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EVALUATION OF THE PROXIMATE, MINERAL AND VITAMIN COMPOSITIONS OF *ACALYPHA WILKESIANA* LEAF

Nwolisah, O.S.[1*], Enemor, V.H.A.[1], Odili, C.E.[1], Ehichanya, C.A.[1], Obih, M.S.[1] and Okochi, C.V.[1]

[1] Department of Applied Biochemistry, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria.

***Corresponding author's email:** ogenwolisah@gmail.com

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ABSTRACT

Acalypha wilkesiana, commonly known as "Copper leaf" and belonging to the *Euphorbiaceae* family, is a tropical shrub renowned for its ornamental beauty and diverse cultural uses. Its leaves are frequently utilized by locals for various medicinal purposes. This study evaluated the proximate, mineral and vitamin compositions of *Acalypha wilkesiana* leaf. Proximate analysis was conducted using standard methods, while the mineral concentrations were determined by means of atomic absorption spectrophotometer, and vitamins were quantified using appropriate colorimetric and titration methods. The percentage proximate composition for moisture, ash, fiber, protein, fat, and carbohydrates were 13.75±1.75, 12.75±0.75, 8.00±0.60, 3.65±0.05, 12.90±0.10, and 48.95±0.45, respectively, while the energy value was 326±1.10 kcal/g. The mineral analysis revealed that the leaf is rich in sodium, potassium, phosphorus, magnesium, manganese, calcium and zinc, with concentrations (mg/g) of 3.96±0.05, 2.89±1.79, 2.73±0.25, 1.94±0.08, 1.40±1.01, 1.31±0.42 and 0.30±0.14, respectively. The fat soluble vitamins ranged from 4.12±0.26 (for vitamin D) to 17.28±1.00 mg/kg (for vitamin E), while the water soluble vitamins ranged from 0.05±0.02 mg/100g (for vitamin B_2) to 69.61±1.77 mg/kg (for vitamin C). The results show that *Acalypha wilkesiana* leaf is a good source of nutrients, minerals and vitamins, and could serve as a dietary supplement for addressing nutritional deficiencies.

Keywords: *Acalypha wilkesiana*, *Euphorbiaceae*, Vitamins, Minerals, Nutrient

INTRODUCTION

Acalypha wilkesiana is a member of the *Euphorbiaceae* family, commonly known as the 'spurge' family. Some of its popular names include copper leaf, Irish petticoat, and Jacob's coat (Christman, 2004). The term "Acalypha" originates from Ancient

Greek, as an alternative to 'Akelephe,' showing its resemblance to nettle. In Nigeria, it's referred to as "Jiwene" and "Jinwinini" by the Hausas, and "Aworoso" by the Yorubas. Despite being native to Fiji and the South Pacific islands, *Acalypha wilkesiana* is now widely cultivated as an ornamental plant across the globe, especially in the tropical regions of Africa, America, and Asia (Sagun et al., 2010).

Acalypha wilkesiana, like other species in its genera, exhibits a wide range of biological activities, spanning from local to systemic effects (Al-Attar, 2010). In West Africa, *Acalypha wilkesiana* is used for treating headache and cold. In Nigeria, the cold leaf extract is applied as an herbal remedy for bathing babies with skin infections (Alade and Irobi, 1993). The essential oils distilled from leaf of the plant have been found to possess phytochemical and microbiological activities against *Staphylococcus aureus* and *Klebsiella aerogenes* (Agu, 1980, Adesina et al., 1980). The expressed juice or boiled concoction of the plant is used for the treatment of gastrointestinal disorders and fungal skin infections, including *Pityriasis versicolor*, *Impetigo contagiosa*, *Candida intetrigo*, *Tinea versicolor*, *Tinea corporis*, and *Tinea pedis*. Additionally, the leaves are consumed as vegetables for managing hypertension in Southern Nigeria (Akinde, 1986, Ogundaini, 2005, Ikewuchi et al., 2008).

Given that the leaves of *Acalypha wilkesiana* are traditionally consumed, it is important to assess its nutritional content. Therefore, the aim of this study was to evaluate the proximate, mineral and vitamin compositions of *A. wilkesiana* leaf.

MATERIALS AND METHODS

Plant Materials Preparation

Fresh *Acalypha wilkesiana* leaves were collected from a garden in Agbor quarters, Alor, Anambra State, Nigeria. The plant sample was identified and deposited in the hebarium (NAUH-241[a]) of the Department of Botany, Nnamdi Azikiwe University Awka. The freshly collected leaves were thoroughly washed in running tap water and rinsed in distilled water before they were cut into small bits, then shade-dried at room temperature for 10 days, ground to powder and preserved in an airtight bottle in a refrigerator at 4 °C until use.

Proximate Analysis

The moisture, ash, crude fiber, crude protein, and crude fat contents of *A. wilkesiana* leaf samples were determined in triplicates using the method described by AOAC (2004). The carbohydrate content was determined by difference method as reported by Yerima and Adamu (2011). The total carbohydrate content was calculated as: Total Carbohydrate (%) = 100 - (% Moisture + % Ash + % Crude Fiber + % Crude Protein + % Fat). The Atwater method of energy calculation was used to determine the calorific value of *A. wilkesiana* leaf as reported by Chaney (2006). The total energy value was calculated as:

Total energy (Kcal) = 4 x (Protein + Carbohydrate) + 9 x (Lipid)

Mineral Analysis

The mineral composition was determined using the method outlined by APHA (American Public Health Association) in 1995.

Preparation of Sample and Digestion Mixture

Two grams (2 g) of finely powdered sample was measured into a porcelain crucible and incinerated at 400°C for 4 hours in a muffle furnace. The sample was removed from the furnace and allowed to cool in a desiccator. Next, 0.5 ml of 1 M HNO_3 solution was added to the ash obtained and evaporated to

dryness on a hot plate. The crucible was returned to the furnace and incinerated at 400°C for 20 minutes until grayish ash was obtained. The sample was allowed to cool in a desiccator. Subsequently, 15 ml of hydrochloric acid (HCl) was added to the ash to dissolve it, and the volume was adjusted to 100 ml with distilled water. The solution was filtered into a 100 ml volumetric flask, and the volume was made up to 100 ml with distilled water.

Determination of Mineral Composition

The sample solution was utilized for the estimation of minerals such as sodium, potassium, phosphorus, zinc, magnesium, calcium and manganese using the Varian AA240 Atomic absorption spectrophotometer. The metal concentration detected was expressed in parts per million (ppm). The concentration of each element in the sample was calculated using the formula:

Metal (mg/100g) = (Concentration of metal in ppm × volume used) / weight of sample.

Quantification of Vitamins

Vitamin A

Vitamin A was quantified using the method described by AOAC (1990). The assay was conducted in the dark to prevent light interference. One gram of the sample was weighed and macerated in 20 ml n-hexane solution. The mixture was refluxed for 10 minutes at a temperature of 60°C. After refluxing, 3 ml of the upper hexane extract was transferred into a dry test tube in duplicates and evaporated to dryness at 60°C. Subsequently, 0.2 ml acetic anhydride chloroform was introduced and mixed with 2 ml of trichloroacetic acid reagent. The absorbance was read at 620 nm in a spectrophotometer (Genesys 10UV). Vitamin A content was expressed as mg/kg.

$$\text{Concentration of Vitamin A in sample} = \frac{\text{Absorbance of sample} \times \text{Conc. of standard}}{\text{Absorbance of standard}}$$

Vitamin D

Vitamin D was assayed according to the method of AOAC (1990). The sample (0.1g) was weighed into a 25 ml volumetric flask. A solution consisting of chloroform and methanol in a 1:9 ratio was rapidly added, mixed and diluted with the solution mixture until it reached the mark. Following that, 1.6 ml of 0.25 N HCl, 0.5 ml of 15% trichloroacetic acid (TCA), and 0.5 ml of 0.375% thiobarbituric acid (TBA) were added to the solution. The absorbance was read at 620 nm.

$$\text{Concentration of Vitamin D in sample} = \frac{\text{Absorbance of sample} \times \text{Conc. of standard}}{\text{Absorbance of standard}}$$

Vitamin E

The estimation of Vitamin E in the sample was conducted using the Emmerie-Engel reaction, as reported by Rosenberg (1992).

Sample Preparation

The sample (2.5 g) was measured in 50 ml 0.1N sulphuric acid, vortexed and left to stand overnight. The contents were vigorously agitated and filtered through Whatman No.1 filter paper. Aliquots of the resulting filtrate were used for the estimation. The sample (1.5 ml) was carefully dispensed into a test tube and centrifuged for one minute. Anhydrous ethanol (1.5 ml) was added and shaken vigorously. Xylene (1.5 ml) was also added and shaken vigorously. The test tube was centrifuged for another 10 minutes. The xylene layer (1.5 ml) was carefully transferred into another test tube. To each of these tubes, 1 ml of dipyridyl reagent was introduced and mixed thoroughly. The mixture (1.5 ml) was dispensed into a

cuvette, and the absorbance was measured at 460 nm.

$$\text{Concentration of Vitamin E in sample} = \frac{\text{Absorbance of sample x Conc. of standard}}{\text{Absorbance of standard}}$$

Vitamins B_1 and B_2

Vitamins B_1 and B_2 were analyzed using the method described by Kirk and Sawyer (1991). In this analytical procedure, 1g of the sample was accurately weighed and placed in a conical flask. It was dissolved by adding 100 ml of deionized water. The solution was shaken thoroughly and heated for 5 minutes. After heating, it was allowed to cool and subsequently filtered to remove any particulate matter. The resulting filtrate was poured into a cuvette. To determine the concentration of each vitamin, the spectrophotometer was set to specific wavelengths: Vitamin B_1 (Thiamine) was measured at 261 nm and vitamin B_2 (Riboflavin) was measured at 242 nm.

$$\text{Concentration (mg \%)} = \frac{\text{A x D.F x volume of cuvette (5)}}{\text{E}}$$

Where A= absorbance; E= extinction coefficient (25); DF = dilution factor

Vitamin B_3

Vitamins B_3 was analyzed using the method described by Kirk and Sawyer (1991). Five grams of the sample was dissolved in 20 ml of anhydrous glacial acetic acid and gently warmed. Subsequently, 5 ml of acetic anhydride was added, and 2-3 drops of crystal violet solution were introduced as indicator. The solution was titrated with 0.1M perchloric acid until a greenish-blue color was attained.

$$\text{Vitamin } B_3 \text{ (mg \%)} = \text{Titre value x } 0.0122/0.1$$

Vitamin B_6

The analysis of Vitamin B_6 was conducted using the method outlined by Kirk and Sawyer (1991). Five grams (5 g) of the sample was dissolved in 5 ml of anhydrous glacial acetic acid and 6 ml of 0.1 M mercury II acetate solution. Two drops of crystal violet were included as indicator, and the mixture was titrated with 0.1 M perchloric acid until it reached a green color endpoint.

$$\text{Concentration (mg\%)} = \frac{\text{A x D.F x volume of cuvette (5)}}{\text{E}}$$

Where A= absorbance; E= extinction coefficient (25); DF = dilution factor

Vitamin B_{12}

The analysis of Vitamin B_{12} was conducted using the method outlined by Kirk and Sawyer (1991). In preparing the sample, 0.1 ml of the sample was weighed into a separator. In the separator, 5 ml of water was added, thoroughly mixed, and then extracted with 5 ml of chloroform. The water layer was discarded, and the chloroform was transferred to a dry 50 ml volumetric flask. It was passed through anhydrous sodium sulfate to remove any residual water and then brought up to a total volume of 50 ml with chloroform.

Assay

Two milliliters (2 ml) of the sample were dispensed into a test tube and 2 ml of a 0.2% solution of phenyl hydrazine (in a mixture of hydrochloric acid and alcohol with a 1:5 v/v ratio) was added, and the contents were thoroughly mixed. Subsequently, the test tube was heated in a water bath until it was nearly dry, after which it was allowed to cool at room temperature. Also, 2 ml of a solution mixture (comprising ammonia and alcohol in a 1:1 ratio) and 1 ml of pyridine were added to the test tube. The absorbance of the solution was measured at 635 nm. Calculation:

$$\text{Concentration (mg\%)} = \frac{\text{A x D.F x volume of cuvette (5)}}{\text{E}}$$

Where A= absorbance; E= extinction coefficient (25); DF = dilution factor

Vitamin C

The analysis of Vitamin C was conducted using the titrimetric method described by Kirk and Sawyer (1991). One gram sample was homogenized in a solution containing 6% EDTA/TCA. After filtration, the resulting mixture was employed for analysis. To this, 20ml of 30% KI solution was added, and it was titrated using 0.1M $CUSO_4$ solution until a black coloration appeared, indicating the endpoint. The concentration of ascorbate in the sample was calculated and expressed in terms of mg/kg of sample. 1ml of 0.1 mole $CuSO_4$ is equivalent to 0.88mg vitamin C.

$$\text{Vitamin C (mg/kg)} = \frac{\text{1 x 0.88 x Titre-Blank}}{\text{Weig}}$$

RESULTS

Proximate composition of *A. wilkesiana* leaf

Table 1 presents the proximate composition of *Acalypha wilkesiana* leaf. The highest composition was observed for carbohydrates (48.95±0.45%), followed by moisture (13.75±1.75%), fat (12.90±0.1%), ash (12.75±0.75%), fiber (8.00±0.60%), with protein being the least (3.65±0.05%). The energy value was 326±1.10 kcal/g.

Table 1. Proximate composition of *A. wilkesiana* leaf

Parameter	Composition (%)
Ash	12.75±0.75
Moisture	13.75±1.75
Crude Fiber	8.00±0.60
Protein	3.65±0.05
Fat	12.90±0.10
Total Carbohydrate	48.95±0.45
Energy (kcal/g)	326±1.10

Values are expressed as means ± standard error of mean in triplicate analysis

Mineral composition of *A. wilkesiana* leaf

The mineral content (mg/g) in the leaf of *Acalypha wilkesiana* is presented in Table 2. The result shows that sodium (3.96±0.05 mg/g) had the highest value, followed by potassium (2.89±1.79 mg/g), phosphorus (2.73±0.25 mg/g), magnesium (1.94±0.08 mg/g), manganese (1.40±1.01 mg/g) and calcium (1.31±0.42 mg/g). However, zinc was found to in the least amount (0.30±0.14 mg/g).

Table 2. Mineral composition of *A. wilkesiana* leaf

Minerals	Composition (mg/g)
Sodium (Na)	3.96±0.05
Potassium (K)	2.89±1.79
Calcuim (Ca)	1.31±0.42
Magnesium (Mg)	1.94±0.08
Manganese (Mn)	1.40±1.01
Zinc (Zn)	0.30±0.14
Phosphorus (P)	2.73±0.25

Values are expressed as means ± standard error of mean in triplicate analysis.

Vitamin content of *A. wilkesiana* leaf

The result of the concentration of vitamins found in *A. wilkesiana* leaf is shown in Table 3. The quantification of vitamin assays revealed the presence of fat and water soluble vitamin content in *A. wilkesiana* leaf. The fat soluble vitamins ranged from 4.12±0.26 to 17.28±1.00 mg/kg, while the water soluble vitamins ranged from 0.05±0.02 mg/100g to 69.61±1.77 mg/kg.

Table 3. Vitamin composition of *A. wilkesiana* leaf

Vitamins	Concentration
Vitamin A (mg/kg)	6.11±1.09
Vitamin D (mg/kg)	4.12±0.26
Vitamin E (mg/kg)	17.28±1.00
Vitamin B_1 (mg/100g)	0.06±0.05
Vitamin B_2 (mg/100g)	0.05±0.02
Vitamin B_3 (mg/100g)	1.17±0.41
Vitamin B_6 (mg/100g)	0.32±0.06
Vitamin B_{12} (mg/100g)	5.66±0.81
Vitamin C (mg/kg)	69.61±1.77

Values are expressed as means ± standard error of mean in triplicate analysis.

DISCUSSION

The proximate composition of *A. wilkesiana* leaf in Table 1, showed that the leaf had a moisture content of 13.75±1.75%. The shelf life and longevity of medicinal plants can be influenced by factors such as moisture, temperature and other environmental conditions (Idris et al., 2019). The moisture content in food serves as an index of its water activity, providing a measure of stability and susceptibility to microbial contamination (Uyoh et al., 2013). According to Hammond et al. (2015), plant materials with moisture levels exceeding 15% are at risk of bacterial or fungal contamination. This shows that *A. wilkesiana* leaf can be preserved with minimal risk of microbial invasion, thereby extending its shelf life. The presence of inorganic constituents in plants is indicated by their ash content, a fact further supported by the mineral compositions (Idris et al., 2019). *A. wilkesiana* showed an ash content of 12.75±0.75%, which is nearly three times the value found in the leaf of *Acalypha hispida* (3.55%) and surpasses the value in the root of *A. hispida* (8.13%) as reported by Okonwu and Ahunanya (2020).

The crude fiber content determined in this study was 8.00±0.60%, a value comparable to that reported for leaves of other Acalypha species such as *Acalypha indica* leaf (8.97%), *Acalypha hispida* (10.25%) and *Acalypha marginata* (11.50%) (Osibote et al. 2020). The presence of crude fiber in plants contributes to the absorption of trace elements in the gut and promotes bowel movement (Abolaji et al., 2007). According to Dillard and German (2000), fiber increases fecal bulk, thereby diluting heightened colonic bile acid concentrations resulting from a high-fat diet. The identified crude fiber content in *A. wilkesiana* supports its potential in managing digestive disorders. Given the edibility of *A. wilkesiana* leaves, they could prove beneficial in addressing ailments related to the colonic environment. The determined crude fat content of *A. wilkesiana* was reported to be 12.9 ± 0.10%. This value is higher than fat contents observed in *A. hispida* roots and leaves, reported by Okonwu and Ahunanya (2020). Fats significantly contribute to the differences in the gross energy of various food substances, providing 9 kcal/g compared to carbohydrates and proteins,

which yield about 5 kcal/g (Idris et al., 2019). Dietary fats also play a role in enhancing the palatability of food and aiding in the absorption and retention of flavors (Antia et al., 2006).

The crude protein content of *A. wilkesiana* leaf obtained from this study was 3.65±0.05%. This value obtained is in tandem with that reported for *A. wilkesiana* leaf by Iyamu et al. (2021), however, it is higher than the protein content of aqueous extract of *A. wilkesiana* reported by Omage et al. (2013). The total carbohydrate content of *A. wilkesiana* leaf was determined to be 48.95±0.45%. This value exceeded the recorded carbohydrate content for various edible vegetables, such as eggplant (2.0%), carrot (6.0%), green chili (5.9%), onion (12.2%) and Tomato (1.4%), as reported by Mina et al. (2016). Consequently, the leaves of A. wilkesiana could serve as a substantial source of energy. The energy value was determined to be 326.5 kcal/100g. This calorific value is higher than values of some wild edible vegetables reported by Datta et al. (2019), which ranged from 80.53 to 148kcal/g. Plants with high calorific values are considered beneficial for a balanced diet (Datta et al., 2019), indicating that *A. wilkesiana* can be utilized as a food source or included as part of dietary supplements.

The mineral composition of *A. wilkesiana* leaf sample shown in Table 2, revealed appreciable amounts of sodium (3.96±0.05 mg/g) and potassium (2.89±1.79 mg/g). Sodium and potassium are primary cations found in both extracellular and intracellular fluids, playing crucial roles in maintaining electrolyte balance within the body (Robert et al., 2003). These values are observed to be moderate yet higher than those reported for *A. hispida* leaf and root (Okonwu and Ahunanya, 2020), and aqueous extracts, ethanol extracts and powdered form of *A. wilkesiana* leaf reported by Omage et al. (2013).

The phosphorus content of *A. wilkesiana* is 2.73±0.25 mg/g. A deficiency of phosphorus can lead to incomplete calcification of teeth, failure of dentin formation, and increased susceptibility to caries (Ghosh et al., 2016). Thus, *A. wilkesiana* can be considered as a source of phosphorus, contributing to dental health. The magnesium content (1.94 ±0.08 mg/g), is in tandem with values obtained from *A. wilkesiana* Muell arg. and *A. wilkesiana* Java white, as reported by Akinloye et al. (2016).

The calcium quantity in *A. wilkesiana* leaf (1.31±0.42 mg/g) closely aligns with values obtained from leaves and roots of *A. hispida and A. wilkesiana*, as reported by Okonwu and Ahunanya (2020). The moderate quantity of calcium present in this plant is considered healthy, as caution must be taken in choosing calcium-rich foods due to the fact that approximately 5% of kidney stones are composed predominantly of calcium compounds (Sodamade et al., 2021). The manganese content (1.40 ± 1.01 mg/g) was higher than values obtained from other Acalypha species, *A. wilkesiana* Muell arg. and *A. wilkesiana* java white as reported by Akinloye et al. (2016). The zinc content in *A. wilkesiana* leaf (0.30±0.14 mg/g) closely aligns with values obtained from ethanol extract of *A. wilkesiana* leaf reported by Iyamu et al. (2021). Zinc deficiency is associated with dental caries (Harris et al., 2008), and delayed healing of wounds (Touger-decker and Loveren, 2003). This shows that *A. wilkesiana* leaf can be considered as a source of manganese and zinc.

The vitamin composition presented in Table 3, revealed the presence of fat and water soluble vitamin content in *A. wilkesiana* leaf. Among the fat-soluble vitamins,

vitamin E exhibited the highest concentration (17.28±1.00mg/kg). The high concentration of fat-soluble vitamin E shows the pharmacological importance of the plant, as it functions as a potent antioxidant, protecting cells from damage by free radicals (Lukaski, 2004). The vitamin A and D contents were 6.11±1.09 mg/kg and 4.12±0.44 mg/kg, respectively, and this suggests that *A. wilkesiana* is a good source of retinol and is essential for bone maintenance.

The vitamin B complex (thiamin, riboflavin, niacin, pyridoxine, and cobalamin) was also detected in the leaf, with cobalamin (B_{12}) exhibiting the highest concentration (5.66±0.81 mg/100g), followed by niacin (B_3) (1.17±0.70 mg/100g). The substantial concentration of vitamin B_{12} enhances the plant's medical utility, supporting nerve tissue health, brain function, red blood cell production, energy levels, eyesight, digestion, hormone regulation, and cardiovascular health, as well as aiding in the treatment of anemia (Callaghan et al., 2014). Vitamin B_2 had the least value (0.06±0.05 mg/100g), but not withstanding it still has a major role to play when taken into the body as it is involved in the energy production for the electron transport chain, the citric acid cycle, as well as the catabolism of fatty acids (Gropper et al., 2009). The analysis indicated a relatively high concentration of vitamin C (69.61±1.77 mg/kg) in *A. wilkesiana* leaf. The ascorbic acid's antioxidant property is crucial for maintaining normal connective tissues, promoting wound healing, and facilitating the absorption of dietary iron from the intestine (Button, 2004). In different Acalypha species, the content of ascorbic acid varied, with *A. wilkesiana* Muell. arg. showing a quantity of 9.7 mg/g and *A. wilkesiana* java white exhibiting 5.9 mg/g, as reported by Akinloye et al. (2016). *A. wilkesiana* is thus identified as a rich source of ascorbic acid.

CONCLUSION

This study shows that *Acalypha wilkesiana* leaf serves as a rich source of proximate nutrients, minerals and vitamins. The analyses show the nutritional significance of *Acalypha wilkesiana* leaf, and its potential in addressing malnutrition and promoting health. The findings suggest that incorporating these leaves into the diet could provide essential nutrients, contributing to improved nutritional status and overall well-being. Additionally, the nutritional profile of *Acalypha wilkesiana* supports its traditional use and shows its potential as a natural dietary supplement. These results pave the way for further research into the therapeutic applications and health benefits of this plant.

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