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Phytochemical, Proximate and Nutritive Composition Analyses of the Seed of *Landolphia Owariensis* P. Beauv. (Apocynaceae)

Odoh Uchenna Estella*, Agbachi Bournaventure C.

***Abstract*— Quantitative phytochemical, proximate and nutritive composition of the seed of *Landolphia owariensis* P.Beauv. (Apocyaceae) was studied using standard analytical method. The result of the quantitative phytochemical analysis indicates the presence of carbohydrates, alkaloids, glycosides, saponins, flavonoids, resins, proteins, steroids and terpenoids. Proximate composition analysis of *Landolphia owariensis* seeds indicates that it contains 1.50, 3.35, 0.40, 11.03, 2.79 and 80.93 % of moisture, ash, fats, protein, fibre and carbohydrate respectively. The elemental analysis shows that it contains 2.297, 0.120, 3.840, 0.836, 0.0598 and 1.081 mg/100g of iron, calcium, magnesium, copper, sodium and potassium respectively with a minute quantity of phosphorus (15.555 ppm) and zinc (1.339 ug/100g). The vitamin contents were 3.102, 0.743, 0.933, 0.736, 0.887, 2.356 and 0.489 mg/100g of vitamins E, B1, B2, B3, B6, C and K respectively. Vitamin A is also present but in a minute quantity (3.942 ug/100g). This result shows that the seed of L. *owariensis* contains appreciable amounts of nutrients that justify its use in treatment of ulcer, worm infestation and rheumatism.**

***Index Terms*— *Landolphia owariensis*, Phytochemical analysis, Proximate analysis, Nutritive composition.**

I. INTRODUCTION

The medicines, food, fibre, shelters and other items that are used by humans are mainly supplied by plants (Hemingway, 2004). The plant parts that are used are leaves, stem, roots, flowers, fruits and seeds. Plants serve as important constituents of human diet supplying the body with carbohydrates, proteins, vitamins, mineral salts, lipids and water. Edible fruits contain various nutritive substances among which the most important are minerals, organic constituents, vitamin C and growth substances, etc. (Malaiesse, 1997; Loura et al., 2000). Seeds have nutritive and calorific values which make them necessary in diets (Odoememlam, 2005).The plant part commonly eaten is the seeds which are either cooked or eaten raw. Some nuts, grains and seeds such as pumpkin seeds, cashew nuts, almonds, buckwheat, coconut, flax seed, millet, oats, peanuts, rye, sunflower seeds and amaranth have high calorific values and are also rich in protein, dietary fiber and minerals – manly potassium and phosphorus (Decupere, 2011).The fruits and seeds contribute to the enhancement of nutrition, a better family income and the economy of the country (Lamien et al., 1996). Moreover, seeds and fruits are good sources of vitamins and minerals. The importance of vitamins and minerals cannot be overemphasized. Vitamins and minerals make peoples bodies work properly. Although you get vitamins and minerals from the foods you eat every day, some foods have more vitamins and minerals than others (Kidshealth, 2011).

Landolphia Owariensis P. Beauv. Is a shrub in the savanna or a huge liane of secondary deciduous and dense forests, attaining 100m long by over 1m girth, occurring from Guinea to West Cameroons and extending across central Africa to Suda, Uganda and South Tanganyika. Landolphia owariensis is known to have history of use for soothing injured mucous membranes in the digestive tract. It serves not only as lubricant but equally more important as a protective barrier between the mucosal surface and the luminal contents (Owoyele et al., 2001). The antiulcer properties and the use of Landolphia owariensis exrtracts for the treatment of ulcerogenesis and gastric mucosal injury is linked to the reported presence of flavonoidal compounds in Landolphia owariensis (Owoyele et al., 2001). The extract from the stem bark of Landolphia owariensis has been found to posses the ability to destroy parasitic worms from the body (Lewis and Lewis, 1977). The crude extract of Landolphia owariensis leaf has been found to exhibit very high anti-inflammatory activity. This activity may be linked with the presence of polyphonolic compounds in the extract of Landolphia owariensis (Owoyele et al., 2001). Liver function tests revealed that the serum activities of alanine aminotransferrase (ALT), aspertate aminotransferase (AST) and alkaline phosphate (ALP) as well as the concentration of total protein and albumin were not significantly ($P>0.05$) affected by the oral administration of the extract. This result suggests that Landolphia owariensis leaf extract is not hepatotoxic (Nwogu et al., 2008). Study has shown that the aqueous methanol and chloroform extracts of Landolphia owariensis leaf have moderate analgesic activity and reduce sensitivity to pain (Owoyele et al., 2001). Ebi and Ofoefule (1997) have validated the folkloric use of Landolphia owariensis as an antimicrobial agent. Its leaves, stem and roots are of great value in the indigenous treatment of various ailments.

The decoction of leaves is used as a purgative and to cure malaria, root is soaked in local gin for about a week and the extract is given two full wine glasses a day to cure gonorrhea

Odoh Uchenna Estella*, Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria
Agbachi Bournaventure C., Department of Pharmacognosy and Environmental Medicines, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria

(Gill, 1992). The latex is drunk or used in French Equatorial Africa is an enema for intestinal worms and in parts of Ivory Coast, the latex forms an ingredient arrow poison (Owoyele *et al.*, 2001). The leaf extracts have also been used for treating ulcer, inflammation and pains. The leaves are boiled in some parts for application to sprains (Owoyele *et al.*, 2001). In Congo, the sap expressed from the leaves is dripped into the eyes and used to wash patient's face in a treatment for giddiness (Owoyele *et al.*, 2001). The sap is also rubbed with massage into scarifications over areas of oedema and rheumatism (Owoyele *et al.*, 2001). The study aimed at investigating the proximate, nutritive and dietary fibre composition of *Landolphia owariensis* seed for the purpose of determining its nutritional status.

II. MATERIALS AND METHODS

Collection and Preparation of Plant Material

The seeds *Landolphia owarienis* were collected during the month of June from Lejja community in Nsukka, Enugu State, Nigeria and authenticated by Mr. A Ozioko of the International Centre for Ethno Medicine and Drug Development (INTERCEDD) Nsukka. The voucher specimen (UNN/PCOG/017/046) was deposited in the Department of Pharmacognosy and Environmental Medicine, University Nigeria, Nsukka. The pulps were separated from the seeds and the seeds were sun-dried. After drying, the seeds were pulverized using an electric blender into a fine powder of 60 mesh size and then used for further studies.

Preparation of standard

Stock solutions of iron, potassium, calcium, sodium, and magnesium were prepared in accordance with standard methods (Association of Official Analytical Chemistry; AOAC, 1995).

Qualititative Phytochemical Analysis

Phytochemical tests were performed on the major secondary plant metabolites of pharmacognostic importance which include: alkaloids, steroids, tannins, sapanins, resins, flavonoids, oils following standard procedures (Trease and Evans, 1985).

Quantitative Phytochemical Analysis

The quantitative determination of the phytoconstituents (alkaloids, flavonoids, cyanogenetic glycosides, saponins, tannins and phytic acid) was done according to the methods of Odoh *et al*., (2012).

Alkaloids determination

To a 5 g of sample in 250 ml beaker, 200 ml of 10 % acetic acid in ethanol was added, covered and allowed to stand for 2 h. It was then filtered and the filtrate was concentrated on a water bath. Concentrated ammonium hydroxide was added drop-wise to the extract. The precipitate formed was collected and washed using dilute ammonium hydroxide and filtered. The residue which is the alkaloid was dried and weighed.

Flavonoids determination

A 10 g of the sample was extracted with 100 ml of 80 % aqueous methanol for 4 times. The filterates obtained were pooled and evaporated over a water bath and weighed to a constant weight.

Cyanogenetic glycosides determination

A portion (5 g) of the sample was made into a paste and the paste was dissolved into 50 ml distilled water. The extract was filtered and the filtrate was used for cyanide determination. To 1 ml of the sample filtrate, 4 ml of alkaline picrate was added and absorbance was recorded at 500nm and cyanide content was extrapolated from a cyanide standard curve.

$$\text{Cyanide (mg/g)} = \frac{\text{Absorbance x GF x DF}}{\text{Sample weight}}$$

Where GF = gradient factor and DF = dilution factor

Saponins determination

A portion (20 g) of the sample was put into a conical flask and 100 ml of 20 % aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at 55 ^{0}C. The mixture was filtered and the residue re-extracted with 200 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at 90 ^{o}C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated on a water bath. After evapouration the samples were dried in the oven to a constant weight. The saponins content was calculated as percentage.

Tannins determination

A portion (500 mg) of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to mark. 5 ml of the filtrate was pipette out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Phytic acid determination

A portion (2 g) of the sample was weighed into 250 ml conical flask. 100 ml of 2 % concentrated hydrochloric acid was used to soak the sample for 3 h. The mixture was filtered and 50 ml of each filtrate was placed in 250 ml beaker and 107 ml of distilled water was added to give proper acidity. 10 ml of 0.3 % ammonium thiocyanate solution was added to the solution as indicator and was titrated with standard iron chloride solution which contained 0.00195 g of iron per ml.

% Phytic acid = y x 1.19 x 100

Where y = titre value x 0.00195

Proximate Analysis

The proximate analysis (moisture, ash, carbohydrates, fats, protein, moisture, fibre, energy and β-Carotenes) was determined by using AOAC (2005) methods. The moisture and ash were determined using weight difference method.

Carbohydrate was determined by difference method: the sum total of the moisture, fat, protein, and ash content of each part of the samples were subtracted from 100 as follows: Carbohydrate content = 100 – [protein (%) + fat (%) + moisture (%) + ash (%)]. The nitrogen value which is the precursor for protein of a substance was determined by Micro-Kjeldahl method. The nitrogen value was converted to protein by multiplying to a factor of 6.25. Crude fat was determined using oven-dried samples from moisture content determination. This was extracted with petroleum ether (boiling point (BP) 40 to 60°C) for 6 h with Soxhlet extractor. After evaporation of ether, drying to constant weight, and cooling, the result was expressed in percentage. The sample calorific value was estimated (in kcal) by multiplying the percentage crude protein, crude lipid, and carbohydrate by the recommended factor (2.44, 8.37, and 3.57, respectively) used in vegetable analysis (Asibey-Berko & Tayie, 1999). Crude fibre content was determined using Gallenkhamp muffle furnace at 550°C and the result was expressed in percentage. The β-carotene was determined by taking the absorbance using a spectrophotometer (model 22UV/VIS) at a wavelength of 436nm.The concentration of β-carotene was calculated using Bear-Lamberts Law, which states that the absorbance (A) is proportional to the concentration(C) of the pigment, as represented by the equation:

A ∞L (if concentration(C) is constant).

A=ECL; C=A/EL

Where:C= concentration of carotene, A= absorbance, E=extinction coefficient,

L= thickness of cuvettes (path length) =1cm, E of β-carotene =1.25x104µg/l

Mineral Analysis

(a) The sample was investigated for mineral composition (sodium, calcium, potassium magnesium, and iron) as outlined by Shah *et al.* (2009) and Shivraj (2009) for plant samples by using atomic absorption spectrophotometer (AAS), Bulk Scientific model AVG 210. A 2 g of the processed sample weighed was subjected to dry ashing in a well-cleaned porcelain crucible at 550°C in a Gallenkhamp muffle furnace. The resultant ash was dissolved in 5 ml of HNO_3/HCl/H_2O (1:2:3) and was heated gently on a hot plate until brown fumes disappeared. To the remaining material in the crucible, 5 ml of de-ionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through Whatman Grade No. 42 filter paper and the volume was made to the mark with de-ionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer (AAS). A 10 cm long cell was used and the concentration of each element in the sample was calculated in the percentage of dry matter, that is, mg/100 g sample. Appropriate working standard solution was prepared for each mineral. The calibration curves were obtained for concentration versus absorbance.

III. VITAMINS ANALYSIS

The vitamin analyses were performed on the plant material following Standard procedures (Pande *et al.*, 2002).

Vitamin A

A 1g of sample was extracted with 50 ml of petroleum ether and concentrated to dryness. The residue was saponified with 5 ml of 0.1 m potassium hydroxide under reflux. 20 ml of petroleum ether was used to extract the unsaponifiable matter and concentrated to dryness. 20 ml of ethanol was added to dissolve the residue. 1 ml was transferred into 3 test tubes and was added 1ml of 0.2% ferric chloride in ethanol and 1 ml of 0.5% dipyridyl in ethanol and made up to 5ml with ethanol. The absorbance taken at 520 nm against a blank.

Vitamin C

A 1 g of the sample was macerated with 20ml of 0.4 % oxalic acid for 5 minutes and filtered. 1 ml of the filtrate was transferred into 3 test tubes and 9 ml of 2, 6 – dichlorophenolindophenol (12 mg / l) was added and the absorbance taken at 520 nm against a blank.

Vitamin E

A 1 g of the sample was extracted with 50 ml of petroleum ether and concentrated to dryness. The residue was saponified with 5ml of 0.1 M potassium hydroxide under reflux. 20ml of petroleum ether was used to extract the unsaponifiable matter and concentrated to dryness. 20 ml of ethanol was added to dissolve the residue. 1ml was transferred into 3 test tubes and was added 1 ml of 0.2 % ferric chloride in ethanol and 1 ml of 0.5 % dipyridyl in ethanol and made up to 5 ml with ethanol. The absorbance was taken at 520 nm against a blank.

Vitamin K

A 1 g of sample was extracted with petroleum ether and concentrated to dryness and 10 ml of petroleum ether added to redissolved the residue, 10 ml of half saturated barium hydroxide solution was added, then the ether layer was collected and washed with 5ml of water the washing was further washed with 10 ml of petroleum ether and the ether layer combined and evaporated to dryness, to the residue was added 2 ml of (1:1) ethanol 1ml of 0.04 % 2-4 dinitrophinylhydrazine mixture was cooled to room temperature and 2ml of 8% NaoH was added followed by amyl alcohol the mixture was heated on the water baths for 2 minutes with ethanol the absorbance was taking at 620 nm against water blank.

Thiamine (B_1)

A 2ml of reagent solution was added to the 2ml of sample and was mixed, 15ml of isobutyl alcohol was added after 1munites and shaken slowly for 2minutes moving the test tube up and down separate the isobutyl alcohol layer, the isobutyl layer was collected and dried by adding a spatular tip of anhydrous sodium sulphate it was shaken and the absorbance at 367nm was determined using isobutyl alcohol as blank.

Riboflavin (B_2)

To 1.5ml of the sample, 6.5ml of distilled water was added, 2ml of designees reagent solution was added also was mixed

and was allowed to stand for 15minutes, filtered if necessary, and the absorbance was taking at 525nm against a reagent blank.

Niacin (B_3)

A 1ml of sample, 6.5 ml of H_2O+0.5 ml of 1.5 % ammonia, 1ml of cyanogens bromide, 1 ml sulphanillic acid, 0.5 ml of concentrated HCL were added and was made up to 10ml with water, the absorbance was read at 43 nm.

Pyridoxine (B_6)

A 1ml of the filtrate was transferred into 3 test tubes; 2ml of water, 0.4ml, of 50 % sodium acetate, 0.1ml of diozotize reagent were added and shaken. 0.2ml. of 5.5 % sodium carbonate was added, mixed and the absorbance taken at 540nm, against reagent blank.

IV. Statistical Analysis

The data were expressed as mean ± standard error mean (SEM). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

V. Results and Discussion

(b) The results of the phytochemical composition indicated that *Landolphia owariensis* seed is rich in the following phytochemicals: carbohydrates, alkaloids, glycosides, saponins, tannins, flavonoids, resins, proteins, steroids and terpenoids (Table 1). The presence of these secondary metabolites has contributed to its medicinal value as well as physiological activity (Omale *et al.*, 2010). For instance, flavonoids have been shown to have antibacterial and anti-inflammatory activities (Omale *et al.*, 2010). Many of these effects have been linked to their known functions as strong antioxidant, free radical scavenger and metal chelators (Omale *et al.*, 2010). A number of medical diseases could be prevented or improved with the use of dietary flavonoids. These include circulatory disorders, hypertension, lung disorders, liver problems, diabetes and eye problems (Sahelian, 2005). Alkaloids, which are naturally occurring plant constituents (Beckett and Stenlake, 1988) act on the nervous systems of the human body. They are used as analgesics, antibacterials, antispasmedics, antiplasmodics and sedatives (Babjide et al., 1999). Alkaloids contribute to plant species fitness of survival. They produce bitter taste that repels insects from feeding on the plant parts where they are concentrated. Tannins bind to both proteins and carbohydrates, and provoke astringent reactions. Tannins precipitate proteins in the gut, reduce digestibility, or inhibit digestive enzymes like trypsin, amylase and lipase, and also inhibit microbial activities (Grifits, 1979). They have been found to be insecticidal, anti-inflammation, antifungal, antiseptic and wound healers (Beckett and Stenlake, 1988). Saponins enhance nutrient absorption and aid in animal digestion (Omale *et al.*, 2010).

The proximate composition analysis shows that the percentage of proteins, carbohydrates, fats and oils, dietary fibre, moisture and ash are 11.03, 80.93, 0.40, 2.79, 1.50 and 3.35 % respectively. The high content of carbohydrate implies that the plant could be a good source of energy. There are certain plants like *croton tiglium* that can yield carbohydrates up to a low amount of 15.51 % (Omale *et al.*, 2010). This showed that *Landolphia owariensis* is relatively a good source of carbohydrate.

Mineral analysis revealed that high concentrations of magnesium and iron have been found in the seed of *Landolphia owariensis*. The plant's seed is not rich in zinc and phosphorus. The increasing order of mineral elements in *Landolphia owariensis* seed is P > Zn > Na > Ca > Cu > K > Fe > Mg. Some plant seeds have low amount of mineral elements. For instance, one table spoon of dried sesame seeds contains 1.31 and 0.7 mg of iron and zinc respectivelyh (Decuypere, 2011). Therefore, *Landolphia owariensis* is a good source of minerals when compared with other plants.

The results of the vitamin analysis of the seeds of *Landolphia owariensis* indicated high amount of vitamins C and E. It contains other vitamins in small amounts with the concentration of vitamin A being the least. In comparison, flax seeds contain 2.6 mg and 0.19 mg per table spoon of vitamins C and E respectively (Decuypere, 2011), showing that *Landolphia owariensis* is one of the good sources of vitamins.

VI. Conclusion

The study showed that there is variation in phytochemical, proximate, elemental and vitamin composition of *Landolphia owariensis* seed. It is of high nutritional and medicinal value because it contains appreciable amounts of nutrients and phytochemicals. This plant could be well integrated into Nigerian food and medicine considering the results of this investigation.

Table 1: The result of the phytochemical analysis carried out on *Landolphia owariensis* seed.

Constituents	**Inference**
Carbohydrates	++
Reducing sugars	-

Alkaloids	+++
Glycosides	+++
Saponins	+++
Tannins	+
Flavonoids	++
Resins	+++
Protens	+++
Oils	-
Steroids	+++
Terpenoids	++
Acidic compounds	-

Key: + = slightly present, ++ = moderately present, +++ = highly present, - = absent

Proximate contents

Table 2: Result of the proximate composition analysis of *landolphia owariensis* seed

Proximate content	**Value (%)**
Proteins	11.03
Fats and oils	0.40
Fibre	2.79
Moisture	1.50
Ash	3.35
Carbohydrates	80.93

Mineral Analysis

Table 3: Result of the mineral composition analysis of *landolphia owariensis* seed

Mineral content	**Value**
Iron	2.2971 ± 0.821mg/100g
Magnesium	3.840 ± 1.010mg/100g
Copper	0.836 ± 0.111mg/100g
Sodium	0.0598 ± 0.014mg/100g
Potassium	1.081 ± 0.032mg/100g

Calcium	0.120 ± 0.013mg/100g
Zinc	1.3390 ± 0.051ug/100g
Phosphorus	15.555 ± 3.022ppm

Values are mean ± SEM, n = 3

Vitamin Analysis

Table 4: Result of the Vitamin composition analysis of *Landolphia owariensis* seed

Vitamin content	**Value**
Vitamin A	3.9421 ± 0.1053 ug/100 g
Vitamin C	2.356 ± 0.5010 mg/100 g
Vitamin E	3.1025 ± 1.0020 mg/100 g
Vitamin K	0.4890 ± 0.1013 mg/100 g
Vitamin B1	0.743 ± 0.1242 mg/100 g
Vitamin B2	0.9330 ± 0.1110 mg/100 g
Vitamin B3	0.7360 ± 0.2120 mg/100 g
Vitamin B6	0.8865 ± 0.2334mg/100 g

Values are mean ± SEM, n = 3

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