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Proximate and Mineral Analysis of Methanolic Leaf Extract of *Napoleona imperialis (mkpodu)*

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Authors' contributions

This work was carried out in collaboration between all authors. Author EOE designed the work and wrote the draft of the manuscript. Author BIO carried out the analysis while author IRO did the extraction. The three authors read the final manuscript and approved accordingly for publication.

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ABSTRACT

Napoleona imperialis is an evergreen non-timber plant that has wound healing and antihypertensive effect. The study was undertaken to evaluate the proximate and elemental analyses of the leaf of *Napoleona imperialis*. The mineral elements were analyzed using Atomic Absorption Spectrophotometer, equipped with air acetylene flame; spectrophotometer and flame photometer. The proximate analyses were carried out using standard methods. The proximate analysis of the leaves of *Napoleona imperialis* showed that it contained moisture 3.78%, crude fiber 8.03%, crude fat 6.45%, ash 5.2%, crude protein 13.13% and carbohydrate 63.44%. The mineral analysis of the leaves showed sodium (16.5947 ppm), potassium (3.3134 ppm), calcium (15.000 ppm), iron (8.350 ppm), copper (0.703 ppm) and zinc (8.0455 ppm). The presence of minerals like Ca, Fe, Cu, and Zn in concentrations admissible for human consumption Vis-a'-Vis World Health Organization Standard suggests its beneficial effect in enhancing metabolic processes via enzyme activation hence its ethno-medicinal potential.

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1. INTRODUCTION

Napoleona imperialis is a wild plant found in south eastern Nigeria. It is an evergreen non-timber plant that grows abundantly in bush fallows, secondary bushes and marginal lands in most of the tropical humid zones of West Africa, [1]. The plant belongs to the family known as the *Lecythidaceae*, along with the cannon ball tree (*Corrupita guianensis*), which grows in most regions of Nigeria, [2-4]. The tree is about 14.5 m high, 10 m in girth with a dense rounded crown and dark green foliage. The bark is grey to pale brown and darkens on exposure. The leaves have stout common stalk with broad and elliptic leaflets mostly rounded at the apex. It flowers between January and March, and is usually yellowish white and about 0.7- 1.10 mm long, crowded in compact branched stout central stalk. It fruits around April and is broadly globular. The fruits which are fleshy structures are attached directly to the main trunks and limbs, [5,6].

Though *Napoleona imperialis* is one of the lesser known plants, its economic importance has partially been reported by Dalziel [2], and Irvine [7]. These include the use of the roots for medicinal purposes and the twigs as traditional chew sticks. It has been reported that, different parts of the plant are used for different purposes in the region including mulching and fodder (leaves and twigs); and firewood, chewing sticks and ethno medicine (stem and root), [8,9]. The juice from the fruits and pods are consumed by man while the seeds are discarded.

Plants and plant products have gone a long way in providing the basic necessities of man. These necessities of man include: clothing, shelter, food as well as medicinal value. Almost every plant can be utilized in one way or the other to provide the materials which are necessary to the existence of man.

The proximate analysis of plant parts, foods, feeding stuffs and other compounds reveals the presence of protein, carbohydrate, fat, crude fiber and moisture in them [10]. 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 [11].

Knowledge of the nutritional and mineral composition of plants is now very essential as the global trend in nutrition and medicine is shifting towards the consumption of plant foods (fruits and vegetables) and medicinal plants (phyto-medicines) respectively, because the plant kingdom is reported to be full of large numbers of beneficial substances to both human and animal health [12-14].

A proper understanding of their proximate minera l and nutrient compositions will lower the over dependence of many communities and Industries on few arable crops for vegetables and herbal drugs. In addition, knowledge of their composition would enable one to know the better type of vegetables to eat or feed to animals at any given time.

2. METHODOLOGY

2.1 Sample Collection

Leaves of *Napoleona imperialis* plants were harvested from bushes in Mgbirichi community in Imo State, South-Eastern Nigeria, and were identified at the herbarium, University of Nigeria, Nsukka.

2.2 Preparation of Extracts

The leaves were removed from leaf branches, air dried at room temperature for 3 weeks until no trace of water was found in it. Leaves after air-drying were reduced to fine powder by milling. The powdered plant materials were subjected to extraction with 80% methanol for 48 hours. The methanolic extracts were concentrated using a rotary evaporator (Büchi, Rotavapor R-200) and allowed to paste using a water bath set at 40℃ and stored at 4℃ until used for mineral analysis. Dried sample of *N. imperialis* leaves were used for proximate analysis.

2.3 Proximate Analysis

The proximate compositions of the *N. imperialis* leaves were determined using standard analytical methods.

2.3.1 Determination of moisture content

The most commonly used method is the indirect distillation method employing drying oven. The

moisture content of the samples was determined using Association of Official Analytical Chemist (AOAC) method [15]. Porcelain/silica crucible was washed thoroughly and placed in oven to dry. 5 g-10 g of the sample was weighed into a pre-weighed, pre-dried and cooled crucible or dish, and then placed in the oven to dry at 70-80℃ for two (2) hours and at 100-110℃ (usually at 150℃) until a constant weight was obtained. Crucible and its content of dried sample were allowed to cool in a desiccator before weighing. The percentage moisture content is calculated using the equation below:

$$\%\text{Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where

W_1 = Initial weight of empty crucible
W_2 = Weight of empty crucible + sample before drying
W_3 = Weight of empty crucible + sample after drying

2.3.2 Determination of ash content

Crucibles were thoroughly washed, cleaned and placed in a hot air-circulation oven for 2 hours and cooled to room temperature in a desiccator. The empty crucibles were then transferred to the muffle furnace to burn off all organic matter and also to stabilize the weight of the crucibles at the temperature range of 550℃-600℃, before cooling at room temperature in a desiccator. The pre-weighed crucible was then transferred into a pre-heated furnace and ash for 3-5 hours at 600℃. Crucible and its content (ash) are reweighed after cooling in a desiccator. Then percentage ash content was calculated as:

$$\%\text{Ash content} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times \frac{100}{1}$$

$$= \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where

W_1 = Weight of empty crucible
W_2 = Weight of empty crucible + Sample before ashing
W_3 = Weight of empty crucible + Ash

2.3.3 Determination of fiber content

The sample (2 g) was weighed accurately in the fiber flash and 100 ml of 0.25N H_2SO_4 was used to dissolve the mixture and heated for 1hr with the heating mantle. The hot mixture was filtered through a fiber sieve cloth. The filtrate obtained was thrown off and the residue was returned to the fiber flash to which 100 ml 0f 0.31N NaOH was added and heated under reflux for another 1 hour. The mixture was filtered through a fiber sieve cloth and 10 ml of acetone was added to dissolve any organic constituent. The residue was washed with about 50 ml hot water twice on the sieve cloth before it was finally transferred into the crucible. The crucible and the residue was oven dried at 105℃ overnight to dry off moisture. The oven dried crucible containing the residue was cooled in a desiccator and later weighed to obtain the weight W_1. The crucible with weight (W_1) was transferred to the muffle furnace for ashing at 550℃ for4 hours. The crucible containing white or grey ash (free of carbonaceous material) was cooled in the desiccator and weighed to obtain W_2. The difference W_1-W_2 gives the weight of fiber AOAC, [16]. The percentage fiber was obtained by the formula:

$$\%\text{Fiber Content} = \frac{W_1 - W_2}{\text{Weight of the sample}} \times \frac{100}{1}$$

Where

W_1 = Weight of empty crucible
W_2 = Weight of empty crucible + Sample before ashing
W_3 = Weight of empty crucible + Fiber after ashing

2.3.4 Determination of crude Fat/Lipids

Using the AOAC [17] method, 2-5 g of finely ground dried sample was weighed into a thimble and place into a soxhlet apparatus containing a glass wool. A dried pre-weighed 500 ml round bottom flask (containing few crystals of anti-bumping chips) was attached to the base of the extractor and clamped to a retort stand. About 300 ml of petroleum ether then was added in the barrel containing the thimble and the assembled unit placed on a electro-thermal heater with the top of the extractor connected to a reflux condenser and source heater to enable the solvent in the flask to boil and extract the lipids in the sample for about 3-6 hours on completion, then the thimble was removed and reclaimed ether by distillation. Flask and the extracted lipid were placed in an oven at 70℃ for few minutes to completely remove all the ether residues and cooled in desiccators before reweighing. The

process of drying and cooling was continued until a constant weight was obtained. The percentage lipid was calculated thus:

$$\text{Crude Lipid (\%)} = \frac{\text{Weight of lipid } (W_1) \text{ X } 100}{\text{Weight of the sample (W)}}$$

Where

W_1 = weight of flask and content after extraction – weight flask before extraction
W_2 = Weight of flask + oil
W = Weight of sample used

2.3.5 Determination of crude protein content

Kjeldahl method was used for the determination of crude protein. Exactly 8 g of the sample and 3 g of copper Sulphate catalyst and 25 mls of concentrated sulphuric acid was heated over a burnsen flame in a fume cupboard to expel any poisonous gas. It was then heated with shaking at intervals for 1 hr until the mixture became clear. 400 ml of distilled water was added followed by the addition of 50 ml of 2% boric acid with 1 ml of methyl red indicator. 75 ml of 50% NaOH was added to make the solution alkaline. The ammonia was distilled into the boric acid solution. 250 mls of the distillate was collected after washing the walls of the receiver and the condenser. The distillate was titrated with 0.1N Sulphuric acid. The percentage protein was calculated from the percentage nitrogen of the sample as follows:

$$\text{\% Nitrogen} = \frac{\text{ml Acid} - (\text{N acid x } 1.4)}{\text{Weight of the sample}}$$

Therefore,

% Crude Protein
= %Nitrogen x 6.25
= (Titre value x Atomic Mass of Nitrogen x Normality of HCl x 4) x 6.25
= (Titre value x 14 x 0.01 x 4) x 6.25

2.3.6 Determination of total carbohydrates (By difference)

By this, the total carbohydrate content of sample is the percentage remaining after the moisture, ash, lipid, fiber and protein contents have been removed.

Mathematically, it is given as;

%Total Carbohydrate = 100% - % (Protein + Lipids + Fiber + Ash + Moisture Content)

2.4 Mineral Analysis

The mineral elements were analyzed using S240 varian Atomic Absorption Spectrophotometer, eq uipped with nitrous oxide oxidant gas, acetylene gas, air oxidant gas and distilled water. All determinations were done in duplicates and values of minerals were reported in ppm.

2.4.1 Reagents

All reagents used were of analytical grade and various elemental standard solutions were prepared for use.

2.4.2 Principle of atomic absorption spectrophotometer

Atomic absorption working principle is based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelength a source lamp composed of that element is used, making the method relatively free from spectral or radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

2.5 Sample Digestion

2 g was weighed out of the dried sample into a digestion flask and add 20 ml of the acid mixture (650 ml conc. HNO_3) 80 ml per choric acid 20 ml concentrated H_2SO_4. Heated the flashed until a clear digest was obtained. Digest is diluted with distilled water to the 25 ml mark Appropriate dilutions were then made for each element.

2.6 Procedure

The sample was thoroughly mixed by shaking, and 100 ml of it was transferred into a glass beaker of 250 ml volume. The sample was prepared into the oxidizing air-acetylene flame or nitrous oxide acetylene. When the aqueous sample was aspirated, the sensitivity for 1% adsorption was observed.

3. RESULTS AND DISCUSSION

Proximate result (Table 1) shows that the moisture content of the leaf is 3.76%. This was however low when compared to the moisture

content of *Napoleona* fruit, pulp, jam and seed which were recorded to be 69%, 87.5%, 80.5% and 4.8%, respectively [18-20]. The high moisture content of a 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 [21]. Thus, low moisture content of this sample would hinder the growth of microorganism and storage life would be high [22].

The ash content of the leaf was 5.20% (Table 1) which is relatively close to the reported value of 3.5% for the fruit, 0.35% for pulp, 0.25% for jam and 3.52% for the seed [18-20]. This result of Ash content determination used as an approximate indicator of the total content of mineral elements [18] shows that *Napoleona* leaf can be used as a source of mineral supplement.

The dietary fiber of the *Napoleona imperialis* leaf extract recorded a value of 8.03% (Table 1). Although comparatively lower than 16% value reported for Napoleona fruit, it is however higher than the reported value of 5.05% for Napoleona fruit-pulp, 2.5% for the jam, and 3.60 for Napoleona seed [18-20], and relatively close to the reported value of 8.8% for *Securinega virosa* leaves [23]. Hence, the high level of dietary fiber in this leaf indicates that it is good for human consumption, as CFP [24] reported that diet low in fiber was undesirable. The dietary fiber can lower serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer [25,26]. The 8.3% crude fiber content of *N. imperialis* leaves give the leaves its medicinal properties. Thus, could act as antihypertensive agents, and be used in management of other health complications.

Crude lipids are the principal sources of energy but should not be more than 30 calories so as to avoid obesity and other related diseases. A diet providing 1-2% of its caloric energy as fat is said to be sufficient in human beings as excess for consumption above 35% as recommended in Dietary Guidelines for Americans 2003, is implicated in certain cardiovascular disorders for such as atherosclerosis, cancer and aging [27]. The proximate composition gives dietary fat of the *Napoleona imperialis* leaf a value of 6.45% (Table 1). Although this is comparatively higher than values reported for *Napoleona* fruit, pulp, jam and seed which were 2.1%, 1.0%, 0.2% and 4.95%, respectively [18-20]. It is however lower than values reported for water spinach leaves and *Senna obtusfolia* which are 11% and 12% respectively [28]. It can therefore be said that the level of fat in this leaf is good as it is within the accepted range (less than 10%/day) described by Committee on Food Protection, National Academy of Sciences, Washington D. C. [24].

The result indicated that *Napoleona imperialis* leaves contained appreciable amount of crude protein content (13.139%) making it to be a good source of protein supplement for man and livestock. This is higher than that reported for *Securinega virosa* leaves which was 1.98% [23], and higher values reported for Napoleona fruit, pulp, jam and seed which were 1.93%, 1.3%, 0.4% and 11.73%, respectively [18-20].

Table 1. Percentage proximate composition of *Napoleona imperialis* leaves (n=3)

Nutrient	Percent value
Moisture	3.76±0.04
Ash	5.20±0.06
Crude fiber	8.03±0.10
Crude fat/lipid	6.45±0.07
Crude protein	13.13±0.18
Carbohydrate	63.44±0.31

The carbohydrate content of 63.44% was recorded, and that is relatively close to the acceptable values for legumes 20 – 60% of dry weight [29]. The result is much higher when compared to the 7.47%, 9.9% and 15.7% values reported for the Napoeona fruit, pulp and jam respectively, but lower than 71.40% value for the seed [18-20]. However this value was closely related to that reported for *Securinega virosa* leaf which was 64.25% [23]. Carbohydrate deficiency causes depletion of body tissues. Sufficiency of Carbohydrate is however necessary for optimum functioning of the brain, heart, nervous, digestive and immune systems, [30]. The leaf is a good source of supplementary carbohydrate for man and livestock.

The result of mineral analysis (Table 2) shows that the leaves of *Napoleona imperialis* leaf contain 15.000 ppm of Calcium. Calcium is good for growth and maintenance of bones, teeth and muscles.

The potassium content of *Napoleona imperialis* leaf (3.3134 ppm) is lower compared with 17.50 g/kg recorded for Napoleona seed [20], and 3.67 mg/100 g found in *Securinega virosa* [23], 14.55 mg/100 g found in *Indigo feraastragelina* leaves. The Sodium content of

Napoleona imperialis is 16.5947 ppm. This lower than 16.10 g/kg of Napoleona seed [20]. Sodium and potassium in concentrations admissible for human consumption (Na: 0.5 to 2.4 g/day and K: 2 g/day), are very essential forosmotic pressure maintenance; maintenance of blood volume and blood pressure [31].

Table 2. Mineral composition of *Napoleona imperialis* leaves (n=3)

Mineral	Value (ppm)
Calcium	15.000±0.010
Potassium	3.3134±0.001
Sodium	16.5947±0.004
Iron	8.350±0.046
Copper	0.703±0.015
Zinc	8.045±0.025

Copper as a coenzyme plays several biochemical roles including assistance in collagen production. It is a very powerful pro-oxidant and catalyzes the oxidation of unsaturated fats and oils as well as ascorbic acid; functions as a cofactor for energy metabolism. Copper content found in the sample was 0.706 ppm. This is higher than 0.47 mg/100 g, 0.02 mg/100 g but lower than 4.39 mg/100 g for *Securinega virosa,* 1.28 mg/100 g for *Mucuna sloanei*, *Indigo feraastragelina* and *T*. *terrestris* leaves respectively [32].

Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and oxidation of carbohydrates, protein and fats [33]. The iron content of *Napoleona Imperialis* is 8.350 ppm which is higher than values reported for some cultivated vegetables such as *lettuce* (0.7 mg/100 g) and Cabbage (0.3 mg/100 g). This shows that N. *imperialis* leaves could be a potential source of Fe supplement for human and livestock and also help in boosting of the blood level in anemic conditions.

Zinc is an essential element in the nutrition of man where it functions as an integral part of numerous enzymes or as a stabilizer of molecular structure of sub-cellular constituents and membrane. Zinc participates in the synthesis and degradation of carbohydrates, lipids, protein and nucleic acids and has shown to play an essential role in polynucleotide transcription and translation and thus in the process of genetic expression [31]. The zinc content of the leaves sample is 8.045 ppm, this is relatively close to 0.85 mg/100 g value *Securinega virosa* leaves, but much higher than 0.25 mg/100 g and 0.11 mg/100 g of *Mucuna sloanei* and *I. astragelina* leaves respectively. The presence of Zn is of special interest in view of the importance of Zn in the diet of animals and humans.

4. CONCLUSION

The study showed that *Napoleona imperialis* leaves contain a high level of beneficial nutrients such as carbohydrate, protein and fiber, thus, the plant might be explored as a viable supplement in both animal and human food. Mineral analysis shows that consumption of the leaves cannot lead to any health hazards but would rather be of beneficial effect to health as shown by the mineral composition (especially the trace elements). The leaf is nutritionally and therapeutically valuable and has the potential of providing precursors for the synthesis of useful bio-molecules e.g. Fe for hemoglobin; hence could be useful in the treatment of anemia. Na and K concentration confirms that the leaf could be useful in maintenance of osmotic pressure thus antihypertensive property of the leaf. Ca is essential for the maintenance and building up of teeth and bone. Cu affects metabolism of Fe. Thus the presence of these minerals in concentration admissible for human consumption indicates the beneficial effects of this leaf in ethno- or phyto-medicines.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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