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Evaluation of the Mineral and Vitamin Compositions of Leaves of *Alchornea cordifolia* and *Thaumatococcus daniellii*

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Authors' contributions

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

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ABSTRACT

The study was conducted to evaluate the vitamin and mineral compositions of leaves of *Alchornea cordifolia* and *Thaumatococcus daniellii*. Standard methods were employed to determine the vitamin and mineral compositions of plant samples. Results obtained from the study showed that the concentration of vitamin B6 (4.46±0.02 mg/100 g) was significantly (P<0.05) higher than those of other vitamins reportedly present in *Alchornea cordifolia* leaf. However, the least in concentration of all the vitamins present being Vit C (0.34±0.02 mg/100 g). Results on the mineral compositions of leaf of *Alchornea cordifolia* revealed that the leaf contains significantly (P<0.05) higher concentration of phosphorus (4.58±0.13 mg/100 g) compared to those of other minerals found present while zinc was found to be available at the least concentration of (0.90±0.04 mg/100 g). *Thaumatococcus daniellii* leaf contains a significantly (P<0.05) higher concentration of vitamin B12 compared to all other vitamins present. Results on the mineral compositions however, revealed that leaf of *Thaumatococcus daniellii* contains considerable amounts of calcium (6.15±0.03 mg/100 g)

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which was not significantly (P<0.05) different from the value reported for phosphorus (5.75±0.12 mg/100 g) which as well was not significantly (P<0.05) different from potassium (5.30±0.14 mg/100 g). However, zinc was reportedly present in the plant sample at the concentration of (1.42±0.02 mg/100 g). In conclusion, findings from this work have expanded the knowledge about *Alchornea cordifolia* and *Thaumatococcus daniellii* while consolidating already established facts on their therapeutic potentials.

Keywords: Vitamin; mineral; Alchornea cordifolia; Thaumatococcus daniellii.

1. INTRODUCTION

Micronutrients are vitamins and minerals which are required in minute quantities for proper cellular functions [1]. Vitamins are organic substances that function as regulators or co-enzymes of enzymatic reactions in the body. They are categorized as fat soluble and water soluble vitamins [2]. While fat soluble vitamins are domiciled in the fatty tissues and liver, water soluble vitamins occupy the aqueous cellular compartments such as the mitochondria where they function in synergy with the enzymes of the respiratory chain to synthesize ATP [3]. Vitamins are considered critical to the overall well being of an individual.

Minerals are essential nutrients and represent about 5-6% of the total body weight. They are categorized as major or macro minerals and the trace or micro minerals and have been found to be useful as structural and regulatory molecules that have also taken active parts in many metabolic and immune functions [3]. Thus, their deficiencies can result to serious health issues.

Micronutrient malnutrition is common among the rural poor populace of developing countries of the world. It diminishes immune function and thus, predisposes a child to infections such as diarrhea, a leading cause of death among children aged <5 years, delays full recovery and increases the probability of development of severe illness [4]. The practice of using plants in the treatment of diverse forms of diseases plaguing mankind is predominant among those in the low income regions of developing countries [5].

The plant *Alchornea cordifolia* of the family Acalypholdeae commonly known as the Christmas Bush is a medium-sized shrubby tree mostly found in marshy areas along the coastal regions of West Africa [6]. Its leaf has been used extensively in the treatment of notable human diseases including diarrhea [7].

Thaumatococcus daniellii, also known as the sweet prayers plant is a tropical rain forest, large rhizomatous flowering herb. The height of a fully grown *Thaumatococcus daniellii* plant is about 3-4cm, bearing some large papery leaves of about 46cm long with pale purple flowers and soft fruits containing a number of shiny black seeds. In Nigeria, the plant grows predominantly in the cocoa growing areas of the south west [8]. It is found throughout the hot humid tropical rain forest and coastal zone of West Africa [9]. In countries like Ghana, Cote d'voire and Nigeria the leaf is used for wrapping food [10]. The plant is a source of thaumatin, an intensely sweet, non-toxic and heat stable protein used as a sweetening agent or taste modifier in beverages, desserts, chewing gums and pet foods. *Thaumatococcus daniellii* has been used widely used in the treatment of diverse diseases ravaging mankind. For instance, the leaf sap of this plant has been used as an anti-dote against venoms, stings and bites, while the leaf and root sap have been used successfully as sedatives and mental therapy [11].

Although, various parts of *Alchornea cordifolia and T. daniellii* have been used to meet several human needs, further researches targeted at some sparingly probed parts of these plants such as the leaf may suggest enhanced potentials to meet more human needs. More so, while the specie *Alchornea cordifolia* is the most studied of the alchornea genus, there is paucity of information on its micronutrient content and hence the need for a study in this regard, is undoubtedly imperative.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Plant Material

About 300 g of fresh mature leaves of *A. cordifolia* and *T. daniellii* used for the study were obtained from a farm land within Achara Uturu community of Isukwuato Local Government Area of Abia State. The plant materials were identified at the herbarium unit of the Department of

Forestry, Micheal Okpara University of Agriculture Umudike, Abia State Nigeria. The leaves were washed clean with tap water and allowed to dry at room temperature. Dried leaves were pulverized and properly stored in an airtight container.

2.2 Vitamin Analysis

Vitamins A, B1, B3, B5, B6, B12 and C content of *A. cordifolia* and *T. daniellii* were evaluated by the official methods of the Association of Official Analytical Chemists [12].

2.3 Determination of Vitamin A (Retinol) Content of Plant Samples

About 1 g each of samples of *A. cordifolia* and *T. daniellii* leaves was weighed and macerated using 20 ml of n-hexane in a test tube for a period of 10 minutes. Then 3 ml of the upper hexane extract was poured into a clean dry test tube in duplicates and evaporated to dryness. Subsequently, 0.2 ml of acetic anhydride chloroform reagent was introduced before the addition of 2 ml of 50% trichloroacetic acid (TCA) in chloroform and absorbance was read at 15 seconds and 30 seconds intervals at 620 nm.

2.4 Determination of Vitamin C (Ascorbic Acid) Content of Plant Samples

About 0.5 g each of *A. cordifolia* and *T. daniellii* leaf sample was weighed macerated with 10 ml of 0.4% oxalic acid contained in a test tube for a period of 10 minutes. They were centrifuged for 5 minutes and the solution filtered. 1 ml of the filtrate generated from each sample was transferred into a dry test tube in duplicates, 9ml of 2, 6- dichlorophenol indophenol was added and absorbance was read at 15 seconds and 30 seconds interval at 520 nm.

2.5 Determination of Vitamin B Complex Content of Plant Samples

HPLC–MS/MS analyses of the vitamin B Complex was carried out with the aid of an Accela liquid chromatography (Thermo Scientific, San Jose, CA) equipped with a diode array detector (DAD), an autosampler and a TSQ Quantum triple quadrupole analyzer (Thermo Scientific). Chromatograph linked to an MS analyzer via an electrospray (ESI) interface Xcalibur software (Thermo Scientific) was used to analyze and store the data. The column used was an ACE-100 C18 (100 x 2.1 mm i.d, 3 µm particle size) (Advanced Chromatographic Technologies, Aberdeen, UK). The method developed to simultaneously separate the various B Complex vitamins in a single run using 10 mM ammonium acetate solution (pH 4.5) as mobile phase A, MeOH with 0.1% acetic acid as mobile phase B and MeOH with 0.3% acetic acid as mobile phase C. The flow rate was 0.2 ml/min whereas the injection volume was 10 µL. The DAD recorded the spectra from 200 to 680 nm. Column and autosampler compartments were thermostated at 20 and 5˚C, respectively. To identify and quantify the Vitamin B Complex (VBC), the mass spectrometer was operated first in the negative ESI mode, for 1.7 min. Spray voltage and capillary temperature were set at 3000 V and 250˚C respectively. A second segment of 10.3 min followed using positive ESI mode to monitor the presence of the other VBC. In this segment the spray voltage and capillary temperature were set at 5000 V and 250˚C respectively. Nitrogen was used as sheath and auxiliary gas at pressures of 40 and 19 a.u respectively, Ion sweep gas pressure was 2 units and collision gas (Ar) pressure 1.3 mTorr. Scan width and scan time were fixed at 0.020 (m/z) and 0.1s, respectively, and the system was operated in selected reaction monitoring (SRM). SRM parameters were optimized by direct injection of standards. Two ion transitions were monitored for identification but only the most intense product ion for each precursor ion was used for quantification. The values corresponding to the tube lens offset voltage and collision energy for each selected ion

2.6 Determination of Minerals

Calcium (Ca), phosphorus (P), potassium (K) and Zinc (Zn) content of *A. cordifolia* and *T. daniellii* leaves were analyzed from solution obtained when 5 g of the samples was separately digested with 10 ml of 5 N concentrated hydrochloride. The resulting mixture was placed on a water bath and evaporated almost to dryness. The solution was cooled and filtered into 100 ml standard flask and diluted to volume with distilled water. Atomic absorption spectrophotometer was used to analyze the minerals separately after acid digestion of the sample, as described by the official method of the Association of Official Analytical Chemists [12].

2.7 Determination of Phosphorus (p) Content of Plant Samples

The plant samples were evaluated for Phosphorus content based on colorimetric

reaction with sodium molybdate (Na2MoO4·2H2O, ACS ≥99%, Sigma-Aldrich 331058, Sigma-Aldrich Ltd, Brazil; Fiske and Subbarow, 1925). This was carried out at 725 nm in a spectrophotometer UV/Visible BEL Photonics 2000 UV (Bel Photonics do Brasil Ltda, Osasco, SP, and Brazil). Phosphorus standard solutions were prepared using monopotassium phosphate (KH2PO4; P.A., ACS, Vetec 1361, Sigma-Aldrich Ltd, Brazil).

2.8 Determination of Calcium (Ca) Content of Plant Samples

About 1 ml each of the processed samples of *A. cordifolia* and *T. daniellii* leaves was introduced into a test tube in duplicate. This was followed by the addition of 3 mls of calcium working reagent and absorbance was read at 512 nm against blank.

2.9 Determination of Potassium (k) Content of Plant Samples

About 5 mls each of the processed samples of *A. cordifolia* and *T. daniellii* leaves was placed in a test tube in duplicate. This was followed by the addition of 2 mls of colbalnitrite into respective test tubes which were shaken vigorously before being allowed to stand for 45 minutes and subsequently centrifuged for 15 minutes. The supernatant was decanted after which 2 mls of ethanol was added to the residue.

The resulting solution was vigorously shaken prior to being centrifuged for about 15 minutes. The resulting supernatant was drained off, followed by the addition of 2 mls of distilled water to the residue. The solution was boiled for 10 minutes with frequent agitation to dissolve the precipitate. About 1 ml of 1% choline hydrochloride and 1 ml of 2% sodium ferric cyanide were added. Then 2 mls of distilled water was also added and the solution was shaken to mix well. The absorbance was taken at 620nm against the blank.

2.10 Determination of Zinc Content of Plant Samples

The Zinc content of processed leaf samples of *A. cordifolia* and *T. daniellii* was evaluated using a GBC Avanta Σ atomic absorption spectrophotometer (Scientific Equipment, Braeside, Victoria, Australia), hollow-cathode lamps (213.9 nm for Zn), a nitrous oxide-acetylene flame for Zn analysis. Standard solution was produced from pure stock solution containing 1000 ppm of the element (Merck 1.09953 Titrisol for Zn, Merck KGaA, Darmstad, Germany).

2.11 Statistical Analysis

Data generated from this study was analyzed using Analysis of Variance (ANOVA). Values were expressed as mean ± standard error of mean (SEM) from three determinations. Differences in mean were compared using Duncan multiple test range. $P<0.05$ was considered significant.

3. RESULTS AND DISCUSSION

Micronutrients are indispensible factors for any functioning living system. Their deficiency has resulted in several debilitating health conditions. They are found in different parts of plants and may be responsible for the therapeutic potentials of most plants. Table 1 shows the vitamin compositions of *A. cordifolia* and *T. daniellii* leaves. Results generated from the analysis carried out on plant samples showed that Vit B6 was present at a concentration of (4.42±0.02 mg/100 g) which was significantly ($P<0.05$) different from the values recorded for Vitmins B3, B12 and A (3.30±0.12 mg/100 g), (2.62±0.01 mg/100 g) and (2.27±0.09 mg/100 g) respectively. However, it is important to note that there was no significant ($P>0.05$) different in the values recorded for Vit B12 and A while all other vitamins evaluated were present at concentrations less than 1.00 mg/100 g. Results from this study are consistent with the findings Ezeokeke et al. [13] which have demonstrated the antioxidant property of *A. cordifolia* leaves which may be attributed to the presence of vitamins such as vitamin B6 etc.

Similar study on *T. daniellii* shows the vitamin compositions of *T. daniellii* leaf. Results generated from the analysis carried out on plant sample showed that leaf of *T. daniellii* contains considerable amount of vitamin B12 (7.87±0.07 mg/100 g) which was considered significantly ($P<0.05$) different from values recorded for vitamins A and C (3.00±0.01 mg/100 g) and (2.25±0.22 mg/100 g) respectively. Other vitamins reportedly present in the leaf of *T. daniellii* are Vit B1, B3 and B5 but were found to be present at concentrations less than 2.00 mg/100 g. Results from this study are consistent with the findings of Shalom et al. [14] which have shown that leaf of *T. daniellii* has antioxidant property which may be attributed mainly to its rich vitamin B12 content.

Table 2 shows the mineral compositions of *A. cordifolia* and *T. daniellii* leaf. The outcome of the analysis showed that *A. cordifolia* leaf contains phosphorus at the concentration of (4.58±0.13 mg/100 g), this was significantly ($P<0.05$) different from the value recorded for potassium and calcium which were reportedly present at concentrations of (2.32±0.05 mg/100 g) and (1.79±0.12 mg/100 g) respectively and zinc being the least at the concentration of (0.90±0.04 mg/100 g). These results are in tandem with the findings of Joseph et al. [15] which showed that extracts of *Alchornea cordifolia* can ameliorate diarrhea, a condition exacerbated in micro-nutrient deficiencies. Similarly, studies on *T. danielii* leaf showed the mineral compositions of *T. daniellii* leaf. The outcome of the analysis conducted on sample material revealed that leaf of *T. daniellii* contains higher concentrations of calcium (6.15 ±0.03 mg/100 g) which was considered significantly different from values recorded for phosphorus and potassium (5.75±0.12 mg/100 g) and (5.30±0.14 mg/100 g) respectively but however has low zinc (1.42±0.02 mg/100 g). These results are in tandem with the finding of Shalom et al. [16]) which showed *T. daniellii* leaf is a dependable source certain of minerals

Table 1. Vitamin compositions of *A. cordifolia* and *T. daniellii* leaves

Vitamins	Concentrations (mg/100 g)	
	A. cordifolia	***T. daniellii***
A	2.27 ± 0.09^{c}	3.00 ± 0.09^{b}
B1	0.84 ± 0.01^{e}	1.07 ± 0.01^{d}
B3	3.30 ± 0.12^{b}	1.32 ± 0.02^{d}
B5	0.36 ± 0.03^{d}	1.11 ± 0.02^{d}
B6	4.42 ± 0.02^{a}	1.34 ± 0.02^{d}
B12	2.62 ± 0.01^{c}	7.87 ± 0.07^{a}
C	0.34 ± 0.02^{d}	2.25 ± 0.22^{c}

Values are expressed as mean ± SEM from three determinations. Values with different superscripts in a row are significantly different (P<0.05)

Table 2. Minerals compositions of *A. cordifolia* and *T. daniellii* leaves

Minerals	Concentrations (mg/100 g)	
	A. cordifolia	***T. daniellii***
Calcium	1.79 ± 0.12^{c}	6.15 ± 0.03^{a}
Phosphurus	4.58 ± 0.13^{a}	5.75 ± 0.12^{ab}
Potasium	2.32 ± 0.05^{b}	5.30 ± 0.14^{b}
Zinc	0.90 ± 0.04^{d}	1.42 ± 0.02^{c}

Values are expressed as mean ±SEM from three determinations. Values with different superscripts in a row are significantly different (P<0.05)

4. CONCLUSION

The knowledge of the presence of micronutrients in *A. cordifolia* leaf has added value to the wealth of existing knowledge about this important medicinal plant and further consolidates findings already established on it especially as it regards its therapeutic potentials. Similarly, *T. daniellii* has been proven through this research to be one of the very few medicinal plants with multiple potentials to function both as therapies for numerous human diseases as well as sources of micronutrients for active cellular metabolic functions and hence may be suitable as livestock feed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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