poultry health that is if it should sorely be used as a feed. The content composition level of HCN could only be considered if the sample is to be used as a feed additive.

The result of the antimicrobial analysis indicates that *Escherichia coli* were significantly susceptible to all the extracts compared to other bacteria studied. The bacterium (*Escherichia coli*) was highly susceptible to the aqueous extract ($p<0.05$). *Pseudomonas aeruginosa* was only susceptible to ethanol and ethyl acetate extracts. *Staphylococcus aureus* was highly susceptible to hexane and ethanol extracts. *Bacillus spp*. and *Candida albicans* showed the least susceptibility to the test extracts. This result is in agreement with findings of Akin-Osanaiye *et al.* [14] who studied the antibacterial efficacy of extract of *Chrysophyllum albidum*. The authors reported that *Chrysophyllum albidum* extract successfully inhibited the growth of *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. Their study indicated that *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were susceptible to ethanol extract at all concentrations studied.

Furthermore, *Bacillus* spp. had poor susceptibility to the extracts of *Chrysophyllum albidum.* This result is in agreement with the findings of Oputah*et al*. [33]. The authors reported that all test bacteria were susceptible to ethanol seed extract of *Chrysophyllumalbidum* except *Bacillus aereus*. Oputah*et al*. [33] also reported that *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* exhibited significant susceptibility to ethanol extract of *C. albidum*.

There was no significant difference in the red blood cell concentration in all the treatment groups. The hemoglobin concentration was significantly higher in groups 3 and 4 ($p<0.05$) compared to other groups. The percentage hematocrit values in group 4 was significantly higher than other group ($p<0.05$). The mean corpuscular hemoglobin concentration was significantly higher in group 4 and lowest in group 5 ($p<0.05$). The platelets was significantly higher in group 5 and lowest in group 2 ($p<0.05$). There was however no significant difference in the mean corpuscular volume in all treatment groups ($p>0.05$).

Hematological parameters reveal the deleterious effect of foreign compounds on the blood constituents of animals. They can also be used to determine possible alterations in the levels of bio molecules, metabolic products, hematology, normal functioning and histomorphology of the organs. The platelets of treatment group 5 were significantly higher than the control and other treatment groups. The Mean corpuscular hemoglobin (MCV), and mean corpuscular hemoglobin concentration (MCHC), which are red blood cell indices used in classifying types of anemia did not show any sign of anemia in experimental animals.

However, the trend in the values of the hematological parameters revealed that the rich mineral (Ca, K, Zn, Cu) and vitamin (B, C) content of the dietary CASC meal positively influenced haematopoiesis, hence favouring blood production.

Organ weight is an important factor of physiological and pathological status in animals. The heart, liver, kidney, spleen, and lungs are the primary organs affected by metabolic reaction caused by toxicant according to Jothy*et al*., [34]. The liver, being a key organ in the metabolism and detoxification of xenobiotics, is vulnerable to damage induced by a huge variety of chemicals. The absence of significant difference in the parameter obtained in this study is an indication that the *Chrysophyllum albidum* did not affect the weight of the organs.

The result of the histopathology analysis in this study is also in agreement with Akin-Osanaiye *et al*. [14]. The authors

reported a normal liver, spleen, kidney, heart and brain matter of control and experimental groups. Study by Ibrahim *et al*. [30] also reported no visible lesion in the organs of Albino rats fed *Chrysophyllum albidum* supplementary diets. *Chrysophyllum albidum* cotyledon extract might be free from any deleterious effect at this level of inclusion. This is indicating that it could probably be used as a viable carbohydrate component in the diet of livestock.

Assessment of the spectra results showed that all contain (-OH) stretching vibration bonds at a range of 3200 - 3600cm^{-1} indicative of either an alcohol or a carboxylic acid functional group in the seed cotyledon of *Chrysophyllum albidum.* The sp^2CH stretching vibration is unique to all the spectra excluding (e) and (f). The absorption takes place at a wave number range between 3000 – 3100cm^{-1} indicative of the presence of a double bond or an olefin in the oil of the seed cotyledon. At a wave number less than 3000 – 2900cm^{-1} is the Sp^3 (CH) stretch indicating the presence of aliphatic hydrocarbons in the entire spectra.

The peak between 1700 – 1800cm^{-1} are due to C=O stretching that can be attributed to the presence of carbonyl compounds in the oil but not present in the spectrum of (g). The C=C stretch is present at a wave number of 1618cm-1 showcasing an absorption peak in spectra (a), (b), (c) and (d).

All the spectra show absorption peaks at a wave number 1350 – 1480cm^{-1}. The vibrations indicate the presence of methyl and methylene (CH_2 vibration and CH_3 symmetrical vibrations). At a range of 1050 – 1300cm^{-1} are peaks indicative of the C-O stretches (Sp^3C-O and Sp^2C-O) summarized in table 11 above.

V. CONCLUSIONS

The seed cotyledon of *Chrysophyllum albidum* as observed in this study exhibited potent antimicrobial effect, high proximate and nutrient composition, high quantities of essential elements and low quantities of toxic metals. These characteristics of the plant material coupled with the no cost of gathering the seed cotyledon and the simple milling procedures makes it a viable candidate as livestock feed additive.

REFERENCES

[1] Fisayo, F., Gbenro, A., Tunde, A and Jesusegun, A. (2016). Chickens forced to skip meals as poultry feed rises by 100 percent. Punch, p.4. Retrieved from http://www.punching.com

[2] Eddy, N. O. and Udo, C. L. (2004). The energy value of some Nigeria soup. *Pakistan Journal of Nutrition*, 3:101-103.

[3] Amaechi, N.C. (2009). Nutritive and Anti – Nutritive Evaluation of wonderful kola (Buccholziacoricea) seeds. *Pakistan Journal of Nutrition*: 8:1120-1122.

[4] Makinde, O.J., Aremu, A., Alabi, O.J., Jiya, E.Z. and Ibe, E.A. (2017). Roasted African star apple (*Chrysophyllum albidum*) kernel meal improves growth performance of growing rabbits. *Tropical and Subtropical Agroecosystems*, 20: 457 – 464.

[5] Amusa, N.A, Ashaye, O.A. and Oladapo, M.O. (2003). Biodeterioration of the African star apple (Chrysophyllum albidum) in storage and the effect on its food value. *African Journal of Biotechnology*, 2: 56-59.

[6] Idowu, T.O., Iwalewa, E.O., Aderogba, M.A., Akinpelu, B.A. and Ogundaini, A.O. (2006). Biochemical and behavioural effects of eleagnine from *Chrysophyllum albidum*. *Journal of Biological Sciences*, 6: 1029-1034.

[7] Akubugwo, I.E and Ugbogu, A.E. (2007). Physicochemical studies on oils from five selected Nigerian plant seeds. *Pakistan Journal of Nutrition*, 6: 75-78.

[8] Olorunnisola, D.S., Amao, I.S., Ehigie, D.O. and Ajayi, Z.A.F. (2008). Antihyperglycaemic and hypolipidemic effect of ethanolic extract of *Chrysophyllum albidum* seed cotyledon in alloxan induced-diabetic rats. *Research Journal of Applied Sciences*, 3: 123-127.

[9] Bada, S.O. (1997). Preliminary information on the ecology of *Chrysophyllum albidum* G. Don in West and Central Africa. Proceedings of National Workshop on the potentials of Star Apple in Nigeria, pp. 16-25.03.

[10] Chowdhury, P. R., McKinnon, J., Wyrsch, E., Hammond, J. M., Charles, I. G. and Djordjevic, S. P. (2014). Genomic interplay in bacterial communities: implications for growth promoting practices in animal husbandry. *Frontiersin Microbiology*, 5: 394: 1–13.

[11] Zeng, Z., Zhang, S., Wang, H. and Piao, X. (2015). Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. *Journal of Animal Science and Biotechnology*, 6(1): 7.

[12] Anibijuwon II, Udeze OA (2009). Antimicrobial activity of Carica papaya (pawpaw leaf) on some pathogenic organisms of clinical origins from South-western Nigeria; Ethno-botanical leaflets, 13: 850 – 864.

[13] Arekemase, M. O., Kayode, R. M. O. and Ajiboye, A. E. (2011).Antimicrobial Activity and Phytochemical Analysis of *Jatropha curcas*Plant against some Selected Microorganisms. *International Journal of Biology*, 3: 52.

[14] Akin-Osanaiye BC, Gabriel AF, Salau TO, MuranaOO (2018). Chrysophyllum albidum seed as an additive in agricultural feed and a potent aktimicrobial. *Direct Research Journal of Agriculture and Food Science*. Vol.6 (5), pp. 107-113, ISSN 2354-4147.

[15] Pavia, L.K. (1976). A contemporary approach, Introduction to organic laboratory techniques, N.B. Saunders company Canada, 46, 50, 358, 599 - 605.

[16] Annongu, A.A. and Abimbola, S. F. (2003). Biochemical evaluation of *Gmelinaarborea* fruit meal as swine feedstuff. Biokkemistri 15 (1), 1-6.

[17] National Institute of Health (NIH) (1985). Guide for the care and use of laboratory Animals. Department of Health Education and Welfare, Pp.85 – 123.

[18] AOAC, (1990). Official Methods of Analysis. 4th Edition, Association of Official Analytical Chemists, Washington DC.

[19] Ekanayake, I.J. (1990). Evaluation of Potato and Sweet potato genotypes for drought Resistance, CIP, Lima, 1-11.

[20] AOAC (Association of Official Analytical Chemists), (2000).Official Methods of Analysis International, 17thed. Washington, DC: AOAC.

[21] Obadoni BO, Ochuko PO (2001).Phytochemical studies and Comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria.*Global J. Pure Appl. Sci. 8: 203-208.*

[22] Van-Burden TP, Robinson WC (1981). Formation of complexes between protein and tannin acid..*Agric Food Chen. 1 J: 77-82.*

[23] Boham AB, Kocipai AC (1994) Flavonoid and condensed tannins from Leaves of Hawaiian vaccininumvaticulum and vicalycinium. Pacific Sci. 48:458-463

[24] Ojinnaka MC, Ebinyasi CS, Ihemeje A, Okorie SU. "Nutritional evaluation of complementary food gruels formulated from blends of soyabean flour and ginger modified cocoyam starch. *Advance Journal forfood science Tech. 2013; 5: 1325-1330.*

[25] Rahman, M.M., Khan, M.M.R., Hosain, M.M. (2007). Analysis of Vitamin C (ascorbic acid) Contents in Various Fruits and Vegetables by UV-spectrophotometry.*Bangladesh Journal Science Industry Research*, 42 (4), 417-424.

[26] Bradbury, M.E., S.V. Egan and J.H. Bradbur (1999). Picrate paper kits for determination of total cyanogens in cassava roots and all tuber products. *J. Sci. Food. Agric*., 79: 595-601.
[27] National Committee for Clinical Laboratory Standards (NCCLS) (1990).Performance Standard for Antimicrobial Disc usceptibility Test. Approved Standard M2 – A4, Wiley, PA, USA.
[28] Baker, F.J., Silverton, R.E. and Pallister, C.J. (1998).Haematological Estimation. Baker and Silverton's Introduction to Medical Laboratory Technology, 356- 360.
[29] Ajayi, I.A. and Ifedi, E.N. (2015). Chemical and preliminary toxicological evaluation of *Chrysophyllumalbidum*seed flour in dietary formulation of Albino Rats. *Journal of Environmental Science, Toxicology and Food Technology*, 9(6): 59-67.
[30] Ibrahim, H.O., Osilesi, O., Adebawo, O.O., Onajobi, F.D., Karigidi, K.O. and Mohammed, L.B. (2017). Nutrients compositions and phytochemical contents of edible parts of *Chrysophyllum albidum* fruit. *Journal of Nutrition and Food Science*, 7(2): 1000579.
[31] Aremu, M.O., Olonisakin, A., Otene, I.W. and Atolaiye, B.O. (2005). Mineral content of some agricultural products grown in the middle belt of Nigeria. *Oriental Journal of Chemistry*, 21: 419-425.
[32] Udedibie, A.B., Enyenihi, G.E., Akpan, M.J., Obasi, O.L. and Solomon, I.P. (2008). Physiochemical nature and nutritive value of dried cassava Fufu meal for laying hens.*Niger. Agric. J., 39: 44-49.*
[33] Oputah, S.I., Mordi, R.C., Ajanaku, K.O., Olugbuyiru, J.A.O., Olorunshola, S.J. and Azuh, D.E. (2016). Phytochemical and Antibacterial Properties of Ethanolic Seed Extracts of *Chrysophyllum albidum* (African Star Apple). *Oriental Journal of Physical Sciences*, 1(1&2): 27-30.
[34] Jothy, S.L., Zakaria, Z., Chen, Y., Lau, Y.L., Latha, L.Y. and Sasidharan, S. (2001). Acute oral toxicity of methanolic seed extract of Cassia Fistulainmice. *Molecules*, 16:5268-5282.