Nutritional Qualities and Phytochemical Constituents of Two Neglected Wild Edible Leafy Vegetables in Akwa Ibom State, Nigeria

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Received: 13 December 2017**; Accepted:** 27 December 2017

Abstract:

Domesticated and non-domesticated green leafy vegetables have numerous dietary and health benefits. Investigations on the nutritional qualities and phytochemical constituents of two neglected wild edible leafy vegetables [*Microdesmis puberula* (*Mp*) and *Bombax buonopozense* (*Bb*)] in Akwa Ibom State were carried-out. The neutraceutical investigations revealed that the vegetables contained bioactive compounds comprising alkaloids (5.824 and 7.23 mg/100g), saponins (1.08 and 1.94 mg/100g), cardiac glycosides (7.48 and 7.96 mg/100g), terpenes (6.20 and 5.27 mg/100g) in *Mp* and *Bb* respectively and tannins (1.05 mg/100g) and flavonoids (4.52 mg/100g) in *Bb*. The bioactive compounds were found in abundance and were significant (p=0.05). Anthraquinones and phlobatannins were not detected in any of the vegetables. *Microdesmis puberula* and *Bombax buonopozense* revealed high nutrient qualities in crude proteins, crude fibre, carbohydrates and caloric value with relatively low concentrations in crude lipid, ash and moisture content. The minerals profile; Fe, Zn, Ca, K, Mg and P of the leafy vegetables were high and fell within the permissible limit but significant (p=0.05) increase were noted in Mg, Fe and P (*Mp*) and K, Fe and (*Bb*). Vitamins A, B_1, B_2 and C were significantly (p=0.05). Also, the vegetables contained minute anti-nutrients which ranged as oxalate (0.003 and 0.00 mg/100g), phytate (0.45 and 0.81 mg/100g), tannic acid (0.00 and 1.05 mg/100g) cyanide (0.00 and 0.005 mg/100g) and saponins (1.08 and 1.94 mg/100g) *Mp* and *Bb* respectively. All neutraceutical qualities tested fell within the FAO/WHO reference daily intake. The two vegetables are neglected species though they have been described as valuable on account of nutrients, minerals and secondary metabolites they contain. Based on their neutraceuticals status, the consumption of these important food supplements should be encouraged and popularized as these could be beneficial resources for prevention, management and treatment of diseases, food security and improve the nutritional quality of human diet.

Keywords:
Anti-Nutrients, *Bombax Buonopozense*, *Microdesmis Puberula*, Minerals, Neutraceutical Status, Phytochemicals

1. Introduction

Domesticated and non-domesticated green leafy vegetables (GLVs) have numerous dietary and health benefits. They are inexpensive, easy to cook and are rich sources of macronutrients, micronutrients, carotenoids and vitamins [1]. Regular consumption of vegetables is also recommended for better health and management of chronic diseases such as cardiovascular complications, diabetes and cancer [2]. Research has shown that indigenous communities in East Africa have dietary additives of wild plant materials that contain antioxidants to degrade cholesterol from their traditional foods of meat, milk and blood [3]. They also pointed out that higher intake of GLVs may increase ingestion of anti-nutrients that impair bioavailability of nutrients such as tannins, phytates and oxalates or directly cause illnesses (or fatality) such as haemolysis and even death due to excessive ingestion of lectins [2]. Despite the actual and potential benefits, research into the GLVs has to a large extent not been a priority concern in some parts of Nigeria. GLVs are yet to emerge as an area of focus in the development sector. The ever widening gap between population growth and production of conventional crops, recurrent food deficits, and higher prevalence of macronutrient and micronutrient malnutrition with increasing cases of chronic diseases make diversification of food sources a worthwhile endeavour. Aspirations of developing countries to create healthy, productive food and nutrition secured communities could come true through effective application of indigenous knowledge [4].

Vegetables play an important role in the maintenance of good health as source of nutrients, which are usually in short supply in daily diets [5]. The nutrients in vegetables can be absorbed and used as regulatory and protective materials, as well as for body building [6]. Vegetables have become significant part of human diets supplying the body with low calories and substantial amounts of necessary minerals and vitamins. The low caloric content of vegetables is of importance; plants produce food in the leaves but do not store food in the leaves. Consequently, vegetables do not supply excess energy to the body. The fibres in vegetables are known to promote digestion and prevent constipation [7]. Fats and oils in vegetables have been reported to lower blood lipids, leading to a reduction in the occurrence of diseases associated with coronary artery failure. Plant materials that possess nutritional and medicinal functional values are referred to as nutraceutical plants [8].

Microdesmis puberula (Euphorbiaceae) is a shrub that grows up to 6 metres tall, though it sometimes becomes a small tree of up to 15 metres tall if not harvested or prematurely cut. The stem is up to 8 cm in diameter. The plant is commonly found in tropical Africa - Nigeria to Central African Republic, Uganda and south to D.R. Congo. *Microdesmis* has its habitat in primary and secondary forests and at forest edges, in fallow land and at elevations from sea-level up to 1,100 metres. The plant can germinate massively in fallow land. It is a dioecious plant, both male and female forms need to be grown if fruit and seed are required [9]. *Microdesmis puberula* is widely used in traditional medicine. The stem, bark, leaves and roots have numerous medicinal uses throughout the plant's distribution area. Very little research has been carried out into the plant although traces of alkaloids have been detected in the stems

and roots [10]. The leaf sap or crushed and burnt twigs and roots, are applied to snakebites or to scarifications. The sap, sometimes together with the twig sap, is commonly taken orally or applied as an enema to treat diarrhoea. It has a rather mild action and is thus prescribed for pregnant women and young children [11].

Figure 1. *Plant showing leaves and fruits of Microdesmis puberula Hook. F. ex Planch.*

Bombax buonopozense belongs to the family Bombacaceae. It is a large tropical tree that grows to 40 m in height with large buttress roots that can spread to 6 m. The bark is covered with large conical spines, especially when young, shedding them with age to some degree. The branches are arranged in whorls; the leaves are compound and have 5-9 leaflets and 5-25 secondary veins. The individual leaflets have entire margins and are also large. The under-side of the leaflets may be glabrous or puberclous. It is widely distributed in Africa, from Ghana to Sierra Leone, Uganda, Gabon and Nigeria [12]. The young leaves and shoots are eaten as vegetable. The flowers are also eaten in soups. The tree is decorative for its shape and red flowers and planted at village squares and city centers. The leaves are good fodder for goats. A red-brown dye from the bark is used in colouring clothing materials and other fibres. Drinks are made from the immature fruits. The timber is grayish white, soft but strong and used to make plywood, veneers, doors, stools, boxes, turnings, carving and handicrafts [13]. It is an important tree in fetish grove as it is venerated by traditional religionists. Sacrifices are usually performed at its base for the release of those afflicted by wizardry [14]. However, the chemical, nutritional and toxicological properties as well as the nutrients present in them still need to be properly established and documented before their use as alternative dietary sources can be advocated.

Figure 2. *Plant showing leaves of Bombax buonopozense P. Beauv.*

2. Materials and Methods

2.1. Collection of Plant Materials and Authentication

Fresh leaves of *Microdesmis puberula* and *Bombax buonopozense* were collected from Mbak Etoi in Uyo Local Government Area in Akwa Ibom State, Nigeria on November 25th, 2015 by the corresponding author, of the Department of Biological Science, Akwa Ibom State University, Nigeria. The plants were identified and authenticated by a Plant Taxonomists. Voucher specimens were deposited at the herbarium of the Botany and Ecological Studies Department, University of Uyo, Uyo.

2.2. Preparation of Plant Extract

The leaves were washed, shade–dried and extracted with 70% ethanol (v/v) by cold extraction for 72 hours. The filtrate was evaporated to dryness by heating in a water bath at 40^0C to yield of semi-dry extract with black colour. This was re-suspended in 250 ml distilled water and stored in 100 ml beaker in a refrigerator until ready for use in phytochemical screening [15].

2.3. Qualitative and Quantitative Phytochemical Screening

This experiment was performed in the Postgraduate Laboratory, Department of Pharmacognosy and Natural Medicine, University of Uyo, Akwa Ibom State. Chemical tests were carried-out on the ethanol extract using standard procedures to identify the secondary metabolites as described by [15], [16].

2.4. Determination of Nutrients, Minerals and Vitamins Compositions

The samples were analyzed chemically for moisture content, crude protein, fat (lipids), crude fibre, carbohydrate and ash content according to the methods of [17]. Tannin, oxalate, phytate and cyanide, vitamins A, B_1, B_2 and C were determined according to the methods of [17], [18]. The minerals such as potassium (K), Magnesium (Mg), calcium (Ca), phosphorus (P), Zinc (Zn) and Iron (Fe) were determined by the atomic absorption spectrophotometer (UNICAM 939 AAS) [17]. The analyses were carried-out in triplicates.

2.5. Statistical Analysis

Data obtained were processed, summarized and expressed as mean ± standard error of the mean of three replicates and was subjected to statistical analysis using two-way analysis of variance (ANOVA) and student' t-test using SPSS version 17.0. Probability limit was set at 95% level of significance (p=0.05) as described by [19], [20].

3. Results

The qualitative phytochemical screening of the two neglected leafy vegetables revealed the presence of alkaloids, saponins, cardiac glycosides and terpenes in both plants. Tannins and flavonoids were detected in *Bombax buonopozense*. Anthraquinones and phlobatannins were not detected in both vegetables. Tannins and flavonoids were not detected in *Microdesmis puberula* (Table 1). The highest concentration of quantitative phytochemical was recorded cardiac glycosides in *Bombax buonopozense* (7.96±0.46 mg/100g) while the lowest was tannins (1.05±0.62 mg/100g) in the same plant (Tables 2).

Table 1. *Phytochemical screening of Microdesmis puberula and Bombax buonopozense ethanolic leaves extract.*

TEST	*Microdesmis puberula*	*Bombax buonopozense*
ALKALOIDS		
Dragendoff's test	++	+
Mayer'stest	+++	++
Picricacidtest	+	++
SAPONNINS		
Frothingtest	+	+
Fehlingtest	+	+
TANNINS		
Ferricchloridetest	ND	+
Brominewatertest	ND	+
FLAVONOIDS		
Shinoda's test	ND	++
CARDIACGLYCOSIDES		
Lieberman'stest	+++	++
Keller Kiliani's test	+++	++
Salkowski's test	+++	+
TERPENES		
Lieberman's Burchard's test	+++	+++
ANTHRAQUINONES		
Free anthraquinones	ND	ND
Combined anthraquinones	ND	ND

Legend:-=Absent,+=Trace,+++=Moderate,++++=Abundance,ND=NotDetected

Table 2. *Quantitative Phytochemical Screening of Neglected Edible Leafy Vegetables(mg/100g).*

Names of Compound	*Microdesmis puberula*	*Bombax buonopozense*
Alkaloids	5.84 ± 0.63^{a}	7.23 ± 0.55^{a}
Tannins	ND	1.05 ± 0.62^{b}
Saponins	1.08 ± 0.16^{b}	1.94 ± 0.00^{b}
Flavonoids	ND	4.52 ± 0.27^{c}
Cardiacglycosides	7.48 ± 0.68^{c}	7.96 ± 0.46^{d}
Terpenes	6.20 ± 0.01^{a}	5.27 ± 0.92^{e}

Results are means of five determinations of dry weight basis ± Standard Error, ND = Not Detected

Mineral contents in *Microdesmis puberula* were Ca (8.13±0.03), Mg (5.82±0.00), K (9.90±0.05), Fe (13.83±0.01), P (6.24±0.51) and Zn (0.24±0.11 mg/kg) while *Bombax buonopozense* recorded Ca (6.63±0.02), Mg (5.91±2.06), K (9.02±0.00), Fe (13.83±0.0), P (6.24±0.51) and Zn (0.10±0.00 mg/kg). Among all the minerals, Zn concentrations were very low in both plants (Table 3). Proximate compositions of the vegetables ranged between 2.60+1.51 – 50.90+0.05%, recorded in crude protein and carbohydrate being the lowest and highest respectively (Table 4).

Table 3. *Mineral Contents of the Neglected Edible Leafy Vegetables (mg/kg).*

Elements	*Microdesmis puberula*	*Bombax buonopozense*
Ca	8.13 ± 0.03^{a}	6.63 ± 0.02^{a}
Mg	5.82 ± 0.00^{b}	5.91 ± 2.06^{a}
K	9.90 ± 0.05^{a}	9.02 ± 0.00^{b}
Fe	13.83 ± 0.01^{c}	13.83 ± 0.01^{c}
P	6.24 ± 0.51^{d}	6.24 ± 0.51^{a}
Zn	0.24 ± 0.11^{e}	0.10 ± 0.00^{d}

Results are means of five determinations of dry weight basis ± Standard Error, [a-e]Means in the same row with different superscripts are significantly different (p=0.05)

Table 4. *Nutrients Composition of the Neglected Edible Leafy Vegetables.*

Nutrients(%)	*Microdesmis puberula*	*Bombax buonopozense*
CrudeProtein	12.70±0.10[a]	10.30±0.44[a]
CrudeLipid	2.60±1.51[b]	5.70±0.16[b]
Ash	5.10±0.30[b]	4.80±0.8[b]
Crude Fibre	25.30±0.66[c]	29.90±0.05[c]
CHO	50.90±0.05[d]	44.10±0.12[c]
MoistureContent	3.40±0.48[b]	5.20±0.72[b]
CaloricValue(Kcal)	352.03±0.17[e]	374.30±0.05[d]

Results are means of five determinations of dry weight basis ± Standard Error, [a-e]Means in the same row with different superscripts are significantly different (p=0.05)

Anti-nutrients concentrations were very low and recorded thus: Oxalate (0.003), phytate (0.45), tannic acid (0.00), cyanide (0.00) and saponins (1.08 mg/100g) in *Microdesmis puberula* while *Bombax buonopozense* showed: oxalate (0.00), phytate (0.81), tannic acid (1.05), cyanide (0.005) and saponins (1.94 mg/100g) Figure 3.

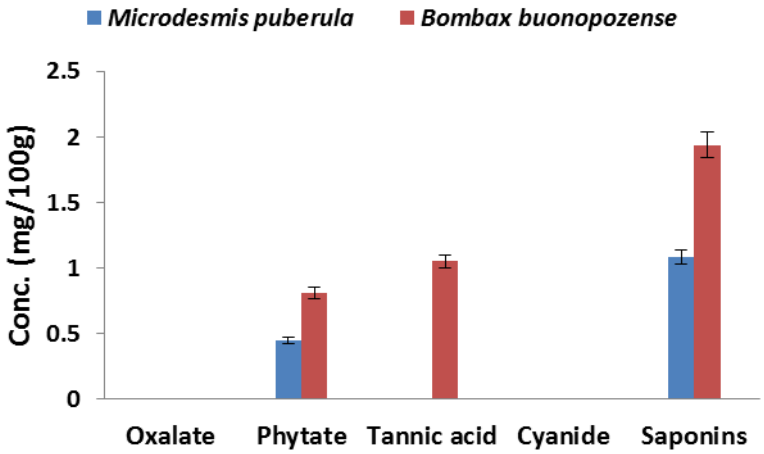

Figure 3. *Quantitative anti-nutrients composition of neglected edible leafy vegetables.*

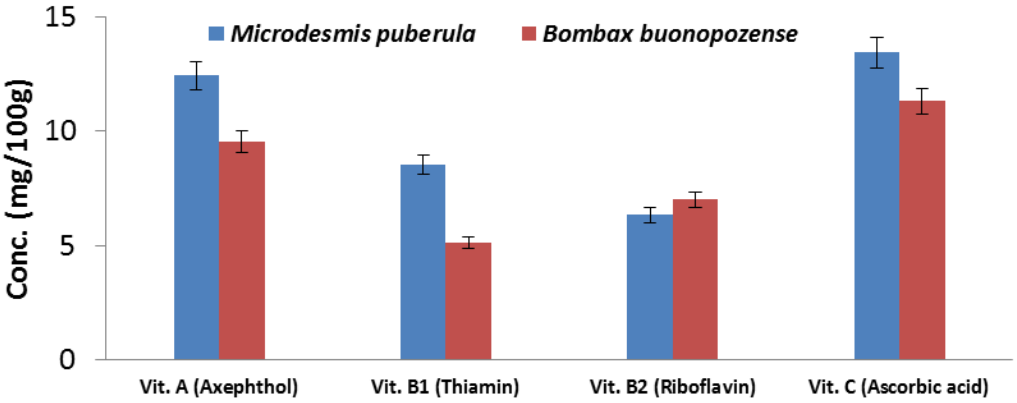

Figure 4. *Quantitative vitamin composition of neglected edible leafy vegetables.*

Vitamin constituents of the two neglected edible leafy vegetables are summarised in Figure 2. The figures showed that the vegetables were relatively rich in vitamin A

(Axerphthol) and vitamin C (Ascorbic acid). Vitamin B_1 (thiamin) and vitamin B_2 (Riboflavin) concentrations were moderate. In vitamin A, *Microdesmis puberula* had the highest concentration (12.44 mg/100ml) while *Bombax buonopozense* had 9.54 mg/100g. Vitamin B_1 concentrations were 8.54 mg/100g and 5.10 mg/100g in *Microdesmis puberula* and *Bombax buonopozense* respectively. Vitamin B_2 was observed as: *Microdesmis puberula* (6.33 mg/100g) and *Bombax buonopozense* (7.01 mg/100g). Vitamin C composition in the vegetables showed 13.45 mg/100g (*Microdesmis puberula*) and 11.32 mg/100g (*Bombax buonopozense*) Figure 4.

3. Discussion

The qualitative and quantitative phytochemical analyses of the edible leafy vegetables showed that they contained bioactive compounds. The concentration of these plant chemicals varied significantly (p=0.05) among the vegetables. Alkaloids, cardiac glycosides and terpenes were found in abundance while flavonoids, tannins and saponins were found in trace amount. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic ailments has been reported [21].

The medicinal value of plants lies in their inherent chemical substances which have a definite physiological action on the human body [22]. Different phytochemicals have been found to possess a wide range of activities, which may help in protection or prevention against chronic diseases. For example, alkaloids are known to protect against chronic diseases (diabetes and liver infections). Saponins protect the body against hypercholesterolemia and possess antibiotic properties while terpenes show analgesic properties [23]. Tannins, flavonoids and cardiac glycosides in *Peristrophe bicalculata* have the potency to promote haemopoietic indices and to restore the lost blood during excessive bleeding [24]. Similar reports in other plant species were obtained from *Morinde citrotolia* and *Digitalis pupurea* [16], [25]. They reported the presence of cardiac glycosides in these plants and the cardiac glycosides can be used in the treatment of diseases associated with the heart. These plants are currently used by herbalists, to treat tumour [26]. Naturally occurring alkaloids and their synthetic derivatives have analgesic, antispasmodic and bactericidal activities [27]. The presence of these phytochemicals in the vegetables may be partly responsible for their medicinal properties.

The mineral contents of Ca, Mg, K, Fe and P were moderate except Zn which shows trace amount. Their concentrations were significant (P=0.05). Potassium plays an important role in the regulation of acid base balance in the cell, water retention and is essential for protein biosynthesis by ribosomes. Calcium is one of the minerals present in the structure of the body and in bones. It is necessary for the normal functioning of cardiac muscles, blood coagulation and milk clotting, and the regulation of cell permeability [28]. It also plays an important part in nerve-impulse transmission and in the mechanism of neuromuscular system [29]. Phosphorus is an essential component of bone mineral. Phosphorus is a constituent of cytoplasm and nuclear protein, phospholipids and nucleic acids, as well as taking important part in carbohydrate metabolism. Phosphorus plays a vital role in normal kidney functioning and transfer of nerve impulse. Deficiency of phosphorus-calcium balance results in osteoporosis, arthritis, pyorrhea, rickets and tooth decay. Calcium and phosphorus are associated with each other for growth and maintenance of bones, teeth and muscles [30]. The calcium level in the leaves studied compares favourably with the value reported in some green leafy vegetables consumed in Nigeria (Ca: 6.41-8.02 mg/kg)

and some wild edible leaves grown in Eastern Anatolia (Ca: 5.33-11.16 mg/kg). For good Ca to P intestinal absorption, Ca/P ratio should be close to unity. The values recorded in this study meet this requirement and as such predicates good Ca to P intestinal absorption [30]. Magnesium is involved in over 300 enzymatic reactions in the body [31], including glycolysis, the Kreb cycle, creatinine phosphate formation, nucleic acid synthesis, amino acid activation, cardiac and smooth muscle contractibility, cyclic *adenosine monophosphate* (cAMP) formation and protein synthesis [28]. Magnesium is also important in the proper utilization of vitamins B and E and functions with other minerals such as calcium, sodium and potassium in maintaining fluid and electrolyte balance [32]. Magnesium is a component of chlorophyll and it is required in the plasma membrane and extracellular fluid, where it helps maintain osmotic equilibrium [33]. Zinc is an essential micronutrient for human growth involved in normal function of the immune system [34]. An estimated 20% of the world population is reported to be at risk of inadequate zinc intake [35]. Studies in Nigerians shows that zinc deficiency affects 20% of children less than five years; 28.1% of mothers and 43.9% of pregnant women [36]. Zinc is very useful in protein synthesis, cellular differentiation and replication, immunity and sexual functions i.e. male and female fertility [37]. Iron is essential trace element for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins, fats and cytochromes [38]. The deficiency of iron has been described as the most prevalent nutritional deficiency and iron deficiency anaemia is estimated to affect more than one billion people worldwide [39]. The high content of minerals recorded in this study could also serve the same purpose in the human system.

Microdesmis puberula and *Bombax buonopozense* had moderate percentage of moisture content and were significant (p=0.05). The values of moisture for the vegetables corroborated with results of [40], [41]. The high water content of African leafy vegetables (ALVs) when eaten raw helps the body as the body does not need to use some of its own water to digest them. This means that the body uses less energy and resources to digest the greens and can then assimilate all the nutrients of the vegetables much faster. In addition, water is needed to separate (by a process called hydrolysis) a phosphate group from adenosine triphosphate (ATP) or guanosine triphosphate (GTP) to get energy [42]. The vegetables in this study contained high amount of crude protein. [43] reported that the leaves of *Moringa oleifera* had high crude protein which indicated that the vegetables can be used for building and repairing of body tissues, regulation of body process and formation of enzyme and hormones. Proteins also aid in the formation of anti-bodies that enable the body to fight infection. It was also reported that 30g of drumstick leaf powder can cover one third of the daily allowance for protein [44]. The neglected leafy vegetables contained moderate amount of crude lipid. Lipids play a very important role in the human body and help in brain function, joint mobilization and even energy production [6]. Significant amount of caloric energy were recorded. The values were in line with work of [45] who reported that diet providing 1-2% of its caloric energy as fat is said to be sufficient to human beings, as excess fat consumption yields to certain cardiovascular disorders such as atherosclerosis, cancer and aging.

The crude fibre contents of the leaves were moderate in *Microdesmis puberula* and were higher in *Bombax buonopozense*. These results agreed with the report of [46] stated that intake of fibres can lower the serum cholesterol level; prevent the risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer.

Thus these vegetables could be valuable sources of fibre in human nutrition. The carbohydrate contents of the vegetables were in moderate amounts. These values compared favourably with values reported for *Momordica balsamina* [47]. These vegetables might be a source of energy to vegetarians. Carbohydrates are pivotal nutrients required for adequate diet [48]. Their prime role is to produce energy required for the smooth functioning of the body.

Oxalates were found in trace amounts in the leafy vegetables. Toxicity of oxalates for humans is set at 2-5 g/day and the consumption of diet high in this anti-nutrient may result in kidney disease [49], [47]. The results of this study suggest that the consumption of large amounts of the fresh leaves of the test plants might have no adverse effect on human health. Phytate concentrations in the leafy vegetables were relatively low as compared with the recommended value (0.003 mg/100g) as reported by [50]. Phytate is anti-nutritional factor which is present in various fruits and vegetables, with high concentrations known to reduce mineral bioavailability in foods. Cyanide was detected in *Bombax buonopozense* in trace amounts. Excessive ingestion of hydrogen cyanide can be very poisonous as it interferes with electron flow in the mitochondria-electron transport chain, thereby inhibiting energy generation [51].

The concentrations of vitamins A, B1, B2 and C were significantly ($p=0.05$) high. These values compared favorably with reference daily intake (3.81-16.53 mg/100ml) reported by [52]. Vitamin A is important for normal vision, gene expression, growth and immune function by its maintenance of epithelial cell functions. In plants, vitamin A occurs in the form of provitamin A carotenoids which amount determines their bioavailability in human diet [53]. Vitamin B_1 maintains good appetite and aids in normal digestion; thiamine is an essential co-enzyme for the decarboxylation of pyruvate to acetyl CoA, in tricarboxylic acid (Krebs) cycle. Thiamine pyrophosphate (TPP) is the co-enzyme for transketolase on the hexose monophosphate shunt pathway and for decarboxylation of α-ketoglutarate to succinate in the Krebs cycle [28]. Vitamin B_2 (Riboflavin) functions as a co-enzyme in tissue oxidation. It serves as a co-enzyme in respiration and also involved in the metabolism of protein, fat and carbohydrate [28], [54]. The green leafy vegetables were rich in ascorbic acid as revealed by the highest value recorded in *Microdesmis puberula*, which indicated that they were vital for body performance. Vitamin C is a powerful anti-oxidant essential for healthy formation of bone and teeth [55].

4. Conclusions

This investigation has revealed that the two leafy vegetables *Microdesmis puberula* and *Bombax buonopozense* are rich in phytonutrients. These properties in addition to its high fibre, protein and ash content make the leaves of these plants suitable for intake as vegetables. Being found in the wild with an ability to highly resist drought, it can provide cheaper source of vegetables as an alternative to common vegetables during the dry season.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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