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Proximate composition, mineral elements and phytochemical contents of seed powder and extract of *Picralima nitida* as a phyto-additive in poultry diets

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***Target Audience:** Animal Scientists, Technologists, Biochemists, Ethno-veterinarians*

Abstract

The phytochemical, proximate and mineral compositions of seed powder and aqueous extract of Picralima nitida as a medicinal plant was investigated. Picralima nitida pods were sourced from Ago Owu Farm Settlement, Ikoyi, Osun State, Nigeria, broken to remove the seeds which were processed into powder form and subsequently extracted using aqueous solution. The percentage yield after extraction was 27.25%. Proximate composition (%) of Picralima nitida seed powder (PnSP) and aqueous Picralima nitida Seed Extract (PnSE) were: crude protein (13.92±5.23% and 16.44±4.13%), ether extract (7.13±1.42% and 2.08±0.15%), crude fibre (7.15±4.26% and 15.00±0.18%), ash (6.82±3.74% and 4.97±0.06%), nitrogen free extract (58.09±5.27% and 51.70±2.82%) respectively. The seed powder and extract of P. nitida showed good contents of macro and micro elements. The seed powder had higher contents of most mineral elements except manganese (4.25±0.45 mg/kg), molybdenum (0.03±0.00 mg/kg) and sulphur (0.04±0.00 mg/kg) which were higher in PnSE. Saponin (267.11±4.37 mg/100g), tannin (6.84±0.41 mg/100g), flavonoid (5.47±0.84 mg/100g), alkaloid (295.19±2.80 mg/100g) and oxalate (124.35±3.78 mg/100g) were highest in PnSE compared to the PnSP. It could be concluded that Picralima nitida has adequate nutrients, minerals and bioactive secondary metabolites that could be exploited for use as a phyto-additive in poultry diets.

***Keywords:** Picralima nitida, Proximate, Minerals, Phyto chemicals and Extraction*

Description of Problem

Despite tremendous progress in human medicine, infectious diseases caused by bacteria, fungi, viruses and parasites are still major threats to public health. Plants in developing countries are making huge impacts as alternative to synthetic medicine due to relative high price of medicines and the emergence of widespread drug resistance (1,2). From historical times, different parts (seed, leaves, bark, stem and roots) of medicinal plants have been used to cure specific ailments (2). In recent years, there has been a gradual revival of interest in the use of medicinal plants in developed and developing countries, phytoadditives have been reported to be safer and without any adverse effect especially when compared with synthetic drugs (3), because some seeds offer multiple advantages by providing nutrients, minerals and phytochemicals with reduced cost of production and less difficulty in processing.

At present, developments have been made on extraction of plants using aqueous and ethanol solutions, but it is not being accorded the attention it deserves in terms of concentration, chemical properties and dosage of the extracts for consumption. This could be due to dearth of information on the different processing techniques in relation to the ethno-medicinal, nutritional and

biochemical properties for different end users and its application (4). *Picralima nitida* (K. Schum) Hallier falls into the category of less recognized and under-exploited seeds despite its promising medicinal values (2, 5, 6).

Most of the end users neither have knowledge of the nutrients and bio-chemicals in the herbs nor have dosage and time values of usage, this study therefore, aimed at providing baseline information on the proximate composition, mineral elements and phytochemical contents of *P. nitida* in tropical environment and hence creates a room for further research.

Materials and Methods

Procurement and Processing of *Picralima nitida* Seed

Picralima nitida pods were sourced from Ago Owu Farm Settlement, Ikoyi, Osun State, Nigeria. The pod and leaves of the plant were taken to Forestry Research Institute of Nigeria (FRIN) Ibadan for identification and authentication in the herbarium taxonomy section and a herbarium number FHI-113021 was obtained. The pods were broken to remove the seeds, processed to powder form as described (7) in the Animal Biosciences Unit of Department of Animal Nutrition and Biotechnology, LAUTECH, Ogbomoso. The seeds were cleaned with distilled water, de-hulled and diced into small sizes with stainless knife to facilitate air-drying in a room temperature for 4 days when constant weight was obtained. The dried seeds were screened to remove extraneous matter and thereafter, was milled with Super Master (SMB-2977) electric blender into powder form and stored in an air-tight container and kept in a cool place until needed for chemical analysis. Pictures taken while processing *P. nitida* are presented in Plates 1a-d

Aqueous extraction

The aqueous extraction was carried out by macerating the seed powder as described by (8, 9). Two hundred grams (200 g) of pulverized *P. nitida* seed powder was macerated with 800 ml distilled water at room temperature for 72 h after which it was filtered using Whatman Number 1 filter paper. The filtrate was concentrated to dryness in a freeze dryer at -40^{o}C, the picture is presented in Plate 1d. The same procedure was repeated for another sample; however, it was not concentrated to dryness. Both the concentrated and un-concentrated extracts were stored at 4^{o}C until needed for chemical analysis. The extraction yield was calculated as percentage weight of the dried powdered sample.

Plate 1a: *Picralima nitida* seed

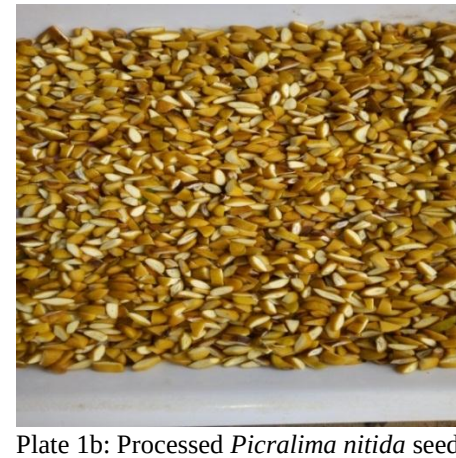

Plate 1b: Processed *Picralima nitida* seed

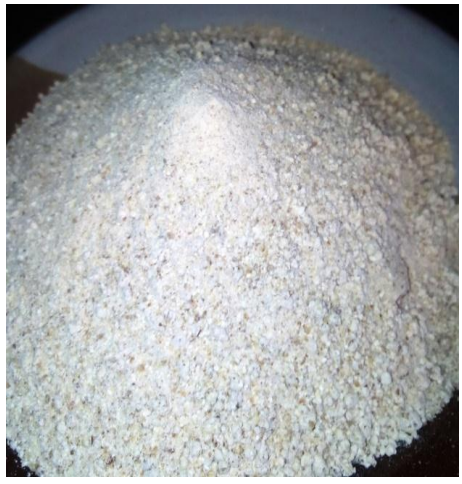

Plate 1c: *Picralima nitida* seed powder

Plate 1d: Concentrated aqueous extract of *Picralima nitida* seed powder

Chemical Analysis

Samples of PnSP and PnSE were analysed for proximate, mineral contents and phytochemicals

Proximate analysis

Picralima nitida seed powder (PnSP) and extract (PnSE) were analysed in triplicates for proximate components namely; dry matter (DM), crude fibre (CF), crude protein (CP), ash and ether extract (EE) using methods of the Association of Official Analytical Chemists (10).

Mineral composition

Macro elements (sodium, potassium, calcium, magnesium, phosphorus and sulphur) were determined in triplicates by flame photometer, while the micro elements (iron, manganese, zinc, selenium, chloride, cobalt, iodine, fluorine and molybdenum) were determined in triplicates using atomic absorption spectrophotometer as described in the methods of the Association of Official Analytical Chemists (10) after appropriate digestion by acids.

Phytochemical composition

The quantitative phytochemical analyses (tannin, saponins, oxalate, flavonoids and alkaloids) of *P. nitida* seed were determined in triplicates using the procedures as follows:

Test for tannins: According to the method described by (11), 0.2 g of the sample was measured into a 50 ml beaker, 20 ml of 50% methanol was added, it was covered with parafilm and placed in a water bath at 77-80^{o} C for 1 hour. It was shaken thoroughly to ensure uniform mixing. The extract was quantitatively filtered using a double layered Whatman Number 4 filter paper into a 100 ml volumetric flask, 20 ml water was added, 2.5 ml folin-denis reagent and 10ml of 17% Na_2Co_3 were also added and mixed properly. The mixture was made up to mark with water and was allowed to stand for 20 min when a bluish–green colour developed. The absorbance of the tannic acid standard solutions as well as sample was read after colour development on a spectronic 21D spectrophotometer at a wavelength of 760 nm. % tannin was calculated using the formula.

Tannin (%) = absorbance of sample X average gradient factor X dilution factor
Weight of the sample X 10,000

Test for saponin: As described (12), 1 g of finely ground sample was weighed into a 250 ml beaker and 100ml of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman Number 1 filter paper into a 100 ml beaker and 20 ml of 40% saturated solution of magnesium carbonate was added. The mixture obtained with saturated $MgCO_3$ was filtered again through a Whatman Number 1 filter paper and a clear colourless solution was obtained. One millilitre (1 ml) of the colourless solution was pipetted into 50 ml volumetric flask and 2 ml of 5% $FeCL_3$ solution was added and made up to mark with distilled water. It was allowed to stand for 30min and blood red colour developed. Zero to ten parts per million (0-10 ppm) standard saponin solutions was prepared from saponin stock solution. The standard solutions were treated similarly with 2 ml of 5% $FeCL_3$ solution as done for 1ml sample above. The absorbance of the sample as well as standard saponin solutions were read after colour development in a Jenway V6300 spectrophotometer at a wavelength of 380 nm.

Saponin (%) = Absorbance of sample X gradient factor X dilution factor
Weight of the sample X 10000

Test for Oxalates: Two grams (2 g) of sample was boiled in 40 ml of water for 30 minutes in a reflux condenser. Ten millilitres (10 ml) of 20% Na_2CO_3 was added and boiled for another 30 minutes. The liquid extract was filtered and washed with hot water until the washed water does not show any alkaline reaction. The combined wash water and filtrate was concentrated to a small volume and cooled. With constant stirring, HCL (1:1) was added in drops until the final acid concentration neutralized to about 4% at the stage which heavy precipitate appears (which is allowed to flocculate). Carefully, the extract was filtered into a 250 ml flask and make up to mark. It was kept overnight, and then the supernatant liquid was filtered through a dry filter paper in a dry beaker. An aliquot of this filtrate was poured in a 400 ml beaker, diluted with water up to 200 ml to make it ammoniacal and re-acidify with lactic acid. In the cold medium, 10 ml of a 10% calcium chloride solution was added, stirred well to obtain calcium oxalate precipitate and it was allowed to settle overnight. Carefully, the clean supernatant liquid was decanted through Whatman Number 42 filter paper, without disturbing the precipitate it was dissolved in HCL (1:1). Oxalic acid was re-precipitated by adjusting the pH with ammonium hydroxide solution. The content was boiled and allowed to settle overnight and oxalic acid was determined by titrating against 0.05N $KMnO_4$ solution (13).

Calculation: 1 ml of 0.05N $KMnO_4$ = 0.00225 anhydrous oxalic acid
Oxalic acid **(**%)
= Titre value x 0.00225 x 100
2 1
Oxalic acid (%) = Titre value x 0.1125.

Test for flavonoids: According to (14). Half gram (0.50 g) of finely ground sample was weighed into a 100 ml beaker and 80 ml of 95% ethanol was added and stirred with a glass rod to prevent lumping. The mixture was filtered through a Whatman Number 1

filter paper into a 100 ml volumetric flask and made up to mark with ethanol. One millilitre (1 ml) of the extract was pipetted into 50 ml volumetric flask, four drops of concentrated HCL was added via a dropping pipette after which 0.5 g of magnesium turnings was added until magenta red colouration was observed. Standard flavonoid solution of 5 ppm was prepared from 100 ppm stock solution and treated in a similar way with HCL and magnesium turning like sample. The absorbance of magenta red colouration of sample and standard solution were read on a digital Jenway V6300 spectrophotometer at a wavelength of 520 nm. The percentage flavonoid was calculated using the following formula:

$$\text{Flavonoids (\%)} = \frac{\text{Absorbance of sample X average gradient factor X dilution factor}}{\text{Weight of the sample X 10,000}}$$

Test for alkaloids: This was carried out using a distillation and titrimetric procedures as described (15). Two grams (2 g) of the sample was weighed into a 100 ml beaker and 20 mls of 80% absolute alcohol was added to give a smooth paste. The mixture was transferred to a 250 ml flask and more alcohol was added to make up to 100 ml and 1g magnesium oxide was added. The mixture was digested in a boiling water bath for 1.5 h under a reflux air condenser with occasional shaking. The mixture was filtered while hot through a small boucher funnel. The residue was returned to the flask and re-digested for 30 min with 50 ml alcohol after which the alcohol was evaporated; hot water was added to replace the alcohol lost. When all the alcohol has been removed, 3 drops of 10% HCL was added. The whole solution was later transferred into a 250 ml volumetric flask, 5 ml of zinc acetate solution and 5 ml of potassium ferrocyanide solution was added and thoroughly mixed to give a homogenous solution. The flask was allowed to stand for a few minutes, filtered through a dry filter paper and 10 ml of the filtrate was transferred into a separate funnel and the alkaloids present were extracted vigorously by shaking with five successive portions of chloroform. The residue obtained was dissolved in 10 ml hot distilled water and transferred into a kjeldahl tube with the addition of 0.20 g sucrose and 10ml concentrated H_2SO_4 and 0.02 g selenium for digestion to a colourless solution to determine %N by Kjeldahl distillation method. Percentage nitrogen obtained was converted to % total alkaloid by multiplying by a factor of 3.26

% alkaloids = %N X 3.26.

Results

The percentage yield after aqueous extraction was 27.25% v/w. The proximate composition and mineral elements of *P. nitida* seed powder (PnSP) and seed extract (PnSE) are presented in Table 1. Proximate composition revealed that PnSP and PnSE contained percentage crude protein (13.92±5.23 and 16.44±4.13), ether extract (7.13±1.42 and 2.08±0.15), crude fibre (7.15±4.26 and 15.00±0.18) and dry matter (93.11±0.77 and 90.19±1.17) respectively. *P. nitida* seed extract had higher values of CP, CF and gross energy (GE).

Both PnSP and PnSE had appreciable amount of mineral elements (Table 1). *PnSP* had higher values (mg/kg) for sodium (0.25±0.01), potassium (0.83±0.01), Calcium (0.24±0.01), Magnesium (0.26±0.00), Phosphorus (0.39%±0.00) iron (17.97±1.55), Zinc (57.30±1.00), Selenium (1.90±0.30), Chlorine (1.69±1.38), Iodine (5.35±0.64) and Fluorine (1.28±1.77). However,

manganese, molybdenum and sulphur were higher in PnSE. The phytochemical contents of *P. nitida* seed powder and extract are presented in Table 1. Saponin, tannin, flavonoid, alkaloid and oxalates were higher in PnSE compared to PnSP. Tannin was not present in PnSP.

Table 1: Proximate composition, mineral elements and phytochemical contents of *Picralima nitida* seed powder (PnSP) and aqueous *Picralima nitida* seed extract (PnSE)

Parameters	PnSP	PnSE
Proximate (%)		
Dry matter	93.11±0.77	90.19±1.17
Crude protein	13.92±5.23	16.44±4.13
Ether extract	7.13±1.42	2.08±0.15
Crude fibre	7.15±4.26	15.00±0.18
Ash	6.82±3.74	4.97±0.06
Nitrogen free extract	58.09±5.27	51.70±2.82
Gross energy	5.91±0.65	6.25±0.09
Macro minerals (%)		
Sodium	0.25±0.01	0.15±0.04
Potassium	0.83±0.01	0.47±0.14
Calcium	0.24±0.01	0.19±0.03
Magnesium	0.26±0.00	0.21±0.03
Phosphorus	0.39±0.00	0.30±0.05
Micro minerals (mg/kg)		
Iron	17.97±1.55	57.97±1.65
Manganese	4.25±0.45	25.33±6.04
Zinc	57.30±1.00	33.45±2.02
Selenium	1.90±0.30	0.06±0.02
Chlorine	1.69±1.38	1.86±0.75
Cobalt	0.41±0.02	0.10±0.02
Iodine	5.35±6.45	4.43±1.19
Fluorine	1.28±1.77	0.27±0.09
Molybdenum	0.03±0.00	2.21±0.26
Sulphur	0.04±0.00	2.12±0.84
Phytochemicals (mg/100g)		
Saponin	7.40±0.04	26.21±2.37
Tannin	0.00±0.00	6.84±0.41
Flavonoid	1.20±0.08	5.47±0.84
Alkaloid	1.88±0.02	29.52±2.80
Oxalate	6.75±0.09	12.83±1.78

PnSP= *Picralima nitida* Seed Powder
PnSE= *Picralima nitida* Seed Extract
± = Standard deviation

Discussion

Extractive yield

The percentage yield of 27.25% after concentrating the aqueous extract into powder is relatively low, supporting previous report that maceration gave lower yield of plant extract compared to Soxhlet extraction (16). However, value obtained is higher than that of 1.67% (ethanol) and 6.80% (cold water) reported by (17) who worked on root,

seed and stem bark extracts of *P. nitida.* The variation could be attributed to the concentration (15 g / 80 mls) and duration of time (18 h) used for maceration compared to 20 g / 80 mls and 72 h for concentration and maceration time respectively, in this study.

Proximate contents

Protein is an essential feed component that is required to build up and repair worn out tissues in the body (6). This study therefore, revealed that the CP value of PnSP was lower when compared with that of PnSE. This implies that nutrients are more released to extraction. The % CP for PnSP (13.92%) was in closer to (13.65%) for De-hulled *Hunteria umbellata* Seed (DHuS) and higher than 9.01% CP for Whole *Hunteria umbellata* Seed (WHuS) reported by (6). The higher value (16.44%) obtained for PnSE is close to 18.41% reported for *Hydrocotyle asiata* (6). The values obtained in this study were lower when compared to 23.30% and 19.40% reported for *Hunteria umbellata* and *Solanum dasyphyllum* respectively (18). However, they were higher compared to *Cola millenii* (12.52%), *Megaphrynium macrostarchyum* (10.78%) and *Rauwolfia vomitoria* (8.65%) reported by (18).

The higher value of ether extract (EE) recorded for PnSP is an indication that there were losses of fat to extraction, corroborating (19). However, (19) concluded that methanol is the best choice of solvent for extraction of *Hunteria umbellata* due to its higher oil yield. The EE value of PnSP (7.13%) was lower than that of whole *Hunteria umbellata* seed (14.97%) reported by (6) and in close range with 8.15% reported for *Hydrocotyle aziata* (18). The EE value of PnSE (2.08%) is in close range with the values 2.87%, 2.65%, 3.25%, obtained for dehulled *Hunteria umbellata* seed, *Solanum dasyphyllum* and *Canna bidentata,* respectively (18).

Appreciable amount of fibre observed in PnSP (7.15%) and PnSE (15.00%) is an indication that *P. nitida* can aid digestion by maintaining normal peristalsis of the intestinal tract (20). *Picralima nitida* could also help to reduce serum cholesterol level, risk of coronary heart diseases and hypertension (21). The values were lower than 26.79% (DHuS) and 23.90% (de-hulled seed of calabash) obtained (6) and (22). The mineral values obtained generally indicated the presence of inorganic elements in *P. nitida* seed as reported by (6). The ash values obtained for PnSP (6.82%) and PnSE (4.91%) were higher compared with DHuS (3.89%) and WHuS (3.45%) reported (6) and (3.36%) for *Prunus persica* seed (23) as well as 4.0% reported for calabash whole seed (22).

The nitrogen free extract (NFE) recorded for PnSP (58.09%) is a bit higher than PnSE (54.95%). The value of PnSP supported the report (6) that WHuS had 58.88% NFE. However, the value was higher than 43.23% (DHuS), 26.58% (*Hunteria umbellata*), 47.48% (*Hydrocotyle asiata*), and 47.55% (*Rauwolfia vomitoria*) as reported by previous authors (6 and 18). The moisture content of PnSP (6.89%) and PnSE (9.81%) after concentration indicated that they can be stored for a longer time without spoilage. Dry matter content of PnSE (90.19%) was in close range with (90.43%) and (89.05%) reported (6) for DHuS and WHuS, respectively, as well as 90.8% (calabash whole seed) and 88.78% (baobab pulp seed) as reported by (22).

Mineral elements

The current study revealed that values recorded for minerals were reduced with extraction, indicating that nutrients were lost to extraction methods. However, the values obtained for PnSP and PnSE indicated that *P. nitida* is an appreciable source of

minerals. There are variations with the reports (6, 18 and 24) who worked on *Hunteria umbellata, Morinda lucida, Euphorbia heterophylla* and some other plants with medicinal potentials. The variation in the composition could be as a result of location at which the plants were grown and plant species involved.

Minerals are essential for overall mental and physical wellbeing; and are useful for the formation of bones, teeth, tissues, muscles, blood and nerve cells (25). These elements are useful for the maintenance of acid-base balance, response of nerves to physiological stimulation and blood clotting (26). Potassium acts as cofactor that helps in the synthesis and activation of protein enzymes, it can be used to balance fluid and nerve transmission. The potassium values (0.83%, 0.47%) for PnSP and PnSE, respectively; were lower than 1.13%, 1.15% and 1.41% for DHuS, WHuS and baobab pulp, respectively, as reported by (6, 22). However, the values were extremely higher when compared to 0.029% obtained for *Blighia sapida* (22).

Calcium help in the formation and stability of cell walls and in maintenance of membrane structure and permeability, activates some enzymes, regulates many responses of cells to stimuli (6). The calcium values obtained in this study for PnSP and PnSE (0.24% and 0.19%, respectively) were higher than 0.08%, 0.08% and 0.007% reported for DHuS, WHuS and *Canna bidentata*, respectively (6, 18). The presence of calcium in *P. nitida* seed will help body structure and bones to be very strong. Guevarra (27) recommended Na:K to be less than one (<1%) in broiler diets, therefore, the Na:K (0.30), PnSP and (0.32) PnSE falls within the recommendation and could be good for hypertensive patients as this support its local usage for hypertensive patients.

Iron acts as component of cytochromes, electron transport, activates some enzymes, and plays a role in chlorophyll synthesis. The presence of iron indicates that *P. nitida* seeds could be useful in prevention of anaemia and other related diseases (18). Iron is an essential trace element for haemoglobin formation, normal functioning of central nervous system and the oxidation of carbohydrate, protein and fats (28). The values of 17.97 mg/kg (seed powder) and 57.97 mg/kg (aqueous extract) were lower than 600 mg/kg for DHuS and 630 mg/kg for WHuS obtained by (6) however (29) obtained lower values (101.2 mg/kg) with *Morinda lucida.* The presence of manganese in diets cannot be overlooked as it helps in formation of amino acids, activation of some enzymes and coenzyme activity; it is also required for water-splitting step of photosynthesis and chlorophyll synthesis (6). Manganese is a microelement essential for human nutrition and it is available in *P. nitida*.

Phytochemical contents

There is paucity of information on quantitative phytochemicals of *P. nitida*. Previous studies (17, 30, 31 and 32) reported qualitative results of *Hunteria umbellata* and related plants.

These results revealed that aqueous extracts had higher values of phytochemicals when compared with powdered seed and this could be attributed to the percentage yield of aqueous extract (27.25%). Nevertheless, PnSP and PnSE both had appreciable phytochemical contents such as saponin, tannin, flavonoid, alkaloid and oxalate except for PnSP that did not have tannin. These compounds have been reported to be active against potentially significant pathogens including those that are responsible for enteric infections (17 and 33). Apart from the fact that they have the potential of antibacterial activity, alkaloids

present in *P. nitida* are known as antimalarial agents, analgesics and can act as stimulants (6).

Glycoside moieties such as saponin and flavonoids can inhibit tumor growth, act as an anti-parasitic agent, and can be used as an anti-depressant (6) which could help to promote growth of livestock animals. Ajayi and Ojelere (18) reported the presence of phytochemicals like saponin, flavonoid and alkaloid in *Caesalpinia bunduc, Hunteria umbellata, Hydrocotyle asiata and Solanum dasyphyllum* and this is in accordance with the result of present study. The absence of tannin in PnSP disagreed with (18) who reported that tannin is present in *Hunteria umbellata, Ranwolfia vomitoria, Sphenocentrum jollyanum and Solanum dasyphyllum*. The variation in tannin could be attributed to the varietal differences.

Conclusion and Applications

It could be concluded from this study that:

1. *Picralima nitida* seed powder (PnSP) and extract (PnSE) contained appreciable amounts of secondary metabolites (alkaloids (7.40 / 26.21mg/100g), flavonoids (1.2 / 5.47mg/100g) and saponin (7.40 / 26.21mg/100g)); mineral elements: sodium (0.25 / 0.15%), calcium (0.24 / 0.19%), iron (17.97 / 57.97mg/kg), zinc (57.30 / 33.45 mg/kg) and chlorine (1.69 / 1.86mg/kg)) and proximate constituents: crude protein (13.92 / 16.44%), ether extract (7.13 / 2.08%) and crude fibre (7.15 / 15.00) that are needed to produce healthy poultry.
2. Aqueous extraction led to reductions in ether extract, ash, NFE sodium potassium, calcium, magnesium and phosphorus. However, manganese, chlorine, molybdenum and sulphur had higher values in PnSE.
3. Aqueous extract of PnSE had higher values of phytochemical contents compared with PnSP. *Picralima nitida* could possibly serve as a useful alternative product for synthetic antibiotics in the livestock industry.
4. Further research on its use as a phyto-additive that could replace antibiotics or other prophylactic drugs in poultry production is advocated.

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