Original Research Article

PROXIMATE AND MINERAL COMPOSITIONS OF THE STEM AND LEAF OF *CNIDOSCOLUS ACONITIFOLIUS*

ABSTRACT

Cnidosculus aconitofilius in the family Euphorbiaceae, is one of the most productive green vegetables in both south western and south eastern of the Nigeria. In this study, investigations were carried out on proximate and mineral constituents of the seed and leaves of the plant parts to ascertain its nutritive and medicinal potentials. Various standard methods were used to determine the availability and quantity of the various proximate (crude protein, fat, moisture, crude fiber, total ash and crude carbohydrate) and minerals (sodium, potassium, calcium, magnesium, iron and zinc). Results of the proximate analysis revealed that the leaves have higher composition of moisture, ash, fiber, ether extract and crude protein while the stem was richer in dry matter. The total proximate composition of leaves (24.28±30.638) is higher than those of the stem (22.40±31.797). The stem has higher mean composition of all minerals (Sodium, Calcium, Magnesium, Iron and Zinc) when compared with that of the leaf. The total mineral compositions of the stem (190.24±174.429) are higher than those of the leaf (127.76±101.255). *Cnidosculus aconitifolius* provides reasonable quantities of most essential nutrients useful for human health maintenance. Minerals and other micronutrients have been shown to be essential in the control of diseases.

Keywords: Euphorbiaceae, Phytonutrients, Proximate, Microelements

INTRODUCTION

Review of extant literatures revealed that about 25% of the global population lacks adequate food for healthy and active life (1). Food prices, drought, climate change and variability have negative effects on agro-ecosystem which invariably results in poverty and hunger across the globe. According to United Nations report, millions of children die every year due to malnutrition (2). Nutrient deficiency has been implicated in the poor development of children.

It is recommended that fruits and vegetables intake should increase to 5 -13 servings per day depending on individuals' calorie needs (3). According to Naude, 2013, at least one serving of dark -green leafy vegetables could provide the daily requirements for vitamins and minerals as well as reduce the burden of nutrition -related disease. In many developing countries, to meet the daily requirements of micronutrients in deficient population, identifying and inclusion of nutritionally rich plants in the diet is the best option (4). Underutilized crops such as *Cnidoscolus aconitifolius* have an underexploited potential to contribute to food and nutrition security, health, income generation and environmental services (5). *Cnidoscolus aconitifolius*, commonly called chaya or sponnach tree is widely consumed in the southern part of Nigeria as vegetables. It is a perennial shrub commonly found in the tropics. It is one of the most productive green vegetables eaten in south western Nigeria, where it is called Iyana Ipaga (6). It is also eaten by inhabitants of South Eastern Nigeria where it is called "Hospital too far" (7). *Cnidoscolus aconitifolius* belongs to the Euphorbiaceae family which is a group of arborescent shrubs with small flowers on dichotomously branched cymes (8). The leaves are large, 32cm long and 30cm wide on chartaceous and succulent petioles. The crop originated as a domesticated leafy green vegetable in the Maya region of Gua-temela, Belize, South east Mexico during Precambrian period (9). It is easy to grow, very hardy and suffers little insect damage. Some varieties have stinging hairs and require gloves for harvesting.

It is cultivated and is used by people with scarce economic resources, commonly consumed by indigenous of Southern Nigeria. Despite the widespread use of this plant across the region, the scientific literature is yet to fully investigate the traditional uses and its nutritional values (10). Inference of most scientific researches show that *Cnidoscolus aconitifolius* is rich in nutrients but there is dearth of information on the mineral and proximate composition of the different parts. This research is conducted to comparatively study the proximate contents of the stem and leaves of *Cnidoscolus aconitifolius*.

MATERIALS AND METHOD

The materials used for this research were young seedlings of the research plant *Cnidoscolus aconitifolius* collected from the Botanical Garden of the University of Nigeria, Nsukka. The seedlings with tender young leaves, roots and stems were planted for maturation at an isolated area around the Dept of Botany Laboratory, Nnamdi Azikiwe University, Awka. The seedlings were planted in plastic buckets filled with soil and the soil samples were sterilized, sieved and mixed with some poultry manure and river sand in the ratio of 3:2:1, the mixture was left for a period of 2 hours after 14days after sterilization were being used to fill the plastic buckets. The buckets were perforated at the bottom and sides in order to allow for proper draining of water and plants toxic wastes from it

Proximate Analysis

Crude Protein determination: This was carried out using the kjeidahl method described by Sáez-Plaza, (11). The total nitrogen was determined and multiplied with the factors 5.25 to obtain the protein. One -half gram (0.5g) of each sample was mixed with 10 mg of concentrated Sulphuric acid, AR grades (Analytics Reagent Grade) in a Kjeldahl digestion flask. A tablet of selenium catalyst was added to it and the mixture was digested (heated) in a fume cupboard until a clear solution was obtained in a separate flask. The acid and other reagent were digested.

All the digests were carefully transferred to a 100 ml volumetric flask using distilled water and made up to a mark in the flask. A 100 ml portion of each digest was mixed with equal volume of 45% NaOH solution in Kjeldahl unit. The mixture was distilled and the distillate collected in 100ml of 4% Boric acid solution containing three (3) drops of the mineral indicators (bromocresol) green, methyl (red) a total of 50 ml distillate was obtained and titrated against 0.02 m H_2SO_4 solution). Titration was done from initial green color to a deep red end point.

The Nitrogen content was calculated as shown below;

$$\%N_2 = \frac{100}{W} \times \frac{N \times 14}{1000} \times \frac{vf}{Va}$$

Where:

W = Not of sample analyzed

N = Conc of H_2SO_4 titrant

VF = Total volume of digest

Va = Volume of digest distilled

Determination of fat content (ether extract)-

Fat content of the sample was determined by the continuous solvent extraction method using a socket apparatus. Five grammes (5.0g)of each sample was wrapped in a porous paper (Whatman No1 filter paper).The wrapped sample was put in a socket on flux flask containing 200 ml of petroleum ether. The upper end of the reflux flask was connected to a condenser. By heating the solvent in the flask through electro thermal heater, it vaporizes and condensed into the reflux flask. Soon the wrapped sample was completely immersed in the solvent and remained in contact with it until the flask filled up and siphoned over thus carrying oil extract from the sample down to the boiling flask. The process was allowed on repeatedly for about 4hrs before the defatted sample was removed and reserved for crude fiber analysis. The solvent was recovered and the extracting flask with its oil solvent was dried in the oven at 60°c for 3 mins (i.e. to remove any residue solvent). After cooling in a desiccator, the flask was reweighed.

By difference, the weight of fat (oil) extraction was determined and expressed as a percentage of the sample weight. It was calculated as:

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

Where:

W1 = Wt of empty extraction flask

W2 = Wt of flask and oil extract

Determination of moisture content: Moisture content was determined by the gravimetric method of Sáez-Plaza *et al*., (11). A measured weight of each sample (5g) was weighed into a weighed moisture can. The can and its sample were dried in the oven at 105°C for 3 hours in the first instance. It was cooled in a desiccation and reweighed. The weight was recorded while the sample was retained to the oven and further drying. The drying, cooling and weighing was continued repeatedly until a constant was obtained. By the difference, the weight of moisture was determined and expressed as a percentage, it was calculated as shown below:

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

Where:

W_1 = Wt of empty moisture can

W_2 = Wt of can before drying

W_3 = Wt of can sample after drying to a constant weight

Determination of Solute Carbohydrate (dry matter): The carbohydrate content was calculated by difference as the Nitrogen free extractive (NFE), a method described by Sáez-Plaza *et al*., (11). The NFE was by % NFE=100-% (a+b+c+d+e)s

Where:

a = protein

b = fat

c = fiber

d = Ash

e = moisture

Determination of Crude Fiber - This was determined by the Wende method Sáez-Plaza *et al*., (11). Five grammes (5g) of each sample here defatted (during fat analysis). The defatted sample was boiled in 200 ml of 1.25% H_2SO_4 solution under reflux for 30 mins. After that the sample were washed with several portion of hot boiling water using a two-fold Muslim-cloth to trap the particle. The washed sample were carefully transferred quantitatively back to the flask and 20ml of 1.25% NaOH solutions was added to it. Again, the samples were boiled for 30mins and washed as before with hot water. Then they were carefully transferred to a weighed porcelain crucible and dried in the oven at 70°C for 3hrs, after cooling in a dessicator, they were reweighed (W2) and then put in a muffle furnace and burn at 550°C for 2hrs (until they become ash). Again they were cooled in a desiccator and weighed.

The crude fiber content was calculated gravimetrically as

$$\% \text{ Crude fiber} = \frac{W_2 - W_1}{W_{t \text{ of sample}}} \times \frac{100}{1}$$

Where:

W^2= weight of crucible + Sample after washing and drying in oven

W1 =weight of crucible + Sample as ash.

Determination of Total Ash - This was done using the furnace incineration gravimetric method. A measured weight (5g) of each fruit sample was in a previous weighed porcelain crucible. The sample in crucible was put in Muffet furnace set at 550 and allowed to burn for 2-3 hours (until the sample become a grey ash)

The sample in crucible was very carefully removed from the furnace taking care not to allow air blow ash away) and cooled in a desiccator. It was reweighed by difference, the weight of ash was obtained and in percentage it was given by the formulae

$$\% \text{ Ash} = \frac{W_2 - W_1}{\text{Wt of sample}} \times \frac{100}{1}$$

Where:

W_1 = Wt of crucible

W_2 = Wt of empty crucible

Determination of minerals

The mineral content of the test sample was determined by the dry ask extraction method following specific mineral element. About 2 grs of the sample was burnt to ashes in a muffle (as in ash determination) the resulting ash was dissolved in 100 mls of78 dilute hydrochloric acid (1ml Hcl and then diluted to 100ml in a volumetric flask using distilled water. The digest so obtained was used for the various analysis.

RESULTS

Proximate Composition of the stem and leaf of *Cnidosculus aconitofolius*

Table 1 indicates that proximate composition of leaves (24.28±30.638) is higher than those of the stem (22.40±31.797). In comparison, the leaves have higher composition of moisture, ash, fiber, ether extract and crude protein while the stem has only higher composition of dry matter.

Table 1: Mean Proximate Composition of Stem and Leaves of *Cnidosculus aconitofolius*

Proximate (%)	Stem	Leaf
Moisture Content	8.88±0.020	9.81±0.030
Dry Matter	91.12±0.020	90.19±0.030
Ash	11.53±0.095	13.92±0.025
Crude fibre	7.87±0.030	9.67±0.030
Ether extract	2.47±0.020	4.18±0.015
Crude Protein	12.52±0.180	17.91±0.050
Total	22.40±31.797	24.28±30.638

Results are in Mean±SD *Differences between groups are significant based on Paired Sample T-test (P<0.05)

Test of Significant Difference of the Proximate Composition between Stem and Leaves of *Cnidosculus aconitofolius*

Table 2 below shows that the calculated t-statistic value of 4.101 is higher than the critical value of 2.109 at 5% of significance. This indicates that there is significant difference in the proximate composition of *Cnidosculus aconitofolius* between the stem and the leaves.

Table 2: Test of Significant Difference of the Proximate Composition between Stem and Leaves of *Cnidosculus aconitofolius*

	Stem	Leaf
Mean	22.39778	24.27889
Variance	1011.075	938.662
Observations	18	18
Df		17
T Stat		-4.10084
P(T<=t) two-tail		0.000745
t Critical two-tailed		2.109816

Mineral Composition of Stem and Leaves of *Cnidosculus aconitifolius*

Table 3 below indicates that the mineral composition of the stem (190.24±174.429) is higher than those of the leaf (127.76±101.255). The stem has higher mean composition of all minerals (Sodium, Calcium, Magnesium, Iron and Zinc) when compared with that of the leaf.

Table 3. Mean Mineral Composition of Stem and Leaves of *Cnidosculus aconitifolius*

Minerals	Stem	Leaf
Na	494.18±0.015	276.53±0.075
K	169.58±0.125	160.88±0.040
Ca	280.55±0.055	163.84±0.000
Mg	194.65±0.055	163.85±0.000
Fe	0.84±0.000	0.62±0.000
Zn	1.67±0.035	0.87±0.020
Total	190.24±174.429	127.76±101.255

Test of Significant Difference of the Mineral Composition of Stem and Leaves of *Cnidoscolus aconitifolius*

Table 4. below shows that calculated t-statistic value of 3.212 is higher than the critical value of 2.109 at 5% level of significance. This indicates that there is significant difference in the mineral composition of *Cnidoscolus aconitifolius* between the stem and the leaves.

Table 4. Test of Significant Difference of the Mineral Composition of Stem and Leaves of *Cnidoscolus aconitifolius*

	Leaf	Stem
Mean	190.2422	127.7644
Variance	30425.55	10252.65
Observations	18	18
t-Stat		3.211848
P(T<=) two-tail		0.005115
T Critical two-tail		2.109816

DISCUSSION

The inference of the proximate analysis carried out on the leaf and stem of *Cnidoscolus aconitifolus* as presented in the results showed that the moisture content 88.8% obtained from the stem was low when compared to 9.81% recorded from the leaf. The relative low moisture content of the stem will hinder the growth of microorganisms and also increase their storage life. This agrees with the findings of Abolaji *et al.* (12).

The relatively higher quantity of ash, crude fiber, ether extract and crude protein recorded from the leaf completely agrees with the observations of Akindahun, *et al.* (13) on *A. viridus, O. gratissum* and *H.`esculenta.*

For the mineral compositions of the stem and the leaves of *Cnidoscolus aconitifolus*, the findings of this research revealed that the stem has more Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe) and Zinc (Zn) contents than the leaf. All the minerals tested were detected in high quantity in both the leaf and the stem. This finding does not agree with the reports of some researchers who stated that low quantity of these minerals were present in the leaf and stem of the plant (14).

It can be deduced from the findings of this work that the leaf has more proximate content than the stem while the reverse is the case in minerals. The rich nutritional compositions of *Cnidoscolus aconitifolius* as observed in this research work agrees with the findings of some researchers (15, 16). The high presence of iron in the stem and fresh leaves of *Cnidoscolus*

aconitifolius, as reported in this study supports the use of the plant parts as a blood builder in some parts of South – East Nigeria, where it has also been nick-named "Blood" (15).

While some edible, leafy green vegetables are usually good sources of proximate and minerals, *Cnidoscolus aconitifolius* provides reasonable quantities of most essential nutrients useful for human health maintenance. Minerals and other micronutrients have been shown to be essential in the control of diseases (10).

CONCLUSION

The proximate and mineral analysis of the fresh leaf and stem of *Cnidiscolus aconitifolius* revealed the presence of moisture, dry matter, ash, crude fiber, ether extract, crude protein, sodium, potassium, calcium, magnesium, iron and Zn. These compounds may contribute to the reputed nutritional values of the plant. Relatively, the leaf has more proximate content while the stem is richer in minerals. The inference of this study supports the usage of *Cnidiscolus aconitifolius* as a nutritive green plant.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

REFERENCES

1. FAO. The State of Food Insecurity in the World: Addressing food insecurity in protracted crises. https://www.fao.org/4/i1683e/i1683e.pdf, 2010; Accessed 24th June, 2024
2. UNICEF, The state of the world's children 2012: children in an urban world. Esocialsciences, 2012
3. Santos, SJ, Hurtado-Ortiz, MT, Armendariz M, VanTwist V, Castillo Y, Obesity-Related Dietary Patterns and Health Status of Diabetes among At-Risk Latino College Students. Journal of Hispanic higher education, 2017; 16(4), 291–313. https://doi.org/10.1177/1538192716653504

4. Tanumihardjo S, Food-based approaches for ensuring adequate vitamin A nutrition. Comprehensive reviews in food science and food safety, 2008; 7: 373 -381.

5. Gobena DA, Shewa AG, Abera S, Neme G, Mahamed W, W/Tsadik K, Chaya (Cnidoscolus aconitifolius) for Enhancing Food and Nutrition Security of Arid Lands of Ethiopia. JNFS; 2023; 8 (3): 461-467. URL: http://jnfs.ssu.ac.ir/article-1-577-en.html

6. Odunmbaku A., Ekute BO, Amadi GK, Groundwater Quality of Iyana-Ipaja Metropolis in Alimosho Local Government Area of Lagos State, Nigeria. ChemSearch Journal, 2023; 13 (2): 72-76

7. Iwalewe, Antibacterial activities and toxicological potentials of crude ethanolic extracts of Eurphobia hirta. African Biotechnology 2008; 6: 2400-2405

8. Panghal A, Shaji AO, Nain K, Garg MK, Chhikara N, Cnidoscolus aconitifolius: Nutritional, phytochemical composition and health benefits – A review. Bioactive Compounds in Health and Disease 2021; 4(11): 1-11 - Online ISSN: 2574-0334; Print ISSN: 2769-2426

9. Rushton, EAC, Whitney BS, Metcalfe SE, A Tale of Maize, Palm, and Pine: Changing Socio-Ecological Interactions from Pre-Classic Maya to the Present Day in Belize. Quaternary, 2020; 3 (4): 30. https://doi.org/10.3390/quat3040030

10. Adeniran OI, Olajide OO, Igwemmar NC, Orishadipe AT, Phytochemical constituents, antimicrobial and antioxidant potentials of tree spinach [Cnidoscolus aconitifolius (Miller) I. M. Johnston]. Journal of Medicinal Plants Research, 2013; 7(19): 1317-1322, DOI: 10.5897/JMPR12.899 ISSN 1996-0875

11. Sáez-Plaza P, Michałowski T, Navas MJ, Asuero AG, Wybraniec S, An Overview of the Kjeldahl Method of Nitrogen Determination. Part I. Early History, Chemistry of the Procedure, and Titrimetric Finish. Critical Reviews in Analytical Chemistry, 2013; 43(4): 178–223. https://doi.org/10.1080/10408347.2012.751786

12. Abolaji OA, Adebayo AN, Odesanmi, OS, Nutritional qualities of three medicinal plants; *Xylopia aethiopicca*, *Blighia sapida* and *Parinari polyandra*. *Pakistan Journal of Nutrition*; 2007; 6: 665-668.

13. Akindahunsi AA, Salawa, SO, Phytochemical screening of nutrients and antinutrient composition of selected tropical green leafy vegetables. *African Journal of Biotechnology* 2005, 4(6):497-501.

14. Oyagbemi AA, Odetola AA, Azeez OI, Phytochemical investigation and proximate analysis on the leaves of Cnidoscolus aconitifolius. J Med Food. 2016; 14(3):322-4. doi: 10.1089/jmf.2008.0321. Epub 2010 Dec 23. PMID: 21182430.

15. Chikezie UN, Chijioke AN, Adjeroh LA, Ogbulie TE, Udensi JU, Oyirioha KC, An Evaluation of the Phytochemical and Nutritional Compositions of Fresh Leaves of *Cnidoscolus aconitifolius* [Miller] I.M.Johnston. *International Journal of Research Studies in Biosciences (IJRSB)* 2016; 4(6): 21-28. ISSN 2349-0357 (Print) & ISSN 2349-036 (Online) http://dx.doi.org/10.20431/2349-0365.0406004 www.arcjournals.org

16. Jansen PCM, *Cnidoscolus aconitifolius* (Mill) I.M. Johnst, In: Grubben GJH & Denton OA (Editors), PROTA 2: Vegetables/Legumes. (CD–ROM). PROTA, Wageningen, Netherlands. 2004.