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Variation of Nutrients and Functional Properties within Young Shoots of a Bamboo Species (*Yushania alpina*) Growing at Mt. Elgon Region in Western Kenya

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Abstract Yushania *alpina* is an indigenous bamboo species growing at Mt. Elgon forest in Western Kenya and its young shoots are consumed by the local people as a vegetable. This study was done to determine some physico-chemical properties and their distribution within the shoot. Each shoot was divided into the upper and lower portions which were analyzed separately, and results were expressed in dry weight basis. On proximate composition, there was no significant difference ($p<0.05$) between the two portions in terms of moisture, protein, ash and fat content, which ranged 92.2-92.4% fresh weight, 33.0-33.4% dry weight basis (dwb), 17.0-17.1% dwb and 2.0% dwb, respectively. Significant difference was found in fiber and carbohydrates, whose content was 23.9 and 23.6% dwb in the upper portion compared to 30.7 and 17.3% dwb in the lower part, respectively. The upper portion contained Ca of 2,670, Mg of 4,300, K of 35,900 and P of 7,630 µg/g dwb, whereas the lower portion had Ca of 1,060, Mg of 1,270, K of 27,600 and P of 4,810 µg/g dwb. The upper portion was found to contain thiamine, riboflavin and vitamin C of 2.2, 8.4 and 78.2 µg/g dwb, respectively, whereas the lower part contained 1.8, 7.3 and 51.2 µg/g dwb, respectively. Fructose was found to differ significantly with the upper portion having 2.19 % against 0.62 % in the lower portion. Total polyphenol and flavonoid content of 27.6 and 24.6 mg/g dwb, respectively, were observed in the upper part compared to 25.9 and 20.1 mg/g dwb, respectively in the lower part. The upper portion was observed to exhibit better antioxidant activity with LC_{50} of 1 mg/ml compared to 5 mg/ml for the lower part, and higher Hunter's L* value.

Keywords: *Yushania alpina, bamboo, nutrients' distribution, minerals, polyphenol, flavonoids, antioxidant activity*

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1. Introduction

Bamboo belongs to the grass family *Poaceae* and has over 1,250 species worldwide, although most of them are found in South East Asia [1]. *Yushania alpina* (*Y. alpina*), formerly known as *Arundinaria alpina* K. Schum and which is also commonly referred to as the alpine bamboo, is an indigenous species found in Kenya and other Eastern Africa highlands at altitudes of between 2,400 and 3,400 m. The species is currently estimated to cover about 150,000 ha and grows on the Aberdare ranges, Mt Elgon, Mt Kenya, Mau escarpment and Cherangany Hills, amongst other areas [2, 3]. This species is also found in Ethiopia on altitudes of 2,200-4,000 m and consumption of the shoots has been reported, although the bamboo is mainly used as construction material [4]. Some exotic varieties belonging to the genera of *Bambusa*, *Dendrocalamus*, *Gogantochloa*, *Oxytenanthera* and others, have also been planted in Kenya [5].

Several researchers [6,7,8] have reported the nutritive value of over 20 edible species of bamboo all over the world and have found them to be very rich in protein, dietary fiber, amino acids, minerals and vitamins, among others. It has been reported that bamboo shoots are good sources of dietary fiber with beneficial effect on lipid profiles in the body and bowel functions in young women [9]. They are low in fat and contain phytosterols forming good diet and maintaining low cholesterol level in the body [1]. Due to their distinct characteristics, bamboo shoots have been termed as vegetable with health promoting functionalities [10,11]. The nutritional composition of shoots of *Y. alpina*, *Dendrocalamus giganteus* and *Bambusa vulgaris* growing in Kenya has been studied and found to possess great potential in fighting malnutrition and ill-health [12].

Traditionally, bamboo has been used by some communities in various ways but its potential as a food source remains

unexploited [6]. In Kenya, bamboo is used for fencing, basketry, making flower props, tooth picks and furniture. However, some consumption of the shoots of *Y. alpina* by local people living around Mt. Elgon has been reported, although its usage as a food has been ranked the least important amongst the different uses mentioned above [5]. The shoots are therefore underutilized resource, despite the fact that they have potential of improving life of the rural population [7]. Kigomo [13] found that lack of adequate technical information on the local bamboo resource was a hindrance to its utilization. Choudhury *et al.* [8] has also pointed out that research on bamboo shoots is inadequate, and that bamboo-based products are lacking in the international market, while Sood *et al.* [14] reported underutilization of bamboo as food.

The shoots of *Y. alpina*, unlike those of the giant bamboo species, are relatively slender. They are harvested after the onset of rain around May and June each year when they are about 60 cm tall. There is however, no scientifically guided approach in harvesting these shoots in order to get the most nutrient-rich part and therefore, variation of nutrients and other bio-actives within the shoot needs to be investigated. Thammawong *et al.* [15] found variation in sugars between different sections of the shoot of *Phyllostachys pubescens* Mazel from the apex to the bottom. The age of the shoots was found to affect the concentration of nutrients and other biochemical constituents of *Dendrocalamus asper*, *D. strictus* and *Bambusa tulda*, particularly for proteins, phenolics, fiber and carbohydrates [16]. There is however, little information available on these aspects of the shoot and more research on bamboo from different agro-ecological regions as a food resource is recommended [6].

This research work therefore, was undertaken to determine the distribution of nutrients and other functional properties within the shoots of *Y. alpina* growing in Kenya, in order to maximize its usage as a functional food beneficial to human health.

2. Materials and Methods

2.1. Sample Preparation

About 40 kg of shoots of *Y. alpina* with heights of about 60 cm were randomly harvested during a peak season in May 2013, inside Mt. Elgon forest in Western Kenya and transported to Food Science Laboratory of Jomo Kenyatta University of Agriculture and Technology. The outer leaves were removed and each of the soft shoot was divided into two parts, making the upper and the lower portions. The upper part was 15 cm long from the apex after peeling, and the lower portion was the remaining part up to the soft part easily cut by a knife as standardized based on local practice. Color and moisture content were determined for the fresh samples and the rest were dried in an air-circulating oven at optimum temperature of 70ºC for 24 hr [17]. After drying, the upper and the lower portions were ground separately, to pass a sieve of 0.84 mm. Each portion was well homogenized and packaged in sealable polyethylene bags and kept at 5°C until analysis. A minimum of three determinations were done for each parameter under consideration and results given in dry weight basis (dwb).

2.2. Reagents and Chemicals

Gallic acid, Follin Ciocalteau reagent and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich, Germany. Sucrose, glucose, fructose, thiamine hydrochloride and riboflavin were from BDH Biochemical, England, whereas L-ascorbic acid was purchased from Panreac, Spain. Mineral standards were purchased from Wako Chemicals, Japan. The rest of reagents/chemicals used were of analytical grade, and deionized and distilled water was used.

2.3. Determination of Proximate and Mineral Composition

Determination of proximate composition was performed using AOAC methods [18], where moisture content was determined by drying 5 g of sample at 105°C to constant weight. Protein content was determined by Kjeldahl method and protein content obtained by multiplying percentage nitrogen by 6.25. Fat was extracted by Soxhlet's method using petroleum spirit and determined gravimetrically. Ash content was determined by incinerating 5 g of the sample at 550°C until constant weight was attained. Fiber was determined by boiling under reflux 2 g of sample in 1.25% H_2SO_4 and 1.25% NaOH, consecutively. The residual after filtering was washed with alcohol and ether. It was then dried and incinerated at 500°C for 1 hr and the difference in weight before and after incineration was calculated as a percentage fiber content. Carbohydrate content was obtained by subtracting the sum of moisture, fat, ash, fiber and protein content from 100.

After digesting ground samples with H_2SO_4-H_2O_2 and diluting to 50 ml with deionized water, minerals were determined by an inductively coupled plasma spectrometer (ICPS 8100, Shimadzu Ltd, Japan).

2.4. Determination of Thiamine and Riboflavin

AOAC methods were used with slight modification [18]. Two grams of the sample were mixed with 20 ml of 0.1 N HCl and heated in boiling water for 60 min. The pH was adjusted to 4.0 and 100 mg of taka-diastase was added, mixed and incubated at 40°C for 18 hr. The digest was made to 50 ml with 2 % acetic acid and centrifuged at 5,000 rpm for 10 min and the supernatant was used for HPLC analysis. In case of thiamine, pre-column oxidation with potassium ferricyanide in NaOH solution to give thiochrome form for fluorescence was done before injecting into HPLC (Shimadzu LC-10A) with a reverse phase C18 column of 250 x 4.6 mm, and fluorometric detection at Ex 365 nm and Em 435 nm (Shimadzu RF 1501). The mobile phase for thiamine was methanol: acetate buffer of pH 4.4 (40:60) and for riboflavin was methanol: water: acetic acid (40:59.5:0.5) at UV wavelength of 270 nm (Shimadzu SPD-10A). Thiamine hydrochloride and riboflavin were used to prepare standard solutions for quantification.

2.5. Determination of Vitamin C

Vitamin C content in the samples was determined by HPLC method [19]. Ten grams were homogenized with 20 ml of 0.8% *m*-phosphoric acid for 30 min and

centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was micro-filtered with a 0.45 µm filter and injected into HPLC (Shimadzu LC-10A) with a photo diode array detector (Waters PDA 2996) set at 266 nm. Separation was achieved using a reverse phase C18 column of 150 x 4.6 mm and 0.8% *m*-phosphoric acid at a flow rate of 0.5 ml/min and L-ascorbic acid was used as a standard.

2.6. Determination of β-Carotene

β-Carotene was extracted by acetone, partitioned with petroleum spirit, and isolated using an open column chromatography of 30 X 2.54 cm packed with silica gel (mesh 200-400) and eluted with petroleum spirit [20]. Absorbance was read at 450 nm and results calculated using the standard curve of β-carotene solutions.

2.7. Determination of Soluble Sugars

Soluble sugars were extracted using the method of Osborne and Voogt [21]. About 10 g of sample were refluxed with 50 ml of 96% (v/v) ethanol for 1 hr at 100ºC. The extract was filtered and the residue washed with 80% v/v ethanol. The filtrate was evaporated to dryness in a rotary vacuum evaporator at 60ºC and the dry residue was re-constituted with 10 ml distilled water and defatted using diethyl ether. Sample was micro-filtered with 0.45 µm filter and 20 µl injected into the HPLC (Shimadzu LC-10A) equipped with a refractive index detector (Shimadzu RID-6A). The mobile phase consisted of acetonitrile:water (80:20) at a flow rate of 0.5 ml/min on NH_2 column of 250 x 4.6 mm.

2.8. Determination of Total Polyphenols, Total Flavonoids and Antioxidant Activity

2.8.1. Determination of Total Polyphenols

Total polyphenol content was determined by the method of Waterman and Mole [22], where 10 mg of ground sample were extracted using aqueous 50% methanol at 80°C for 1 hr. One milliliter of the extract was reacted with Follin Ciocalteau reagent and the absorbance was read at 760 nm against gallic acid as a standard.

2.8.2. Sample Extraction for Flavonoids and Antioxidants

The method by Harborne [23] was applied for sample extraction for analysis of flavonoids and antioxidant activity. Five grams of ground sample were weighed into 250 ml flasks and 100 ml of methanol added. The flasks were closed with parafilm, covered with aluminum foil and shaken for 3 hr. The samples were kept in the dark to extract for 72 hr. They were then filtered and concentrated to 20 ml, and kept in closed vials until analysis. Working solutions were prepared from these extracts.

2.8.3. Determination of Total Flavonoids

The method according to Jagadish *et al.* [24] was used to determine flavonoid content. In a 10 ml volumetric flask, 4 ml of distilled water and 1 ml of plant extract were added. After 3 min, 0.3 ml of 5% sodium nitrite solution were added and left to stand for 3 min, after which 0.3 ml of 10% aluminum chloride was added and held for 5 min. Two milliliters of 1 M NaOH were added and the volume was made up to 10 ml with distilled water. The absorbance was read at 415 nm using a spectrophotometer. Total flavonoids were calculated from a calibration curve prepared using quercetin as a standard.

2.8.4. Determination of Free Radical Scavenging Activity

The antioxidant activity of the extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined using the method of Molyneux [25]. Concentrations of the extract were prepared in 0.01, 0.1, 1.0, 2.0, 5.0 and 10.0 mg/ml in methanol, and the results were expressed on dry matter basis. L-ascorbic acid was used as a standard at the same concentrations as the extracts. One milliliter each of the extract and the standard was put in a test tube and 3.0 ml of methanol added, followed by 0.5 ml of 1 mM DPPH in methanol. A blank solution was prepared using methanol and DPPH. Absorbance was measured at 517 nm after keeping the mixture in the dark for 30 min. The DPPH radical scavenging activity was calculated using Equation 1 below.

$$DPPH\ radical\ scavenging\ activity = \frac{(Ab - Aa)}{Ab} \times 100 \quad (1)$$

Where Ab is the absorbance of the reference (blank) and Aa is the absorbance of the sample.

2.9. Measurement of Color

Color was measured using a color spectrophotometer NF 333 (Nippon Denshoku, Japan) using the CIE L*a*b* color scale. The color of the sample was measured and expressed in terms of Hunter's L* (lightness), a* (redness/greenish) and b* (yellowness/bluish). Hue angle and chroma values were computed from a* and b* values.

2.10. Data Analysis

All samples were analyzed at least in triplicates and data presented as mean ± standard deviation ($n \geq 3$). The means were subjected to simple ANOVA for significance test ($p<0.05$) using SPSS version 17.0.

3. Results and Discussions

3.1. Proximate Composition

The proximate composition was determined for the upper and the lower portions of the shoot and is shown in Table 1 below. There was no significant difference ($p<0.05$) in moisture, protein, fat and ash content between the two portions. Analysis of similar species from three different sites in Ethiopia revealed moisture content of 91.9-93.3%, protein content of 25.9-38.9%, fat content of 0.64-1.50% and ash content of 14.2-17.1% on dry weight basis [4]. These values compare well with the findings of this study even though their focus was not in different sections of the shoot. However, fiber and carbohydrates contents were found to have significant difference between the two portions ($p<0.05$), where fiber content in the upper and the lower portions were 23.9 and 30.7% dwb, respectively. Higher fiber content observed in the lower portion was due to toughening near the base as the culm develops for structural support [26].

Table 1. Proximate Composition of Upper and Lower Portions Expressed in % dwb

Parameter	Upper portion	Lower portion
Moisture *	92.2±0.3a	92.4±0.2a
Protein	33.4±3.0a	33.0±1.3a
Fat	2.0±0.2a	2.0±0.1a
Ash	17.1±0.1a	17.0±0.0a
Fiber	23.9±0.2b	30.7±0.2a
Carbohydrates	23.6±3.1b	17.3±1.3a

Data are presented as mean ± SD (n=3). Mean values within each row followed by different letters differ significantly at p<0.05.
*Fresh weight basis.

3.2. Mineral Composition

The shoots were found to contain high ash content of up to 17% in both the upper and the lower portions as shown in Table 1 above. However, a comparison of specific minerals contained in the upper and the lower portions, as shown in Table 2, indicates presence of significant differences (p<0.05) for all except iron.

Table 2. Composition of Minerals in Upper and Lower Portions Expressed in µg/g dwb

Type of Mineral	Upper portion	Lower portion
Calcium	2,670±40^a	1,060±10^b
Magnesium	4,300±420^a	1,270.6±70.6^b
Potassium	35,900±2,000^a	27,600±1,200^b
Phosphorus	7,630±170^a	4,810±10^b
Zinc	52.1±2.7^a	38.1±2.4^b
Manganese	29.8±1.5^a	20.3±0.8^b
Iron	26.2±2.2^a	22.2±1.6^a
Copper	12.3±0.7^a	3.7±0.3^b

Data are presented as mean ± SD (n=3). Mean values within each row followed by different letters differ significantly at p<0.05.

The results show that the upper portion contains large quantities of all major minerals and these mineral contents reduce towards the lower part of the shoot. The quantities of calcium, magnesium and copper in the upper portion were more than double the content in the lower part. Potassium, which is the major mineral in the shoot, was found to be about 1.3 times higher in the upper portion than in the lower. Therefore, by selecting the right portion of the shoot during harvesting time, the shoot can help to boost the intake of minerals towards meeting the recommended daily allowance (RDA) for calcium, magnesium and potassium, which are 1,000 mg, 350 mg, and 2,000-6,000 mg, respectively [27]. Potassium is a vital electrolyte charged with maintaining proper flow of water inside and outside body cells and is important particularly for athletes. This high amount of potassium may also provide protective effect against hypertension [28].

3.3. Vitamin Composition

Some vitamins found in the shoots of *Y. alpina* are shown in Table 3 below.

Significant difference (p<0.05) was noted in the content of thiamine, vitamin C and β-carotene. The upper portion contained more thiamine and vitamin C, while β-carotene was significantly more in the lower. It is thus more beneficial to consume the upper part of the shoot. Sood *et al.* [14] reported vitamin C content of 5.3 mg/100g in fresh bamboo samples. The value obtained in our study is lower than reported by Sood *et al.* [14] because the samples were dried before analysis and therefore, some heat labile vitamins such as vitamin C may have been degraded.

Table 3. Comparison of Vitamins in Upper and Lower Portions Expressed in µg/g dwb

Parameter	Upper portion	Lower portion
Thiamine	2.2±0.2^a	1.8±0.1^b
Riboflavin	8.4±0.2^a	7.3±0.7^a
Vitamin C	78.2±3.8^a	51.2±2.4^b
β-Carotene	9.9±0.3^a	15.7±1.2^b

Data are presented as mean ± SD (n=3). Mean values within each row followed by different letters differ significantly at p<0.05.

3.4. Sugar Content

Three sugars namely, fructose, glucose and sucrose were found in the shoots as shown in Table 4 below. Fructose was found to be the dominant sugar in the upper portion and varied significantly from 2.19% to 0.62% in the lower (p<0.05). However, sucrose content was not significantly different between the two portions.

Table 4. Comparison of Sugars in Upper and Lower Portions Expressed in % dwb

Parameter	Upper portion	Lower portion
Fructose	2.19±0.19^a	0.62±0.06^b
Glucose	0.65±0.10^b	0.77±0.08^a
Sucrose	0.68±0.06^a	0.58±0.06^a

Data are presented as mean ± SD (n=3). Mean values within each row followed by different letters differ significantly at p<0.05.

Other researchers found significant difference in sugar content between four portions of shoots measured from the apex to the basal part of *Phyllostachys pubescens* Mazel, where higher glucose, fructose and total sugars were found at the basal part of the shoots underground [15]. This report shows the opposite of our findings, where fructose was found to be higher in the upper portion. However, our results agree with their observation that there was no significant difference in sucrose content in different portions of the shoots, which had just emerged from the ground.

3.5. Total Polyphenols, Flavonoids and Antioxidant Activity Content

The total polyphenol and flavonoid contents were determined and results are shown in Table 5 below. There was no significant difference in total polyphenol content between the upper and the lower portions. However, the total flavonoid content varied significantly (p<0.05) between the two portions, where the upper portion contained 24.6 mg/g dwb compared to 20.1 mg/g dwb in the lower part. It was also observed that the upper portion had higher antioxidant activity with LC_{50} of 1.0 mg/ml compared to LC_{50} of 5.0 mg/ml in the lower portion as shown in Figure 1 below.

This high inhibition against the DPPH radical may be due to synergic effect caused by higher vitamin C, polyphenol and flavonoid contents observed in the upper portion of the shoot.

Table 5. Total Polyphenol and Flavonoid Contents Expressed in mg/g dwb

Parameter	Upper portion	Lower portion
Polyphenol*	27.6±1.4[a]	25.9±0.4[a]
Flavonoid**	24.6±2.0[a]	20.1±1.5[b]

Data are presented as mean ± SD (n=3). Mean values within each row followed by different letters differ significantly at p<0.05.
*Gallic acid equivalent; ** Quercetin equivalent.

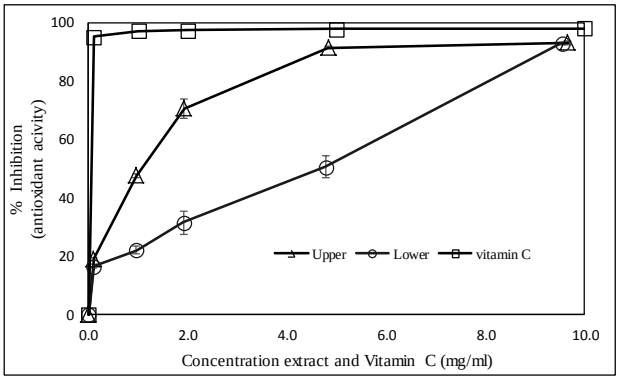

Figure 1. Antioxidant activity of the extracts of the upper and the lower portions of *Yushania alpina* expressed in dry weight basis

3.6. Color Variation within the Shoot

The Lab Hunter's values were applied for color measurement. The L* value measures the lightness or brightness of a substance and ranges from 0 for black surface to 100 for white. Hue angle indicates whether the object is red, orange, green, blue or violet and ranges from 0 to 359°, whereas chroma or saturation value is a measure of color intensity and defines a range from pure color (100%) to gray color (0%) at a constant lightness [29]. The results for color variation are shown in Table 6 below. Significant difference between the upper and the lower portions (p<0.05) was noted for L*, b*, Hue angle and chroma values. The upper portion with L* value of 82.1 was therefore, found to be brighter than the lower portion. Negative values of a* and positive values of b* were also observed, indicating that the color was in the yellow-greenish region, which was confirmed by the value of Hue angle. The chroma value shows that greenish-yellow color was stronger in the lower portion than the upper. This means that the color near the tip is more whitish than the lower part. These results are consistent with the fact that the lower portion was found to contain more β-carotene than the upper, and thus influencing the apparent color.

Table 6. Color Variation

Parameter	Upper portion	Lower portion
L*	82.1±2.1[a]	74.6±2.7[b]
a*	-1.34±0.12[a]	-1.35±0.11[a]
b*	14.0±1.4[b]	22.1±2.0[a]
Hue angle	66.4±0.7[b]	68.4±0.4[a]
Chroma value	14.1±1.4[b]	21.9±2.2[a]

Data are presented as mean ± SD (n=3). Mean values within each row followed by different letters differ significantly at p<0.05.

3.7. Conclusion

The upper portion of the shoot of *Y. alpina* was found to contain less fiber and higher carbohydrates, minerals, sugars and vitamins than the lower. It also had higher polyphenols and flavonoids, and exhibited higher antioxidant activity than the lower. The color of the upper portion was brighter than the lower part, and this implies that whiter shoots are potentially rich in nutrients and superior to the greenish ones. Furthermore, the result of the present study provides important baseline information on *Y. alpina* as valuable vegetable source, as well as potential nutrient supplement.

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