The Proximate Composition and Anti-nutritive Content of \textit{Cnidoscolus aconitifolius} Leaves

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2020/v29i830213

Received 10 August 2019
Accepted 17 October 2019
Published 28 August 2020

ABSTRACT

The nutritional composition, mineral content as well as anti-nutritional of Tree spinach (\textit{Cnidoscolus aconitifolius}) leaves were investigated using standard methods. The results of the investigation showed the presence of moisture to be 5.64±0.30%, ash- 9.27±0.16%, crude protein- 8.43±0.34%, crude lipid- 4.43±0.16%, crude fibre- 16.73±0.20%, carbohydrate- 55.50% and caloric value- 276.04%. The Mineral elements determined were calcium- 1.48±0.12 mg/100 g, phosphorus- 0.18±0.03 mg/100 g. Compounds or substances which acts to reduce nutrients intake, intake digestion, absorption and utilization and may cause adverse effects are referred to as anti-nutrients or anti-nutritional factors. The anti-nutritive content of \textit{Cnidoscolus aconitifolius} was classified into three categories; fresh leaves, blanched leaves and cooked leaves which decreased significantly. The anti-nutritional content includes; oxalate- which decreased from 62.71±0.21 mg/100 g in fresh leaves, 40.07±0.09 mg/100 g in blanched leaves and 30.04±0.05 mg/100 g in cooked leaves. Phytate- which decreased from 77.17±1.84 mg/100 g in fresh leaves, 62.02±0.16 mg/100 g in blanched leaves and 28.64±0.88 mg/100 g in cooked leaves. Hydrogen cyanide- decreased significantly from 171.22±0.44 mg/100 g in fresh leaves, 113.00±0.08 mg/100 g in blanched leaves and 0.00 mg/100 g in cooked leaves. Boiling increased the saponin content to 220.30±0.47 mg/100 g in cooked leaves from 218.50±0.50 mg/100 g in fresh leaves and 218.50±0.50 mg/100 g in blanched leaves.

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1. INTRODUCTION

The importance and awareness of nutrient in public health issues have resulted in the increasing demand for knowledge of nutrient in vegetables [1]. Awareness of the significance of vegetable consumption plays an important role in maintaining good health, and in reducing the risk of illness (Kalt, 2005).

Nutritional factors constitute of vitamins, minerals, high dietary fibres, carbohydrate, low fat and water [2,3]. A vitamin is an organic compound and a vital nutrient that an organism requires in a limited amount [4,5,6]. Fats are one of the three main macronutrients, along with carbohydrates and proteins. Fats are also known as triglycerides and are esters of three fatty acid chains and alcohol glycerol. Minerals are any naturally occurring inorganic material that has a definite chemical composition and characteristic physical properties [7]. Anti-nutrients or anti-nutritional factors may be defined as those substances generated in natural feedstuffs by the normal metabolism of species and by different mechanisms (for example inactivation of some nutrients, diminution of the digestive process or metabolic utilization of feed) which exerts an effect on contrary to optimum nutrition [8]. These anti-nutritional factors are also known as ‘secondary metabolites’ in plants and they are highly biologically active [9]. Anti-nutritional factors may be divided into two major categories. They are Proteins (such as lectins and protease inhibitors) which are sensitive to normal processing temperatures [10,11]. Other substances which are stable or resistant to these temperatures and which include, among many others, polyphenolic compound (mainly condensed tannins), non-protein amino acids and galactomannan gums [12]. The major ones include toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins (phytohaemagglutinins), protease inhibitors, chlorogenic acid and amylase inhibitors [13].

Vegetables form an important part of the human diet and are always regarded as food. A vegetable includes roots, stem, leaves, flowers, buds, tubers, seeds, and fruits [14]. Leafy vegetables are traditionally cooked and eaten as a relish together with a starch stable food usually in the form of porridge [15]. These micronutrients are essential food nutrients useful for the body as a protective agent against diseases, thus necessary for health and growth (Erta et al., 2002, Falade et al., 2003). The importance of the nutritional status of vegetables by Nigerians has resulted in the increased demand for knowledge of the nutrient of food [16,17]. Green leafy vegetables are an important component of the dietary regime of humans because they provide the necessary vitamins, and minerals [18]. They however also contain anti nutrients which reduce the bioavailability of these nutrients [19,20]. Aletor and Adeogun [21] however, reported that some anti-nutrients exhibits protective effects thus making them serve a dual purpose.

One of the under-utilized plant genera is Chaya leaf (Cnidoscolus aconitifolius), an herbaceous plant belonging to the family, Euphorbiaceae with green or dark green leaves [22]. It is broadly distributed throughout the tropics and can be found throughout the tropics and can be found throughout Nigeria, East Africa particularly in Kenya. It is an ornamental, evergreen drought-tolerant shrub up to 6 m in height with alternate pinnate lobed leaves, milky sap and small flowers on dichotomously branched cymes. The leaves are large, 32 cm long and 30 cm wide on chartaceous and succulent petioles [23,24]. Chaya is a large, fast-growing leafy perennial shrub reaching a height of about 2-3 meters (6-9 feet). It resembles the hibiscus or the cassava plants. It is probably native to the Yucatan Peninsula of Mexico where it is known as Chaya [25,26]. The leafage used was a matured Cnidoscolus aconitifolius plant.

2. AIM AND OBJECTIVES OF THE STUDY

i. The study aims to carry out analysis on the leaves of Cnidoscolus aconitifolius (Chaya) with a view of identifying the

Keywords: Nutritional composition; anti-nutritional factor; Cnidoscolus aconitifolius.
nutrients and anti-nutritive content present. Thus, the objectives of this study are;

ii. To determine the nutrient content of *Cnidoscolus aconitifolius* leaves.

iii. To determine the presence and level of anti-nutrient content of *Cnidoscolus aconitifolius* leaves.

iv. To compare the results obtained with standard values

### 2.1 Sample Collection and Preparation

Fresh leaves of *Cnidoscolus aconitifolius* were harvested fresh from the garden in a polythene bag at Jos, Plateau state. The samples were washed with clean tap water first to remove dirt and any soil particle. It was further washed with distilled water (Lawal and Audu, 2011). The vegetable samples were subjected to three (3) processing technique (fresh, cooked and blanched). The cooking of the leaves was done for 20 minutes. The blanching of the leaves was done by pouring boiled water on the leaves and left for 5 minutes. The samples were oven-dried at 50°C and crushed into powder before storing in clean and clear labelled polythene bag. The dried powdered samples were used in all the analyses.

### 2.2 Apparatus/Equipments

Crucibles, desiccator, analytical weighing balance, muffle furnace, soxhlet extractor apparatus, fume cupboard, Kjeldahl apparatus, 100 ml volumetric flask, 400 ml beakers, measuring cylinder, oven, Whatman filter paper, 100 ml conical flask, magnetic stirrer, centrifuge, UV spectrophotometer, water bath, desiccator, 500 ml distillation flask, condenser, beaker, 250 ml separatory funnel, burette, retort stand, centrifuge.

### 2.3 Reagents

Potassium iodide (KI), silver nitrate (AgNO₃), hydrochloric acid (HCl), n-hexane, sulfuric acid (H₂SO₄), sodium hydroxide (NaOH), boric acid, trichloroacetic acid, potassium permanganate (KMnO₄), nitric acid (HNO₃), ethanol, molybdovanadate reagent, n-butanol, diethyl ether, folindenis reagent, tannic acid, ammonium oxalate (NH₄OH).

All the reagents used were of analytical grade and were used without any further purification.

### 2.4 Statistical Analysis

The statistical tool used was One-way Analysis of Variance (ANOVA) and the package used was Statistical Package for Social Science (S.P.S.S.), version 23.

### 3. RESULTS AND DISCUSSION

#### 3.1 Proximate Analyses

The estimation of the various parameters namely; moisture content, total ash content, crude protein, crude lipids, crude fibre, total carbohydrate and calorific value were carried out according to standard procedures.

#### 3.2 Results

The results obtained from the proximate analysis carried out on a dried sample of fresh *Cnidoscolus aconitifolius* obtained from in and around Jos metropolis are presented in Table 1. The proximate composition of *Cnidoscolus aconitifolius* as shown in Table 2, the moisture content of 5.64±0.30 was low when compared to those of *O. gratissimum* (81.60%) [27] and *Xylopia aethiopica* (16.04%) [28]. The low moisture content of the leaves would hinder the growth of microorganisms and also increase their storage life.

| Parameter                        | Concentration (% dry matter) |
|----------------------------------|------------------------------|
| Moisture content                 | 5.64 ± 0.30                  |
| Ash                              | 9.27 ± 0.16                  |
| Crude protein                    | 8.43 ± 0.34                  |
| Crude lipid                      | 4.43 ± 0.16                  |
| Crude fibre                      | 16.73 ± 0.20                 |
| Available carbohydrate           | 55.50 ± 0.24                 |
| Calorific value(kcal/100 g)      | 276.04 ± 0.45                |

Values are means ± standard deviation (SD) triplicate
The ash content of 9.27±0.16 was low when compared to the value reported for the leaves of A. viridus (22.84%) (Pandey et al., 2006). The result is higher than O. gratissimum (8.00%) and H. esculentus (8.00%) [19]. The ash content is a reflection of the mineral contents preserved in the food material.

The protein content of C. aconitifolius was gotten to be 8.34±0.34 which is lower than the value reported for C. olitoriusL which was found to be 13.70±0.05 (www.eajournals.org). The crude protein was higher when compared with Ocimum gratissimum (3.33%) [27]. However, this value was low when compared with (20.59%) Amaranthus caudatus and (31.00%) Talinum triangulare [19]. The loss in protein could be attributed to the mild heating effect associated with the drying conditions which could result in the unzipping of hydrophobic forces leading to a partial distribution of the primary, secondary, tertiary and quaternary structure of the protein molecule (Ngoddy, 1985). Plants foods that provide more than 12% of their calorific value from protein are good sources of protein [29]. This shows that the leaf of this plant is a good source of protein.

Lipid content of fresh leave was 4.43±0.16. This vegetable is highly palatable since fat increased the palatability of food by absorbing and retaining flavours [30]. Excess fat consumption is implicated in a certain cardiovascular disorder such as atherosclerosis, diabetes, cancer and ageing [30].

The fibre content of the leaf was 16.73±0.16. The value gotten fell within the range of (8.50-20.9%) reported for some Nigerian vegetables [31]. Dietary fibre can reduce serum cholesterol level, hypertension, diabetes, breast cancer and constipation [32]. Thus, chaya leaves (C. aconitifolius) could be regarded as good sources of dietary fibre.

The carbohydrate content of 55.50 was also obtained in this study, and it compared favourably within the range of 40.4-8.2% as reported for some leafy vegetables [33]. The high value obtained for carbohydrate in this study makes the leaf a good source of energy for both man and animal.

The calorific value of 276.04 (kcal/100 g) was low when compared with (343.08 kcal/100 g) for O. gratissimum by Idris et al., [27].

### 3.3 Mineral Content

The mineral analysis was carried out on C. aconitifolius in Table 2, for the Phosphorus, the value gotten was 0.18±0.03 which is the same value gotten in Sclerocarya birrea fruit juice 0.18±0.12 (Hassan et al., 2010) and lower than the value gotten for the seed kernel of Sclerocarya birrea fruits which 2.87±2.10 (Hassan et al., 2010).

The Calcium content of the freshly harvested C. aconitifolius obtained in the analysis was 1.48±0.12 which is very low compared to the value reported for freshly harvested leafy vegetable; Yannin (120.00±0.74 mg/100 g), Ewuroodu (98.00±0.00 mg/100 g), Efrin (89.00±0.33 mg/100 g) by Oduse et al. [34] and prior data obtained for C. aconitifolius (63.00±0.49 mg/100 g) by (Aye, 2012), and that of Soko (188.00±0.00 mg/100 g) as reported by Gupta et al. [35]. The report of this study showed that consumption of this vegetable would not supply the adequate amount of calcium needed by the body, to play in muscle contraction and relaxation blood clotting, synaptic transmission and absorption of vitamin B12 in the body.

### 3.4 Procedure for Determining Calcium and Phosphorus

#### 3.4.1 Determination of calcium

##### 3.4.1.1 Determination of calcium by AOAC method

**Ashing and extraction:** About 2 g sample was weighed and ashed in a muffle furnace to carbon-free at 600°C for 3 hours. Cooled, the residue was moistened with a little distilled water and transferred to a beaker. 8.5 ml 25% HCl was added and 3-4 drops of nitric acid, heated to boiling. Cooled and filtered into a 100ml volumetric flask and makeup to mark.

**Principle:** The calcium in the sample was converted to calcium oxalate by addition of

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Table 2. Mineral composition of *Cnidoscolus aconitifolius* leaves in mg/100 g

| Nutrients     | Concentration |
|---------------|---------------|
| Calcium       | 1.48 ± 0.12   |
| Phosphorus    | 0.18 ± 0.03   |

Values are means ± standard deviation (Sd) triplicate
ammonium oxalate which was easily precipitated out and titrated against standard potassium permanganate solution.

**Precipitation:** A 4 ml of the filtrate was taken into a conical centrifuge tube, 3 ml 4.2% Ammonium oxalate solution precipitate forms were added and this was accelerated by boiling. Allowed to stand overnight for the precipitate to settle centrifuge at 3000 rpm for 5 minutes. The supernatant was removed making sure that the tube was not tilted, the mouth was wiped to remove any excess of NH₄oxalate. It was washed again with 3 ml 2% NH₄OH and centrifuge. The supernatant was discarded. A 2 ml of bench sulphuric acid (20%) was added into the tube containing the precipitate and then put into a beaker containing water. About 70°C was heated and titrated with 0.01N KMnO₄.

3.4.1.2 **Determination of phosphorus**

Determination of phosphorus using Pearson’s chemical analysis of foods.

**Principle:** Acid solutions containing orthophosphates when treated with molybdic acid forms a stable orange-yellow coloured complex of Vanadomolybdophosphoric acid (H₃PO₄, VO₃11M0₉H₂O). The colour intensity was proportional to the concentration of phosphate in the sample.

**Procedure:** A 2 g of sample was weighed (or any known quantity) and ashed in the furnace at 600°C for 3 hours. Allowed to cool and the residue was moistened with little distilled water in a beaker. 8.5 ml of 25% HCl was added. Depending on the nature of the sample, effervescence may occur and the reaction can be accelerated by adding 2 drops of conc. HNO₃ and heated to boiling. Allowed to cool and filtered into a 100 ml volumetric flask. Mark up to mark with distilled water. Take 1 ml of sample filtrate, 5 ml molybdovanadate reagent was added and allowed to stand for 10 minutes and makeup to 25 ml with distilled water. Absorbance was read at 470 nm. For standard, 0.2 ml of 1 mg/ml phosphorus solution was taken. A 19.8 ml distilled water was added and then 5 ml molybdovanadate reagent was added for blank, 20 ml distilled water was taken and 5 ml molybdovanadate reagent was added. The blank was used to zero the calorimeter. Absorbance was read at 470 nm.

3.5 **Anti-Nutritive Content**

The anti nutritive components of fresh, blanched and cooked leaves of *C. aconitifolius* is shown in Table 3. The oxalate content of *C. aconitifolius* decreased significantly from 62.71±0.21 in fresh leaves to 40±0.09 in blanched leaves and 30.04±0.05 in the cooked leaves. This showed that application of heat reduced the total content of oxalate in the leaf. Therefore, boiling significantly reduced the oxalate content of vegetables. Oxalate is known to interfere with calcium absorption by forming insoluble salt of calcium. This insoluble salt of calcium is capable of passing through the excretory system and interrupts with the efficient working of the kidney, otherwise causes a disease called kidney stone [36].

Phytatechelates minerals in the body. Thus, makes certain important mineral such as calcium and magnesium biologically unavailable in the body [37]. Its content significantly decreased from 77.17±1.84 mg/100 g in fresh leaves to 64.02±0.16 in blanched leaves and 28.64±0.88 which is in agreement with the report of Bawa and Yada (1986), who also reported a decrease in the phytate content of *C. aconitifolius* to 12.5 and 18.75 mg/100 g in boiled leaves.

The Hydrogen cyanide content decreased form 171.22±0.44 mg/100 g in the fresh leaves to 131.00±0.08 mg/100 g in the blanched leaves and 0.00 in the cooked leaves.

| Anti-nutrient          | Fresh leaves | Blanched leaves | Cooked leaves |
|------------------------|--------------|-----------------|---------------|
| Oxalate                | 62.71±0.21a  | 40.07 ± 0.09b   | 30.04 ± 0.05b |
| Phytate                | 77.17±1.84a  | 64.02 ± 0.16b   | 28.64 ± 0.88b |
| Hydrogen cyanide       | 171.22±0.44a | 113.00 ± 0.08b  | 0.00C         |
| Saponin                | 218.50±0.50a | 218.50 ± 0.50b  | 220.30 ± 0.47b |
| Tannins                | 18.30±0.16a  | 11.62 ± 0.15b   | 7.86 ±0.05d   |

Values are means ± standard deviation (SD) triplicate; means with different superscript in the same row were significantly different (P<0.05)
Table 4. Anova table for tannin

|                  | Sum of squares | Df | Mean square | F       | Sig. |
|------------------|----------------|----|-------------|---------|------|
| Between Groups   | 167.868        | 2  | 83.934      | 3155.409| .000 |
| Within Groups    | .160           | 6  | .027        |         |      |
| Total            | 168.027        | 8  |             |         |      |

The Saponin content increased from 218.50±0.50 mg/100 g in fresh leaves harvested and blanched leaves to 220.30±0.47 mg/100 g in the cooked leaves. Fagbohun et al., [23] reported that it had anti-hyper cholesterol, anti-inflammatory, cardiac depressant properties, and appears to inhibit cancer cells without destroying the normal cells.

Saponins are phytochemicals which can be found in most vegetables. Saponins have many health benefits. Several studies have illustrated the beneficial effect on blood cholesterol levels, cancer (antioxidant), bone health and stimulation of the immune system. The increase in this research was as a result of the heat produced during the cooking process.

The tannin content reduced from 18.30±0.16 mg/100 g in fresh leaves to 11.62±0.15 mg/100 g in blanched leaves and 7.86±0.05 in the cooked leaves respectively. The presence of tannin in vegetables suggests the ability of the plant to help as an antioxidant and anti-haemorrhoidal agent [38].

The P-value obtained from one-way ANOVA is less than 0.05, it means that it is statistically significant and will, therefore, reject $H_0$ and accept $H_1$ and conclude by saying that the treatments have different means. The 0.00 implies that it is statistically significant.

Table 4 made a comparison between their means. It is said to be statistically significant because the P-value (Sig.) is less than 0.05. Hence, we reject the null hypothesis, $H_0$ and accept the alternate hypothesis, $H_1$ and conclude by saying that there is a significant difference between their means.

Table 5 made a comparison between their means. It is said to be statistically significant because the P-value (Sig.) is less than 0.05. Hence, we reject the null hypothesis, $H_0$ and accept the alternate hypothesis, $H_1$ and conclude by saying that there is a significant difference between their means.

Table 6 made a comparison between their means. It is said to be statistically significant because the P-value (Sig.) is less than 0.05. Hence, we reject the null hypothesis, $H_0$ and accept the alternate hypothesis, $H_1$ and conclude by saying that there is a significant difference between their means.

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Table 5. Anova table for oxalate

|                  | Sum of squares | Df | Mean square | F       | Sig. |
|------------------|----------------|----|-------------|---------|------|
| Between Groups   | 1671.313       | 2  | 835.657     | 4876.740| .000 |
| Within Groups    | 1.028          | 6  | .171        |         |      |
| Total            | 1672.341       | 8  |             |         |      |

Table 6. Anova table for hydrogen cyanide

|                  | Sum of squares | Df | Mean square | F       | Sig. |
|------------------|----------------|----|-------------|---------|------|
| Between Groups   | 45473.327      | 2  | 22736.664   | 231508.058| .000 |
| Within Groups    | .589           | 6  | .098        |         |      |
| Total            | 45473.916      | 8  |             |         |      |

Table 7. Anova table for phytate

|                  | Sum of squares | Df | Mean square | F       | Sig. |
|------------------|----------------|----|-------------|---------|------|
| Between Groups   | 3780.387       | 2  | 1890.194    | 900.216 | .000 |
| Within Groups    | 12.598         | 6  | 2.100       |         |      |
| Total            | 3792.985       | 8  |             |         |      |
Table 8. Anova table for saponin

|                  | Sum of squares | Df | Mean square | F      | Sig. |
|------------------|----------------|----|-------------|--------|------|
| Between Groups   | 7.476          | 2  | 3.738       | 14.690 | .005 |
| Within Groups    | 1.527          | 6  | .254        |        |      |
| Total            | 9.002          | 8  |             |        |      |

Table 7 made a comparison between their means. It is said to be statistically significant because the P-value (Sig.) is less than 0.05. Hence, we reject the null hypothesis, H₀ and accept the alternate hypothesis, H₁ and conclude by saying that there is a significant difference between their means.

Table 8 made a comparison between their means. It is said to be statistically significant because the P-value (Sig.) is less than 0.05. Hence, we reject the null hypothesis, H₀ and accept the alternate hypothesis, H₁ and conclude by saying that there is a significant difference between their means.

4. CONCLUSION

It could be concluded that freshly harvested leaves of *Cnidoscolus aconitifolius* contained all nutrients (macronutrient) in adequate quantity needed by the body. Boiling as a pre-treatment method for *C. aconitifolius* significantly decreased the quantity of the antinutrients; tannins, phytate, hydrogen cyanide and oxalate as a result of processing such as blanching and cooking but increased the saponin contents of the leaf to 220.30 from 218.50 mg/100 g. The leaves should not be over-cooked. However, pre-treating this vegetable would greatly decrease the anti-nutrients in it.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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