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Original Research Article

Comparative Analysis on Nutritional and Antinutritional factors in Raw and Blanched *Crotalaria laburnoides* Klotzsch leaves from Iramba District, Tanzania

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Abstract: This research aimed at investigating the nutritional and antinutritional contents of the leaves of wild edible plant, *Crotalaria laburnoides* (commonly known as *'Nsonga'* by natives), in order to provide knowledge on the nutritional implication of feeding on *C. laburnoides* diet. A comparative analysis on the nutritional and antinutritional factors of raw and blanched leaves of *C. laburnoides* was carried out using standard analytical methods, where levels of some nutrients and antinutrients were determined. Results obtained showed the percentage (%) moisture contents as 15.25 and 14.90, ash as 8.37 and 9.56, crude fiber as 12.91 and 12.87, crude protein as 7.61 and 10.37, crude fat as 1.41 and 1.64, and carbohydrate as 54.45 and 50.14 for raw and blanched samples respectively. The findings also revealed that β-carotene contents as 10.50 and 7.93 mg/100 g, Vitamin B_1 as 1.77 and 1.02 mg/100 g, Vitamin B_2 as 7.71 and 1.83 mg/100 g for raw and blanched leaves respectively whereas Vitamin C was not detected in both samples. Mineral analysis revealed that there were high amounts of Calcium as 5999 and 11700 ppm and Manganese 105 and 110 ppm for raw and blanched leaves respectively. Also the findings revealed the presence of metal contaminants ("heavy metals") in both samples. The contents of antinutritional factors (ANFs); oxalates were 52.94 and 40.0 mg/100g, phytate were 4.30 and 2.02 mg/100g and tannin were 0.115 and 0.073 mg/100g for raw and blanched leaves respectively. The results of this study revealed that *C. laburnoides* leaves are good nutritionally, containing proximate components, minerals and vitamins in amounts comparable to cultivated species. However, they have high contents of ANFs, but these are reduced significantly (p<0.05) during cooking (as seen in phytate and oxalate in blanched samples), hence cannot prevent their utilization as food sources. This reduction could be accounted by the heat effect/preparation method. Despite the popular consumption and good results presented in this paper, the suitability *Crotalaria laburnoides* leaves for nutritional purposes will depend on the ongoing toxicity studies.
Keywords: *Crotalaria laburnoides*, Nutritional contents, Antinutritional factors, Iramba, Tanzania.

INTRODUCTION

The use of wild edible plants in different localities provides optimum source of nutrients [1], they supply the body with minerals, vitamins and certain hormone precursors as well as energy and proteins [2]. Unfortunately, vegetables contain larger amounts of some antinutritional factors such as phytates and oxalates capable of affecting the availability of desirable nutrients [3, 4]. However, traditional cooking methods rid vegetables of most soluble oxalate when the cooking water is discarded. This would otherwise complex with divalent metals in the diet rendering them unavailable for human absorption, also the cooking rids vegetables of cyanogenic glycosides [5]. On the other hand, prolonged cooking not only denatures protein and destroys heat sensitive nutrients such as vitamin C, but also it leads to significant loss in protein, fat and vitamins [6].

Despite the importance of leafy vegetables as sources of nutrients to diets of most people in developing countries including Tanzania, the concern about horticulture and economic development is still little. *C. laburnoides* (fam: Fabaceae), is among important leafy edible vegetable in Tanzania, yet there is no information about its nutritional and antinutritional composition [7].

Therefore, this research aimed at investigating the nutritional content and antinutritional factors of *C. laburnoides* leaves, in order to provide knowledge on the nutritional implication of feeding on low nutritive quality, which will help to ensure better health condition of people in developing countries.

Photograph of the plant *Crotalaria laburnoides* Klotzsch taken from Iramba District

MATERIALS AND METHODS

Plant materials collection, authentication preparation

The leafy parts of the plant *Crotalaria laburnoides* were harvested in fresh condition from the wild in Iramba district, Tanzania. The leaves were washed with spray jets water to remove sand and other adhering soil, dirt and contaminants. The sample was divided into two portions: One portion was blanched at 80^{o}C for 15 minutes (S_2) and the other was left raw (S_1). Both samples were allowed to be air dried under the shade in a room for 12 days. Herbarium specimen was prepared for verification at the Herbarium unit in the Department of Botany, University of Dar es Salaam, where voucher specimen [**FMM 3697 (B)**] was deposited. The dried leaves of the two samples were then pulverized separately to form a course powder and stored in different airtight containers at room temperature prior to extraction and further laboratory analyses.

Chemicals and reagents

The methods used in this work for the various determinations of the samples were either standard methods or adopted from previous researchers, whereas all the reagents used in this work were of analytical grade reagents. **(**4% Boric acid, Anhydrous Diethyl Ether, n-Hexane, Ethyl Acetate, Acetonitrile (HPLC grade), Methanol (HPLC grade) and Formic Acid were obtained from **BDH Company** (**ACS reagent grade**). Standard references β-carotene, thiamine (Vitamin B_1), Ascorbic acid (Vitamin C), and riboflavin (Vitamin B_2) were obtained from **Sigma-Aldrich chemicals)**.

Proximate Analysis

Proximates (moisture content, ash value, crude fiber, and crude fat and total carbohydrate content) were determined by official methods of analysis as described by AOAC [8], while crude protein was determined by the Auto - Kjeldahl method for nitrogen analysis as described in the application note of Foss Tecator Auto-Kjeltec -2300.

Analysis of Vitamins

Analysis of β-Carotene

β-carotene was analyzed by HPLC method as previously described by [9]. The sample was prepared depending on the matrix of the product, whereas exactly 10.0 g of well blended small size sample was weighed.

Preparation of Clear Solution:

This was prepared by adding 10 mL of n-Hexane 95% to 10.0 g homogenized/ hydrated sample in 50 mL polytetrafluoroethylene (PTFE) centrifuge tube and then shaken for 1 minute. About 4 g of magnesium sulphate ($MgSO_4$), 1 g of sodium chloride (NaCl), 1 g Citric acid + $2H_2O$, 0.5 g $Na_2HCitrate$ + 1.5 H_2O were added, and shaken vigorously for 10 minutes then centrifuged for 5 minutes at 4,000 r.p.m. 6 mL aliquot of the supernatant was transferred to a dispersive centrifuge tube containing 150 mg PSA and 900 mg $MgSO_4$ and shaken for 1 minute then centrifuged for 5 minutes at 4,000 r.p.m. About 5 mL aliquot of the supernatant was transferred in the vial ready for injection in the HPLC.

HPLC conditions

i. Mobile phase composition:
   A: 88% of Acetonitrile HPLC grade, B: 10% of Methanol HPLC grade,
   C: 2% Ethyl Acetate.
ii. Model: Low pressure gradient.
iii. HPLC Column: Discovery (R) Supelco-C8; 12.5 Cm x 4.0 mm, 5µm.
iv. Analytical; cat no. 581324-U. Col: 110156-01; BL: 6853.
v. Flow rate: 1.50 mL/ min
vi. Temperature: 25^{o}C – 45^{o}C
vii. Injection Volume: 10 µL
viii. Wavelength: 436 nm
ix. Running time: 6.5 minutes with estimated retention time 3.378 for β-carotene

Mathematical Calculation

Since a dilution was made for a clear solution, the final result was calculated as:
Result (mg/Kg) = HPLC concentration reading X dilution factor

Analysis of water soluble vitamins

Vitamins B_1, B_2 and C were estimated by HPLC method [11].

Analysis of Ascorbic Acid (Vitamin C)

Preparation of vitamin C Stock Standard Solution

Due to limited stability of vitamin C (ascorbic acid), a stock solution of 2.0 mg/ mL was freshly prepared before analysis by accurately weighing 20 mg of the standard vitamin powder and adding distilled water to a total of 10 mL to form a stock solution of 2.0 mg/ mL. The working standards containing vitamin in 0.015% formic acid were prepared on the day of use from this stock solution.

Preparation of working samples

Plant materials consist of many components that may cause chromatographic interferences with the vitamins. The samples were prepared by solid phase extraction (SPE) to enable separation of water soluble vitamins from most of the interfering components.

In this method, the raw dried sample (S_1) was prepared by taking 6 parts of distilled water (30 g) into 1 part of the sample (S_1), 5 g (dilution factor, F = 7), whereas, the blanched dried sample (S_2) was prepared by taking 4 parts of distilled water (20 g) into 1 part of the sample (S_2), 5 g (dilution factor, F = 5). The mixture of each sample was homogenized using homogenizer at a medium speed for 1 minute. The homogenized samples centrifuged for 20 minutes at a speed of 4,000 r.p.m. The SPE method was used for the extraction of water soluble vitamins. At the end, the residue from each sample was dissolved in the mobile phase A (0.015% formic acid). Before HPLC analysis, all samples were filtered through 0.45 µm pore size (axiva nylon filters) at 7 bar max.

HPLC Conditions

i. Mobile Phase
   A: 0.015% formic acid in distilled water; **B:** Methanol/Acetonitrile (17:83 v/v)
ii. Gradient: 100% Mobile Phase B for 3 minutes, 0 - 45% Mobile Phase A in 5 minutes, 45 - 100% Mobile Phase A in 0.1 Minute.
   100% Mobile Phase A for 16.9 minutes, 5 minutes of equilibration at 100% Mobile phase A prior to injection.
iii. Model: Low pressure gradient
iv. HPLC Column: BDS Hypersil C-18; 4.6 Cm x 150 mm (Thermo Scientific, SN-0972769Q).
v. Flow rate: 1.00 mL/ min
vi. Temperature: 45^{o}C
vii. Injection Volume: 5 µL
viii. Wavelength (Noise): 210 nm, 280 nm and 350 nm
ix. Running time: 16.9 minutes
x. System backpressure: 4.0 MPa at 100% Mobile Phase A, 1.9 - 2.0 MPa at 100% Mobile Phase B
xi. Detection: Photodiode array; 210 nm - 350 nm

Analysis of Thiamine (Vitamin B_1) and Riboflavin (Vitamin B_2)

Preparation of Extraction Solutions

Extraction solution was made by mixing 50 mL of acetonitrile with 10 mL of glacial acetic acid and the volume was finally made up to 1000 mL with double distilled water.

Preparation of Buffer and Mobile Phase

To prepare buffer, 1.08 g of hexane sulphonic acid sodium salt and 1.36 g of potasium dihydrogen phosphate were dissolved in 940 mL HPLC water and 5 mL of triethylamine was added to it and the pH was adjusted to 3.0 with orthophosphoric acid. For the preparation of mobile phase, buffer and methanol were mixed in a ratio of 96:4 and filtered through 0.22 µm membrane filter and sonicated for degassing in an ultrasonic bath.

Standard Preparation

Standard stock solutions for vitamin B_2 (riboflavin) and vitamin B_1 (thiamine) were prepared by dissolving 6.9 mg of riboflavin in 100 mL of extraction solution.

Preparation of Samples

A weight of 2.5 g of each sample was weighed and made into homogenized in mortar with pestle and transferred into conical flask and 25 mL of extraction solution was added, kept on shaking water bath at 70°C for 40 min. Thereafter, the sample was cooled down and centrifuged at a speed of 4000 r.p.m for 20 minutes. The supernatant was drawn out and finally the volume was made up to twice the volume of supernatant (approximately 50 mL) with extraction solution, where the 150 mg of PSA were added in each sample then the samples were vortexed for 1 minute then centrifuged for another 10 minutes at speed of 4000 r.p.m. Then each sample was filtered through 0.45 µm filter tips and aliquots of 20 µL from the resulted solution of each sample were injected into the HPLC by using autosampler.

HPLC Conditions

Analytical reversed phase C-18 column (ODS column, 250 × 4.6 mm, 5 µm, Phenomenex, Inc.) was used for the separation. Mobile phase consisting of a mixture of buffer and methanol in the ratio of 96:4 (v/v) was deliv-ered at a flow rate of 1 mL/min with UV detection at 210 nm. The mobile phase was filtered through 0.22 µm membrane filter, sonicated and degassed before use. Analysis was performed at room temperature (~28°C) temperature. All the prepared sample solutions were first chromatographed to ensure that interfering peaks were not present. 20 µL aliquots of the standard solutions and sample solutions were injected.

i. Mobile Phase: Consisting of buffer and methanol (HPLC grade) in the ratio of 96:4 (v/v)
   Buffer was prepared by mixing hexane sulphonic acid sodium salt and potasium dihydrogen phosphate and dissolved in HPLC water and triethylamine was added to the mixture in stated proportions and the pH was adjusted to 3.0 with orthophosphoric acid.
ii. Model: Low pressure gradient
iii. HPLC Column: Analytical reversed phase C-18 column (ODS column, 250 × 4.6 mm, 5 µm, Phenomenex, Inc.)
iv. Flow rate: 0.600 mL/ min
v. Temperature: Room temperature (~28^{o}C)
vi. Injection Volume: 10 µL
vii. Wavelength: 210 nm (190 - 300 nm)
viii. Running time: 12 minutes with estimated retention time for Vitamin B_1 and B_2 at 3.68 and 5.30 min respectively
ix. System backpressure: 2.0 MPa
x. Detection: Photodiode array; 210 nm - 350 nm

Determination of the mineral content (Mineral Analysis)

Minerals were determined by X-Ray fluorescence (XRF) spectrometer whereby the application note by Innov X-systems (2003) was adopted. The procedure was as follows; the samples for analysis (S_1 and S_2) were prepared by grinding into mortar and pestle then was ground in a mixer mill (Romer analytical sampling mill) to a very fine powder which was then passed through a sieve of 250 µm mesh. An amount of 60 - 70 mg of the ground powder of each sample was weighed, transferred into respective XRF test cups which were then covered tightly with nylon material. The XRF test cups containing samples were then brought to the analyzer (XRF spectrometer) for analysis.

Analysis of Antinutritional factors (ANFs)

i. Determination of Tannin contents

Tannins were determined by method adopted from previous researchers [12]. Briefly, 400 mg of each sample was placed into separate conical flasks and 40 mL diethyl ether containing 1% acetic acid (v/v) was added, the mixtures were properly mixed to remove the pigment materials. Each supernatant was carefully discarded after 5 minutes. 20 mL of 70% aqueous acetone was added and the flasks were sealed with cotton plug covered with aluminum foil, then kept in electrical shaker for 2 hours for extraction. The content in the flasks was filtered through Whatman filter paper no. 42 and sample filtrates were used for analysis. 50 mL of the filtrate (tannins extract) from each sample was taken into test tubes and the volume of each was made up to 1.0 mL with distilled water. 0.5 mL Folin Ciocalteu reagent was added to each and mixed properly. Then, 2.5 mL of 20% sodium carbonate solution was added and mixed. The mixtures were kept for 40 minutes at room temperature, after which absorbance was determined using spectrophotometer and concentration was estimated from the tannic acid standard curve.

ii. Determination of oxalate content

A standard method [13] was adopted in the determination of tannins. Briefly, 1.0 g of each sample was placed in a 250 mL volumetric flask, 190 mL of distilled water and 10 mL of 6M Hydrochloric acid were added. Each mixture was warmed on a water bath at 90ºC for 4 hours and the digested samples were centrifuged at a speed of 2,000 r.p.m for 5 minutes. The supernatant were then diluted to 250 mL. Three 50 mL aliquots of each supernatant were evaporated to 25 mL, the brown precipitate was filtered off and washed. The combined solution and washings were titrated with concentrated ammonia solution in drops until Salmon pink color of methyl orange changed to faint yellow. The solutions were heated on a water bath to 90ºC and the oxalate was precipitated with 10 mL of 5% calcium chloride ($CaCl_2$) solution. The solutions were allowed to stand overnight then centrifuged. Each precipitate was washed into a beaker with hot 25% H_2SO_4, diluted to 125 mL with distilled water and after warming to 90ºC it was titrated against 0.05 M Potassium permanganate, $KMnO_4$.

iii. Determination of Phytate content

Phytate amount was quantified by method as described by [12]. Briefly, 2.0 g of each sample was weighed separately into 250 mL conical flask. 100 mL of 2% Hydrochloric acid was used to soak each sample in the conical flask for 3 h and then filtered through a double layer of hardened filter papers. 50 mL of each filtrate was placed in 250 mL beaker and 100 mL of distilled water was added to each to give proper acidity. 10 mL of 0.3% ammonium thiocyanate solution was added into each solution as indicator. Each solution was titrated with standard iron chloride solution. The end point color was slightly brownish-yellow which persisted for 5 minutes. The percentage phytic acid was calculated.

DATA ANALYSIS

The proximate composition, minerals and ANFs were estimated in triplicates, and the means were compared in pair wise depending on the way of preparation of the plant, i.e. whether as fresh or blanched sample by using One-Way ANOVA in Microsoft Office Excel 2007. In both analyses, the *p*-value cut point used was 0.05, that is, the results were considered as statistically significant if the *p*-value was <0.05.

RESULTS AND DISCUSSION

Proximate composition

Table 1: Proximate composition of *Crotalaria laburnoides*

Parameter	Raw dried sample (S_1) (%)	Blanched dried sample (S_2) (%)	*p*-values
Moisture content	15.25±0.28	14.90±0.31	$p>0.05$
Total Ash	8.37±0.06	9.56±0.32	$p<0.05$
Crude Fiber	12.91±0.05	12.87±0.03	$p>0.05$
Crude Fat	1.41±0.06	1.64±0.01	$p<0.05$
Crude protein	7.61±0.01	10.89±0.02	$p<0.05$
Carbohydrate	54.45 ±0.006	50.14 ±0.006	$p<0.05$

***Mean±S.D of triplicate determinations**

The results of proximate composition of both samples are represented in **Table 1**. The moisture content in both raw and blanched samples was less than 10%. Similar results have been reported from other leguminous leafy vegetables like *Uraria picta* (moisture 8.70%) [14]. This accounts for minimal chances to microorganisms' growth (contamination) in the dried vegetable hence preventing their deterioration [15].

Ash content, which is an index of mineral content in both raw and blanched samples (8.37 and 9.56%), compares well to that reported in the leaves of *Senna occidentalis* (8.35%) [16]. But it was higher than values obtained in *Crotalaria odoratum*, 7.88% [17]. Higher ash content is a reflection of the mineral contents preserved in the food materials. The result therefore suggests a high deposit of mineral elements in the leaves of this plant and is in agreement with the fact that, leguminous plants are rich in mineral elements [18].

Regarding the fat content, *C. laburnoides* is a poor source of lipid when compared with other leguminous leafy vegetables, such as *Senna occidentalis* whose reported content was 5.15% [18]. However, a diet providing 1-2% of its caloric energy as fat is said to be sufficient to human beings as excess fat consumption is implicated in certain cardiovascular disorders such as atherosclerosis, cancer and ageing [19].

Crude protein values were higher compared to the related leguminous leafy vegetable such as *Senna occidentalis*, 3.83% [16], but lower than vegetables of related species; *Crotalaria walkerii* (12.5%) and *Crotalaria fysonii* (17.19%) [18]. After blanching there was an increase in protein value by 4.28%. This might be due to elimination of tannin by the heat leading to free protein formally bound to tannins [20].

C. laburnoides leaves were found to be rich in fibres, with comparable values of raw and blanched samples with $p>0.05$, suggesting little or no heat effects on the fibres and matches with the previously report from another study [20]. Although no disease is caused solely by fibre-free diet; constipation, hemorrhoids and high cholesterol levels are among the medical complications resulting from not eating adequate fibre containing foods [14]. They are also reported to lower risks of diabetes [16].

Carbohydrate content of *C. laburnoides* slightly decreased after blanching (54.45% - 50.14%). Our findings are contrary with previous study that showed an increase in carbohydrate. This could be linked with the destruction of antinutritional factors binding carbohydrates in the raw sample [20]. In present study, ANFs had little or no effect on destruction of the type of carbohydrate found in *C. laburnoides* leaves.

Vitamin content

Table 2: The vitamin contents in raw and blanched samples of *Crotalaria laburnoides*

Vitamin Analyzed	Concentration in S_1 (mg/100 g)	Concentration in S_2 (mg/100 g)	Recommend daily intake by FAO/WHO
β-carotene (pro-vitamin A)	10.50	7.93	-
Thiamine (Vitamin B1)	1.77	1.02	1.1mg to 1.5mg
Riboflavin (Vitamin B2)	7.71	1.83	1.1mg to 1.6mg
Ascorbic Acid (Vitamin C)	ND	ND	

ND = Not detected

Our findings revealed that, *C. laburnoides* contain higher amounts of β-carotene, 7.93 mg/100 g and 10.45 mg/100 g in S_1) and S_2 respectively. These values are comparable with those reported in other leafy vegetables commonly used in Tanzania such as *Crotalaria brevidens, Amaranthus sp* [*"mchicha"*in Swahili], *Cucurbita sp*, ("*majani ya maboga*" or *"msusa"* in Swahili, and *"Kwipa"* in Nyiramba) whose botanical name was not determined. With the exception of the later, their β-carotene contents of the edible portions are reported to be more than 5 mg/100 g [21]. This underlines its important role as a source of vitamin A.

Vitamin C was not detected in both samples of *C. laburnoides*. However, this vitamin is unstable and thermo labile. Our work used raw dried leaves and blanched samples. These may have facilitated the loss of vitamin C [6]. Considering the methods employed to prepare the vegetable i.e. boiling and frying are not in the favour of retaining Vitamin C.

Observed vitamin B_1 in S_2 was lower than the recommended FAO/WHO nutrient daily intake. But for the case of vitamin B_2, the value is slight higher than the recommended values as stated by FAO/WHO [22]. However, it is unlikely to cause toxicity due to limited intestinal absorption of vitamin B_2 [23] and amounts of vegetable the consumed in a single day.

Mineral composition

Table 3: Mineral composition of *Crotalaria laburnoides* leaves

Element	Amount in S_1 (ppm)	Amount in S_2 (ppm)	*P* – value
Macro Elements			
Calcium	**5999**	**11700**	***p*<0.05**
Iron	**174**	**374**	***p*<0.05**
Chlorine	**1509**	**9286**	***p*<0.05**
Sulphur	**3130**	**2034**	***p*>0.05**
Micro Elements			
Manganese	**105**	**110**	***p*>0.05**
Zinc	**36**	**30**	***p*<0.05**
Metal Contaminants			
Arsenic	**8.1**	**7.1**	***p*>0.05**
Rubidium	**58**	**42**	***p*<0.05**
Strontium	**122**	**170**	***p*>0.05**
Cadmium	**ND**	**ND**	

***Mean of triplicate determinations, ND = Not detected**

Raw and blanched *Crotalaria laburnoides* leaves contain higher level of calcium, sulfur and chlorine when compared with related species: *C. semperflorens*, *C. clarkei*, *C. paniculata*, *C. fysonii* and *C. walkerii* [18]. Calcium is essential for healthy bones, muscles, nerves, teeth and blood. It is also required for the absorption of dietary vitamin D, synthesis of the neurotransmitter acetylcholine, and activation of enzymes such as the pancreatic lipase [24]. The recommended daily allowance (RDA) of calcium for children is between 500 and 1000 mg and for adults 800 mg, the vegetable is thus a useful source of calcium.

Concerning trace elements, Manganese and Zinc were detected. The permissible limit of Mn is 5.50 ppm (mg/kg) and that of Zn is 5 ppm (mg/kg) per day [23]. In our findings, the amounts of both Zn and Mn were beyond the limits. Excessive of these trace elements in the diet, especially for prolonged daily intake, as in case of Zn ranging 150 - 450 mg/day is detrimental to human health [25]. Under normal circumstances, either raw or blanched vegetable taken per day is unlikely to exceed safe limits of these metals.

Detected metal contaminants (heavy metals) are rubidium (Rb), strontium (Sr) and arsenic (As). Although rubidium is not among the essential metals, some evidences suggest its role in reducing free radical pathology and serve as a mineral transporter across defective cell membranes especially in cells associated with aging. Also, having a close physiochemical relationship with potassium and it may have the ability to act as a nutritional substitute. Strontium is of health benefits especially in the women in their postmenopausal age as in moderate dietary levels it promotes of calcium uptake into bones [26] thus slowing the thinning of the bones and by allowing new bone to rebuild and strengthen [27]. Arsenic is the most toxic of all and was found to be by 31 and 27.3 times higher than the permissible limits [22]. Despite the fact that, amount taken per day is unlikely to exceed safe limits, a systematic review and risk assessment have associated low-level arsenic exposure in drinking water with cardiovascular disease [28]. This crops a serious concern on consumption of *C. laburnoides* as a vegetable.

After blanching there was a statistical significant increase in the amounts of Ca, Fe, Mn and Cl. This finding is in agreement with previous study by [20]. In fresh vegetables ANFs such as phytates do complex with several minerals. However, blanching frees the minerals and these explanations matches well with the observed decrease of the phytate after blanching which was statistically significant. However, the decrease in S was not statistically significant with $p>0.05$.

Antinutritional factors (ANFs)

Results are as presented in **Table 4.** The quantification of antinutritional factors revealed higher amounts of tannins, oxalates and phytates in S_1 than S_2 suggesting a significant reduction effect, by heating process.

Table 4: The results for quantification of ANFs in *Crotalaria laburnoides*

S/No	Parameter	Raw dried sample (S_1) (mg/100 g)	Blanched dried sample (S_2) (mg/100 g)	*p*-value
1.	**Alkaloid**	**57.5±0.05**	**10.5±0.01**	***p*<0.05**
2.	**Tannins**	**0.115±0.02**	**0.073±0.03**	***p*>0.05**
3.	**Oxalate**	**52.94±0.20**	**40±0.20**	***p*<0.05**
4.	**Phytate**	**4.30±0.02**	**2.02±0.02**	***p*<0.05**

The reduction of tannins content after blanching was not significant. Tannins are phenolic compounds that bring about their antinutritional influence by precipitating or binding dietary protein and digestive enzymes to form complexes not readily digestible [29]. The observed amounts of tannins in our study are relatively lower compared to that reported by other researchers, but it should be noted that, even a low level of tannin is undesirable from the nutritional point of view. Fortunately, processing methods (like blanching, cooking and frying) have been shown to reduce the tannin content of food products [30].

Observed phytate contents in both samples were higher than values reported from previous studies on other leguminous leafy vegetable like *Senna occidentalis* leaves (0.2705±0.05 mg/100 g) [16]. Phytate content of food products has been known to lower the bioavailability of minerals, the solubility, functionality and digestibility of carbohydrates and inhibition of several enzymatic activities [31] and inhibition of protein digestion by forming complexes with them. However, the phytate content can further be lowered by processing [32], and this accounts for the increase in crude protein and some minerals (Ca, Mn, Fe) in blanched sample.

After blanching, oxalate content reduction was significant. Similar results were reported from the leaf vegetable *Colocasia esculenta* [20]. This further proves that, heat treatment of vegetable product prior to consumption reduces antinutrients. Oxalates have deleterious effects on human nutrition and health, mainly by decreasing calcium absorption and aiding the formation of kidney stones [33]. Oxalate also forms insoluble salts with minerals such as zinc, calcium and iron preventing their utilization. Cooking methods that involve discading the decoction after boiling is advantageous, it leaves cooked vegetables with low content of antinutrients thus enhancing the absorption of essential elements [34].

CONCLUSION

Analysis of the leaves of *C. laburnoides* showed presence of valuable nutritional factors (proximates, vitamins and minerals) and antinutritional factors in appreciable amounts as well as metal contaminants. From nutrition point of view, oxalates, phytates and tannins present in *C. laburnoides* are likely to interfere with other nutrients especially minerals, protein and the functioning of various enzymes. Theoretically, long term /excessive use of this plant are likely subject users to health problems/risks including kidney stone due to its large quantity of oxalates. Fortunately, cooking has shown to reduce these ANFs (as seen in phytate and oxalate) significantly ($p<0.05$), hence cannot prevent *C. laburnoides* utilization as food sources. The suitability of *C. laburnoides* leaves for nutritional purposes will depend on results from toxicity studies and especially that it contains high levels of the arsenic which among the most toxic metals.

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