

Proximate and phytochemical compositions of *napoleona vogelii* hook fruit

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-----ABSTRACT-----

Chemical analyses were carried out to determine the proximate and phytochemical compositions of *Napoleona Vogelii* fruits locally sourced from Ebonyi State of Nigeria. Results for proximate analysis of the fruit showed the moisture content of 69%, protein 1.93% fat 2.1%, crude fibre 16%, ash 3.5% and carbohydrate 7.47%. There was a low level of phenol, saponin and HCN with the values of 3.8 mg/kg, 0.75% and 3.382 mg/kg respectively, and a significant presence of flavonoid, alkaloid and tannin, which were 4.65%, 0.8% and 333.4 mg/kg respectively. These nutrients and phytochemicals present in the wild fruit indicated the beneficial effects of the plant. It is recommended that industries should boil the fruit to reduce the level of toxicants in the fruit when using it as supplement.

KEYWORDS: *Napoleona Vogelii*, AOAC, food, proximate, phytochemical, nutrient

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I. INTRODUCTION

Napoleona Vogelii Hook fruit is a sweet and edible fruit found in a holly- a tree with hard prickly leaves all through the year and the ripe fruits in the dry seasons (Igidi, O. J.; Omaka, N. O. and Nwabue, F. I., 2012). The trees bearing these fruits are grown wildly on a prickly rocky bush and farmlands in most parts of Ebonyi State of Nigeria, particularly along the Akpoha- Amasiri axes in Afikpo North L.G.A and Amuzu-Echara axes in Ezza South L.G.A of the state. Habitants, mainly farmers and bush animals eat these fruits often. Thus, because of the edibility of the species there is need to identify the nutritional and anti-nutritional components of the fruit and assess their cumulative adverse effects on the consumers. The tropical rainforest of Nigeria, where Ebonyi State falls, is full of some plant species producing edible fruits, seeds or leaves (Dike, M. C., 2010), and hence demands scientific investigation. Okaka, J. C; Enoch, N. J; and Okake, N. C. (1992) reported that some plant species were in the process of being lost. Recently, 30 plant species producing edible fruits, seeds and leaves in South- Eastern Nigeria have been reported as endangered (Meregini, A. O. A., 2005). Moreover, within the rainforest 115 plants species whose uses were not classified have been reported endangered (Dike, 2010). Many of these edible fruits are collected mainly from the wild and their habitats are currently threatened. There is paucity of literature on the proximate, phytochemical and nutritional composition of most of these plants, including *Napoleona Vogelii* Hook fruit. There is need to understand their suitability for either food or fodder. A proper understanding of their proximate, phytochemical and nutrient compositions will lower the over dependence of many communities and Industries on few arable crops for fruits, seeds and vegetables. In addition, knowledge of their composition would enable one to know the better type of fruits to eat or feed to animals at any given time. Some species due to their aroma and delicacy are eaten irrespective of their composition (Dike, 2010). There is the possibility that some fruits could contain very small quantity of either anti-nutritional or poisonous chemicals. In most developing Countries of the world, Nigeria inclusive, majority of livestock-farmers rely on traditional healthcare practices to keep their animals healthy. Mainly the local practitioners of folklore ethnoverinary medicine who depend solely on the use of medicinal plants provide these traditional healthcare services (Makoshi, M, S and Arowolo, R. O. A., 2011). Most of these herbal plants have been used for centuries in the management and prevention of a wide range of livestock diseases by traditional healers and have been employed for same purpose in both animals and humans. The use of herbal and ethnoverinary in most developing Countries has been found to be of great value in areas where allopathic or orthodox medicines are often beyond the reach of populace and poor livestock producers (Liener, I. E., 1994 & 1995). The methanol and n-hexane leaf extracts of *Napoleona Vogelii* has been investigated for anti-ulcer related properties using three experimental ulcer models induced by ethanol, indomethacin and hypothermic-restraint stress in rats (Akah, P. A.; Nnaeto, O; Nwonu, C. S.; and Ezike, A. C., 2007). The anti-ulcer related properties of the extract such as gastrointestinal transit, the activity on isolated gut tissue preparation and the

antimicrobial activities have also been determined. The extracts appear to exhibit better protection against indomethacin and ethanol induced ulcer than against the stress ulcer (Akah *et al.*, 2007). It did not produce lethality or signs of acute toxicity in the mice administered after 24 hrs. Flavonoids, tannins, saponin, carbohydrate, terpenes, resins, steroids and alkaloid were found present in the extract of *Napoleona Imperialis* (Akah, *et al.*, 2007). Previous studies have shown that seeds extracted from fruits contain nutrients, calorific value and anti-nutritional factors. Extracted saponin from the seed was used for erythrocyte fragility tests (heamolysis). The investigation therefore shows that the seeds of *Napoleona Imperialis* are rich sources of commercial haemolytic saponin. In addition, saponin extraction and cyanide detoxification would make the seed meal a good raw material source for livestock feed (Ukpabi, U. H. and Ukpabi U. J., 2003). Studies on the proximate analysis of the dried milled seeds of *Napoleona Imperialis* show that the seed meal is a possible good source of protein. In many food chemistry laboratories, bulk of analysis comprises the methods of proximate analysis. Proximate analysis of food is the determination of the major components of food, which includes moisture, fats, protein, carbohydrate and fibre. In practical terms, the methods used for the determination of the different food components do not only vary according to the food material being studied but also in detail of evaluation procedures (Onwuka, G. I., 2005). These methods were evolved by thorough studies of the inherent properties of the component of interest and exploring the unique advantage such properties have over others, thus allowing the component to be isolated or eliminated. Phytochemicals are bioactive components found in natural tropical food materials; some of which are known to cause toxicity in human when taken in high amount (Onwuka, 2005). Anti nutritional factors are the main draw back limiting the nutritional and food values of plant (Liener, I. E., 1976).

Aim and Objectives

The aim of this research is to extract the fruit juice and determine/evaluate the proximate composition and phytochemical content of the fruit.

II. EXPERIMENTAL AND METHODS

Sampling, Pre-treatment and analysis of samples

Fresh fruits of *Napoleona Vogelii* samples were collected from a prickly rocky bush between Akpoha in Afikpo North and Amuzu and Echara axes in Ezza South Local Government Areas of Ebonyi State. Sample collections and treatment were carried out following standard procedures recommended by Abu, A. E.; Ango, K. M.; Bawa, G. S.; Chindo, P. S.; James, D. B. and Takubu, L. B. (2005); Onwuka, G. I. (2005); Pearson, D. (1976); James, C. S. (1995); and Association of Official Analytical Chemists (AOAC 1990).

Determination of the Moisture Content of the Sample (Pearson, D. 1976)

Procedure: A Petri-dish was washed and dried in an oven and taken into a desiccator to cool. The Petri dish was weighed after it has been cooled and 2.0g of the un-dried sample weighed out into the pre-weighed Petri-dish. The sample in the dish was placed in the oven to dry at 105°C for about four hours when a constant weight was gotten. The dried sample was transferred to the desiccators using a crucible tong, cooled and the dry weight of the sample plus dish taken. The values generated were used to calculate the percentage moisture content.

Determination of Ash content of the sample (James, C. S. 1995)

Procedure: 2.0g of the finely ground sample was weighed out into a preweighed porcelain crucible. The sample was chard on the heater inside a fume cupboard - to drive off most of the smoke. The sample was transferred into a carbolite furnace at 550°C, left at this temperature until a light gray ash resulted. When the residue became black in colour it was moisten with small amount of water and dried in an oven, the ashing process was repeated and cooled in a desiccator. After cooling, the sample was reweighed and the values generated were used to calculate the percentage ash content of the sample.

Determination of crude fibre content of the sample (Onwuka, G. I. 2005)

Procedure: 2.0g of the sample was weighed out into a beaker. The sample was defatted with petroleum ether for about 3 hours, and boiled under reflux for 30 minutes with 200 mL of 1.25% H₂SO₄ solution. The solution was filtered and washed with boiling water and the residue transferred into a beaker and boiled for 30 minutes with 200 mL of 1.25% NaOH solution. After boiling, the mixture was filtered using a weighed filter paper and the residue obtained was washed with hot distilled water and was allowed to drain. The drained sample was carefully transferred to an oven and dried to a constant weight, the weight was recorded and sample was further transferred into a weighed crucible and incinerated in a furnace. The ashed sample was cooled in a desiccator and reweighed, and the percentage crude fibre calculated.

Determination of Fat Content of the Sample (James, C. S. 1995)

Procedure: Using continuous solvent extraction method, 10.0 g of the sample was weighed out into a labelled filter paper and placed in the thimble of Soxhlet apparatus, mounted on a weighed, dried, cleaned, and cooled boiling flask containing 200 mL of n-hexane. The extraction thimble was plugged lightly with cotton wool and the Soxhlet apparatus assembled and allowed to reflux for about 4 hours on an electro-thermal heater. The sample was removed and the solvent recovered. The flask, free of n-hexane, was dried in an air oven (Griffin Oven) at 60°C for 30 minutes; the sample was transferred from the oven into a desiccator, allowed to cool and reweighed. The values generated were then used to calculate the percentage fat content of the sample.

Determination of Protein content of the sample (Kjeldahl method, AOAC, 1990)

Procedure: Finely ground 0.5g sample was weighed into a Kjeldahl digestion flask and anhydrous Na₂SO₄ and 1g of CuSO₄ added. 20 mL of conc. H₂SO₄ was also added and the mixture allowed digesting by heating under a fume cupboard until a clear solution was obtained. The digest was carefully transferred into a distillation flask and 100 mL of distilled water, 60 mL of 40% NaOH and piece of zinc added to digest in the distillation. The mixture was then distilled into a 250 mL conical flask containing 4% boric acid, 100 mL distilled water and 3-drops of screen methyl indicator. A total of 250 mL distillate was collected and titrated against 0.1 M HCl. The experiment was done in triplicate to reduce experimental error and the values generated were used to calculate the percentage of crude protein in the sample.

Determination of Carbohydrate content of the sample (Pearson, 1976)

Procedure: The carbohydrate content of the sample was determined by estimation using arithmetic difference. The Carbohydrate was calculated and expressed as the Nitrogen free extract as shown below:

% CHO = 100 - % (A + B + C + D + E), where A = Protein, B = Fat, C = Ash, D = Crude Fibre, and E = Moisture.

Phytochemical analysis

Determination of Alkaloid content of the sample (Harbone, 1973)

Procedure: Applying alkaline precipitation gravimetric method, 5.0g of the sample was weighed out into a conical flask; 200ml of 10% acetic acid in ethanol was measured out and added into the conical flask containing the sample. The mixture was allowed to stand at room temperature for 4 hours before it was filtered through a filter paper. The filtrate was then reduced to a quarter of its original volume by evaporation over a steam bath. The alkaloid in the extract was precipitated by drop wise addition of concentrated NH₄OH until full turbidity was obtained. The precipitate was washed with 1% NH₄OH solution, dried in the oven at 100°C for an hour; it was cooled in a desiccator and reweighed. By difference, the weight of alkaloid was determined and expressed as a percentage of the sample analyzed.

Determination of Flavonoid (Bohm and Kocipai, 1994)

Procedure: Using ethyl acetate precipitation method, 20g of the sample was weighed out and hydrolyzed by boiling in 100 mL of 2M hydrochloric acid solution for about 35 min. The hydrolysate was filtered to recover the filtrate which was treated with ethylacetate. The precipitated flavonoid was recovered by filtration using weighed filter paper (W₁). After drying in the oven at 100°C for 30min, it was cooled in a desiccator and reweighed (W₂). The values gotten were used to calculate the percentage of flavonoid in the sample.

Determination of tannin content of the sample (Van Buren, J. P. and Robinson, W. B. 1981)

Procedure: 5g of the finely ground sample was weighed and transferred into 250 mL conical flask and 50 mL of distilled water added and shook vigorously for an hour. The resulting solution was filtered into a volumetric flask and 5 mL of the filtrate pipetted out into a test tube. 0.1g of tannic acid was dissolved in 100 mL of water to form tannic acid solution. 5 mL of the tannic acid solution was pipetted out into another 50ml volumetric flask. A blank sample was also prepared using 5ml of distilled water. The three samples were incubated for 1.5 hours at 20 - 30°C and the sample was then filled with distilled water up to mark of 50 mL of the volumetric flask. The absorbance of the three samples was measured at 760 nm using spectronic 21D. The values generated were used to calculate the tannin content.

Determination of saponin (Obadoni and Ochuko, 2001)

Procedure: Using double solvent extraction method, 20g of the finely ground sample was weighed out into a conical flask. 100 mL of 20% aqueous ethanol was added into the sample, it was heated over a water bath for 4hrs with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 100 mL of 20% ethanol. The combined extract was reduced to about 40 mL over a water bath at 90°C, and the concentrate transferred into 250 mL separating funnel and 40 mL of pet-ether was added and shook vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. The

saponin was extracted with 60 mL of normal butanol. The combined extract were washed with 5% aqueous NaCl solution, and evaporated to dryness in a pre-weighed beaker (W_1) and was dried at 60°C in the oven and reweighed (W_2). The saponin content was determined by difference and calculated as a percentage of the original sample.

Determination of Phenol content of the sample (AOAC, 1990)

Procedure: Using the spectrophotometric method described by AOAC (1990), 5g of the finely ground sample was weighed out into a conical flask, and 50 mL of petroleum ether measured out and added into the same conical flask, and left to stand for 3hours. The mixture was filtered to obtain the residue. 50 mL of diethyl ether was added into the residue, the mixture were covered and heated in a water bath for 15 minutes. 5 mL of the extract was pipetted into a 50 mL flask, followed by 10 mL distilled water and 2 mL ammonium hydroxide solution. The mixtures were made up to mark and left to react for 30 minis for colour development. This was measured at 505 nm; the values generated were used to calculate the phenol content.

Determination of Hydrogen Cyanide (Onwuka, G. I., 2005)

Procedure: 20g of the sample was weighed out into a conical flask. 200 mL of distilled water was added into the sample and allowed to stand for 2 hours. The cyanide was distilled into a 20 mL of 10% NaOH solution by the aid of a condenser. 25 mL of the distillate was pipetted out, 25 mL of distilled water and 25ml of NH_3 solution were added into the distillate. It was titrated against 0.1M $AgNO_3$ and a persistent yellow colour observed. The titer value gotten was used to calculate the HCN content.

III. RESULTS AND DISCUSSION

Table 1 represents the result of proximate composition of the *Napoleona Vogelii* fruit. The result shows that the **moisture** content of the fruit is 69%. This was extremely high when compared to the moisture content of legumes, which ranges between 7.0 and 11.0% (Arkroyed and Doughty, 1984). However this value was closely related to those reported for fresh fruits in *Xylopi Acthiopica* which was 68.49 (Dike, 2010). The high moisture content of this sample implies that storage for a longer period will lead to spoilage, since higher moisture content could lead to food spoilage through increased microbial action (Onyeike, E. N; Olungwe, T. and Uwakwe, A. A., 1995).

The result also indicates that the **protein** content of the *Napoleona Vogelii* fruit is 1.93%. This is lower than in such protein rich food as beans, cowpea, pigeon peas, melon and pumpkin all ranging between 23.1 – 33.0 % (Olaofe, I. O. 1994). However, the result is within the same range as that reported for *Solanum Aethopicum*, which was 1.6% (Akubugwu, I. E.; Obasi, A. N.; Ginika, S. C. 2007). It can be deduced from the study that the low level of protein in this wild fruit could be due to high level of moisture content (Onwuka, 2005).

The proximate composition gives **crude fat** of the *Napoleona Vogelii* fruit a mean value of 2.1% (Table 1). This is relatively lower than values reported for soya beans, cashew nut and *beniseed* which are 23.5%, 36.7% and 42.3% respectively (Aremu *et al*, 2006 and Njoku *et al*, 2010). However, this is higher than that reported for *Solanum Aethopicum* and *Solalanun Macrocarpon*, which are 0.1% and 1.0% respectively (Shalom, *et al*, 2011). It can therefore be said that the level of fat in this wild fruit is good as it is within the accepted range described by Committee on Food Protection, National Academy of Sciences, Washington D. C. (CFP, 1996).

The **crude fibre** of the *Napoleona Vogelii Hook* fruit extract recorded a value of 16%, which is relatively higher than the values reported for *beniseed* and *African elemi* pulp with 6.2% and 1.39% respectively (Ekoh, U. P. 2009). Hence, the high level of crude fibre in this wild fruit indicates that it is good for human consumption, as CFP (1966) reported that diet low in fibre was undesirable.

The **ash** content of the fruit was determined to be 3.5% (Table 1) which is relatively close to the reported values of 3.7% and 4.2% for sesame and canarium album respectively (Qin, *et al*, 2009). The result implies that *Napoleona vogelii* fruit is a good source of minerals.

The **carbohydrate** content of 7.47% was recorded, and that is relatively low compared with the acceptable values for legumes 20 – 60% of dry weight (Arkroyed and Doughty, 1984). The low carbohydrate value of the sample could be due to high levels of moisture and crude fibre (Onwuka, 2005).

Phytochemical composition of the *Napoleona vogelii*

Table 2 represents the result of **phytochemical** composition of the *Napoleona Vogelii* fruit. The result shows that the **Alkaloid** content of the fruit is 0.8%. This is similar to the value of 0.850% reported for *African elemi* pulp (Ekoh, 2009), but above the permissible limit of 20 mg/100g, equivalent to 0.04% (Liener, 1994). Caution should be taken in the consumption of plant materials with very high concentration of Alkaloid because they could inhibit certain mammalian enzymes activities such as those of cyclic adenosine monophosphate (AMP) (Kalu *et al.*, 2011). Cooking lowers the alkaloid content of foods by 40 – 50% (Hotpkins, 1995). European Food Safety Authority (EFSA, 2011) stated that since cooking only lowers alkaloid content of foods by 40 – 50%, highly sensitive individuals should avoid this category of food entirely. Thus , as high as 0.8% alkaloid content of *Napoleona Vogelii* fruit implies that it is toxic and should be boiled or cooked to reduce the percentage alkaloid.

The **phytochemical** result indicates the **flavonoid** content of *Napoleona vogelii* Hook fruit is 4.65%. This value is relatively high compared to the value of 1.0% reported for *African elemi* pulp (Ekoh, 2009). However, George *et al* (2012) reported that high amounts of flavonoid help protect blood vessels from rupture or leakage, enhance the power of vitamin C; protect cells from oxygen damage and prevent excessive inflammation. High amounts of flavonoid do not appear to cause unwanted side effects. The level of flavonoid found in the *Napoleona Vogelii* fruit shows that it is essential for human consumption

The value obtained for **tannin** in this fruit was 333.4mg/kg (1.667mg/5g, Table 2). This value is higher than that reported for legume (Liener, 1994). The high tannin content in the sample implies severe nutritional challenge to animals or humans due to its affinity for certain digestive enzymes.

The **saponin** value is 0.75% (Table 2). This is quite high compared with the value of 0.370% reported for raw African elemi pulp (Eko, 2009). A high Saponin diet can inhibit dental and platelet aggregation in treatment of Hypercalciuria in human (excessive urinary calcium excretion, an antidote against acute lead poisoning (Shi, *et al* 2004). Saponin also decreases blood lipids, lower cancer risks and blood glucose response as well as posses antioxidant activity. Toxicology studies of saponin using relevant experimental models have established that even at an upper concentration of 3.5%, saponin was safe and failed to cause systemic side effect (Qin *et al*, 2009). More also, Muralidhara *et al* (1999), reported that when *Ferungreek seeds* containing 1% saponin was fed to rats, after 90 days of subchronic study, there was no side effect. Thus, it can be deduced from the above that the level of saponin in this edible plant is safe for man and animal consumption.

The **phenol** content of *Napoleona Vogelii* fruit showed a value of 3.8 mg/kg (0.019 mg/5g) (Table 2). This value is lower compared to what is reported for *S. nigrum*, *L. Var Virginium* leaf and seed (Akubugwu, *et al*; 2007). Phenols are unique properties that can be found in high levels in certain food that seem to affect children with Autism and individuals with sensitive digestive and immune system (Haslam, 1989). Hence, the low level of phenol in this fruit implies that it is consumable without the above listed side effects, since it is below the described oral lethal dose of approximately 70mg/kg (Haslam, 1989).

The value obtained for **hydrogen cyanide** in *Napoleona Vogelii* fruit is 3.342mg/20g and is lower than the 36mg/100g considered lethal dose for man (Mgbagwu, *et al* 2010). Hydrogen cyanide does not occur free but combines with sugars to form a compound known as cynogenic glycoside. Thus, *Napoleona Vogelii* fruit can be said to be safe for man and animal's consumption in terms of **hydrogen cyanide** level, as it is within permissible limits. Besides, HCN can be significantly reduced by boiling, heating and soaking (Siddhuraju, *et al*, 1996).

IV. CONCLUSION.

The study showed that *Napoleona Vogelii* Hook grown in farmlands in Ebonyi State of Nigeria contain a high level of beneficial nutrients such as minerals and fibres, and phytochemicals such as alkaloid, flavonoid, saponin and tannin. The study further revealed low level of toxicants such as phenol and hydrogen cyanide, with a high level of flavonoid, tannin, alkaloid and saponin. The fruit also showed a high level of moisture and crude fibre. The composition of *Napoleona Vogelii* fruit shows that the wild fruit is nutritionally and therapeutically valuable and have the potential of providing precursors for the synthesis of useful chemicals.

Table 1: Percentage Proximate Composition of *Napoleona Vogelii*

Nutrient	Percent value
Moisture	69
Protein	1.93
Fat	2.1
Crude fibre	16
Ash	3.5
Carbohydrate	7.47

Table 2: Phytochemical composition of *Napoleona Vogelii*

Parameter	Value
Alkaloid	0.8%
Flavonoid	4.65%
Tannin	333.4mg/kg
Saponin	0.75%
Phenol	3.8mg/kg
Hydrogen Cyanide	3.382 mg/kg

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